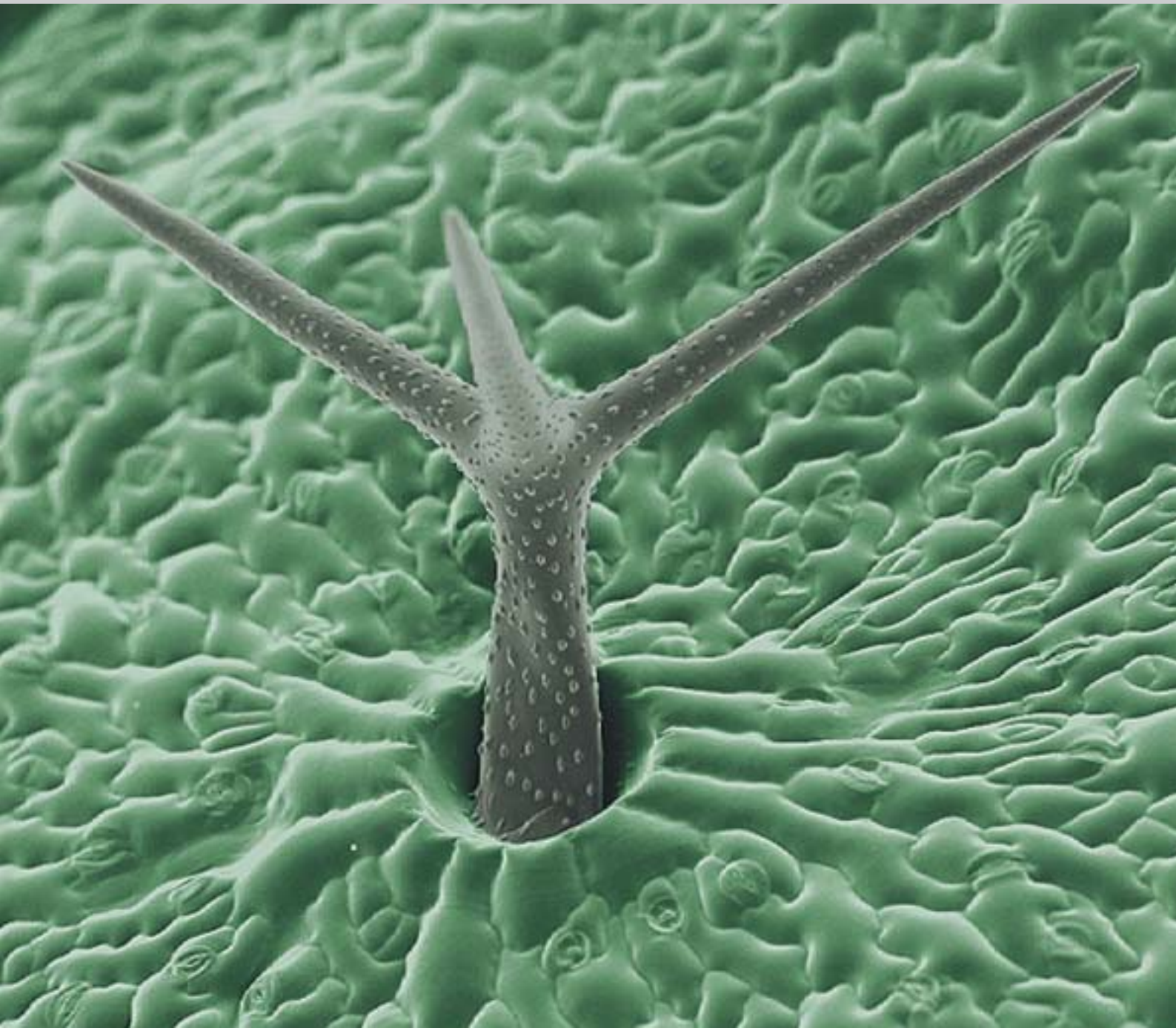
MAX-PLANCK-GESELLSCHAFT

Scientific Report 2003

Max Planck Institute for
Plant Breeding Research
Cologne



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Cover picture:	Arp Schnittger, University of Cologne, with kind help of the REM-Laboratory, Basel, Switzerland Re-colored scanning electron micrograph showing an Arabidopsis leaf hair (trichome) undergoing cell death. Wild-type trichomes do not die, and in the depicted trichome, cell death was induced by misexpressing trichome-specifically the plant homolog of p27Kip1, the cyclin-dependent kinase inhibitor KRP1. This finding opens a new link between cell-cycle and cell-death control in plants.

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Introduction

This year, the Max Planck Institute for Plant Breeding (MPIZ) is celebrating its 75th anniversary. The Institute was originally founded as the Kaiser-Wilhelm-Institute für Züchtungsforschung in Müncheberg with Erwin Baur as its first director. Even at the time of its foundation, core activities were already focussed on plant genetics with model plants, e.g. the genetics of the flowering plant *Antirrhinum majus*. In 1955, the Institute moved to its current location and became part of the Max Planck Society (MPG). At this time, major activities were still dedicated to classical breeding. In 1978, Jozef Schell was appointed as head of a department and with him molecular biology and molecular genetics rapidly evolved as powerful tools for the dissection of fundamental plant processes. Clearly, the discovery of plant transformation by Agrobacterium and the modification of the process for plant breeding remains an outstanding achievement of Jozef Schell and his colleagues. It serves as a convincing example as to how basic research can have a major impact on applied plant breeding.

Plant biology has undergone a revolution over the last 10 years. The study of fundamental problems in model species using genetic approaches and the ability to isolate genes marked by mutations has provided information on molecular building blocks that control many plant processes. Large-scale genome projects culminating in the sequencing of the complete Arabidopsis genome provided a catalogue of the 25,000 genes required to make a plant and presented a dramatic picture of how different the gene complements of plants and animals are. Systematic analysis of gene function and the interactions between genes and the proteins they encode promise another leap forward in our understanding of plant biology. Furthermore, developments in information technology and relational databases enable recognition of novel connections between different molecular layers including DNA sequences, gene expression, protein and small molecule profiles. However, to date, the tremendous increase in knowledge in plant science has not resulted in a sustained impact on plant breeding, and plant breeding is still far from being a rational science.

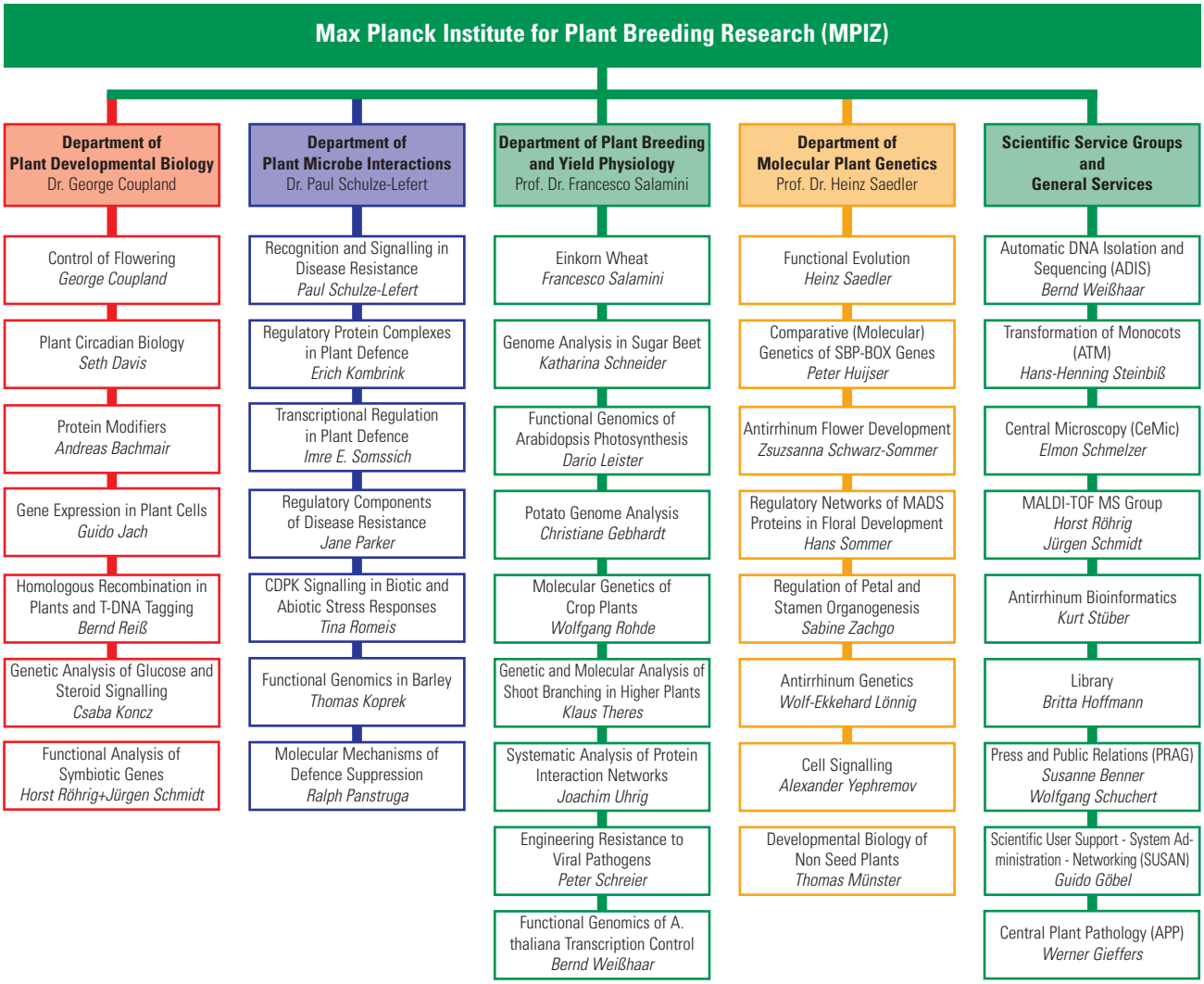
Future discovery oriented plant biology at the MPIZ will utilise integrated approaches to elucidate networks of fundamental biological processes in plants. Multidisciplinary approaches bridging genetics, biochemistry, cell biology and bioinformatics will be essential for in depth understanding of the molecular mechanics of traits that are relevant to plant breeding. This approach will allow a multidimensional understanding of selected plant traits allowing intricate questions to be posed: How many components contribute to a particular trait and how are their functions inter-related? How many components within the

network can be used to create variation in the trait? How many of these can be changed without pleiotropic effects? Primary identification and analysis of these pathways will be carried out wherever possible using intensely studied model species, mainly Arabidopsis.

However, striking examples exist in which the activities of pathways established in Arabidopsis are modified in other species to generate radically different phenotypes. To capture and exploit natural genetic diversity that cannot be generated by Mendelian genetics in Arabidopsis, our research programmes include a selected number of other genetically amenable species including crops. Studying such 'Darwinian diversity' will both test the validity of trait models formulated on the basis of a single species, and provide indications of how flux through and topologies of networks can be modified to generate novel variation in the context of breeding programmes. Thus, one aspect of our work is dedicated to gain systematically insights in trait (network) evolution.

While genetic methods have proven invaluable for the dissection of complex plant traits, small molecules can significantly complement this toolbox. Indeed, there is a clear need to develop 'plant pharmacology' as a novel research branch for understanding the molecular mechanics of traits. In principle, it should be possible to identify specific agonists and antagonists from existing chemical compound libraries for virtually any plant process. The MPIZ will initiate future research programmes that aim to develop technologies and bioassays for the identification of pharmacologically active compounds. This could also have practical implications and might offer alternative avenues to modulate plant traits by chemicals.

Prof. Paul Schulze-Lefert, Acting Director



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Department of Plant Developmental Biology

Director: George Coupland

The Department is focused on understanding molecular mechanisms that underlie the flexibility of plant development in response to environmental stimuli. Our research is based on genetics and gene isolation, but increasingly we have used protein biochemistry, cell biology based approaches and comparisons between species to extend our understanding of these processes.

Last year, the Department was strengthened by the appointment of two new group leaders, Seth Davis and Andreas Bachmair. Their interests are respectively in circadian clock function and protein modification, and extend the research programmes of the Department. The circadian clock is an endogenous timing mechanism that controls daily rhythms in gene expression and protein activity, and enables plants to anticipate daily or seasonal changes in their environment. Since the last report, members of the Department have made significant contributions to understanding the mechanisms by which rhythms are generated in plants by studying the roles of proteins required to create these rhythms. Furthermore, one of the processes regulated by this timing mechanism is the control of flowering, which occurs through a circadian clock controlled transcriptional cascade. This cascade is studied extensively in the Department, and recently we have demonstrated the importance of exposure to light in regulating this pathway at the post-transcriptional level by allowing the accumulation of a key protein in the nucleus. Additionally, we have shown the involvement of long-distance, systemic signalling within the pathway.

Protein modifications by covalent attachment of small peptides, such as ubiquitin or the related protein SUMO, play an important role in many plant signalling pathways. We have illustrated that SUMOylation has an important role in regulating flowering, although the SUMO targets responsible for this are unknown. Two groups in the Department have programmes aimed at identifying these SUMO substrates and understanding how SUMO attachment regulates their function. Similarly, the roles of Snf1-related protein kinases in regulating the activity of ubiquitin ligases that attach ubiquitin to substrate proteins and the wider roles of ubiquitination in regulating processes, such as cell death, are studied in two groups.

The model species Arabidopsis is employed in all research groups as the system of choice for gene discovery and for establishing interaction networks. However, there is tremendous variation between plant species in environmental responses, such as the control of flowering-time, and work on Arabidopsis cannot explain all important flowering phenotypes. We have therefore initiated comparative approaches in other species to understand whether the flowering network identified in Arabidopsis is conserved in these species, and if so, how it is modified to generate distinct phenotypes. Flowering of Arabidopsis is triggered by long days and not short days, whereas many species show the reverse response. We have therefore initiated a comparative programme in the classical physiological short-day model plant *Pharbitis nil*. Similarly, Arabidopsis is an annual plant, and we are beginning to use the related species *Arabis alpina* as a model for perennialism. In a related approach, homologues of key Angiosperm flowering-time genes have been isolated in the department from *Physcomitrella patens* to analyse the ancestral functions of these genes.

Recently, the Department has established new technologies to underpin these programmes. We have created a facility for high throughput analysis of dynamic luciferase expression patterns *in planta*, to extend our analysis of circadian clock and light-regulated gene expression. A MALDI-TOF mass spectrometer was purchased and established within the Service Group led by Jürgen Schmidt and Michael John to support our analysis of protein modification by SUMO and ubiquitin. We purchased jointly with the Department of Plant Microbe Interactions a new confocal microscope, which we are using to analyse the dynamic patterns of sub-cellular localisation of regulatory proteins and to describe protein-protein interactions *in vivo*. These new technologies are already having an impact in our research programmes and we expect this to become even more apparent in the next reporting period.

Control of Flowering

George Coupland

PROJECT 1: The Control of Flowering by Photoperiod in Arabidopsis and Pharbitis

Introduction

The transition from vegetative development to flowering is often triggered by environmental cues, enabling plants to synchronise their life cycle with the changing seasons and to adapt to life at particular latitudes. Since the 1920s, photoperiod (or daylength) has been known to be one of the environmental signals that trigger flowering. For over 50 years after the initial discovery of photoperiodism, physiological approaches were used to describe in detail the response of many plants to daylength. We have used forward genetics in Arabidopsis to define a genetic pathway that promotes flowering in response to long days. We have shown that three genes (*GIGANTEA* (*GI*), *CONSTANS* (*CO)* and *FT*) represent an output pathway from the circadian clock that promotes flowering in a daylength-dependent fashion. This daylength dependence is due to accumulation of *FT* transcript only under long days. The activation of *FT* is dependent on *CO* activity, and is proposed to be daylength dependent because circadian-clock control of *CO* transcription causes its mRNA to accumulate late in the day, and post-transcriptional regulation of CO by light leads to its activation only under long-day conditions.

Since the last report, we have extended our analysis of this pathway to study the post-transcriptional regulation of CO using approaches based on biochemistry, confocal microscopy and yeast two hybrid. We have also performed misexpression experiments to identify the cells in which the pathway acts to regulate flowering and to study the molecular interactions between the photoperiod pathway and other flowering-time pathways. In addition, we have initiated a new project in the short-day plant *Pharbitis nil* to study the conservation of the pathway in species showing different responses to daylength.

New Results

Post-transcriptional regulation of *CO* by light

We have devised a novel transgenic system to follow the post-transcriptional regulation of *CO* indirectly by following the activity of a reporter of *FT* expression. This utilises a fusion of the 35S promoter to *CO* (*35S::CO*), to exclude the circadian clock regulation of *CO* transcription, and a fusion of the *FT* promoter to the luciferase marker gene. In these plants the *CO* transcript is present throughout the normal daily light/dark cycle, but luminescence associated with luciferase activity is only detected when plants are exposed to light. This illustrates the post-transcriptional regulation of *CO* that is triggered by light, and occurs when plants are exposed to far-red and blue light, but not when plants are exposed to darkness or red light. We have tested which photoreceptors are required for this activation of CO by introducing mutations that impair photoreceptor function into the *35S::CO* background. Inactivating the blue light receptors cryptochrome 1 and cryptochrome 2 prevents the activation of *FT* expression in blue light, whereas inactivating phytochrome A prevents *FT* activation in far-red light.

To try to explain the basis of the post-transcriptional regulation of *CO*, we have studied CO protein levels in *35S::CO* plants under different light conditions using antibodies raised to CO or followed CO::GFP fusion proteins. Both of these approaches indicate that CO protein accumulates in the nucleus when plants are exposed to white, blue and far-red light, but not in red light or darkness. In these experiments, therefore, there is a direct correlation between accumulation of the protein in the nucleus and activation of the target gene, suggesting that regulation of nuclear import/export or the rate of protein degradation in the nucleus are major control points. To determine whether photoreceptors reduce CO function by impairing these processes, we compared the abundance of CO protein in the nucleus in *cry1 cry2 35S::CO* and in *35S::CO* plants. CO protein was present at similar levels in the nuclei of both plants, suggesting that the role of the cryptochromes is not to regulate nuclear accumulation of CO but a subsequent step. Explaining these later steps will require an understanding of the protein complexes in which CO acts to regulate transcription; we are approaching this question using yeast two-hybrid analysis, and *in vivo* epitope-tagged versions of CO, forward genetic screens, comparative analysis of CO-like proteins in Arabidopsis along with characterisa-

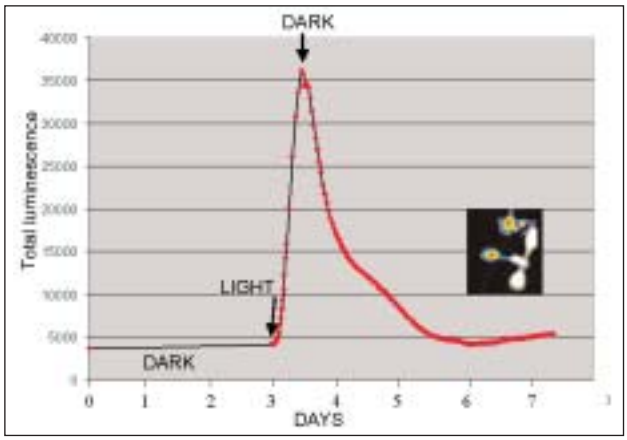

Fig. 1. Transcription of the flowering-time gene *FT* is activated by CONSTANS only when plants are exposed to light. Transgenic plants containing *35S::CONSTANS* and *FT::LUCIFERASE* are shifted from dark to light and back to dark. Expression of *FT::LUCIFERASE* is dependent on CONSTANS activity and is detected by the luminescence of the seedlings (inset), which can be quantified in a scintillation counter.

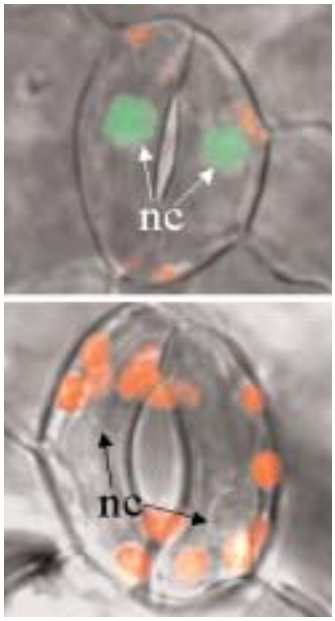

Fig. 2. Nuclear accumulation of CONSTANS protein occurs when plants are exposed to light. Stomatal guard cells of a transgenic plant carrying *35S::CO:GFP* exposed to light for 8 hours (top) and then shifted to darkness for a further 2 hours (bottom). nc: nucleus.

tion of the sequences that confer CO reponsiveness on the *FT* promoter. (Fig. 1+2).

Spatial regulation of photoperiod response

The genes of the photoperiod pathway show general patterns of expression. For example, *CO* is expressed in the shoot apical meristem, throughout young leaf primordia and in the vascular tissue of old leaves. These general patterns of expression have made it difficult to assess in which cells these genes act to regulate flowering. To approach this problem, we developed a misexpression strategy based on the GATEWAY recombination system. We inserted 12 promoters that drive well-characterised patterns of expression within GATEWAY entry clones. In parallel, the open reading frames of the five flowering-time genes were inserted in a binary vector adjacent to a GATEWAY destination site. We then recombined each promoter into the destination site upstream of the open reading frames. These fusions were introduced into the appropriate mutants by Agrobacterium-based transformation. The experiments demonstrated that despite the general pattern of expression of the wild-type gene, complementation of the *co* mutation only occurred when *CO* was expressed in the phloem companion cells. Detailed characterisation of the pattern of expression of the wild-type *CO* gene within the vascular tissue confirmed that it is expressed in the phloem. Furthermore, expression of the CO target gene *FT* in the phloem also complemented the *co* mutation. These experiments suggest that in wild-type plants, CO acts in the phloem companion cells of mature leaves to promote the expression of a long-distance signal that induces flower development at the apex of the plant.

Interaction between the photoperiod and other flowering pathways

Flowering of Arabidopsis is promoted by vernalisation, extended exposure to low temperature as well as long photoperiods. Vernalisation promotes flowering by repressing the expression of the MADS-box transcription factor FLC, a repressor of flowering. The vernalisation and photoperiod pathways regulate the expression of the downstream genes *FT* and *SOC1*, and FLC represses the capacity of CO to activate expression of these genes. We have developed genetic and molecular approaches to study the mechanism of the convergence of the two pathways, by analysing the antagonism between CO and FLC. To study this genetically, we have constructed plants carrying *35S::CO 35S::FLC SOC1::GUS*, which flower at a time intermediate between the extreme early flowering of *35S::CO* plants and the very late flowering of plants carrying *35S::FLC* and show no GUS expression due to the repression of *SOC1* by FLC. We have mutagenised this line and identified mutants that flower earlier than the progenitor and show increased expression of the GUS marker. The expression of the endogenous *SOC1* and *FT* genes is also increased in these mutants compared to the transgenic progenitor line. The mutations might cause an increase in the capacity of CO to activate *SOC1* and to promote flowering, or a reduction in the repression of both processes by FLC. We are in the process of mapping these mutations and will clone the affected genes in the mutants showing the strongest effect. These genes should provide insight into how the activities of pathways activated by different environmental stimuli are integrated.

Diversity in photoperiod response: CONSTANS and FT regulation in *Pharbitis nil*

Arabidopsis is a long-day plant in which *FT* expression is activated by CO specifically under long days due to the post-transcriptional activation of CO protein by light. However, many plant species show the reverse response and flower in response to short days. *Pharbitis nil* was used as a model short-day plant in many physiological experiments that characterised the response to short days.

We have cloned the *CO* and *FT* orthologues from Pharbitis (*PnCO* and *PnFT*, respectively) and characterised their expression under long and short days. *PnFT* is expressed in Pharbitis only under short-day conditions and therefore, shows the reverse response to *FT* in Arabidopsis. Furthermore, the expression of *PnFT* occurs in extended periods of darkness in Pharbitis. Both *PnCO* and *PnFT* are regulated at the transcriptional level by the circadian clock in Pharbitis, and the peak in *PnCO* expression precedes that of *PnFT*, implying that PnCO does activate *PnFT* in Pharbitis, analogous to the relationship between CO and *FT* in Arabidopsis. However, *PnFT* expression occurs in extended darkness in Pharbitis, suggesting that activation of PnCO function by light is not required.

Future Developments

Our long-term objective is to describe the signalling pathway that activates flowering in response to photoperiod in Arabidopsis, from the photoreceptors that detect long days in the leaf to the activation of gene expression in the shoot meristem. We aim to explain how this pathway is regulated as well as how it is modified in other species to generate distinct responses to photoperiod. Over the next reporting period, we expect to progress in all the areas described above as well as in understanding other parts of the signalling network, which are not described here in detail, such as the function of the GIGANTEA protein in linking *CO* expression to the circadian oscillator.

PROJECT 2: Protein Modification by SUMO and the Control of Flowering Time in Arabidopsis

Introduction

SUMO is an ubiquitin-like protein that is covalently attached to target proteins. It is essential for completion of the cell cycle in yeast, and is involved in the modification of many animal proteins, altering their stability, affinity for other proteins or their cellular location. SUMO is covalently attached to substrate proteins by SUMO-activating and conjugating enzymes similar to those that activate ubiquitin. Despite the intense interest in SUMO in yeast and animal systems, almost nothing is known of the role of SUMOylation in plants. Nevertheless, genes predicted to encode SUMO isoforms as well as conjugase and protease enzymes predicted to be components of the SUMO system have been described within the Arabidopsis genome. Furthermore, Western blots using SUMO antibodies detect many high molecular weight proteins, suggesting that SUMO modification is prevalent in plants.

Our interest in SUMOylation emerged from our analysis of the *early in short days 4* (*esd4*) mutant. We isolated early flowering mutants under short days to describe the mechanisms by which flowering is repressed under these conditions, and *esd4* was the most extreme of these mutants. The early-flowering phenotype of *esd4* is caused, at least in part, by increasing the abundance of the mRNAs of the flowering-time genes *SOC1* and *FT*. Furthermore, this increase in *SOC1* and *FT* expression is partly caused by a reduction in the expression of the floral repressor FLC, a MADS-box transcription factor.

We isolated *ESD4* by map-based cloning. The sequence of the gene indicated that the predicted protein product showed homology to SUMO proteases from yeast and animals. These proteases carry out two regulatory functions. They are endoproteases that cleave the SUMO precursor to generate mature SUMO, revealing the C-terminal glycine that becomes attached to a lysine residue of target proteins. They are also isopeptidases that recycle SUMO from conjugates by cleaving the isopeptide bond linking the C-terminal glycine of SUMO to the lysine residue in the target protein. Within the reporting period, we have studied the role of ESD4 protease *in vivo* and *in vitro*, and generated materials to identify proteins conjugated to SUMO *in vivo*.

New Results

ESD4 encodes a SUMO protease

We tested the predicted function of ESD4 *in vivo* and *in vitro*. ESD4 and a SUMO substrate protein were purified from *E. coli* as HIS fusions. When co-incubated for 30 minutes, the purified HIS-ESD4 protein cleaved the SUMO substrate to generate mature SUMO. A mutant form of HIS-ESD4 in which a cysteine residue predicted to be part of the active site was converted to a serine did not induce cleavage of the substrate protein. Similarly, cleavage was prevented by conversion of the two glycine residues that define the cleavage site of the substrate protein to alanines. These experiments demonstrate that *in vitro* ESD4 shows SUMO endoprotease activity.

We have also raised antibodies to the three SUMO isoforms of Arabidopsis. In wild-type plants, these antibodies detect a protein of approximately 17 kD, which probably represents free SUMO, and many larger proteins that are likely to be SUMO conjugates. In the *esd4* mutant, no protein corresponding to free SUMO was detected, although the pattern of conjugates was similar to that of wild-type plants. This result suggests that in wild-type plants, the predominant role of ESD4 is to act as an isopeptidase that recycles SUMO from conjugate proteins, so that in the *esd4* mutant, less free SUMO is detected because it tends to accumulate in conjugates. (Fig. 3)

Transgenic approaches suggest the predominant role *in vivo* of ESD4 is as a SUMO isopeptidase

ESD4 protease is predicted to have two functions in the regulation of SUMOylation: (1) as an endoprotease that

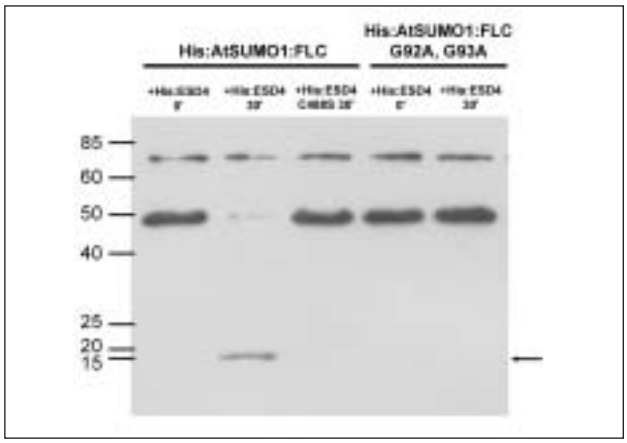

Fig. 3. An *in vitro* assay demonstrates that ESD4 protein is a protease able to generate mature SUMO from its precursor. His:ESD4 (ca. 75 kD) and a derivative of SUMO precursor, HIS:AtSUMO1:FLC (ca. 50 kD) were produced in *E. coli* and the gel was probed with anti-HIS antibody. Co-incubation of these proteins (Track 2) leads to cleavage of HIS:AtSUMO1:FLC to generate mature SUMO (marked with an arrow). Mutations in the active site of ESD4 protease (Track 3) prevent this cleavage, as does mutation of the cleavage site within the recognition site of SUMO (Track 5).

matures SUMO precursor; and (2) as an isopeptidase that recycles SUMO from conjugates.autom.

In collaboration with Jürgen Dohmen (Universität zu Köln), we tested the function of ESD4 in yeast. We generated fusions of the GAL1 promoter to the *ESD4* open reading frame and introduced these into the yeast *ulp1* and *ulp2* mutants, which are both impaired in SUMO proteases. These experiments demonstrated that expression of ESD4 complements the *ulp2* mutation, but not *ulp1*. This supports the proposal that ESD4 is a SUMO protease. Furthermore, this also suggests that ESD4 may mainly act as an isopeptidase, because this is the major role of ULP2, while ULP1 acts mainly as an endoprotease.

We have also generated transgenic plants to distinguish between these two protease activities. We constructed two transgenes in which the 35S promoter was fused to an open reading frame encoding either the SUMO precursor or the mature form of SUMO. If the predominate role of SUMO is as an endoprotease then the expression of the mature form of SUMO may be expected to complement the *esd4* mutation; whereas, if its major role is as an isopeptidase, then overexpression of mature SUMO might be expected to enhance the *esd4* phenotype due to the hyperaccumulation of conjugates. The transgene had no effect on the phenotype of wild-type plants. In contrast, transgenic *esd4* mutants carrying the transgene showed an enhanced phenotype; the transgenic plants exhibited smaller leaves and shorter stems than the *esd4* mutant. The phenotype of these plants also suggests that the major role of ESD4 is to recycle SUMO from conjugates.

Marking SUMO conjugates with epitope-tagged SUMO in Arabidopsis

The analysis of the *esd4* mutant suggests that the mutant phenotype is caused by the over-accumulation of particular SUMO conjugates. We have initiated a biochemical approach to identify SUMO conjugates in Arabidopsis, and particularly, to identify those that are present at higher abundance in the *esd4* mutant. We have introduced into *esd4* mutants and wild-type plants, genes encoding epitope-tagged forms of SUMO. These modified SUMO proteins carry at the N-terminus tandem 10xHis and HA tags. We extracted proteins from these transgenic plants and then passed them over a TALON column which enriches proteins carrying the 10xHis tag. The proteins were released with imidazole and then purified a second time over a column containing the HA antibody. The protein was released under acidic conditions, separated through a gel and transferred onto a filter which was subsequently probed with both HA and SUMO antibodies. Both antibodies recognised SUMO and higher molecular weight conjugates, indicating that the tandem epitopes can be used to purify conjates. We will now increase the scale of this experiment to allow the identification of individual conjugates by protein mass spectrometry.

The Arabidopsis SUMO protease family

In addition to *ESD4* six other Arabidopsis genes encode proteins closely related to SUMO proteases. We are interested to determine whether these genes also have roles in flowering-time regulation, or whether only ESD4 regulates SUMO conjugates associated with flowering. To assess whether loss of function alleles of these genes affect flowering, T-DNA insertions were identified. Populations of plants segregating for each of the insertions were grown, and all flowered at the same time as wild-type plants. These experiments suggest that ESD4 has a specific role in regulating SUMO conjugates that affect flowering.

Future Developments

A major future direction will be to identify Arabidopsis proteins that are targets for SUMO conjugation by using plants expressing the epitope-tagged versions of SUMO to purify conjugates. We will carry this out as a collaboration with Andreas Bachmair. Initially, we will identify SUMOylated proteins at random, but will then focus this analysis on obtaining conjugated proteins that accumulate to higher levels in *esd4* mutants than in wild-type plants and may therefore represent conjugates that are targets for the ESD4 protease. The importance of SUMOylation of these proteins in flowering time regulation and whether SUMOylation activates or inactivates the conjugated protein will be tested genetically, and by constructing transgenes in which the target lysine residue has been altered, preventing SUMOylation. We will also explore the basis of the specificity between ESD4 and the other members of the protease family, by studying the spatial expression pattern and cellular location of each member of the family and by using SUMO antibodies to determine whether mutations in other members of the family also cause depleted levels of free SUMO, indicative of a role in recycling SUMO.

PROJECT 3: Genetic Models for the Control of Flowering in Perennial Plants

Introduction

The molecular control of flowering time has been studied extensively in Arabidopsis and, to a lesser extent, in other species such as rice and maize. These model systems span some of the diversity in the control of flowering observed in the Angiosperms, such as short- and long-day species, vernalisation-requiring and non-requiring plants, and dicotyledonous and monocotyledonous species. However, there is still a need to develop model systems in which the unique features of perennial plants can be studied.

Perennial plants flower repeatedly and their life cycle extends over several or many years; whereas, annual plants enter reproductive development once and their life cycle is contained within one year. Annual plants are typically monocarpic, when they are induced to flower all shoot meristems enter reproductive development. In contrast, in perennial plants some meristems undergo the transition to reproductive development, while others are conserved as vegetative meristems, allowing the plant to initiate further periods of vegetative growth. Similarly, after flowering, the developing seeds of annual plants become strong sink tissues and the leaves senesce rapidly; whereas, the majority of the vegetative tissues of perennial plants stay green after flowering and allow further vegetative development.

We aim to extend our understanding of the regulation of flowering to perennial plants. To this end, we are developing a perennial model system based on a relative of Arabidopsis, *Arabis alpina*. In addition, in collaboration with Professor Nick Battey at the University of Reading, we are cloning the *SEASONAL FLOWERING LOCUS (SFL)* of *Fragaria vesca*.

New Results

Molecular mapping of the *SEASONAL FLOWERING LOCUS* (*SFL*) of *Fragaria vesca*

Fragaria vesca is a diploid perennial plant that has two forms with distinct reproductive behaviours. The typical form of the species (*Fragaria vesca* f. *vesca*) is seasonal and flowers only in the spring whereas the other form (*Fragaria vesca* f. *semperflorens*) is perpetual and flowers over an extended period, mainly during the spring and summer months. Flowers in the seasonal flowering form are induced in the autumn under short days (SD) and cool temperatures (<15ºC). In contrast, the perpetual flowering form is believed not to have any environmental regulation of flowering. Classical genetic analysis based on segregating populations (F2 and BC1) derived from these two forms showed that they differ at one locus (*SFL*), at which dominant alleles generate the seasonal flowering behaviour. Cloning of this locus will help understand how perennial plants balance vegetative growth and reproductive effort in yearly cycles.

Fig. 4. Perpetual and seasonal varieties of *Fragaria vesca* growing under long-day conditions. The perpetual variety (left) carries the recessive allele of the *SEASONAL FLOWERING LOCUS* (*SFL*) and flowers rapidly under long-days, whereas the seasonal variety contains the dominant *SFL* allele and does not flower under long-day conditions (right).

Nick Battey's group (University of Reading) previously created a local map around *SFL* using PCR-based markers. At present, we are using these markers to increase the number of recombinants in this interval by screening a new BC1 population. *Fragaria vesca* has a very short juvenile period and this allows us to rapidly score the phenotype of our mapping progenies. In long day and high temperature growth chambers, only progenies that are homozygous for the recessive allele at the *SFL* locus set flowers within a period of two months from seed. We have also enriched the interval spanned with the original ISSR markers with AFLP markers as well as having generated five new AFLP markers that are genetically linked to *SFL*. We are converting the most closely linked AFLP markers to DNA probes that can be used to screen the *Fragaria vesca* f. *vesca* BAC genomic library that was recently made by Nick Battey's group with whom we are collaborating on this project. (Fig. 4)

Arabis alpina, a rapid cycling perennial relative of Arabidopsis

We have explored the use of several relatives of Arabidopsis as potential perennial model species, and believe that *Arabis alpina* shows many of the characteristics required. It is a diploid species, which simplifies genetic analyses and contrasts with many perennials that are polyploid. *Arabis alpina* also self fertilises efficiently, allowing the propagation of pure stocks and reducing the labour involved in mutagenesis-based approaches. Its relatedness to Arabidopsis should also make mapping strategies faster and allow more straightforward comparative analysis with the annual flowering network of Arabidopsis. We have obtained stocks of *Arabis alpina* from Spain and from Bonn. All described varieties of *Arabis alpina* are perennial, and our objective is to use a mutagenesis strategy to identify mutations that disrupt perennial traits. (Fig. 5)

Fig. 5. *Arabis alpina* flowers in response to vernalization. The plant on the left is 5 months old, has not been vernalised and is still growing vegetatively. The plant on the right is four months old; when 4 weeks old it was given an 8 week vernalisation treatment and then returned to the greenhouse.

Most perennial species have extended juvenile phases and do not flower for several years after germination. Similarly, in the greenhouse, we have observed that *Arabis alpina* will grow vegetatively for at least one year after germination. We have therefore tested whether we can accelerate flowering of this species with a vernalisation treatment, i.e. exposure to low temperatures for an extended period of time. *Arabis alpina* plants were given a vernalisation treatment of 8 weeks at 4°C. The plants were vernalised as seed, or grown for 4, 8, 12, 16 or 20 weeks in the greenhouse prior to vernalisation. The plants exposed to vernalisation at seed stage did not flower 13 weeks after vernalisation and therefore, *Arabis alpina* is probably unresponsive to vernalisation when vernalised as seed. In contrast, the other plants all flowered within four weeks of vernalisation. Therefore, using a vernalisation treatment, *Arabis alpina* can be induced to flower within 16 weeks of germination. We have used the vernalisation-treated plants to bulk seed from these stocks, so that we can initiate mutagenesis strategies. We have also used these vernalised plants to characterise the phenotype of *Arabis alpina* and to describe perennial characters that may be modified by mutations.

Arabis alpina shows striking phenotypic differences to *Arabidopsis thaliana*. For example, each node on the main shoot gives rise to a branch that develops from the axil of the leaf, and grows laterally from the main shoot. This branching habit rapidly gives rise to a complex morphology, and contrasts with Arabidopsis, where at most vegetative nodes side-branch outgrowth is suppressed. In addition, the flowering behaviour of the primary shoot meristem and the side branches of *Arabis alpina* often differ; this seems to depend on the age of the plant at the time of vernalisation. For example, when plants are vernalised 4 weeks after germination, flowers are formed only from the primary shoot meristem up to at least 2 weeks after the vernalisation treatment. In contrast, vernalisation of 8 week-old plants generates flowers at the primary apex and on approximately half of the side branches within 2 weeks after vernalisation. These phenotypic experiments suggest that this differential induction of flowering could form the basis of an analysis of polycarpy in a perennial and allow the identification of mutants that disrupt this character.

Future Developments

In the *Fragaria vesca* project, we will increase the resolution of the genetic map in the vicinity of *SFL* by increasing the number of available recombinants carrying crossovers in the region and by initiating the construction of a physical map of the interval. To construct the physical map, we will isolate DNA fragments corresponding to AFLP markers in the region and use these to screen the BAC libraries. Fragments derived from the physical map will then be located on the genetic map of the region. The genome size of *Fragaria vesca* is estimated at 164 Mb, whereas the closest markers on the genetic map are 0.5 cM on the north side and 0.4 cM on the south side of the gene. We will create a mutant population of *Arabis alpina* by mutagenising seeds and self-fertilising M1 plants to create an M2 population. The M2 population will be screened for characters that impair the perennial character. Initially, we will focus on screening for mutations that prevent continuation of vegetative growth after flowering, the polycarpic character. These mutations might, for example, cause all meristems to enter the reproductive programme soon after vernalisation, and prevent the continued vegetative development of some side shoots that is observed in the wild-type plants. We will also clone homologues of Arabidopsis flowering-time genes and compare their function with those of Arabidopsis.

Scientific Publications

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Structure of the group

Group leader — **Prof. Dr. George Coupland**

PROJECT 1

Postdoctoral fellows
- **Dr. Hailong An** since September 2001
- **Dr. Laurent Corbesier** since March 2003
- **Dr. Frédéric Cremer** since September 2001
- **Dr. Ryosuke Hayama** since April 2002
- **Dr. José Le Gourrierec** since September 2001
- **Dr. Aidyn Mouradov** since July 2001
- **Dr. Paul Reeves** Januar 2002 - May 2003
- **Dr. Iain Searle** since April 2002
- **Dr. Wim Soppe** since September 2002
- **Dr. Federico Valverde** since January 2002
- **Dr. Jochen Winter** July 2001 - June 2003

PhD students
- **Hugo Konijn** since September 2002
- **Dean Ravenscroft** since January 2002
- **Clotilde Roussot** since January 2002
- **Stephan Wenkel** since January 2003
- **Louisa Wright** since January 2002

Technical assistants
- **Coral Freialdenhoven** since June 2001
- **Sandra Kröber** since November 2002
- **Elisabeth Luley** since March 2002

PROJECT 2

Postdoctoral fellows
- **Dr. Yong-Fu Fu** since January 2002
- **Dr. Paul Reeves** January 2002 - May 2003

PROJECT 3

Postdoctoral fellow
- **Dr. Maria Albani** since February 2002

Guest scientists

András Viczián, Institute of Plant Biology, Biological Research Centre, Szeged, PhD student

Dr. Paula Suarez-Lopez, Dept. Genetica Molecular, Instituto de Biologia Molecular de Barcelona (CSIC), Barcelona, Postdoctoral fellow

Grants, external funding

EU project for shared-cost RTD actions: CONFLOW (QLK5-CT2001-0412): 1 Postdoctoral fellow

EU project: REGIA (QLGC-1999-00876): 1 Postdoctoral fellow

Marie Curie Individual Fellowship (MCFI-2001-02002): 1 Postdoctoral fellow

TMR Marie Curie Research Training Grant (HPMFCT-1999-00075): 1 Postdoctoral fellow

Dusberg-Bailly Fundation, University of Liège: 1 Postdoctoral fellow

Alexander von Humboldt-Stiftung (V-8121/UKR/1019479): 1 Postdoctoral fellow

IMPRS: 1 PhD student

Collaborations

Control of flowering in Pharbitis: CSIRO Division of Plant Industry, Canberra, Dr. Rod King

Role of LHY and CCA1 in controlling flowering time: Gene Experiment Center, Tsukuba University, Tsukuba, Dr. Tsuyoshi Mizoguchi

The function of plant SUMO proteases: Institut für Genetik, Universität zu Köln, Dr. Jürgen Dohmen

Flowering of *Fragaria vesca*: Department of Horticulture and Landscape, University of Reading, Dr. Nicholas H. Battey

Invited lectures given

Shanghai Institute of Plant Physiology and Ecology, CAS, Shanghai (22.-26.09.2001), ComBio2001, National Convention Centre, Canberra (30.09-04.10.2001), Institute of Biology, University of Freiburg (13.10.2001), Max Planck Institute of Molecular Plant Physiology, Golm (07.11.2001), Wissenschaftszentrum Weihenstephan der TU München, Weihenstephan (13.12.2001), Plant, Animal & Microbe Genomes X, San Diego (12.-16.01.2002), Department Molecular Cell Biology, Utrecht University (22.01.2002), Special Seminar Programme in Genetics and Cellular Biology, University of Cologne (31.01.2002), Institute of Plant Genetics and Crop Plant Research, IPK, Gatersleben (26.02.2002), Society for Research on Biological Rhythms, Amelia Island, Jacksonville (22.-26.05.2002), XIII

International Conference on Arabidopsis Research, Sevilla (28.06.-02.07.2002), ELSO 2002 Meeting, Nice (29.06.-03.07.2002); CSHL Summer Courses, Cold Spring Harbour (12.-15.07.2002), ASPB Meeting, Denver (03.-07.08.2002), FASEB Summer Research Conference, Vermont Academy Saxtons River, Vermont (10.-14.08-2002), 13th Congress of the Federation of European Societies of Plant Physiology, Hersonissos, Crete (31.08.-02.09.2002), Botanikertagung, Universität Freiburg (25.-27.09.2002), Department of Plant Systems Biology, Universiteit Gent (09.10.2002), EMBO New-Members-Meeting, Oslo (11.-15.10.2002), KWS Saat AG, Einbeck (19.11.2002), Sektionssymposium, Berlin (28.-29.11.2002); Biological Research Center, Plant Biology Institute, Szeged (02.-04.12.2002), Institut des Sciences du Végétal, CNRS, Gif-sur-Yvette (12.-13.12.2002), Università degli Studi di Milano, Milan (13.-14.02.2003), Instituto Juan March de Estudios e Investigaciones, Madrid (23.-26.02.2003), 54. Mosbacher Kolloquium, GMB, Mosbach (28.-30.03.2003) ■

Plant Circadian Biology

Seth Jon Davis

Introduction

Plant growth and development is strongly modulated by daily exposure of sunlight. An intimate network of light-perception pathways and an internal circadian clock are integrated in anticipation of the changing day. Over the last years, our work on the model plant *Arabidopsis thaliana* has focused on both the phytochrome family of photoreceptors and genes that integrate light perception to the clock. With my move to Germany, we will continue and expand our studies on the clock mechanism and how the clock is integrated to the environmental-perception pathways.

There is a relationship between light signalling and the circadian clock in plants. These reciprocal interactions include the entrainment of the clock by light, and regulating the modulation of the response to light by the clock. To unravel the molecular processes coordinating light perception and the circadian oscillator, we have exploited transgenic plants containing luciferase (LUC) fusions under the control of circadian-regulated promoters. The low-light emissions from LUC have been shown not to activate endogenous photoreceptors or alter rhythmic behaviours. As such, the LUC system provides an excellent non-invasive marker for the co-ordinated action of light and the clock on transcriptional regulation.

Work by many labs has revealed that genetic defects in either light signalling or circadian function alters photoperiodic induction of flowering. Thus, our work on the clock leads to parallel insights into the mechanisms used in this developmental switch.

New Results

A number of photoreceptors are important to sense the light environment, including the phytochromes and the cryptochromes. These photoreceptors also play specific roles in the light-input pathway that resets the circadian clock. We identified *SRR1* (*SENSITIVITY TO RED LIGHT REDUCED 1*) which is important for both phytochrome B-mediated light signalling and regulation of multiple outputs of the circadian clock. *srr1* and *phyB* mutants display a number of similar phenotypes such as early flowering in short days, reduced chlorophyll content and decreased sensitivity to red light. Genetic analyses suggest that not all *SRR1*-mediated functions require a functional phyB photoreceptor. In addition to photomorphogenic phenotypes, *srr1* mutants have a short period in all clock-regulated genes tested and a short-period rhythm of leaf movement. The *SRR1* gene was identified and shown to encode a protein conserved in numerous eukaryotes. This might suggest that a function of *SRR1* in the circadian clock is conserved between plants and animals.

Many plants use daylength as an environmental cue to ensure the proper timing of the switch from vegetative to reproductive growth. Daylength sensing involves an interaction between the relative length of day and night, and endogenous rhythms controlled by the plant circadian clock. Thus, plants with defects in circadian regulation are unable to properly regulate the timing of the floral transition. We described *EARLY FLOWERING 4* (*ELF4*), a gene involved in photoperiod perception and circadian regulation. *ELF4* promotes clock accuracy and is required for

sustained rhythms in the absence of daily light/dark cycles. *elf4* mutants show attenuated expression of *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)*, a gene believed to function as a central oscillator component. In addition, *elf4* plants transiently display output rhythms with highly variable period lengths prior to becoming arrhythmic. Mutations in *elf4* result in early flowering in noninductive photoperiods. This is likely due to elevated expression of *CONSTANS* (*CO*), a gene that promotes floral induction.

We isolated the *time for coffee (tic)* mutant that disrupts circadian gating, photoperiodism, and the amplitude and period of multiple circadian rhythms. *TIC* is distinct in physiological functions and genetic map position from other rhythm mutants and their homologous loci. Detailed analysis of rhythmic gene expression shows that the circadian clock requires *TIC* function around dawn, in contrast to *EARLY-FLOWERING 3* (*ELF3*), which functions around dusk. *tic* mutants misexpress genes that are critical for circadian timing in the early day, consistent with our functional analysis. We thus identify *TIC* as a negative factor in the clock gene circuit. In contrast to *tic* and *elf3* single mutants, *tic elf3* double mutants are completely arhythmic. Even the robust circadian clock of plants cannot survive with defects at the two opposite phases of a light/dark cycle.

Future Developments

New mutant screens

Our research programme initiated a high-throughput screen for new mutations that alter the *A. thaliana* clock; this is in collaboration with laboratories in England and Hungary. The screen employs luciferase technology typical of all three labs. We have collectively identified ~60 mutations that alter proper circadian function. Many of these mutants are allelic, but by no means is the genome saturated for lesions that alter clock function. As such, these screens will continue. We have started the analysis of three new loci that regulate the circadian system. Preliminary evidence suggests that the gene products defective in several of these lines have been established. Continuing analyses of these mutants and genes will further our understanding of the clock.

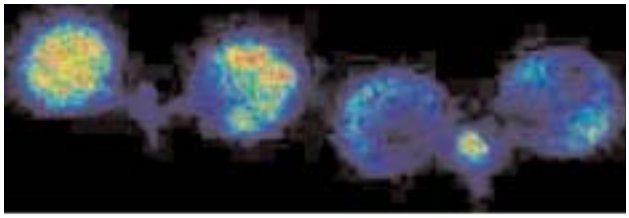

Fig. 1. Bioluminescent image of *LUCIFERASE*-containing *Arabidopsis thaliana* plants.

Continuing work on ELF4

Our first report on *ELF4* did not describe mechanistically how this gene functions, at either a genetic or molecular level. Our current work is beginning to establish the genetic and physiological mechanisms of this gene. We have uncovered via ”jetlag“ experiments that like *TIC*, but unlike *ELF3*, *ELF4* functions at the morning phase of a diurnal cycle. *ELF4* also has direct roles in light-input to the clock, and has a role in red-light phototransduction. We are thus testing epistatic relationships between *elf4* mutant and mutations in *tic*, *elf3*, and the red-light receptor *phyB*. We imagine that the analyses of these respective double mutants will define the genetic pathways of *ELF4* function.

Insights into *ELF4* action require molecular-genetic tools to probe its function. We have generated transgenic plants that misexpress *ELF4*. These plants have distinct circadian defects and thus, we have a system to probe the molecular biology and biochemistry of the ELF4 polypeptide. Appropriate experiments are underway to address these issues.

Continuing work on TIC

Using a positional-cloning strategy, our group is close to isolating the *TIC* gene. This gene will provide a valuable

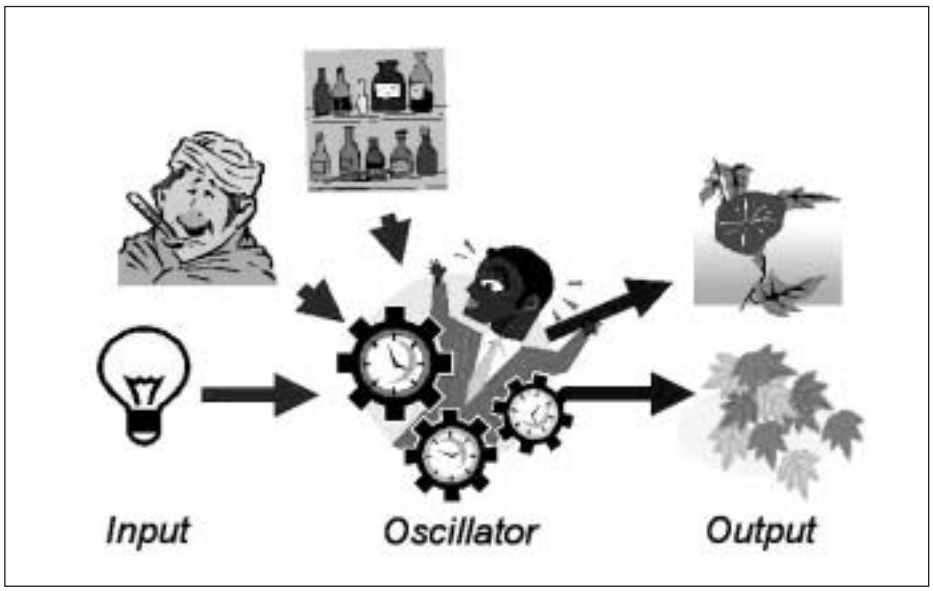

Fig. 2. Cartoon representation of the plant circadian system. The plant circadian system can be thought of in three distinct phases. In the input phase, light, temperature and endogenous ”chemicals“ contribute towards clock entrainment. The central oscillator then generates a biological rhythm that occurs once per day. Finally, physiological outputs from the clock are manifest, which in plants includes daily changes in metabolism and seasonal changes of growth and development. We are interested in all three phases of the plant clock.

tool towards uncovering the molecular events of *TIC* function, and based on its nature, appropriate experiments will be devised. Additionally, new physiological analyses of this mutant are being conducted in isolation and in multiple mutant combinations. The genetic, physiological and molecular characterisation of this *TIC* locus will clarify the events that coordinate light input into the clock.

New early-flowering mutant lines: connection to the clock

We have worked in conjunction with Dr. Richard Amasino to attempt to establish a formal link between the circadian system and photoperiodic induction of flowering time. Dr. Amasino conducted a screen for *A. thaliana* lines that flower earlier than wild type under non-inductive photoperiods. From a collection of ~50 mutants obtained by his laboratory, we established that six of these lines have defective circadian rhythms of leaf movement. This two-stage screen has provided new genes that are either promoters or repressors of normal circadian function, respectively. These lines will be further characterised geneticall-y, physiologically and most importantly, within a molecular framework of the circadian clock.

Scientific Publications

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Structure of the group

Group leader	**Dr. Seth Jon Davis**
Postdoctoral fellow	**Dr. Shigeru Hanano**
Ph.D. student	**Malgorzata Domagalska**
Administrative assistant	**Amanda Davis**

Guest scientists

Andras Viczian, Ph.D. student with Dr. Ferenc Nagy, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Grants, external funding

Life Sciences Research Foundation fellowship
IMPRS: Ph.D. studentship

Collaborations

Circadian gating of light responsiveness, University of Warwick, UK, Dr. Andrew Millar

Isolation of genes regulating circadian rhythms and flowering time, University of Wisconsin-Madison, USA, Dr. Richard Amasino

Characterization of the circadian gene *SRR1*, University of Geneva, Switzerland, Dr. Christian Fankhauser

Light entrainment of the circadian clock, Biological Research Center of the Hungarian Academy of Sciences, Hungary, Dr. Ferenc Nagy; University of Buenos Aires, Argentina; Dr. Jorge Casal

Hormonal regulation of flowering time, Biological Research Center of the Hungarian Academy of Sciences, Hungary, Dr. Ferenc Nagy

Invited lectures given

John Innes Centre, Norwich (2001),
Max Planck Institute for Plant Breeding, Cologne (2001), Texas Agricultural and Mechanical University, College Station, TX (2001), Linkoping University, Linkoping, Sweden (2002), Lund Life Symposia, Lund, Sweden (2002), University of Freiburg, Freiburg (2003), University of Bielefeld, Bielefeld (05.03)

Protein Modifiers in Plants

Andreas Bachmair

Introduction

Modification of proteins by covalent attachment of other proteins is used pervasively in eukaryotic cells to regulate the turnover, location or function of substrate proteins (Fig. 1). We are investigating the role of two modifiers, the prototype modifier ubiquitin, and SUMO, a protein with similarity to ubiquitin ("**s**mall **u**biquitin-like **mo**difier"). Ubiquitin is best known for its role in selective protein turnover, but has also non-proteolytic functions. Much less is currently known about SUMO. Recent evidence suggests that SUMO and ubiquitin can act antagonistically to influence the same biological process.

We try to incorporate both biochemical and genetic methods in order to understand mechanisms and consequences of the processes under investigation. The availability of Arabidopsis T-DNA insertion lines from a variety of sources is particularly beneficial for analysis of protein modifiers, because many candidate components of modification pathways can be identified by sequence similarity. The role of these components in plants, on the other hand, remains poorly defined at this stage and is the subject of our research efforts.

New Results

Substrates and functions of sumoylation in plants

SUMO is essential in eukaryotes, yet little is known about plant substrates for sumoylation. We have expressed tagged versions of SUMO1 in Arabidopsis to detect SUMO conjugates. The affinity tags will be used to purify enough conjugates to allow identification of substrates by mass spectrometric methods. We are working together with the group of George Coupland in order to identify SUMO conjugates that play a role in the initiation of flowering. In collaboration with Dr. C. Hardtke, University of Toronto, Canada, we have analysed Arabidopsis plants that express a dominant negative version of SUMO conjugating enzyme. These plants have a decreased capacity to add SUMO to substrates. One specific phenotype of these plants is early flowering under short day conditions (Fig. 2).

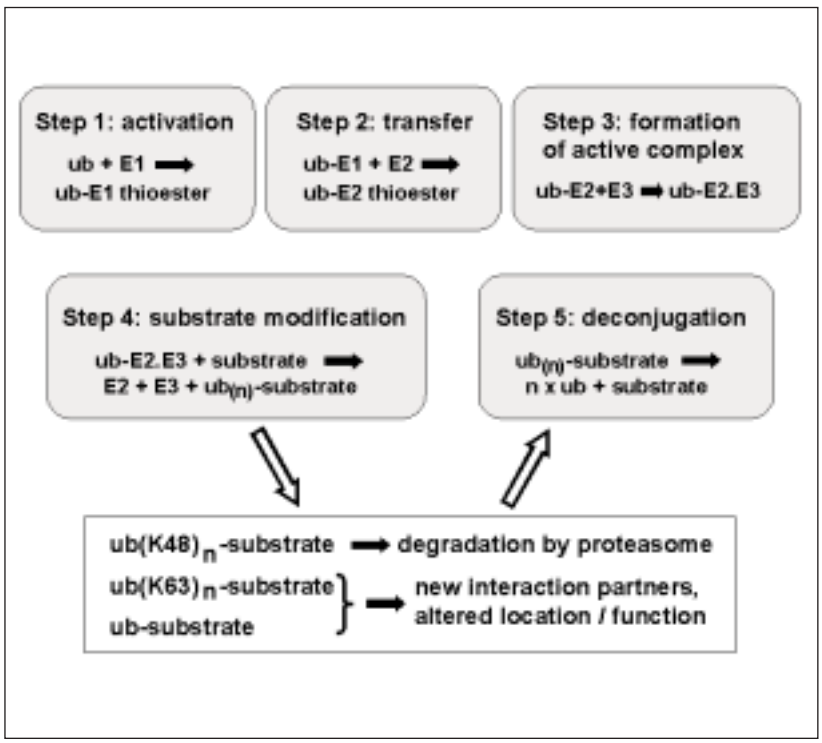

Fig. 1. Conjugation cycle of protein modifiers. The modifier ubiquitin is activated by formation of a thioester bond to ubiquitin activating enzyme (E1) in an ATP-consuming reaction (Step 1). From there, ubiquitin is transferred to a set of ubiquitin-conjugating enzymes (E2s; Step 2), again forming a thioester linkage. E2s with attached ubiquityl moiety associate with ubiquitin protein ligases (E3s; Step 3). Substrates are recruited to this complex and ubiquitylated, forming an isopeptide bond between ubiquitin´s C-terminus and an internal Lys residue of the substrate (Step 4). Several ubiquitin moieties can be linked to each other, resulting in multi-ubiquitylated substrates. The modified substrate has changed properties. A prominent fate of proteins carrying multi-ubiquitin linked via K48 is destruction by the proteasome. Ubiquitin can be removed by de-conjugating enzymes (Step 5) to restore the previous functional state of the substrate, and to recycle ubiquitin for further use.
Conjugation of the protein modifier SUMO is analogous to the ubiquitin cycle, with some differences. Step 2 can directly lead to Step 4 (i.e., sumoylation may occur by direct binding of SUMO conjugating enzyme to the substrate). In contrast to ubiquitin-ubiquitin linkages, SUMO-SUMO linkages are rare and proteolytic destruction is not a known consequence of sumoylation.

Fig. 2. Inhibiton of sumoylation leads to alterations in growth and flowering time. Plants overexpressing either wild-type SUMO conjugating enzyme (left) or an inactive (dominant negative) variant (right) were grown under short day conditions. Overexpression of the wild-type enzyme has no obvious consequences, whereas the dominant negative inhibitor leads to an early flowering phenotype.

Analysis of ubiquitin conjugating enzymes in plants

Arabidopsis contains 37 ubiquitin-conjugating enzymes (UBCs) and 7 so-called ubiquitin enzyme variants (UEVs; UBC homologues without active site Cys residue). Most of them can be grouped into families of two to four members with expected functional redundancy. We have started to combine mutant alleles by crossing lines with single locus T-DNA insertions in order to eliminate function of complete families. Most advanced are efforts with the RAD6 family of UBCs and with the MMS2 family of UEVs. In animals and in yeast, RAD6 is involved in the N-end rule pathway (see below), in DNA repair, in chromatin silencing and in gametogenesis. MMS2 plays a role in formation of Lys 63 linked multiubiquitin chains. The latter chains serve a function in regulatory processes and are not involved in protein degradation. Other UBC families are being investigated in a similar fashion.

The plant N-end rule pathway

The N-end rule pathway of protein degradation recognises proteins with a bulky first amino acid residue (other than Met) and leads to their destruction. In eukaryotes, degradation occurs via the ubiquitin-proteasome pathway. We have previously isolated PRT1, a ubiquitin protein ligase from this pathway that is apparently specific for plants. Based on expression studies in yeast, PRT1 recognises only a subset of bulky first residues, namely the aromatic amino acids Phe, Tyr and Trp. However, other first residues such as Leu or Arg also destabilise a plant protein, suggesting that there is another ubiquitin protein ligase in the plant N-end rule pathway. A candidate ligase from Arabidopsis with homology to yeast and mammalian enzymes is currently under investigation. A different component of the pathway, arginyl tRNA protein transferase, was recently shown by another laboratory to play a role in senescence. T-DNA insertion alleles in identified or presumed pathway components are combined with newly made indicator transgenes in order to prove association of the respective genes with the N-end rule pathway. Furthermore, double and triple mutants are constructed to explore epistatic relationships and to facilitate analysis of specific roles of the N-end rule pathway in plants.

Role of ubiquitin in cell death

Multi-ubiquitin chains with ubiquitin units linked via Lys 48 are essential for proteolysis. Formation of such chains can be inhibited by expression of a ubiquitin variant with Arg instead of Lys at position 48 (ubR48). A characteristic consequence of *in planta* expression of the ubiquitin variant is initiation of cell death. A role for ubiquitin in cell death inhibition is well documented in animals. To better understand the contribution of ubiquitin to cell death suppression in plants, we have isolated Arabidopsis mutants that tolerate higher levels of the ubiquitin variant ubR48. One of the mutants, *sud1* (*s*uppression of *u*bR48-induced cell *d*eath), was selected for further characterisation.

The retrotransposon Tto1

Retrotransposons are mobile genetic elements that multiply via an RNA intermediate that is reverse transcribed into a DNA copy and subsequently inserted into the host genome. Tto1 from tobacco has a broad host range and specifically inserts into transcribed genes. However, its use as a tool for insertional mutagenesis is restricted by the fact that efficient induction occurs only in tissue culture. In collaboration with Dr. H. Hirochika, National Institute of Agrobiological Resources, Japan, we try to understand the biology of the element. We use Arabidopsis as the plant host where we can monitor the complete life cycle of the element. Baker´s yeast is used as another host, where early steps in retrotransposition can be monitored more conveniently. The element seems to have an unusual mode of translation. For instance, several small reading frames precede the long ORF, suggesting that Tto1 RNA is not translated by the usual ribosome scanning mechanism. Changes in the element´s mRNA allowed us to induce transposition events in whole plants (as opposed to tissue culture cells).

Future Developments

In Summer 2002, I moved from the University of Vienna, Austria, to the MPIZ. Approximately half of the research activities are a continuation of projects carried out in Vienna, whereas the other half are new. All efforts benefit from the infrastructure of the MPIZ. For one of the newly

initiated projects, analysis of UBC families, we will decide whether to focus on a smaller number of families than currently surveyed. This decision depends, in part, on whether combinations of insertional mutations in genes with presumably overlapping functions are viable (and thus amenable to in depth analysis). Regarding the retrotransposon Tto1, we are encouraged by transposition events in Arabidopsis during the normal plant life cycle, which we obtained with modified elements. However, modifications tested so far do not yet provide a transposition frequency high enough for convenient insertional mutagenesis.

Scientific Publications

Bachmair, A., K. Garber, S. Takeda, K. Sugimoto, T. Kakutani and H. Hirochika: Biochemical analysis of LTR retrotransposons. In: Mobile Genetic Elements: Protocols and Genomic Applications (W. Miller and P. Capy, eds.). Humana Press, Totowa, NJ (in press).

Bachmair, A., M. Novatchkova, T. Potuschak and F. Eisenhaber: Ubiquitylation in plants: a post-genomic look at a post-translational modification. Trends Plant Sci. **6**, 463–470 (2001).

Hayashi, M., A. Miyahara, S. Sato, T. Kato, M. Yoshikawa, M. Taketa, M. Hayashi, A. Pedrosa, R. Onda, H. Imaizumi-Anraku, A. Bachmair, N. Sandal, J. Stougaard, Y. Murooka, S. Tabata, S. Kawasaki, M. Kawaguchi and K. Harada: Construction of a genetic linkage map of the model legume *Lotus japonicus* using an intraspecific F_2 population. DNA Res. **8**, 301–310 (2001).

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Sandal, N., L. Krusell, S. Radutoiu, M. Olbryt, A. Pedrosa, S. Stracke, S. Sato, T. Kato, S. Tabata, M. Parniske, A. Bachmair, T. Ketelsen and J. Stougaard: A genetic linkage map of the model legume Lotus japonicus and strategies for fast mapping of new loci. Genetics **161**, 1673-1683 (2002).

Schlögelhofer, P., and A. Bachmair: A test of fusion protein stability in the plant, *Arabidopsis thaliana*, reveals degradation signals from ACC synthase and from the plant N-end rule pathway. Plant Cell Rep. **21**, 174-179 (2002).

Schlögelhofer, P., V. Nizhynska, N. Feik, C. Chambon, T. Potuschak, E.-M. Wanzenböck, D. Schweizer and A. Bachmair: The upstream Sal repeat-containing segment of *Arabidopsis thaliana* ribosomal DNA intergenic region (IGR) enhances the activity of adjacent protein coding genes. Plant Mol. Biol. **49**, 655–667 (2002).

Diploma theses

Gudrun Böhmdorfer (2002, University of Vienna) Heterologous expression of the tobacco retroelement Tto1 in yeast and in Arabidopsis thaliana

Claudia Kerzendorfer (2002, University of Vienna) Ubiquitin-dependent cell death in Arabidopsis thaliana

Dissertations

Susanne Stary (2002, University. of Vienna) Characterization of the ubiquitin protein ligase PRT1 of Arabidopsis thaliana

Peter Schlögelhofer (2002, University of Vienna) Analysis of ubiquitin-dependent proteolysis in Arabidopsis thaliana

Structure of group

Group leader	**Dr. Andreas Bachmair**
Postdoctoral fellow	**Dr. Xiao-jun Yin**
PhD students	**Ruchika Budhiraja**
	Gudrun Böhmdorfer
	Marcus Garzón
Technical assistants	**Kerstin Luxa**
	Angelika Krull

Grants, external funding

DFG/Austrian Science Foundation FWF (2 grants): 2 PhD students
IMPRS: 1 PhD student

Collaborations

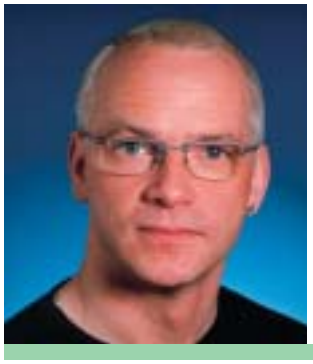
Analysis of retrotransposon Tto1, National Institute of Agrobiological Resources, Japan, Dr. H. Hirochika

Analysis of sumoylation in *Arabidopsis*, University of Toronto, Canada, Dr. C. Hardtke

Ubiquitin-dependent processes, University of Vienna, Austria, Dr. P. Schlögelhofer

Invited lectures

University of Cologne (5.11.2002), University of Bielefeld (10.03.2003) ■



Genes and Tools for Molecular Breeding of New Crops

Guido Jach

Introduction

Even in the most advanced crop management systems, diseases caused by viruses, bacteria, fungi and nematodes continue to limit both productivity and quality. Improving the natural resistance of crops to phytopathogenic organisms has been a major objective in agriculture during the past century and remains a major priority today. However, current protection strategies still rely heavily on the use of agrochemicals to defeat plant diseases and avoid yield and quality losses. Because of negative impacts on the environment, this approach on its own is considered untenable for long-term sustainable production. Indeed, there is a pressing need to reduce the damage potential of 'patho-cides' by limiting environmental exposure and by implementing effective new strategies of integrated pest management.

In all crops, new pest management strategies will be strictly dependent on the availability of either agents or compounds being able to replace the conventional agrochemicals or, in the ideal case, (new) crop varieties displaying resistance towards diseases and pests. Generally, genetically engineered resistant crop varieties might be generated by providing the chosen target plant with new properties or by strengthening of the plant's own defence mechanisms. For this purpose, several strategies are available, with the most promising being: (1) establishment of expression and production of protective natural products (PNPs) in the chosen crop plant; or (2) improvement of endogenous PNP production to gain higher yields. Protective natural products include several classes of compounds with inhibitory activity against phytopathogens, such as peptides, proteins and metabolic compounds.

New Results

Hunting for new genes

In the frame of an EC-project, work was carried out to identify novel genes encoding improved anti-microbial proteins, to find molecular switches and new resistance genes, to provide the basis for map-based cloning of virus resistance genes, to establish an experimental procedure for differential display of proteins and to allow for the *in vitro* analysis of the novel proteins.

In co-operation with partners from Spain and Argentina, 35 Solanum wild species were screened by PCR for the presence of genes belonging to the following protein classes: AP24 (osmotin-like) proteins, resistance-gene-like (RGL) proteins, basic chitinases, basic glucanases and Snakin proteins, the latter being a recently described defensin-like small protein from potato tubers. During the reporting period, 25 new genes have been isolated and subcloned by this approach. For 19 genes, the complete DNA sequences was determined and analysed *in silico*. Further sequencing is in progress as well as establishment of expression systems to produce recombinant protein for *in vitro* analysis of the proteins. In addition, first data on the expression patterns of these genes in the wild species were obtained by RT-PCR-based methods.

Cloned Snakin genes from wild species were placed under the control of the constitutive CaMV 35S promoter and subcloned into plant transformation vectors. Using these vectors, Snakin transgenic tobacco plants were raised and analysed on DNA and RNA level. Bioassays with these plants are in progress. Furthermore, an intronless Snakin gene was cloned. *In vitro* data indicate a two-fold higher protein expression for this gene compared to the wild-type gene.

Furthermore, two SSH libraries from Solanum wild species were generated and new molecular markers linked to a virus resistance gene have been obtained for *Solanum commersonii*. Work is in progress to generate a BAC library and to employ the new markers for map-based cloning of the resistance gene.

Continued work

Trichoderma chitinases

The work to optimise the codon-usage of the previously cloned Trichoderma chitinase genes was finished. The resulting genes were subcloned into plant transformation vectors, which then were used to generate transgenic tobacco plants. Work is in progress to finish the molecular analysis of the resulting chitinase transgenic plant lines and to investigate their resistance levels in bioassays with selected fungal pathogens.

Tools for high-level gene expression and plant transformation

In continuation of previous experiments, an extensive deletion analysis of the 124 bp translational enhancer element (TL) of the tobacco etch virus (TEV) was carried out

to define the *cis*-acting elements needed for its activity. In addition, the activity of TL was compared to the TMV omega element, another prominent translational enhancer. The results indicate that the activity of TL is based on the joint action of two *cis*-elements and that the overall activity of the TL elements exceeds that of the TMV omega element by a factor of two. Oligomerisation of the TL *cis*-elements as well as combinatorial use of TL and omega elements did not result in further increases of the enhancer activity.
Cloning work initiated during the last reporting period to construct new size-minimised binary plant transformation vectors was finished. Vectors sets were cloned containing empty expression cassettes for transcriptional and translational fusions to target protein expression to cytosol, ER, vacuole and cell wall.

Tools for promoter trapping
Previous work already demonstrated the suitability of the red-fluorescent protein from Discosoma sp. (DsRED) as reporter for gene expression studies in plants; however, it also revealed some limitations of this new reporter protein, namely its very slow maturation and its oligomerisation and tendency to aggregate. Meanwhile, a couple of mutant DsRED proteins (DsRED2, DsRED.T3, DsRED.T4, RedStar) with improved properties were published, but their performance *in planta* is not known yet. Using site directed mutagenesis, genes encoding these mutants were created based on the wild-type DsRED gene sequence. Gene expression studies in tobacco BY2 suspension culture cells (transient expression) as well as in stably transformed tobacco and Arabidopsis plants are in progress to investigate the relative "performance" of these genes. Similar experiments are in progress to characterise a recently published monomeric form of the DsRED protein (mRFP1).
In addition, the collection of fluorescent proteins under investigation was enlarged by introducing genes obtained from anthozoan species like *Anemonia sulcata* (asFP499, asCP562), *Entacmea quadricolor* (eqFP611) and *Heteractis crispa* (HcRED). Gene expression studies are in progress. First results are available indicating that asFP499, eqFP611 and HcRED are well suited for plant expression.

Future Developments

Using stably transformed tobacco plant lines, the genes cloned from Solanum wild species will be investigated with respect to their ability to confer resistance towards fungal plant pathogen. Furthermore, the generated SSH libraries will be used to isolation new genes specifically induced by fungi and nematode attack. Work will be carried out to characterise the encoded proteins *in vitro*.
Analysis of the fluorescent reporter proteins will be continued to identify the best-suited protein for promoter trapping studies in dicot and monocot plants. Using this/these, reporter tagging vectors will be constructed and used for promoter trapping studies. Positive events will be analysed in more detail (isolation of the sequence, functional analysis, deletion analysis etc.).

Scientific Publications

Jach, G: Genetics of Resistance and Plant Breeding. Encyclopedia of Pest Management (in press).

Jach, G., E. Binot, S. Frings, K. Luxa and J. Schell: Use of red fluorescent protein from *Discosoma sp*. (dsRED) as a reporter for plant gene expression. Plant J. **28(4)**, 483-491 (2001).

Structure of your group

Group leader	**Dr. Guido Jach**
Postdoctoral fellows	**Dr. Birgit Reintanz** since July 2002
Technical assistants	**Sabine Frings** **Kerstin Luxa** until July 2002

Grants, external funding

BMBF-project: 1 Postdoctoral fellow
EU-project (INCO-program): 1 Postdoctoral fellow, 1 technical assistant

EU-project (FAIR-program): 1 Technical assistant

Collaborations

Plant improvement for resistance to biotic and abiotic stress using agricultural biotechnologies

Hebrew-University of Jerusalem, Israel, Prof. Ilan Chet

Otto-Warburg-Center, Jerusalem, Israel, Prof. Arie Altman

MPIZ, Cologne, Germany, Prof. Dorothea Bartels

Bethlehem University, Palestine, Dr. Moein Kanaan, Dr. Naim Iraki, Dr. Radwan Barakat

National Research Center, Egypt, Prof. Mokthar Satour, Agricultural Research Center, Egypt, Prof. Esmat Hassan,

Resistant wild potatoes as source for novel genes mediating resistance against fungal, viral and nematode diseases

NEIKER, Vitoria, Spain, Dr. Enrique Ritter

University of Costa Rica, San Jose, Costa Rica, Dr. Roberto Valverde

INTA, Buenos Aires, Argentina, Dr. Esteban Hopp, Dr. Alejandro Tozzini

Antimicrobial peptides: studies aimed at application in food and food products

ATO-DLO, Wageningen, Netherlands, Dr. Arie an der Bent

NOVOzymes, Copenhagen, Denmark, Dr. Hans-Henrik Kristensen

Leatherhead Food Research Association, Leatherhead, Great Britain, Dr. Jonathan Rhoades, Dr Paul Gibbs

Invited lectures given

DLR workshop, Buenos Aires (06.09.2001), Biologische Bundesanstalt, Braunschweig (27.11.2001), BMBF workshop, Cairo (04.04.2002)

Department of Botany, University of Kiel (23.10.2002), 2nd CIMbios Symposium, CICY, Merida (25.11.2002), INTA, Balcarce, Argentina (06.12.2002)) ■

Basic Biological Processes in Plant Development

Bernd Reiss

Introduction

Development can be defined as the construction of a three-dimensional, multi-cellular organism from a single cell during time. T-DNA and transposon-tagged mutants have contributed considerably to the understanding of a variety of developmental processes in plants in recent years. We have screened a new population of T-DNA activation tagged mutants for genes involved in two developmental processes: determination of flowering time and plant morphogenesis. In another approach, we compared the role of known and well-defined genes in two organisms with different developmental patterns, i.e. the lower, non-flowering plant *Physcomitrella patens* and the higher, flowering plant *Arabidopsis thaliana*, in order to understand general principles in the determination of flowering time and the role of genetic recombination in plant development.

New Results

Genes affecting the Arabidopsis body plan

Screening of the ZIGIA population of T-DNA activation tagged lines resulted in the identification of ten dominant and three recessive mutants with easily detectable morphological alterations. These mutants were stable and the T-DNA tag co-segregated with the mutant trait. The T-DNA tagged genes of most of those mutants were isolated by plasmid rescue or genomic IR-PCR. The genes identified using this procedure are likely to have caused the mutant phenotype since Southern blotting analyses demonstrated co-segregation of the T-DNA insertion with the mutated gene. In addition, the mutated genes were over-expressed in the dominant mutants, as shown by Northern blots. To date, this approach has resulted in the identification of the two early flowering mutants described below and a variety of mutants in non-annotated genes. This work will contribute new, previously unidentified genes that participate in a variety of plant developmental processes, like basic functions necessary for plant morphology, stem development, flower arrangement and plant growth. In the recessive mutants, three previously unannotated genes of unknown function were found to be tagged. The phenotypes suggest that these genes have a role in cell-cell communication.

Control of the time of flowering

The entire ZIGIA T-DNA tagged population was screened for dominant flowering time mutants and a sub-population of 2000 plants was re-screened in pools for recessive mutants. These screens identified two dominant, early flowering mutants. The isolation and identification of the tagged genes showed that one of them was *FT*, and the other one *AGAMOUS*. Both genes were over-expressed in the mutants. Since both genes are well known to reduce the time of flowering when over-expressed, these mutants demonstrate the potential of the ZIGIA population. However, they also suggest that screening for flowering time mutants by this method will identify mutations in many previously characterised genes.

The *CONSTANS* gene is a well-described flowering time gene in Arabidopsis; however, its role is not well understood in other plants. The moss *Physcomitrella patens* is a lower plant and phylogenetically distant from Arabidopsis. In addition, mosses are not flowering plants, but rather sporulate and produce spores. To analyse whether *CONSTANS* could be involved in this process, database searches were performed to search for homologues in *Physcomitrella*. This work resulted in the identification of a variety of ESTs with homology to *CONSTANS*. Since in Arabidopsis, *CONSTANS* is a member of a large gene family with different degrees of relatedness, more refined analyses were used to find the closest homologue. This analysis resulted in three closely related genes, the existence of which was confirmed in Southern blots. In addition, a low stringency Southern blot analysis indicated that no further genes with this degree of homology exist.

Recombination as a developmental process

Recombination in concert with mismatch and DNA-damage repair is responsible for the maintenance and stability of the genome, but is a also the driving force for genetic variability, suggesting that this process is highly regulated. To fulfil this function, recombination is interlaced with cell-cycle regulation via a variety of checkpoints. This interrelationship ensures that only DNA damage-free genomes can pass through mitosis and thus, are propagated to the next generation of cells. The *RAD51* gene is involved in such a checkpoint in animals as suggested by

the lethality of *RAD51* gene knock-outs in the mouse and chicken as well as the phenotypes observed in conditional knock-outs. In contrast, *RAD51* in the lower eukaryote yeast is necessary for meiosis and DNA-damage repair, but not cell cycle progression. To determine whether RAD51 has also a role in cell cycle control in other higher eukaryotic, multi-cellular organisms, we analysed the role of this gene in the development of a lower (*Physcomitrella patens*) and a higher (*Arabidopsis thaliana*) plant.

The Physcomitrella genome contains two, intronless *RAD51* genes, *Pprad51A* and *Pprad51B*, which encode nearly identical proteins. The A-gene is preferentially expressed in dividing cells to a relatively high level while the B-gene is expressed at low level in all protonema cells, suggesting specialised, but overlapping functions for these genes. In targeted knock-outs of both genes, neither knock-out alone showed a phenotype, as expected from the gene redundancy. However, the double knock-out plants were fully viable and even produced spore capsules and spores, although the spores were not viable. Therefore, RAD51 function is necessary for meiosis, but not for cell-cycle progression in the lower plant Physcomitrella. This phenotype is not restricted to lower plants since also an Arabidopsis *RAD51* gene knock-out mutant was fully viable and produced normal flowers. However, pollen and ovule development is aborted at a developmental stage immediately following meiosis. Therefore, in sharp contrast to animals, plants need RAD51 function only for meiosis, but this gene is not involved in cell cycle control. Thus, plants differ from animals in basic mechanisms of DNA-damage recognition and signalling as well as the regulation of cell cycle progression.

Future Developments

In contrast to studying a gene involved in a developmental process in a single organism, future research will be extended to study the role of the same gene or process in different organisms. We will especially use our expertise and resources in Arabidopsis and Physcomitrella to analyse basic principles involved in the determination of flowering time and genetic recombination. In addition, international co-operations will be used for comparative genomics studies to analyse the mechanisms of genetic recombination and DNA-damage repair across the borders of organisms. Furthermore, we will focus on the role of chromatin in the regulation of basic biological processes like determination of flowering time and genetic recombination.

Scientific Publications

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Hadi M.Z., E. Kemper, E. Wendeler and B. Reiss: Simple and versatile selection of *Arabidopsis* transformants. Plant Cell Rep. **21**, 130–135 (2002).

Markmann-Mulisch, U.,. M.Z. Hadi, K. Koepchen, A. Alonso, V.E.A. Russo, J. Schell and B. Reiss: The organisation of Physcomitrella patens rad51 genes is unique among eukaryotic organisms. Proc. Natl. Acad. Sci. USA **99**, 2959-2964 (2002).

Reiss, B.: Homologous recombination and gene targeting in plant cells. Int. Rev. Cytol. A Survey of Cell Biology (in press).

Masters thesis

Sebastian Haesler (2002) Identifizierung ausgewählter Rekombinationsgene aus Physcomitrella patens und Nicotiana tabacum

Structure of the group

Group leader	**Dr. Bernd Reiss**
Research associates	**Dr. Ulrich Markmann-Mulisch**
	Ernesto Olmos
	until December 2002
Postdoctoral fellow	**Dr. Anzhela Kyryk**
	since July 2001
Masters student	**Sebastian Haesler**
	until October 2001
Ph.D. students	**Oliver Zobell**
	since April 2002
Technical assistants	**Elke Kemper**
	Ellen Mahlow
	until August 2002
	Edelgard Wendeler

Funding from sources other than MPG

BMBF ZIGIA: 1 Research associate, 1 technical assistant

EU QLRT-2000-00365, PREGENE, Precision engineering of plant genes: 1 Research associate, 1 masters student

DFG, Graduiertenkolleg "Molekulare Analyse von Entwicklungsprozessen": 1 PhD student

Collaborations

Friedrich Miescher Institut, Prof. Dr. Barbara Hohn

Friedrich Miescher Institute, Dr. Jurek Paszkowski

UMR CNRS – GEEM – BIOMOVE, Universite Blaise Pascal, Dr. Charles White

Department of Genetics, Wageningen Agricultural University, Prof. Dr. Christa Heyting

Campus Universidad Autonoma de Madrid, Dr. Juan C. Alonso

Vienna Biocenter, University of Vienna, Prof. Dr. Erwin Heberle-Bors

November AG, Gesellschaft für Molekulare Medizin, Dr. Hans Kosak

Department of Genetics, The University of Leeds, Prof. Dr. David J. Cove

BASF Aktiengesellschaft, Dr. Ralf Badur

Leeds Institute for Plant Biotechnology, and Agriculture (LIBA), The University of Leeds, Prof. Dr. Peter Meyer

Laboratoire de Phytogénétique Cellulaire Institut d'Ecologie – UNIL, Prof. Dr. Jean-Pierre Zrÿd

SunGene GmbH & Co. KgaA, Dr. Christian Biesgen

Invited lectures given

ESF Workshop "New science for increasing biosafety of GM plants", Braunschweig (8.-12.03.2003), Zellbiologisches Kolloquium, University of Kiel (28.04.2003) ■

Functional Analysis of Regulators of Sugar and Stress responses in Arabidopsis

Csaba Koncz

General Introduction

Current studies of our laboratory focus on two research areas:

- Characterisation of signalling functions that regulate glucose, hormone and stress responses in interaction with the PRL1 regulatory WD-protein and AMP-activated SnRK protein kinases
- Analysis of functions of CYP90A1 steroid hydroxylase and its interacting partners in regulation of brassinosteroid responses and identification of steroid-binding proteins.

Here, we report on the genetic dissection of signalling cascades that, through interaction with the nuclear regulatory PRL1 WD-protein and SnRK protein kinases, modulate glucose repression of transcription and sensitivity of plants to abscisic acid (ABA), ethylene, auxin and cytokinin hormones.

PRL1, a key element of this regulatory pathway encoded by the *Pleiotropic Regulatory Locus 1*, was identified using a forward genetic screen for T-DNA insertion mutations causing hypersensitivity to glucose and sucrose. Characterisation of the *prl1* mutation showed that PRL1 is a highly pleiotropic nuclear regulator, which influences several signalling pathways and interacts with multiple signalling factors, including Arabidopsis homologues of yeast Snf1 and animal AMP-activated protein kinases (AMPKs, Németh *et al.,* (1998) Genes Dev. **12**, 3059). We have shown that PRL1 is a negative regulator of Arabidopsis SnRKs (Snf1-related kinases) AKIN10 and AKIN11 (Bhalerao *et al.,* (1999) Proc. Natl. Acad. Sci. USA **96**, 5322).

Similarly to yeast Snf1 and animal AMPKs, type I plant SnRKs are implicated in the control of essential cellular functions, including the regulation of activity and stability of key metabolic enzymes (i.e. acetyl-CoA carboxylase, 3-hydroxymethyl-glutaryl-CoA reductase etc.) Through the control of isopentenyl and mevalonate pathways, SnRKs are thought to affect the biosynthesis of several hormones (ABA, cytokinin, gibberellin, brassinosteroids). De-repression of numerous glucose-regulated genes in the *prl1* mutant (which correlates with increased SnRK activity) suggests that stress-regulated activation of SnRKs modulates transcriptional activation and repression. To dissect the regulatory functions of SnRKs, we have performed the characterisation of genes encoding SnRK catalytic (alpha), targeting (beta) and activating (gamma) subunits (Ferrando *et al.*, (2000) Plant J. **22**, 553; Ferrando *et al.*, (2001) Nucleic Acids Res. **29**, 3685; Kleinow *et al.*, (2000) Plant J. **13**, 1; Lumbreras *et al.*, (2000) EMBO Rep. **2**, 55-60). In addition, we have identified numerous SnRK interacting proteins. We have found that SnRKs AKIN10 and AKIN11 occur in stable association with SKP1/ASK1, a common subunit of several SCF E3 ubiquitin ligases (e.g. SCF^{TIR1}, SCF^{COI1} etc.). We showed that SKP1 and both AKIN10 and AKIN11 interact with the alpha4/PAD1 subunit of 26S proteasome. These data indicate that SnRKs are stable SCF ubiquitin ligase components, which probably play a role in phosphorylation of SCF substrate proteins (i.e. phosphorylation is obligatorily required for the recognition of SCF substrates by F-box proteins and their subsequent ubiquitination by SCF complexes). In addition, our data showed that SKP1/ASK1 and SnRKs mediate proteasomal targeting of SCF complexes. Finally, we demonstrated that the SnRK-inhibitor PRL1 protein interrupts the interaction of SnRKs with proteasome and SCF complexes, and thus, probably regulates SnRK-mediated phosphorylation of SCF substrate proteins (Farrás *et al.*, (2001)).

In correlation with the data indicating that SnRK-SCF-proteasome complexes are implicated in SCF-regulated degradation of transcription regulatory factors, we have found that PRL1 interacts with a histone arginine methylase PAM1, which methylates histone H2B and the spliceosome assembly factors RSZp33 and RSZp22 on their RGG motives. Remarkably, RSZp33 was identified as an interacting partner and phosphorylation substrate of SnRKs, and later we found that SnRKs co-immunoprecipitate and co-purify with PAM1. These data suggest that SnRKs are associated with a co-activator complex that contains a conserved histone arginine methyltransferase.

We have shown that PRL1 orthologues (PLRGs) are conserved from fission yeast to humans. Other laboratories found that AMP-activated kinases (representing animal and human orthologues of plant SnRKs) occur in co-activator complexes with histone arginine methylases CARM1 and PRMT1, p300 histone acetylase, p160 co-activator and several nuclear receptors. This analogy raised the question whether AMPKs and histone methylases would occur in association with animal orthologues of PRL1. To address

this question, we have initiated a close collaboration with Dr. Jens Brüning (Universität zu Köln), whose studies aim to reveal whether PRL1 orthologues would also interact with co-activator complexes in mammals. Thus far, we failed to identify a PRL1 orthologue in budding yeast. However, yeast carries a PAM1 histone methylase orthologue, Hmt1/Pmt1, that methylates the CBP80 and CBP20 subunits of mRNA cap-binding complex. Synthetic lethality of *hmt1* and *cbp80* mutations indicates a genetic interaction between the capping complex and protein arginine methylase Hmt1. The fact that Cbps carry RGG motives and are conserved in plants along with the observation that Arabidopsis PAM1 is a suppressor of yeast *hmt1*, *cbp80* synthetic lethality strongly suggests a possible involvement of PAM1 in the regulation of nuclear RNA export. (Fig. 1)

New Results

During this project period, our efforts have concentrated on the development of genomics and proteomics approaches to facilitate the functional characterisation of subunits of protein complexes identified in the PRL1-SnRK regulatory pathway. The key results are listed below.
To provide a basis for the genetic studies, we have constructed and characterised a new Arabidopsis mutant collection (Rios *et al.* (2002); Szabados *et al.* (2002). This and other public collections were used for the identification of T-DNA insertion mutants, which yielded new *prl1* alleles as well as knock-outs affecting the AtSNF4 activating and AKINbeta substrate targeting SnRK subunits, the alpha4 proteasome subunit, six cullin and several SKP subunits of SCF ubiquitin ligases, the RSZp33 spliceosome assembly factor, the PAM1 histone methylase and its close homologue PAM2. Insertion mutations were similarly identified in all other genes that code for PRL1 and SnRK interacting proteins identified in our previous yeast two-hybrid screens (see MPIZ Scientific Report 2001). Genetic analysis of all knock-out mutants has been initiated.
New studies were started on the functional characterisation of PRL1-interacting amidase, CRK1 protein kinase and Ufd1 proteins as well as on a SnRK-binding potential upstream activating kinase.
Constructs for expression of above-described signalling proteins in an epitope-labelled form (using either native promoters of corresponding genes, or CaMV 35S promoter, or an oestradiol-inducible system) were generated and introduced into plants and/or cell cultures. Dominant positive and negative forms of SnRK catalytic subunits, potential upstream activating kinase and PRL1-binding CRK kinase have been or are being similarly generated. Preliminary cellular localisation studies with these epitope-tagged proteins were performed.
To interrupt and regulate protein interactions, isolation of domain-specific PRL1 and SnRK mutations was initiated.
Six cullin and 19 SKP1 subunits of SCF complexes are being expressed in plants using epitope-tagged forms and all possible combinations to facilitate the analysis of subunits and substrates of SnRK-associated SCF complexes.
Using a HA-epitope-tagged form of AKINbeta2-subunit, an SnRK kinase complex was purified for mass spectrometric subunit analysis.
Using HA-tagged PAM1 methylase, a PAM1-SnRK complex was purified from transgenic plants for mass spectrometric subunit analysis.
A transcription activator of glucose-induced aspartokinase 1 (*ASK1*) gene was identified and found to be identical with the G-box-binding bZIP factor DPBF4/EEL1, a dimerisation partner of ABI5. Over-expression of DPBF4 and ABI5 was demonstrated to activate the *ASK1* promoter *in vivo*. DPBF4 was shown to undergo phosphorylation. Studies have been initiated to examine whether DPBF4 is an SCF substrate.

Future Developments

Our studies on the regulation of brassinosteroid (BR) signalling (which are not discussed here due to space limitation) revealed that BRs abolish the transcription of certain ABA-, salt- and sugar-regulated genes (Ábrahám *et al.*, (2003)). To determine how sugar, stress and BR signalling modulates the phosphorylation, methylation, ubiquitination and degradation of gene-specific transcription activators and repressors (labelled as "chromatin-associated substrates" in Fig. 1), we shall exploit CHIP (chromatin-immunoprecipitation) studies using epitope-labelled transcription factors (e.g. DBPF4), PAM1, PRL1, SnRK and SCF F-box subunits. In addition to these new studies, we shall continue the characterisation of PRL1-SnRK signalling pathway using the above described functional genomics and proteomics approaches.

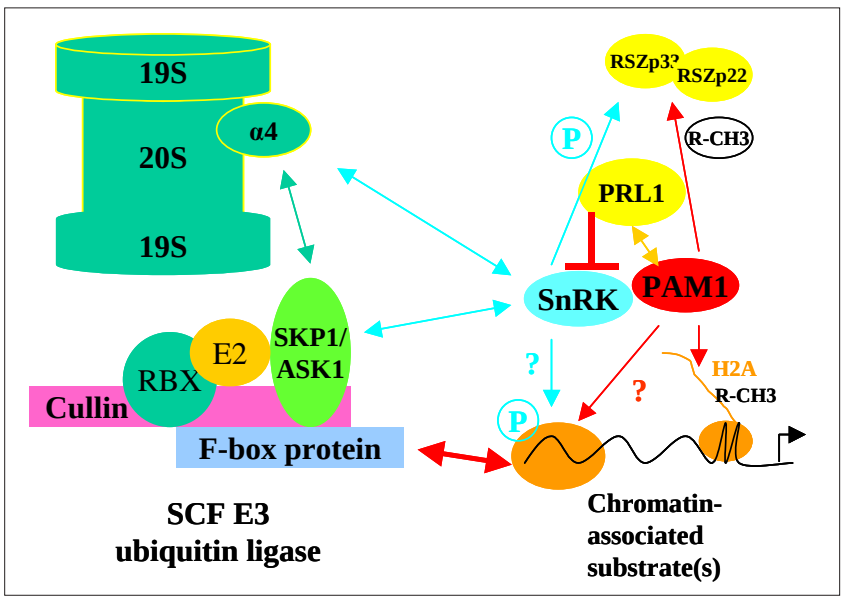

Fig. 1. Elements of PRL1-SnRK signalling pathways identified in our studies.

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Dissertations

Frank Breuer (2000) Untersuchungen zur Funktion zweier PRL-WD-Proteine und deren Proteininteractionspartnern in pflanzlichen Glukose- und Streßsignaltransduktionswegen (University of Cologne)

Tatjana Kleinow (2000) Identifizierung und Charakterizierung von Potentiellen Komponenten der Stress- und Glukose-Signaltransduktion von *A. thaliana* (University of Cologne)

Rosa Farrás I Rivera (2001) Proteasomal components of *Arabidopsis* glucose signalling pathway. (University of Barcelona)

Rossana Adrea Novo Lopes Henriques (2002) Studies on iron and zinc transporter proteins (ZIP family) and on CYP90A2 (homolog of CYP90A1) in *Arabidopsis thaliana* and *Camellia japonica*. (University of Lisbon)

Structure of the group

Group leader	**Dr. Csaba Koncz**
Postdoctoral fellows	**Dr. Zsuzsa Koncz** since September 1984
	Dr. Jan Jásik since January 1999
	Dr. Gabino Rìos since September 1999
	Dr. Attila Oberschall since March 2001
	Dr. Isabella Kovács since January 2003
	Dr. Gergely Molnár since September 2001
	Dr. Luca Eckstein ince January 2003
PhD students	**Kenneth Berendzen** until January 2004
	Vijaya Shukla until September 2004
	Marcel Lafos until January 2005
	Dóra Szakonyi until January 2006
	Mihàly Horvàth until April 2006
Technical assistants	**Sabine Schäfer**
	Andrea Lossow
	Melanie Broisch
	Christiane Gerdes
Gardener	**Ingrid Reinsch**

Grants, external funding

DFG SPP (1067) “Molekulare Analyse der Phytohormonwirkung” project KO 1438/3-3, “Kommunikation zwischen der Kohlenstoff-Katabolit-Repression und dem Auxinsignalweg: Interaktion eines SNF1-Kinase-Inhibitors mit einem neuen Amidase-Komplex”: 1 Postdoctoral fellow

DFG *Arabidopsis* Funtional Genetics Network (AFGN), project KO 1438/9-1, Functional analysis of plant Snf1-related protein kinase signaling complexes using proteomics and reverse genetics in *Arabidopsis*: 2 PhD students

EU GVE (Growth, Vigour, Environment: Molecular breeding for plant growth and yield) QLRT-2000-01871 project: 1 Postdoctoral fellow, 1 PhD student

EU ROST (Molecular breeding for improvement of plant drought, salt and cold stress tolerance) QLRT-2001-00841 project: 1 Postdoctoral fellow

EU Research Training Network HPRN-CT-2002-00333 project UBITARGET (Training Network on Functional genomics and proteomics of *Arabidopsis* ubiquitin ligases and their targets in signaling): 1 Postdoctoral fellow

HFSP project RG0162/2000-M, Regulation of steroid hormone biosynthesis and signaling in plants: 1 Postdoctoral fellow

MINNA James Heinemann Foundation Project, Regulation of lysine, threonine and proline biosynthesis by glucose and stress in plants: 1 PhD student

Collaborations

DLRZ (Deutsche Zentrum für Luft- und Raumfahrt e.V., Internationales Büro des BMBF) WTZ project HUN 01/004,

"Regulation von Arabidopsis Proteinkinasen in der katabolischen Kohlenstoffrepression": Dr. László Szabados, Institute of Plant Biology, Biological Research Center of Hungarian Academy of Sciences, Szeged (until February 2004)

DLRZ (Deutsche Zentrum für Luft- und Raumfahrt e.V., Internationales Büro des BMBF) WTZ project CZE 01/006, "Identification von pflanzlichen Sterol-bindenden Proteinen durch Affinitätsaufreinigung und reverse genetics": Dr. Ladislav Kohout, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague (until February 2004)

DAAD (Deutscher Akademischer Austausch Dienst) PROCOPE Project PKZ D/0122859, "Functional genomics von SCF- und APC-Ubiqutinligasen": Dr. Eva Kondorosi, CNRS Institut des Sciences Vegetales, Gif sur Yvette (until January 2004)

DAAD (Deutscher Akademischer Austausch Dienst Projektbezogenen Personenaustausch) PPP project 324/ssch, "Study of protein interactions involved in brassinosteroid signalling": Dr. Agnes Cséplö, Institute of Plant Biology, Biological Research Center of Hungarian Academy of Sciences, Szeged (until January 2004)

Invited lectures given

Research Institute of Molecular Pathology, Vienna (14.05.2001), Conference of Hungarian Biochemical Society, Sárospatak (17.05.2001), Technische Universität München, Munich (24.01.2002), University of Tel Aviv (29.04.2002), ZOMESII, Antalya (01.05.2002), CNRS Institut des Sciences Végétales, Gif sur Yvette (17.05.2002), AMPK meeting, Dundee (13.09.2002), 48th National Congress of Genetics, Sao Paulo (17.09.2002), University of Stuttgart (11.02.2003), Juan March Foundation, Madrid (25.02.2003), Institute of Experimental Botany, Prague (13.04.2003), Congress of Hungarian Genetic Society, Siófok (15.04.2003)

Department of Plant Microbe Interactions

Director: Paul Schulze-Lefert

Research in the department engages in fundamental processes underlying interactions between plants and microbes. Although much of our work is focussed on molecular processes that permit plants to defend themselves against pathogen attack, we exploit the opportunity to utilise pathogens also as 'probes' to gain insights into diverse plant regulatory pathways that are targeted by microbes during pathogenesis. Since the ability of plants to cope with biotic and abiotic stresses is often interconnected, a significant part of our research programme is dedicated to find out how plants adapt to multiple stresses and integrate these into co-ordinated responses.

The majority of research projects in the department are 'process oriented' rather than 'molecule oriented'. This reflects our ability to apply systematically genetic approaches for the establishment of skeletal frameworks underlying diverse biological phenomena in plant microbe interactions. While in the past mutational approaches have been powerful for this purpose, there is an increasing demand to understand biochemical functions of identified regulatory proteins, the molecular mechanics of processes that are regulated by these components as well as molecular dynamics upon pathogen attack. This necessitates future integrated approaches combining genetic, biochemical, molecular biology and cell biology methods. Indeed, major investments have been made in the reporting period to establish mass spectrometry facilities and non-invasive imaging technology towards this objective. We believe that the application of novel optical methods, such as fluorescence resonance energy transfer and fluorescence correlation spectroscopy, will greatly facilitate analysis of protein dynamics and protein-protein interactions in living cells in a non-invasive and quantitative manner. Ralph Panstruga has been appointed as the new group leader and part of his work programme will be dedicated to developing quantitative optical methods to study protein dynamics in single cells upon pathogen attack.

For the majority of research projects, Arabidopsis is used as the model for genetic and molecular analysis. Complementary research is conducted in tobacco, the cereal barley and the moss Physcomytrella. This has mainly two reasons. First, the breadth of available genetic and genomic tools in Arabidopsis is unrivalled; however, certain *in planta* technologies that are critical for rapid structure/function analysis of proteins are easier to conduct in tobacco or barley, e.g. systemic gene silencing, transient gene expression for the production of large quantities of proteins, single cell imaging and expression assays. Second, I believe that on a longer term, comparative analysis of gene functions and regulatory networks will become important to understand evolutionary aspects of plant microbe interactions. In this context, Thomas Koprek's group is developing basic genetic tools in barley that are expected to facilitate comparative analysis of gene functions between dicots and monocots.

Several groups in the Department study interactions with biotrophic pathogens such as the oomycete *Peronospora parasitica* and the ascomycete *Erysiphe*. There is increasing evidence that biotrophs, unlike necrotrophs, utilise sophisticated molecular methods and infection structures (e.g. haustoria) to actively re-programme host cells for the establishment of biotrophy, a phenomenon that is not understood at the molecular level. The Department does not yet host a group dedicated to work on pathogenesis, mainly because obligate biotrophic pathogens are still intractable to routine analysis by molecular biology methods. We are collaborating with various groups to establish basic molecular methods for the analysis of biotrophic fungi. Additionally, we are considering possibilities to adopt a hemibiotrophic fungal pathogen, such as *Colletotrichum*, to gain insights into molecular mechanisms underlying biotrophy.

Recognition and Signalling in Plant Disease Resistance

Paul Schulze-Lefert

Introduction

Our research focuses mainly on three aspects of plant microbe interactions: (1) How plants recognise pathogens; (2) mechanisms that limit the host range of pathogens; and more recently (3) host compatibility factors. In our experiments, we mainly utilise the model plant Arabidopsis and the cereal barley to study interactions with biotrophic powdery mildew pathogens (*Blumeria* and *Erysiphe* species).

(1) The ability of plants to combat pathogens is often conferred by disease resistance (*R*) loci. These *R* genes encode proteins that detect, directly or indirectly, pathogen effector molecules (encoded by *Avr* genes). The complex barley *Mla* locus is unusually polymorphic and encodes 27 known *R* gene specificities to the powdery mildew fungus (*Blumeria graminis* f sp *hordei*). A subset of *Mla R* genes requires for their function RAR1 and SGT1, two proteins that might exert a chaperone-like activity in resistance reactions (Fig. 1). The molecular isolation of several *Mla R* genes provided the basis to initiate functional studies using a single cell transient gene expression assay. Complementary biochemical approaches are mainly based on functional epitope-tagged MLA derivatives. A major future objective will be the characterisation of recently detected large MLA-containing protein complexes (~750 kD) in non-challenged plants (S. Bieri, S. Mauch, Q.-H. Shen, S. Pajonk).

(2) The host range of pathogens can vary greatly. The underlying molecular basis of this phenomenon is not understood. We hypothesised that plant factors limit the host range of biotrophic pathogens and searched for Arabidopsis mutants that permit partial growth of the barley powdery mildew fungus. The grass powdery mildew does not produce disease on wild-type Arabidopsis or any other tested dicot plant. The mutational approach led to the

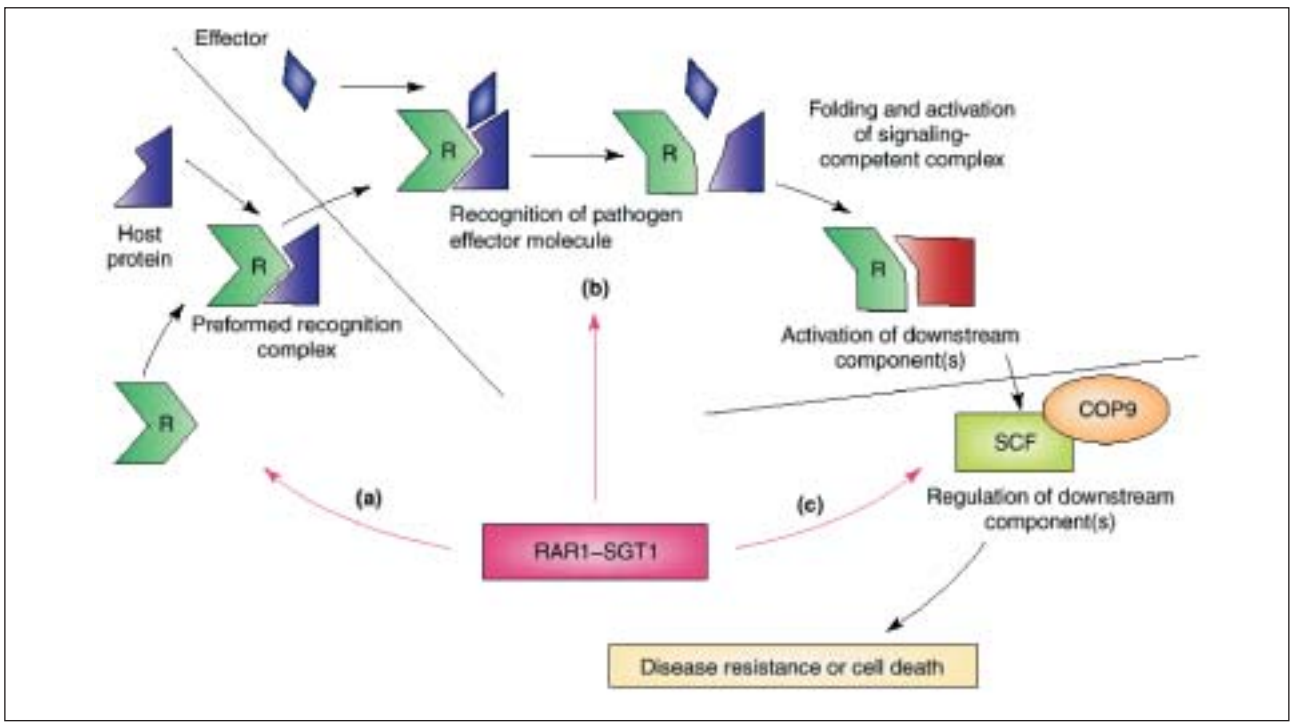

Fig. 1. Possible action points for RAR1–SGT1 chaperone-like activities in disease-resistance signalling. (a) Formation of resistance (R) protein complex with host protein(s). (b) Pathogen effector molecules are recognised by the assembled R protein complex and subsequent conformational changes activate downstream components. (c) Regulation of downstream signalling can occur by removing negative regulators and/or activating positive regulators by SCF and COP9 complexes. Further reading: Shirasu and Schulze-Lefert, 2003.

discovery of conserved SNARE secretion machinery components that mediate cell wall penetration resistance against the non-host Arabidopsis (PEN1) and the host barley (ROR2, SNAP34). These findings point to a role for specific vesicle-mediated exocytotic events in establishing effective barriers to fungal ingress. A long-term objective will be the characterisation of PEN1/ROR2 containing SNARE complexes, the establishment of methods permitting enrichment of corresponding 'defence vesicles', and profiling of vesicle cargo (V. Lipka, N. Collins, J. Dittgen, S. Bau, A. Freialdenhoven, P. Bednarek).

(3) The existence of plant compatibility factors facilitating pathogenesis is controversial and difficult to prove experimentally. We identified an Arabidopsis callose synthase isoform that is necessary for callose accumulation at haustorial complexes, a specialised intracellular feeding structure of many biotrophic fungi. Lack of callose in haustorial complexes leads to growth cessation of several tested powdery mildew species and of the pathogenic oomycete *Peronospora parasitica*, indicating a critical role of the corresponding callose synthase in the maintenance of biotrophy during fungal infection (A. Jacobs, V. Lipka).

New Results and Future Developments

Structure, function, and evolution of MLA NB-LRR proteins

Four molecularly isolated *Mla* resistance specificities each encode predicted cytoplasmic proteins containing a N-terminal coiled coil (CC) structure, a central nucleotide-binding (NB) domain, a leucine-rich repeat region, and a C-terminal (CT) non-LRR region lacking known structure/function motifs. The unusual sequence conservation (>90% identity) of the gene products and an identical gene structure suggest that many, if not all, genetically characterised powdery mildew *R* genes at *Mla* are variants of a single gene at the complex locus. By taking advantage of the high sequence relatedness of *Mla R* genes, candidates of five additional resistance specificities were isolated using RT-PCR methods (S. Pajonk). This provided for the first time a robust basis to examine patterns of sequence diversification in MLA proteins by computational methods. The analysis revealed in the LRR region, both structural diversity and hyper-variability of solvent exposed residues that might contribute to recognition specificity. An important conclusion from this is that different MLA proteins might have the capacity to interact with structurally diverse partners although they share more than 90% identical amino acids.

We have carried out a functional analysis of MLA-mediated pathogen recognition by generating reciprocal domain swaps between different MLA resistance specificities. This showed that the LRRs and corresponding CT domains are critical for the recognition of cognate avirulence determinants. Unexpectedly, this work also revealed that recognition of pathogen determinants can be uncoupled from RAR1/SGT1 dependence in the MLA chimeras. Thus, subtle differences of MLA protein sequence can dramatically alter RAR1 and SGT1 use without changing recognition specificity (Q.H. Shen).

A major part of our current and future biochemical approaches is based on the availability of transgenic barley lines expressing functional epitope-tagged MLA1 or MLA6 variants (S. Bieri). Successful immunoprecipitation (IP) of these proteins from leaf extracts demonstrates accessibility of the epitope tags. By means of blue native gel electrophoresis, we could identify a large ~750 kD complex containing the 108 kD MLA1 protein in non-challenged plants (S. Mauch). We are pursuing various combinations of size exclusion chromatography followed by IP experiments to purify presumed MLA1- and MLA6-containing recognition complexes. We plan to use MALDI-TOF and Q-TOF mass spectrometry to identify components present in these complexes.

Conserved SNARE secretion machinery components mediate cell wall penetration resistance

The molecular isolation of Arabidopsis *PEN1* and *PEN2* as well as barley *ROR2* is considered as a major breakthrough in the reporting period because it provides for the first time a molecular concept for penetration resistance against fungal pathogens (N. Collins, V. Lipka, A. Freialdenhoven). *PEN1* and *ROR2* are required for penetration resistance to the grass powdery mildew fungus in the non-host dicot Arabidopsis and the host monocot barley, respectively. The genes encode highly sequence-related and functionally homologous syntaxins belonging to the SNARE superfamily of proteins that mediate membrane fusion. dsRNAi gene silencing and protein-protein interaction studies identified a SNAP25 homologue as a potential cognate partner of plasma membrane localised ROR2 in resistance promoting SNARE complexes (N. Collins, S. Bau). This and ROR2-dependent congregation of vesicles at incipient sites of fungal entry in plant cells strongly implicate vesicle-mediated exocytosis in establishing effective barriers to powdery mildew ingress. Our findings indicate a common mechanistic basis for host-basal and non-host penetration resistance across the plant kingdom.

Arabidopsis *PEN2* encodes a predicted family 1 glycosyl hydrolase. Analysis of *pen1 pen2* double null mutants showed an accumulative effect, indicating that PEN1 and PEN2 may be components of two separate penetration resistance pathways against powdery mildew ingress. Interestingly, grasses apparently lack PEN2 homologues. Experiments are underway to find out whether catalytic activity is required for PEN2 function (P. Bednarek).

We are focusing our future activities on identifying presumed vesicle resident SNARE (v-SNARE) partners of the ROR2/PEN1 syntaxins. For this purpose, we will perform dsRNAi-mediated single-cell gene silencing in barley for each of the 27 v-SNARE homologues present in grasses. We predict that the products of v-SNARE genes that are required for penetration resistance can be used for

making fusions with fluorescent marker proteins and/or epitope tags to trace vesicles interacting with ROR2/PEN1 syntaxins.

An Arabidopsis callose synthase, AtGSL5, is required for wound callose formation and maintenance of biotrophy during fungal infection

We identified an Arabidopsis callose synthase isoform, encoded by *AtGSL5*, which is required for wound callose formation and appears to be necessary for the establishment of compatible interactions upon challenge with different powdery mildew species or with the oomycete biotroph *Peronospora parasitica*. GSL5 callose accumulates at haustorial complexes, a complex intracellular structure of many biotrophic fungi that is crucial for nutrient uptake. At present, we cannot discriminate whether GSL5 callose serves as a protective shield against antimicrobial compounds, is part of a stealth mechanism to 'hide' haustoria from host recognition, or whether GSL5 callose synthase is a regulatory component of a cell wall integrity pathway that is critical to maintain haustoria function. Future experiments aim to identify characteristics of AtGSL5 that distinguish this isoform from 11 other callose synthases present in the Arabidopsis genome. Plant homologues of known cell wall integrity pathway components in yeast will be examined for their potential role in maintaining haustoria function (V. Lipka). (Fig. 1)

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Devoto A., A. Hartmann, P. Piffanelli, C. Elliott, C. Simmons, G. Taramino, C.-S. Goh, F.E. Cohen, B.C. Emerson, P. Schulze-Lefert and P. Panstruga: Molecular phylogeny and evolution of the plant-specific seven transmembrane MLO family. J. Mol. Evol. **56**, 77-88 (2003).

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Structure of the group

Group leader	**Prof. Dr. Paul Schulze-Lefert**
Postdoctoral fellows	**Dr. Volker Lipka** since May 2000
	Dr. Andreas Freialdenhoven since October 2000
	Dr. Pawel Bednarek since December 2002
	Dr. Stefan Bau since August 2000
	Dr. Stephane Bieri since October 2000
	Dr. Nicholas Collins since August 2000
	Dr. Christina Neu since February 2003
PhD students	**Quian-Hua Shen** since January 2001
	Jan Dittgen since April 2002
	Stefan Mauch since August 2002
Diploma student	**Simone Pajonk** since November 2002
Technical assistants	**Anja Reinstaedler** since August 2002
	Heidrun Häweker since July 2002

Guest scientists

Dr. Min Chul Kim, Biotechnology Research Centre, Gyeongsang National University, Korea, postdoctoral fellow

Dr. Andrew Jacobs, University of Adelaide, Australia, postdoctoral fellow

Francesca Ceron, University of Pisa, Italy, PhD exchange student

Grants, external funding

EUREKA grant: 1 Postdoctoral fellow, 1 technical assistant

GABI-NONHOST, BMBF-funded consortium-based functional genomics initiative on plant non-host disease resistance: 1 Postdoctoral fellow, 1 technical assistant

SYNGENTA grant on broad-spectrum disease resistance: 1 Postdoctoral fellow

BASF grant on family 1 glycosyl hydrolases: 1 Technical assistant

Swiss National Fond: 1 Postdoctoral fellow

IMPRS: 1 PhD student

Collaborations

Interactions between MLO and calmodulin: Biotechnology Research Centre, Gyeongsang National University, Korea, Dr. Moo Je Cho

Non-host resistance to Phythophthora infestans: Institute of Plant Biochemistry, Halle, Dr. Dierk Scheel

Family 1 glycosyl hydrolases: Virginia State University, Dr. Asim Esen

RAR1 and SGT1 functions in disease resistance; Sainsbury Laboratory, Norwich, Dr. Ken ShirasuSingle cell expression profiling: Max Planck Institute for Molecular Plant Physiology, Golm, Dr. Julia Kehr

Callose synthase in disease compatibility: University of Adelaide, Dr. Geoffrey Fincher

Invited lectures given

The Banbury Center, Cold Spring Harbor Laboratory, New York (08.-11.04.2001), IACR-Rothamsted, Harpenden (08.05.2001), MPI für terrestrische Mikrobiologie, Marburg (23.06.2001), University of Wisconsin, Madison (10.-14.07.2001), Syngenta Disease Resistance Symposium, San Diego (18.-18.07.2001), Institut für Genetik, Universität zu Köln, Cologne (23.07.2001), Gordon Research Conference, West Kingston (29.07.-03.08.2001), Jahrestagung der Gesellschaft für Genetik, Halle (04.-06.10.2001), Biologisches Kolloquium, Philipps-Universität, Marburg (25.10.2001), Institute of Plant Physiology, RWTH Aachen (16.11.2001), Bayer AG, Pflanzenschutz, Monheim (30.11.2001), Gesellschaft für Pflanzenzüchtung, Fulda (12.12.2001), Riso National Laboratory, Roskilde, Denmark (13.12.2001), 5th Schloessmann Seminar, Martinsried/Munich (16.-19.12.2001), Botanisches Institut, Universität München, Munich (17.01.2002), KWS Saat AG, Einbeck (04.03.2002), Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben (07.-11.03.2002), Centro Nacional de Biotecnologia, Campus de Cantoblanco, Madrid (15.-17.03.2002), Albert-Ludwigs-Universität Freiburg, Lehrstuhl für Botanik, Freiburg (20.03.2002), Polish Academy of Sciences, Goniadz Symposium, Poland (09.-11.05.2002), Institute for Molecular Biotechnology, Jena (06.06.2002), International Conference on Arabidopsis Research, Sevilla (28.06.-02.07.2002), Gyeongsang National University, Chinju, Korea (06.-08.07.2002), Gordon Research Conference, Boston (09.-14.07.2002), Virginia Polytechnic Institute and State University, Blacksburg Virginia (14.-15.07.2002), Nara Institute of Science and Technology, Nara, Japan (28.-31.07.2002), Kyoto University (31.07.-02.08.2002), Molecular Biology Institute, Copenhagen University (13.-14.09.2002), GATSBY Charitable Foundation, Eynsham Hall, Oxford (23.-24.09.2002), Technische Universität Darmstadt (09.10.2002), EPSO Conference, Brünnen, Switzerland (27.-29.10.2002), Technische Universität München, Munich (06.-07.11.2002), Pflanzenbiologisches Kolloquium, Universität Münster (19.-20.11.2002), Eucarpia Symposium, Salsomaggiore, Italy (22.-23.11.2002), Stanford University, San Francisco (10.-11.01.2003), Symposium Plant and Animal Genome XI, San Diego, California (11.-16.01.2003), Institut für Genetik Universität Köln, Cologne (22.01.2003), ICPP 2003, Christchurch (02.-08.02.2003), 16. Tagung Molekularbiologie der Pflanzen, Wermelskirchen-Dabringhausen (27.02.2003), Polish Academy of Sciences, Institute of Biochemistry and Biophysics, Warsaw (20.-23.03.2003), Keystone Symposia, Utah (10.-16.04.2003)

Pathogen Defence-Related Promoter Elements and Secondary Products

Klaus Hahlbrock

General Introduction

The period from the last Scientific Advisory Board meeting in 2001 until the closure of the laboratory due to my retirement on 30 November 2002 was devoted to completing unfinished projects. Apart from two MPIZ-internal collaborations on the WIP subfamily of zinc-finger proteins (Sagasser *et al*., 2002; see report by B. Weisshaar) and on leucine zipper-containing WRKY proteins (Cormack *et al*., 2002; see report by I. E. Somssich), our work focused mainly on the three topics summarised below under New Results. Preliminary accounts of some, but not all, of these results were included in a 'Colloquium paper' (Hahlbrock *et al*., 2003) giving a general, retrospective overview of a major part of our work during the past two decades.

A project on the involvement of promoter elements and transcription factors in the cross-talk among stress responses, which had been largely completed before the last SAB meeting and was almost fully covered in the last report, was soon thereafter terminated and published (Logemann and Hahlbrock, 2002).

Interactions with the public on various 'bioethical' questions, particularly questions concerning the application of gene technology in crop plant breeding, continued to receive special attention. One typical lecture on this topic, given at a meeting of the Union of the German Scientific Academies in Göttingen, was recently published in the 'Akademie Journal' (Hahlbrock, 2002).

New Results

The *At*CMPG family – A newly discovered family of regulatory proteins

This project falls into two parts, both of which originated from the previously reported discovery of an exceptionally fast and unusually selectively elicitor-responsive promoter element, E17, from the parsley *PcCMPG1* gene (Kirsch *et al*., 2001; see last report). We have now analysed the *CMPG* gene family and the occurrence of E17-related promoter elements in *Arabidopsis thaliana* and have shown that the *A. thaliana* genome contains at least 50 predicted *AtCMPG* genes. The encoded protein family is defined by a common, novel type of domain possessing four strictly conserved amino acid residues [cys, met, pro and gly (= CMPG)] that designate the family. Two members, *AtCMPG1* and *AtCMPG2*, with high sequence similarity to *PcCMPG1,* were selected for analysis of their expression modes and defence-related promoter elements. Among the most striking similarities with *PcCMPG1* were immediate-early transcriptional activation on infection or elicitor treatment, and the functional importance of a W box-containing *AtCMPG1* promoter element, F, with close functional, and considerable structural, similarity to E17. Remarkably, both F and E17 did not respond to wounding, in contrast to most other known elicitor-responsive elements. At least some of the encoded *At*CMPG proteins are likely to be involved in regulatory processes (A. Heise).

The second part of the project was aimed at questions concerning the practical application of these and other promoter elements in gene technology-assisted disease resistance breeding of crop plants. The long-term goals of these studies are to identify promoter elements suitable for the construction of genes that efficiently confer disease resistance and to work out proper conditions for their application in field tests. Of all elements identified so far, E17 and F seem to be best suited for this purpose because they combine all four of the most highly desirable properties: rapid, strong, local and selective activation at pathogen infection sites in transgenic plants. Prior to fusing them with a structural gene that encodes the actual defence or defence-mediating reaction, optimised conditions for the design of a synthetic promoter had to be established. Our results (manuscript in preparation) revealed an optimal element position around 90 nt upstream of the transcription start site, demonstrated that promoter efficiency was independent of the tilt angle between element and transcription start site, and suggested that combinations of two either identical or (better?) structurally distinct, elicitor-responsive elements with defined spacing may be most suitable (A. Heise, V. Secker).

4-Coumarate:CoA ligase – Pivotal branch point in phenylpropanoid metabolism

Phenylpropanoid derivatives are among the most important and chemically diverse classes of plant secondary metabolites. 4-Coumarate:CoA Ligase (4CL) regulates the flow of phenylpropanoid building blocks into various branch pathways and thus, determines to a large extent the

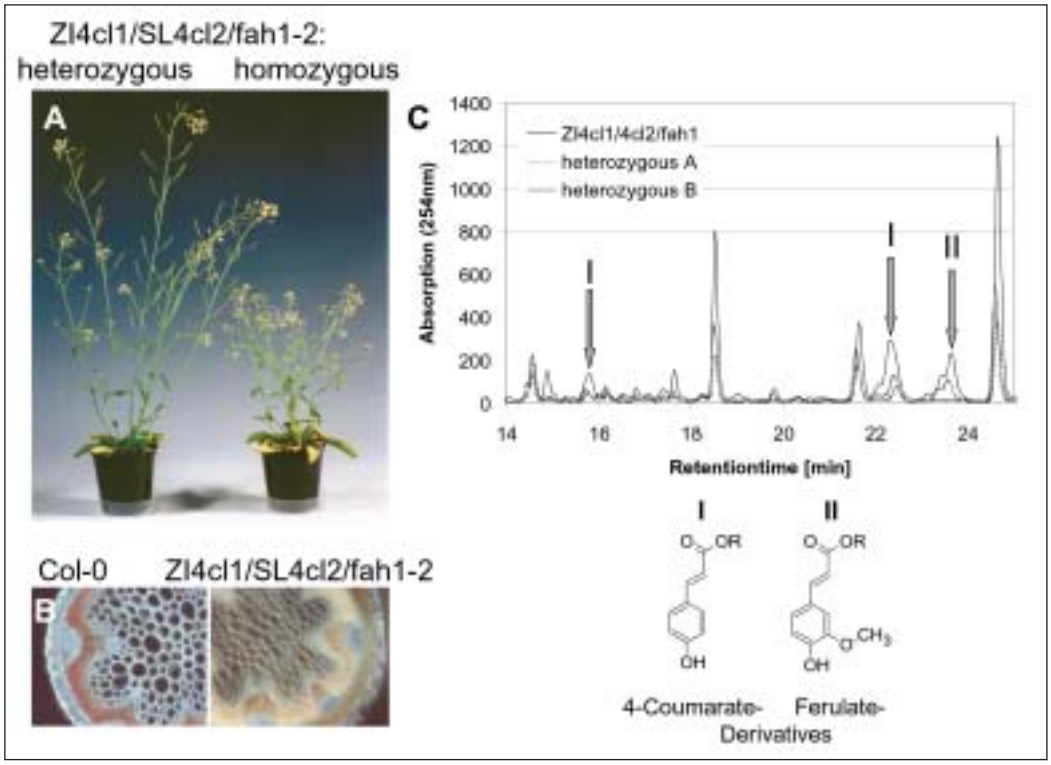

Fig. 1. Morphological (A), histochemical (B) and biochemical (C) phenotypes of the *At4CL1/At4CL2/F5H* knock-out line. (A) Ten-week-old plants. Left (control): heterozygous *At4CL1/At4CL2* combination; right: homozygous triple knock-out line. (B) Lignin-specific Meule staining of stem cross-sections. Left (control): Columbia-0 wild type; right: homozygous triple knockout line. (C) HPLC profiles of soluble aromatic secondary metabolites. Three prominent peaks were identified as I and II by their UV spectra.

sub-class profile and substitution patterns of accumulating end products. To further define the pivotal role of 4CL in *A. thaliana*, we attempted to analyse in detail the *At4CL* gene family and establish the functional affiliations of all individual *At*4CL isoforms with the various metabolic branches.

In silico analysis first led to the discovery of a previously undetected, fourth isoform, *At*4CL4, with unusual substrate specificity, including the extremely rarely observed activation of sinapate. This isoform most probably completes the *At*4CL family. A model for the evolution of the family through a combination of gene duplications and chromosomal rearrangements was derived from an extensive genome analysis, and an evolutionary tree was proposed for all known class I and II 4CLs in plants. The developmental and stress-induced expression patterns of *At*4CL4 were similar to those of *At*4CL1 and *At*4CL2. All four *At4CL* gene promoters contained the characteristic P/L set of regulatory elements.

Knock-out lines for all four *At4CL*s were generated and morphologically and biochemically characterised. A particularly clear-cut result from HPLC analyses was the apparent involvement of *At*4CL3 in UV light-dependent flavonoid biosynthesis. Multiple mutants were established by crossing single knock-out lines (*tt4 [AtCHS]*, *fah1-2 [AtF5H]*) with *At4CL* knock-out lines in an attempt to overcome possible functional redundancies. A particularly striking result was the arrest of growth of the *At4CL1/At4CL2/fah1* knock-out line after five weeks of fairly normal development. This, together with the absence of histochemically detectable lignin and with the accumulation of 4-coumarate and ferulate derivatives (Fig. 1) suggested a function of *At*4CL1 and *At*4CL2 in the biosynthesis of lignin precursors (B. Hamberger).

Secondary products – Key players in pathogen Defence?

In our last SAB report, we presented data indicating that nearly all major soluble, pathogen-induced compounds in *A. thaliana* leaves were indolic metabolites. Various constitutively present phenylpropanoid derivatives were either repressed (sinapoyl malate) or remained unchanged (several flavonoid glycosides). For comparison and for a more comprehensive overview, we have now extended these studies to *A. thaliana* roots and to wall-bound metabolites in both leaves and roots. The following major results were obtained.

The patterns of induction and repression of soluble aromatic secondary metabolites on pathogen infection were basically the same in *A. thaliana* roots and leaves. That is, in both cases, numerous indolic compounds were induced and constitutively present phenylpropanoid compounds were repressed. The structural identification of various glucosinolate breakdown products and of several putative or established intermediates of camalexin biosynthesis allowed us to further develop, and partly modify, the complex metabolic grid proposed for indolic secondary metabolism in *A. thaliana* (P. Bednarek).

By contrast, all but one (indole-3-carboxylate) of the pathogen-induced, wall-bound aromatic secondary compounds were benzoate or cinnamate, that is, phenylpropanoid, derivatives in *A. thaliana*. Remarkably, the chemical nature of this group of induced metabolites appears to be the same in all plant species analysed, including parsley (*Petroselinum crispum*), the other model organism investigated in parallel with *A. thaliana*. As this apparent uniformity is in sharp contrast to the large chemical diversity of induced, soluble compounds (e.g., phenyl-

propanoids and betaketides in *P. crispum* versus indole derivatives in *A. thaliana*), the clear-cut distinction illustrated in Fig. 2 for these two species may well be a universal phenomenon throughout the plant kingdom (Hahlbrock *et al*. 2003; Tan *et al*., manuscript in preparation) (J. Tan, P. Bednarek).

Already at the beginning of these investigations, a surprising observation was the strong induction of one particular compound, X, exclusively in compatible interactions of *A. thaliana* with *Pseudomonas syringae* pv tomato. Essentially the same result was now obtained with tomato (*Lycopersicon esculentum*), the natural host of *P. syringae*, suggesting that the unusual confinement of the induction to compatible interactions may be exploitable as a diagnostic tool. Structural analysis by MS/NMR identified X as a novel type of natural product, 3'O-β-D-ribofuranosyl adenosine (P. Bednarek, B. Hamberger, J. Tan).

All of these data resulted from a very fruitful, efficient and close co-operation with the MPI of Chemical Ecology, Jena (Oldham, Schneider, Svatos). Three joint manuscripts on the results are in preparation.

Although functionally decisive "defense compounds" have not been identified in these and most other studies, it is expected that the combination of extensive analyses of secondary metabolite profiles with detailed studies of the disease resistance potential of defined mutants will reveal important clues as to the significance of the universally observed induction of these types of compounds. Most probably, they will turn out to act in concert rather than as single, decisive agents (Hahlbrock *et al*., 2003).

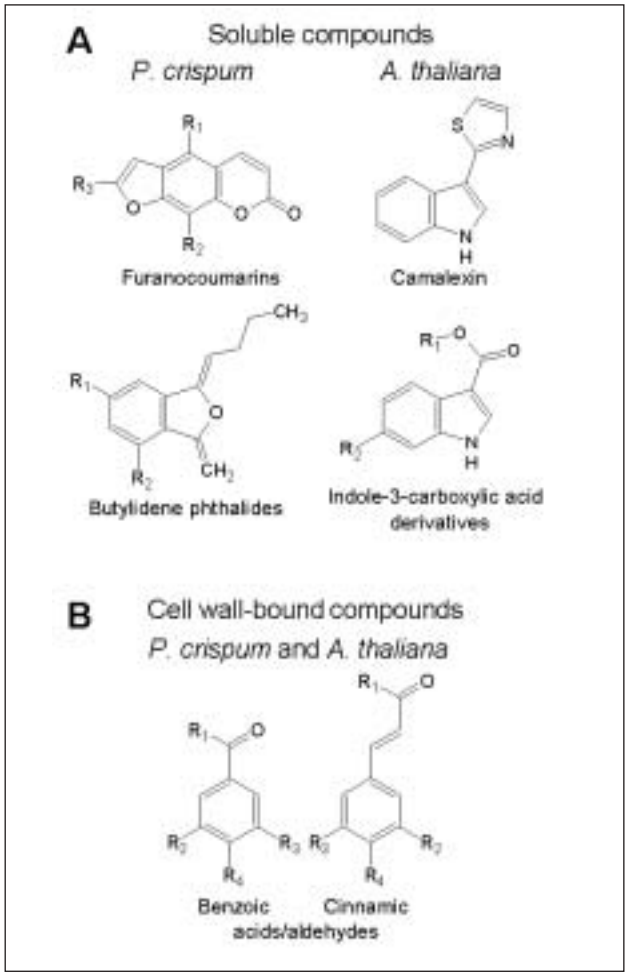

Fig. 2. Chemical structures of major, characteristic classes of compounds accumulating on infection or elicitor treatment in *P. crispum* and *A. thaliana*. (A) Soluble and (B) cell wall-bound material was extracted and analysed by HPLC, MS and NMR.

Future Developments

Due to the closure of the laboratory, all three projects will be continued elsewhere, although some with modifications. The collaboration with KWS Saat AG on the usage of synthetic promoters for gene technology-assisted disease resistance breeding will be carried on at the MPIZ by Drs. Somssich and Schulze-Lefert; the functional analysis of the four *At*4CL isoforms, particularly an extended analysis of the recently generated, single and multiple mutant lines, will be continued by Björn Hamberger during postdoctoral work in the laboratory of Carl Douglas at Vancouver, Canada; and Pawel Bednarek will continue to work on some unsolved questions of his natural products project, including the analysis of mutants, during an extension of his stay at the MPIZ.

Scientific Publications

Cormack, R.S., T. Eulgem, P.J. Rushton, P. Köchner, K. Hahlbrock and I.E. Somssich: Leucine zipper-containing WRKY proteins widen the spectrum of immediate early elicitor-induced WRKY transcription factors in parsley**.** Biochim. Biophys. Acta **1576,** 92-100 (2002).

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Hahlbrock, K., P. Bednarek, I. Ciolkowski, B. Hamberger, A. Heise, H. Liedgens, E. Logemann, T. Nürnberger, E. Schmelzer, I.E. Somssich and J.-W. Tan: Non-Self Recognition, Transcriptional Reprogramming and Secondary Metabolite Accumulation during Plant/Pathogen Interactions. Proc. Natl. Acad. Sci. USA (in press).

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Logemann, E., and K. Hahlbrock: Crosstalk among stress responses in plants: Pathogen defense overrides UV protection through an inversely regulated ACE/ACE type of light-responsive gene promoter unit. Proc. Natl. Acad. Sci. USA **99**, 2428-2432 (2002).

Sagasser, M., G.-H. Lu, K. Hahlbrock and B. Weisshaar: *A. thaliana* TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. Genes Dev. **16**, 138-149 (2002).

Dissertations

Andreas Heise (2002) Analyse pathogen–responsive Mitglieder der CMPG-Genfamilie in *Arabidopsis thaliana*

Jian-Wen Tan (2002) Secundary Metobolites Induced by Pathogens in *Arabidopsis thaliana* and Isolated from Six Higher Fungi

Structure of the group

Group leader	**Dr. Klaus Hahlbrock** since January 1983
Postdoctoral fellows	**Dr. Pawel Bednarek** January 2001 – November 2002 **Dr. Andreas Heise** July 2001 – January 2003
PhD students	**Jianwen Tan** March 2001 – November 2002 **Björn Hamberger** September 1999 – November 2002
Technical assistants	**Elke Logemann** since December 1983 **Vera Secker** July 2001 – October 2002

Guest scientists

Drs. Dariusz Michalczyk and Wioletta Pluskota, University of Olstyn, Poland, postdoctoral fellows

Grants, external funding

KWS Saat AG, Einbeck: 1 Postdoctoral fellow, 1 technical assistant

Fonds der Chemischen Industrie, Frankfurt, Germany: Running costs.

Collaborations

Structural identification of secondary metabolites (MS, NMR): MPI of Chemical Ecology, Jena, Prof. B. Schneider, Drs. N. J. Oldham and A. Svatos; Botany Institute, Chinese Academy of Sciences, Kunming, Prof. J. Liu

Promoter elements for disease resistance breeding: KWS Saat AG, Einbeck, Drs. D. Stahl and G. Strittmatter

Functional Analysis of Plant Defence Responses

Erich Kombrink

Introduction

Plants have evolved a large variety of defence mechanisms to prevent colonisation of their tissues by microbial pathogens. Preformed physical and chemical barriers constitute the first line of defence. Considered more important are inducible mechanisms, which are engaged after successful recognition of the invading pathogen. Key features of active defence are the rapid cell death at the initial infection site, the so-called hypersensitive response (HR) and the induction of host gene expression and protein synthesis in neighbouring cells such as pathogenesis-related (PR) proteins (including chitinases and 1,3-β-glucanases) and enzymes of the general phenylpropanoid pathway. The latter group of enzymes participates in the synthesis of diverse plant secondary products that fulfil important functions in protection against biotic and abiotic stress and plant development.

We are interested in the identification of signalling components controlling early plant defence responses and in the structure-function relationship of 4-coumarate:CoA ligase, an enzyme of plant secondary metabolism that may contribute to the synthesis of antimicrobial compounds.

New Results

Biochemical approaches to identify regulators of HR cell death

Cell death associated with HR is a common defence reaction of plants to infection by pathogens. Efficient HR formation frequently depends on the presence of specific plant disease resistance genes (*R* genes) and their interaction with pathogen-derived molecules such as elicitors or avirulence factors (*avr* gene products). Recent evidence revealed that cell organelles (mitochondria, chloroplasts) are important regulatory units integrated into the signalling network leading to HR cell death. Further evidence from several systems indicates that targeted degradation of negative regulatory factors is a prerequisite for the execution of HR cell death and that a direct connection between ubiquitination, protein degradation and plant resistance may exist.

To identify regulatory components of HR cell death, we are currently utilising two experimental systems. (1) The race/cultivar-specific interaction of barley (*Hordeum vulgare*) with powdery mildew (*Blumeria graminis* f. sp. *hordei*) is being used to search for proteins that are involved in the execution of rapid cell death. In this system, race-specific resistance and HR cell death is dependent on the functional *Rar1* gene. At present, our strategy is protein profiling by 2-D gel electrophoresis and identification of candidate proteins by mass spectrometry. Since regulatory proteins are not expected to be highly abundant, we are concentrating on specific subcellular protein fractions for comparative analysis such as the proteins of the plasma membrane. Current constraints of this approach are the sequencing of low abundant proteins form silver-stained gels and the low number of cells developing HR symptoms upon inoculation, thereby limiting the amounts of altered proteins. (2) To circumvent these constraints, we are utilising an inducible system to evoke an HR response in *Arabidopsis thaliana*. Chemically (estradiol) induced expression of a bacterial avr gene (*avrRPM1*) in plants harbouring the *RPM1* resistance gene leads to massive induction of HR symptoms. Again, comparative protein profiling of subcellular fractions (chloroplasts, mitochondria) of genetically defined plants (e.g. harbouring *RAR1* or *rar1*) that are capable or compromised in HR development led to the detection of candidate proteins, which are currently under investigation. Finally, we have set up a system to identify regulatory proteins that are destined for degradation by the proteasome by inducible expression of modified and tagged ubiquitin variants. For this purpose, we have introduced an estradiol inducible HIS-HA-tagged ubiquitin derivative (K48R) into the above-mentioned Arabidopsis lines, with the aim of purifying proteins that are ubiquitinated during HR formation.

The substrate-specificity determining amino acid code of 4-coumarate:CoA ligase

4-Coumarate:CoA ligase (4CL) is a key enzymes of the general phenylpropanoid metabolism from which a large variety of secondary products is derived such as lignin, wall-bound phenolics and flavonoids. We have recently characterised three 4CL isoforms from Arabidopsis (At4CL1-3) and experimentally verified a close structural and functional relationship to other adenylate-forming enzymes such as luciferases, non-ribosomal peptide synthetases and fatty acid activating enzymes. The Arabidopsis 4CL isoform 2 (At4CL2) is unusual in that its preferred substrate is caffeic acid rather than coumaric acid, whereas the structurally related ferulic acid is not converted. Cinnamic acid is a poor substrate of all Arabidopsis 4CLs, while sinapic acid is not activated at all.

To unravel the structural principles determining 4CL substrate specificity, the crystal structure of the phenylalanine activation domain PheA of gramicidin S synthetase was used as a template for homology modelling. According to this model, 12 amino acid residues are located at a distance of not more than 6 Å to caffeate fitted into the substrate-binding pocket (SBP) of At4CL2 (Fig. 1A). These residues are supposed to function as signature motive determining 4CL substrate specificity. We corroborated this assumption by creating three types of gain-of-function mutants. Targeted substitution of selected bulky residues by smaller ones resulted in At4CL2 variants that use ferulate instead of caffeate as their preferred substrate (Fig. 1B). Deletion of selected amino acids generated sinapic acid activating At4CL2 variants (Fig. 1C). Substitution of charged or polar residues by hydrophobic amino acids strongly enhanced the capacity of At4CL2 to activate cinnamate. These results demonstrate that a size exclusion mechanism controls the accessibility of the At4CL2 SBP for mono- and dimethoxylated 4-hydroxycinnamic acid derivatives, whereas activation of cinnamic acid itself is regulated by the overall hydrophobicity of the SBP.

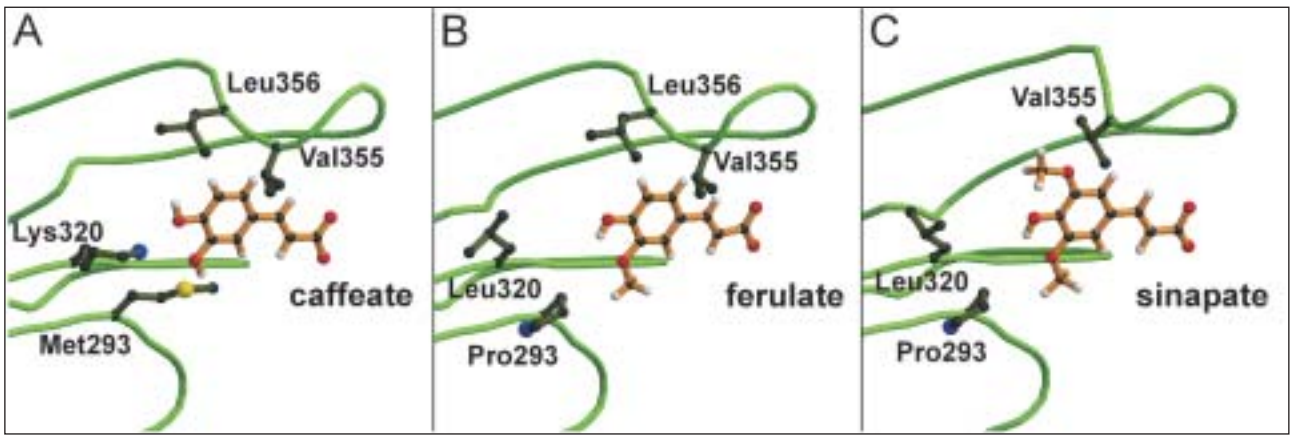

Fig. 1. 3-D models of substrate binding pockets (SBP) of different At4CL2 variants. For each enzyme variant, the bulkiest substrate that can be fitted into the respective SBP is depicted. For clarity, only four out of twelve amino acids lining the SBP are shown. (A) SBP of wild-type enzyme with caffeic acid. (B) SBP of double mutant M293P+K320L with ferulic acid. (C) SBP of triple mutant M293P+K320L+ΔL356 with sinapic acid.

Future Developments

Identification of proteins involved in regulation of HR cell death

Protein profiling by 2-D gel electrophoresis has proven a successful approach for the detection of putative regulatory proteins of HR cell death. Furthermore, mass spectrometry is the method of choice for protein identification with high sensitivity and medium to high throughput. However, we need to improve the sensitivity of the current methods for the identification of low-abundant proteins that are detectable on silver-stained gels. Likewise, dynamic and automated data acquisition and database searching will help to enhance the throughput of the methodology. In barley, alternative methods of protein enrichment, selection and labelling will assist in establishing comprehensive proteomes of particular cell-types, organelles or subcellular fractions. In Arabidopsis, post-translational modifications by ubiquitin or sumo as observed during the onset of HR may allow the enrichment of proteins of interest.

Analysis of 4CL and 4CL-like enzymes from Arabidopsis

Although the structural model of the At4CL2 has proven extremely valuable for the design of enzyme variants with new catalytic properties, the *bona fide* structure for any 4CL enzyme is still unknown. In collaboration with the research group of Prof. D. Schomburg (Biochemistry Department, University of Cologne), we will continue our efforts to generate high quality crystals of At4CL2.

In addition to four *bona fide* 4CL, the Arabidopsis genome encodes more than 25 4CL-like proteins. In collaboration with Prof. C. Douglas (Botany Department, University of British Columbia), we want to elucidate the biological function of these enzymes by expression profiling and identification of their natural substrates. We are using two approaches to determine their *in vitro* enzymatic activity. For selected proteins, 3-D models of their SBPs have been established, which allow screening for putative substrates by *in silico* docking. For experimental verification, we are also developing a general activity assay for adenylate-forming enzymes that will allow the high-throughput analysis of multiple substrates.

Scientific Publications

Ancillo, G., E. Hoegen and E. Kombrink: The promoter of the potato chitinase C gene directs expression to epidermal cells. Planta (in press).

Hoegen, E., A. Strömberg, U. Pihlgren and E. Kombrink: Primary structure and tissue-specific expression of the pathogenesis-related protein PR-1b in potato. Mol. Plant Pathol. **3**, 329-345 (2002).

Kombrink, E.: Alkaloids. In: Plant Sciences, Vol. 1 (R. Robinson, ed.). Macmillan Reference USA, New York pp. 32-34 (2001).

Kombrink, E., and E. Schmelzer: The hypersensitive response and its role in local and systemic disease resistance. Eur. J. Plant Pathol. **107**, 69-78 (2001).

Pietrowska-Borek, M., H.-P. Stuible, E. Kombrink and A. Guranowski: 4-Coumarate:coenzyme A ligase has the catalytic capacity to synthesize and reuse various (di)adenosine polyphosphates. Plant Physiol. **131**, 1401-1410 (2003).

Schneider, K., K. Hövel, K. Witzel, B. Hamberger, D. Schomburg, E. Kombrink and H.-P. Stuible: The substrate specificity-determining amino acid code of 4-coumarate:CoA ligase. Proc. Natl. Acad. Sci. USA **100**, 8601-8606 (2003).

Stuible, H.-P., and E. Kombrink: Identification of the substrate specificity-conferring amino acid residues of 4-coumarate:coenzyme A ligase allows the rational design of mutant enzymes with new catalytic properties. J. Biol. Chem. **276**, 26893-26897 (2001).

Stuible, H.-P., and E. Kombrink: The hypersensitive response and its role in disease resistance. In: Fungal Disease Resistance in Plants – Biochemistry, Molecular Biology and Genetic Engineering (Z. K. Punja and W. Y. Luck, eds.). Haworth Press, New York, (in press).

Structure of the group

Group leader	**Dr. Erich Kombrink**
Postdoctoral fellow	**Dr. Hans-Peter Stuible**
	Janine Gielbert
PhD student	**Katja Schneider**
Diploma students	**Astrid Böckelmann**
	Astrid Höppner
Technical assistants	**Brigitte Pickel**
	Roswitha Lentz

Grants, external funding

DFG Research Grant (Einzelantrag, Ko 1192/6-1 and Ko 1192/6-2): 1 PhD student

Collaborations

Elucidation of the crystal structure of *Arabidopsis* At4CL2 in complex with its substrates (ATP and coumarate) to uncover the structural basis of substrate specificity: Biochemistry Department, University of Cologne (AG Prof. Schomburg), Dr. Klaus Hövel

Capacity of different At4CL2 variants for the synthesis of diadenosine oligophosphates (Ap_nA) and adenosine oligophosphates (p_nA) and analysis of their regulatory role in cell division and DNA replication: Katedra Biochemii I Biotechnoligii, Poznan, Poland, Prof. Andrzej Guranowski

Functional characterization and expression patterns of 4CL-like proteins of *Arabidopsis thaliana*: Department of Botany, University of British Columbia, Vancouver, Dr. Jürgen Ehlting and Prof. Carl Douglas

Invited lectures given

6th International Workshop on Pathogenesis-Related Proteins in Plants, Spa, Belgium (20-24.05.2001), Institute for Plant Biochemistry, Halle (Saale) (04.10.2001), NCCR Plant Survival in Natural and Agricultural Ecosystems, University of Neuchâtel, Switzerland (24-26.04.2002), Workshop on Plant Stress Biology, Minho University, Braga, Portugal (18-21.06.2003)

Transcriptional Regulation of Defence Genes via WRKY Transcription Factors

Imre E. Somssich

Introduction

Plants have evolved complex signalling networks that allow them to respond optimally to changes in their environment. A crucial part of these multifaceted responses centres on the ability of plants to transcriptionally modulate temporal and spatial gene expression patterns. This may be the reason why plants devote a substantial portion of their genome capacity to transcription; for example, the Arabidopsis genome codes for more than 1600 transcription factors.

Our current research is focussed on the role of a family of plant-specific transcription factors, termed WRKY factors, in activating defence responses towards phytopathogens. WRKY factors contain a novel zinc-finger DNA-binding domain and bind specifically to W box elements (T/CTGACC/A) in the regulatory regions of several genes. WRKY factors are capable of both activating and repressing the expression of genes under their control. Furthermore, the expression of several *WRKY* genes themselves are controlled by WRKY factors, suggesting the existence of autoregulatory feedback loops. Nearly nothing is known concerning the involvement of WRKY factors in distinct defence signal transduction pathways and about the target genes that they control. Similarly, there is no information about which other proteins WRKY factors interact to modulate gene expression, nor have any upstream signalling components been identified that can directly act on selective WRKY members. Thus, our studies are aimed at addressing four important areas: (1) To specify more precisely the promoter-binding sites of WRKY factors: (2) to define WRKY family members involved in distinct defence pathways; (3) to identify WRKY-interacting partners; and (4) to identify direct downstream target genes of WRKY factors.

New Results

DNA-binding characteristics of WRKY proteins

WRKY-W box interactions have been demonstrated both *in vitro* and *in vivo*. In spite of the stereotypic binding preferences of WRKY proteins for W boxes, their affinities for certain types of arrangements of this element can vary. Sequences flanking the invariant W box TGAC core may be partly responsible for the observed specificity. EMSA was used to define more precisely the minimal length and optimal sequence binding sites for different WRKY group members. As expected, single base pair exchanges within the TGAC core completely abolished binding to all members. However, base pair exchanges directly adjacent to the core motif or at neighbouring sites differentially influenced the binding of individual WRKY factors (I. Ciolkowski).

Expression of the Arabidopsis *WRKY* genes

The expression of the *WRKY* gene family was monitored by macroarray analysis or by RT-PCR using plants or cell cultures treated with pathogens or under defence-inducing conditions (e.g. treatment with salicylic acid, flagellin, methyljasmonate). Current data revealed that expression of up to 49 out of 74 *WRKY* genes (66% of total) are induced under such conditions (Fig. 1). In an initial study, we analysed the expression patterns of the group III *WRKY* genes in different pathogen interactions and mutants, and found that distinct subsets are activated by different queues along defined signal transduction pathways (Kalde *et al.*, 2003).

Analysis of WRKY loss-of-function mutants

We have identified and partly characterised numerous putative *AtWRKY* knock-out lines from several sources (SLAT, ZIGIA, SALK, GABI-Kat). Although the majority of such mutants have no obvious phenotypes when grown under normal conditions, they have not yet been characterised under altered stress-related environments (B. Ülker, B. Lippok, M. Kalde and I. Ciolkowski). Nevertheless, preliminary experiments indicate that in some cases, single *WRKY* knock-outs do result in plants being either more or less resistant to bacterial or oomycete pathogens (L. Jorda and B. Ülker).

WRKY-interacting partners

*At*WRKY52 (=RRS1) is a unique member of the Arabidopsis WRKY family. The protein contains several motifs typical of resistance gene products (TIR-NBS-LRR) in addition to a C-terminal WRKY domain. Genetic studies have demonstrated that *RRS1* confers broad-spectrum resistance to *Ralstonia solanacearum*. In collabora-

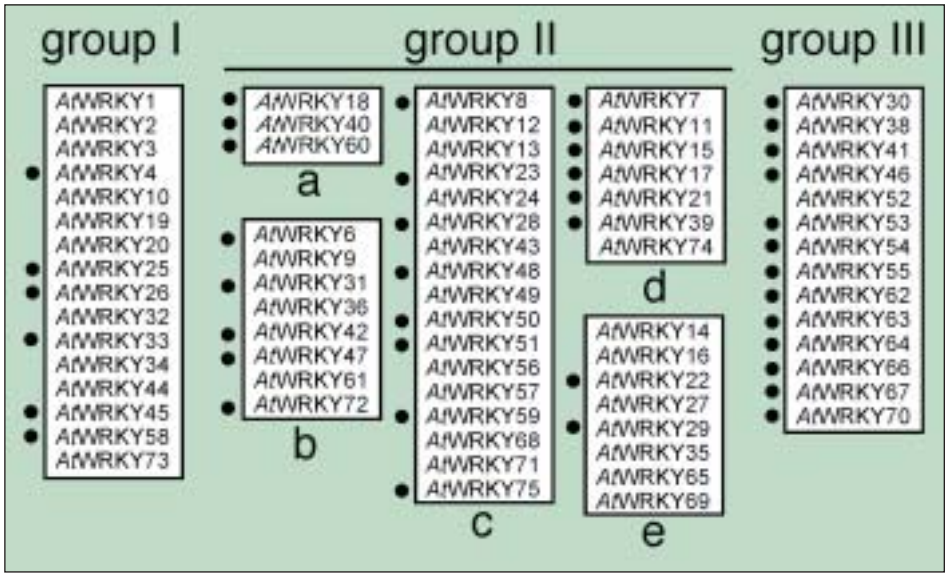

Fig. 1. The Arabidopsis *WRKY* gene family. Division into various groups was based on phylogenetic analyses using the WRKY domains. Closed circles mark those genes whose expression was shown to be modulated under defence-inducing conditions.

tion with Y. Marco and C. Boucher, we showed that *At*WRKY52 interacts with PopP2 (a *R. solanacearum* type III effector protein) and that upon interaction both proteins are localised to the plant cell nucleus (Deslandes *et al.*, in press). Two other *At*WRKY52-interacting partners have been isolated in a split-ubiquitin screen and are currently being analysed (L. Deslandes).

The interaction of WRKY factors with other transcription factors (TFs) is also being tested in a yeast two-hybrid screen (REGIA consortium, B. Ülker). Several WRKY factors were found to interact with other WRKY factors but also with some additional TFs. These preliminary results will require further verification both *in vitro* and *in vivo*.

Target genes of WRKY factors

Recently, we have identified an *At*WRKY6 target gene encoding a receptor kinase, designated *SIRK*, involved in a signalling pathway leading to leaf senescence (Robatzek and Somssich, 2002). Almost simultaneously, another laboratory also identified *SIRK*(=*FRK1*) as a target gene in a defence signalling pathway triggered by the bacterial flagellin peptide Flg22. Flg22 is perceived by a membrane-associated receptor kinase (FLS2), the signal is transduced via a MAP-kinase cascade and *FRK1* activation is mediated by two WRKY factors (*At*WRKY22 and 29). Since the *SIRK* promoter contains numerous W box elements, we tested whether activation of this gene occurs via a converging common step between the two pathways. Our data show that *SIRK* activation by *At*WRKY6 is mediated by a different set of W box elements than those required for activation by Flg22, *At*WRKY22 or *At*WRKY29. Thus, *SIRK* appears to be a common target of at least two distinct pathways employing different subsets of WRKY factors and utilising distinct W box promoter elements (A. Zhou, B. Ülker, and L. Deslandes).

To identify WRKY target genes *in vivo*, we have established the chromatin immunoprecipitation (ChIP) technique (F. Turck). Using this method, we verified *in vivo*, the previously identified putative interaction of parsley WRKY factor 1 (*Pc*WRKY1) with its target gene *PcPR1-1*.

WRKY genes in *Physcomitrella patens*

Sixteen different WRKY genes have been identified in *P. patens* (D. Wanke). Interestingly, we failed to find any group III WRKY members, although orthologous genes to the other groups are present. Phylogenetic studies lend support to the notion that group III WRKY genes have evolved relatively late during evolution. A detailed analysis of a *PpWRKY20* (orthologous to *AtWRKY15*) mutant, generated by homologous recombination, failed to reveal any phenotypes throughout its life cycle.

Future Developments

We have initiated a more extensive expression study to define the role of specific WRKY factors in distinct defence signalling pathways (M. S. Mukhtar). For this purpose, a large set of Arabidopsis mutants defective in key components of different defence signalling pathways are being employed. From these studies, we plan to select candidate WRKY genes for further analyses, e.g. the use of respective *WRKY* knock-out lines, the generation of inducible *WRKY* transgenics and the employment of cDNA-AFLP methods (E. Logemann), in order to identify putative target genes.

The set of *WRKY* knock-out lines will be systematically analysed for their sensitivity to diverse stress-related conditions; particularly for their response to various pathogens. Candidate lines showing clear differences will be given priority for detailed studies including metabolic analyse (J. Brümmer).

Defining WRKY-interacting partners involved in modulating defence pathways will remain a major goal. In particular, our future aim will be to link specific WRKY factors to defined upstream signalling components.

Publications

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I.E., Genin, S., and Marco, Y.: Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. USA, **100**, 8024-8029, (2003).

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Kalde, M., Barth, M., Somssich, I. E. and Lippok, B.: Members of the *Arabidopsis* WRKY group III transcription factors are part of different plant defense signaling pathways. Mol. Plant-Microbe Inter. **16**, 295-305, (2003).

Robatzek, S. and Somssich, I.E.: Targets of *At*WRKY6 regulation during plant senescence and pathogen defense. Genes Dev. **16**, 1139-1149, 2002.

Cormack, R.S., Eulgem, T., Rushton, P.J., Köchner, P., Hahlbrock, K., Somssich, I. E.: Leucine zipper containing WRKY proteins widen the spectrum of immediate early elicitor-induced WRKY transcription factors in parsley. Biochim. Biophys. Acta, **1576**, 92-100, (2002).

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S. Robatzek and Somssich I.E.: A new member of the Arabidopsis WRKY transcription factor family, *At*WRKY6, is associated with both senescence- and defense-related processes. Plant J. **28**, 123-133, (2001).

Diploma thesis

Monika Kalde (2002) Untersuchungen zur Expression ausgewaehlter Vertreter der WRKY Multigenfamilie.

Octave M. Noubibou Doudieu (2001) Untersuchung von durch Pathogene und Elizitoren induzierbare Promotoren von *Arabidopsis* und Petersilie.

Isabell Herrmann (2001) Analysen zur möglichen überlappenden Funktion von WRKY-Transkriptionsfaktoren einer speziellen Subgruppe in *Arabidopsis thaliana*.

Dissertations

Dierk Wanke (2003) Studien zur pflanzenspezifischen WRKY-Transkriptionsfaktorfamilie: Vergleichende Analyse zwischen dem Moos, *Physcomitrella patens*, und höheren Pflanzen sowie eine gesamtgenomische Betrachtung von WRKY-DNA-Bindungsstellen.

Silke Robatzek (2000) Beteiligung eines Vertreters der *Arabidopsis* WRKY-Multigenfamilie an pflanzlichen Entwicklungsprozessen.

Structure of the group

Group leader	**Dr. Imre E. Somssich**
Postdoctoral fellows	**Dr. Janna Brümmer**
	Dr. Laurent Deslandes
	Dr. Hikaru Seki
	(until April 2002)
	Dr. Franziska Turck
	(until March 2003)
	Dr. Bekir Ülker
	Dr. Aifen Zhou
	(until January 2003)
PhD students	**Ingo Ciolkowski**
	Muhammad Shahid Mukhtar
	Dierk Wanke
	(until June 2003)
Diploma student	**Monika Kalde**
	(until January 2002)
Technicians	**Lydia Bollenbach**
	Petra Köchner
	(until May 2002)
	Elke Logemann

Guest scientist

Jiri Libus, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic, Ph D student

Grants, external funding

DFG-funded AFGN Network: 1 Postdoctoral fellow
REGIA, EU: 1 Postdoctoral fellow
KRUPP Foundation (granted to Dr. A. Zhou)
DFG-Fellowship (granted to Dr. F. Turck)
JSPS (granted to Dr. H. Seki)
DFG-funded Graduiertenkolleg GK-GRK 306: 1 Ph D student
DFG: 1 Ph D student
IMPRS: 1 Ph D student

Collaborations

RRS1(WRKY52)-interacting partners: CNRS-INRA, Castanet-Tolosan, France, Dr. Yves Marco
WRKY genes involved in the Flg22 response pathway: FMI Basel, Switzerland, Dr. Silke Robatzek

Invited lectures given

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague (08.06.2001), Symposium "Signal Transduction Meets Functional Genomics", Copenhagen (11-12.07.2001), China-Germany Bilateral Symposium on Plant Molecular Biology, Shanghai (25-26.09.2001), Vienna Biocenter (11.03.2002), MGH, Harvard Medical School, Boston (16.10.2002), Boyce Thompson Institute, Cornell University, Ithaca (17.10.2002), Friedrich Miescher Institute (FMI), Basel (16.01.2003), Department of Biology, University of Fribourg, (19.03.2003) ■

Plant Defence Signalling in Response to Pathogens

Jane Parker

Introduction

Our research focus is to elucidate regulatory mechanisms in plant defence against biotrophic pathogens using Arabidopsis as the model for genetic and molecular analysis. Additionally, we are extending protein and metabolite biochemistry studies to other plant systems such as tobacco. Our current interest is in the biochemical activities, *in planta* modifications and interactions of four defence regulatory proteins (EDS1, PAD4, SGT1 and RAR1) as well as the molecular changes in these proteins upon triggering of the plant resistance pathway. Towards these goals, we have invested time to develop a transient expression-based "read-out" for the activities and interaction dynamics of wild-type regulatory proteins and engineered mutant forms of these proteins. We have started to investigate metabolite changes in vascular and systemic plant tissues during induction of systemic immunity, initially using comparative analysis of Arabidopsis wild-type profiles with those of *eds1* or *pad4* mutants that fail to induce systemic immunity. Highlights from 2002-2003 include: (1) the identification of another Arabidopsis lipase-like protein as an EDS1 interactor in plant tissues by MALDI- and Q-TOF-MS analysis (unpublished, Dr. Bart Feys, Sainsbury Laboratory, Norwich, biochemistry continued by Dr. Steffen Rietz, MPIZ); and (2) the demonstration that Arabidopsis SGT1b, but not RAR1 or SGT1a, is required for SCF^{TIR1} E3 ligase-mediated degradation of AUX/IAA transcriptional activators in response to the phytohormone auxin (Dr. Paul Muskett, MPIZ, in collaboration with Dr. Bill Gray, University of Minnesota).

New Results and Future Developments

Project 1: EDS1 and PAD4 expression and interaction dynamics

Dr. Bart Feys, Lisa Moisan [Norwich];
Dr. Nieves Escobar, Marcel Wiermer

Previous work established that the Arabidopsis defence regulators EDS1 and PAD4 interact in healthy (pathogen unchallenged) and pathogen-challenged leaf extracts. Our subsequent studies showed that only a small pool of EDS1 is bound to PAD4. We are currently investigating the stability and interaction dynamics of EDS1 and PAD4 in wild type and respective *eds1* and *pad4* mutant backgrounds using a combination of gel filtration chromatography, co-immunoprecipitations, affinity enrichment of epitope-tagged EDS1 followed by MALDI- and Q-TOF/MS identification of interactors, and FRET analysis. What emerged was that EPH1 (an EDS1/PAD4 homologue) associates with EDS1. EPH1 does not interact with PAD4 in a yeast two-hybrid assay; however, it remains to be shown whether this is also the case *in planta*. We are analysing *eph1* knock-out mutants for defence-related phenotypes but have so far found none. Several approaches to assess EDS1/PAD4/EPH1 activities and associations in transient expression assays are being explored. In our hands, Agrobacterium-based transient expression (Ag-TE) works poorly in Arabidopsis. Initially, we are using Ag-TE in tobacco and single-cell bombardment of Arabidopsis epidermal cells to assess functionality of EDS1 variants attached to fluorescent protein (FP) or epitope tags. We have obtained promising FRET signals from combinations of EDS1, PAD4 and EPH1 and their interactions in different cell compartments are being measured. A number of EDS1 variants that no longer associate with PAD4 in a yeast two-hybrid analysis have been made. Their stability, defence signalling activity and interaction with PAD4 will be assessed in the bombardment assay and in stable transformants. Targeted EDS1 and PAD4 amino-acid exchanges will be made in conserved motifs based on comparison of monocot and dicot sequences. These variants will be characterised for loss- or (deregulated) gain-of-function in transient assays and, if necessary, in stable transgenic lines.

Project 2: EDS1, PAD4, EPH1 lipase activities and structural biochemistry

Dr. Bart Feys [Norwich]; Dr. Steffen Rietz;
Dr. Adriana Cabral

Site-directed mutations in the predicted lipase catalytic sites of EDS1 or PAD4 do not impair their resistance functions. A fresh examination of their primary and predicted secondary and tertiary structures has been initiated with Dr. Kimmen Sjolander (Univeristy of California, Berkeley) using algorithms based on consensus protein sequences; this should reveal important residues or sub-

strate-binding pockets. Heterologous expression of soluble EDS1, PAD4 and EPH1 in *E. coli* is being attempted for two reasons. First, we want to assess whether these proteins have intrinsic a/b hydrolase capabilities. Second, we want to produce large amounts of protein for physico-chemical determinations and crystal production. A complementary, and possibly superior, approach is to produce large amounts of protein *in planta.* Therefore, suitable expression systems are now being explored. A number of N- and/or C-terminal tagged forms of EDS1 and PAD4 have been produced and a priority will be to examine respective protein modifications in healthy and defence-triggered plant tissues using the MPIZ mass spectrometry facilities.

Project 3: Profiling of systemic immune responses

Dr. Lucia Jorda, Michael Bartsch

We are testing out different elicitor molecules and mutant lines for synchronous induction of the EDS1/PAD4 pathway without pathogen inoculation. This induction system will allow us to do comparative metabolite profiling of wild-type and mutant tissues. One approach is to express conditionally a suitable Avr protein. Another approach is to apply a chemical that triggers the EDS1/PAD4-dependent response. Additionally, we have obtained constitutive gain-of-resistance mutant lines that are completely dependent on EDS1 and PAD4. We have also demonstrated that both EDS1 and PAD4 are necessary for systemic immune responses and are now collaborating with Anita Maldonado and Prof. Chris Lamb (JIC, Norwich) to compare the defects of *eds1* and *pad4* with those of the SAR-deficient mutant, *dir1*, of a putative lipid transfer protein. The defence signalling deficiencies of *eds1* or *pad4* are more extreme than *dir1*. Thus, DIR1 may be one of several systemic "signalling" or "transport" components that are engaged downstream of EDS1/PAD4.

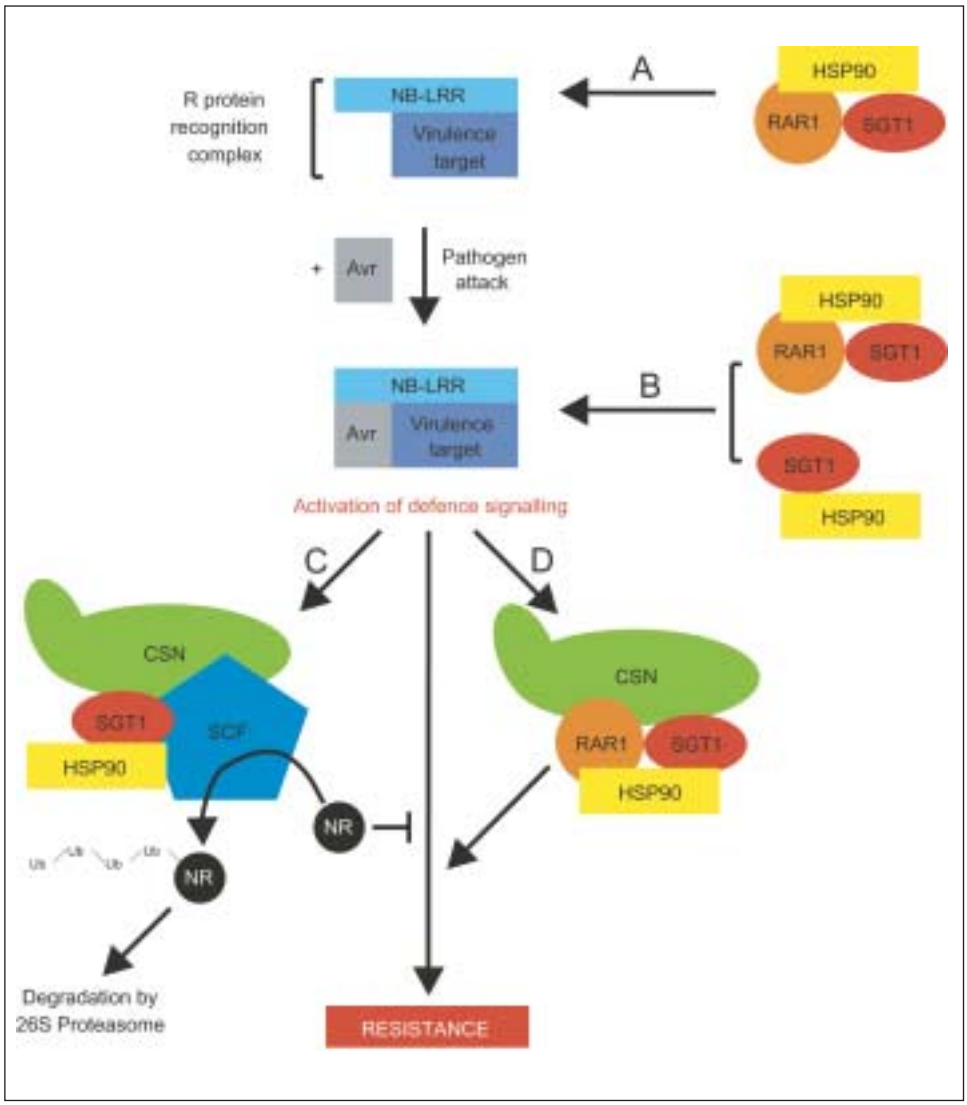

Fig. 1: Possible roles for SGT1 and RAR1 in R protein-mediated resistance. (A) The formation of R protein recognition complex. Possible co-operation with HSP90. (B) Assisting correct folding or intermolecular associations of R protein recognition complex following activation by Avr effector. Possible co-operation with HSP90. (C) Downstream signalling functions of SGT1 in SCF-mediated ubiquitination to remove negative regulators (NR) of the resistance response. (D) A downstream function of RAR1 and CSN (COP9 signalosome), possibly as part of a non-SCF-type E3 ligase complex that could remove negative regulators or activate positive regulators. (Figure provided by Paul Muskett).

Project 4: Non-host resistance in Arabidopsis to *Peronospora*

Dr. Adriana Cabral, Jacquiline Bautor

Here, we are using a genetic approach to unravel pathways of non-host (species level) resistance in Arabidopsis to the obligate biotroph pathogen, *Peronospora*. We have chosen a *Peronospora* isolate that infects Brassicas (evolutionarily related to Arabidopsis) and one that infects tobacco, a solanaceous host. Our aim is to identify and molecularly characterise components that condition durable resistance to these pathogens and ascertain whether these genes govern non-host resistance to other pathogens. Non-host resistance is likely to be

controlled at multiple levels. One scenario that we wish to explore is whether major components of non host-pathogen recognition are related to genes mediating race-specific recognition. The components EDS1 and PAD4 appear to contribute, at least in one Arabidopsis background (Ws-0), to non-host resistance to the Brassica-infecting *Peronospora*. This provides a starting point for unravelling further non-host defence processes.

Project 5: Molecular interactions of SGT1 and RAR1

Dr. Laurent Noël, Shigeyuki Betsuyaku,
Dr. Paul Muskett

Arabidopsis *RAR1* and one of two *SGT1* genes (*SGT1b*) are positive regulators of *R* gene-mediated resistance. RAR1 is a small protein with two zinc-binding domains. SGT1 is conserved among eukaryotes and in budding yeast interacts genetically and molecularly with SKP1, a core component of SCF E3 ligases that target proteins for ubiquitin-mediated degradation. Further studies in budding and fission yeast revealed multiple cellular functions of SGT1 that may be aligned to its structural resemblance to Hsp90 co-chaperone proteins that assist in the assembly or conformational activation of protein complexes. A substantial body of genetic and molecular data from several groups now points to roles of RAR1 and SGT1 in post-translational control of R protein function (Fig. 1). They likely assist the formation of conformationally competent R protein complexes but may also participate in SCF-mediated processes, e.g. to remove a negative regulator of R protein recognition or signalling. We are generating molecular tools and performing genetic screens to investigate the molecular functions of RAR1 and SGT1 in *R* gene-triggered resistance more thoroughly.

Scientific Publications

Austin M.J., P.R. Muskett, K. Kahn, B.J. Feys, J.D.G. Jones and J.E. Parker.: Regulatory role of *SGT1* in early *R* gene-mediated plant defences. Science **295**, 2077-2080 (2002).

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Feys B.J., L.J. Moisan, M.A. Newman and J.E. Parker: Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. EMBO J. **20**, 5400-5411 (2001).

Muskett P.M., K. Kahn, M.J. Austin, L.J. Moisan, A. Sadanandom, K. Shirasu, J.D.G. Jones and J.E. Parker: Arabidopsis *RAR1* exerts rate-limiting control of *R* gene-mediated defences against multiple pathogens. Plant Cell **14**, 979-992 (2002).

Parker J.E., N. Aarts, M.A. Austin, B.J. Feys, L.J. Moisan, P. Muskett and C. Rustérucci: Genetic analysis of plant disease resistance pathways. In: Rice Biotechnology: improving yield, stress tolerance and grain quality. Wiley, Chichester (Novartis Foundation Symposium 236) pp. 153-164 (2001).

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Varet A., J.E. Parker, P. Tornero, N. Nass, T. Nuernberger, J.L. Dangl, D. Scheel and J. Lee: *NHL25* and *NHL3*, two *NDR1*/*HIN1*-like genes in Arabidopsis thaliana with potential role(s) in plant defence. Mol. Plant-Microbe Interact. **15**, 608-616 (2002).

Group Structure

Group leader	**Dr. Jane Parker**
Postdoctoral fellows	**Dr. Adriana Cabral**
	Dr. Nieves Escobar
	Dr. Lucia Jorda
	Dr. Paul Muskett
	Dr. Laurent Noel
	Dr. Steffen Rietz
PhD students	**Michael Bartsch**
	Shigeyuki Betsuyaku
	Marcel Wiermer
Technical assistant	**Jacquiline Bautor**

Grants, external funding

Alexander von Humboldt Stiftung “Sofja Kovalevskaja” Award: 1 Group leader, 2 postdoctoral fellows, 2 PhD students

BMBF “GABI-NONHOST”: 1 Postdoctoral fellow, 1 technical assistant

EU Marie Curie: 1 Postdoctoral fellow

Alexander von Humboldt Stifting: individual postdoctoral fellow

Collaborations

Role of SGT1 in Arabidopsis SCF^{Tir1}-mediated auxin response: University of Minnesota, Dr. Bill Gray

Requirements for EDS1 and PAD4 in systemic acquired acclimation to excess light: University of Stockholm, Dr. Stanislav Karpinski

Metabolite profiling of phloem extracts in systemic immunity: Max-Planck Institute for Molecular Plant Physiology, Golm, Germany, Dr. Julia Kehr

Lipid profiling of wild type and mutant tissues during defence: University of Göttingen, Dr. Ivo Feussner

Invited lectures given

1st IOBC conference, Wageningen (26-28.04.2001), University of Copenhagen (06.2001), University of Utrecht (27-29.08.2001), University of Fribourg (19-20.10.2001), Institute of Plant Genetics, Gatersleben (11.2001), University of Aachen (02..2002), University of Münster (06.2002), BASF symposium,

CDPKs – Switchboard in Early Plant Stress Signalling

Tina Romeis

Introduction

Plants are constantly exposed to changes in their environment and need to integrate a variety of biotic and abiotic stress cues such as pathogen attack, wounding, cold and drought. Common to all these forms of stress is the induction of rapid changes of intracellular calcium levels. Calcium-dependent protein kinases (CDPKs) form a plant-specific class of serine/threonine protein kinases that are implicated as potential sensors that decode and translate changes in calcium concentrations to protein kinase activity. This triggers downstream signalling. My group addresses the following aspects:
(1) Can distinct CDPK isoforms be allocated to defined biological functions; (2) How are CDPKs activated *in vivo;(3)* How are CDPKs integrated in a signalling cascade and what are their downstream phosphorylation targets; (4) Do CDPKs interact with other signalling pathways?

New Results

Function of CDPK isoforms

Previously, we have identified two tobacco CDPK isoforms, NtCDPK2 and NtCDPK3, that function in biotic and abiotic stress signalling (see below). In particular, NtCDPK2 was shown to become rapidly biochemically activated. To allow the integration of genetic approaches and the combination with mutant lines affecting distinct plant signal transduction events, we are in the process of establishing CDPK research in *Arabidopsis thaliana*. Our intention is to select AtCPK isoforms (from the gene family of 34 members) that become biochemically activated after early plant pathogen-related or abiotic stress stimuli. This project can be subdivided in three parts. Phase 1, which is currently under investigation, encompasses the amplification and cloning of AtCPK cDNAs from available cDNA libraries. About two-thirds of the gene family has been isolated. In phase 2, we envisage to express these AtCPK genes in a (heterologous) plant system and subsequently, analyse isoform-specific biochemical parameters of isolated isoforms upon (pathogen-related) stress treatment. So far, about 50% of the gene family has been successfully expressed *in planta* and first indications for post-translational modifications of some isoforms could be observed. In phase 3, candidates selected in phase 2 will be studied for their biological function in their natural host plant. Here, we are currently working on two strategies. First, we apply different transient expression protocols for tagged proteins in *A. thaliana* cell cultures, protoplast systems and leaves. Second, we produced anti-peptide antibodies for some AtCPK isoforms, which should facilitate the analysis of endogenous *A. thaliana* CDPK enzymes and thus, allow the determination of CDPK activation in different mutant lines.

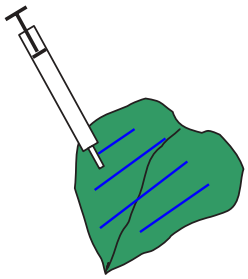
In vivo activation mechanism of NtCDPK2

The biochemical activation of CDPKs *in vivo* is mediated via post-translational modifications on several levels. This includes the binding of calcium to the calmodulin-like domain, a conformational change that disengages the autoinhibitory domain out of the kinase active site, phosphorylation at several sites of the kinase itself and potentially, also a change in CDPK subcellular localisation. We could show for NtCDPK2 that protein phosphorylation is required for enzyme activation *in vivo*. Phosphorylation events could be visualised on Western blots as a shift in electrophoretic mobility. One major aim was to identify phosphorylation sites of NtCDPK2 that are required for the increase in protein kinase activity and are responsible for triggering downstream signalling events. Our strategy is based on the transient expression of NtCDPK2 (and variants) in *N. benthamiana* (Fig. 1). Either before or after stress exposure, the protein is purified from plant extracts and cleaved by proteases into peptides. These are subsequently subjected to 2-dimensional ESI Q-TOF tandem mass spectrometry yielding information about their amino acid sequence and post-translational modifications. Finally, Ser/Thr phosphorylation sites determined by MS (or in addition, selected by an educated guess) were replaced either by Asp (to mimick the negative charge of the phosphate group) or Ala (to block its phosphorylation). The modified NtCDPK2 variants were then analysed for biological function using the transient expression assay in *N. benthamiana* (Fig. 1). We tested several C-terminal tag systems that facilitate a rapid isolation of CDPK from *N. benthamiana* extracts in quantities that were sufficient for biochemical analysis. Furthermore, the proteolytic digestion procedure with different proteases and sample preparation for Q-TOF MS/MS analysis have been optimised

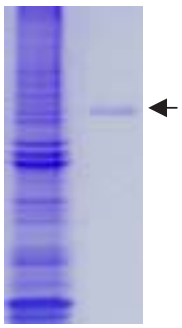
for plant material. To date, we have obtained an almost complete sequence coverage of NtCDPK2, suggesting at least five phosphorylation sites. For two of these sites, a biological function could be shown *in vivo* after site-directed mutagenesis. One of these sites is likely involved in autophosphorylation, the other is probably a substrate to an upstream kinase, Also, membrane localisation seems to be necessary for NtCDPK2 regulation and function.

In addition to changes in the enzyme's phophorylation pattern, we address *in vivo* the conformational changes during the CDPK activation process. Based on the modular structure of CDPKs, one can assume that in its resting state, the autoinhibitory junction domain interacts with the active site in the protein kinase domain. Upon calcium binding, a conformational change within the calcium-sensor domain may then cause an interaction with the junc-

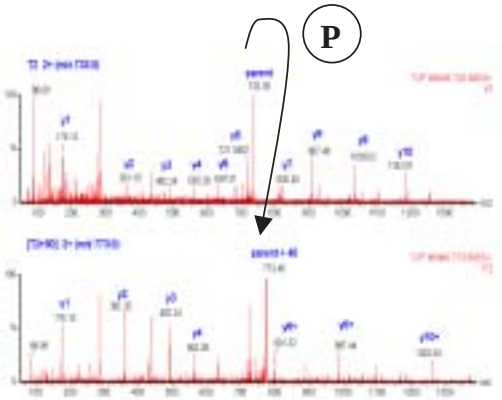
1 Transient expression of NtCDPK2 in *N. benthamiana* leaves



2 Purification of enzyme before and after stress exposure using affinity-tag

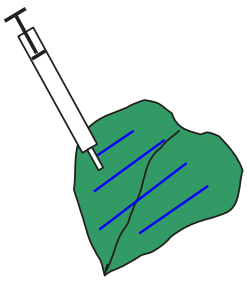


3 MS/MS analysis of selected tryptic phosphopeptides to obtain amino acid sequence



NGLFQSV**S**AAMWR

4 Analysis of NtCDPK2 variants carrying amino acid substitutions in identified P-sites



Function: downstream responses ?

Biochemical properties ?

Fig. 1. Characterisation of NtCDPK2 *in vivo* phosphorylation sites and their role in plant stress signal transduction using ESI tandem mass spectrometry.

tion peptide that results in its disengagement out of the active site. We assess whether we can detect either of these mutual interactions between the junction peptide and its neighbouring domains *in vivo*. Currently, we are testing different experimental strategies in yeast and in plant.

Identification of the NtCDPK2 signalling cascade

To study downstream processes that are triggered by NtCDPK2, we applied a gain-of-function approach that caused prolonged CDPK signalling; when expressing a truncated variant of the enzyme that lacked its regulatory junction and calcium-binding domains, a non-biotic stimulus induced some biotic stress responses such as an HR-like cell death. This cell death response was dependent on the presence of SGT1 and was accompanied by a rapid activation of reactive oxygen species. Furthermore, the analysis of early induced defence genes suggested that NtCDPK2 triggered the JA/ethylene signalling pathways. This could be corroborated by recording changes of different phytohormones upon stress exposure over time.

Our strategy for the identification of direct phosphorylation targets of NtCDPK2 is based on the use of phosphoproteomics. In collaboration with Dr. Janine Gielbert, we acquired know-how in tandem ESI MS/MS analysis to identify peptide sequences of phosphorylated proteins. In parallel, we established and tested experimental procedures for labelling proteins with ^{32}P *in vivo* based on the transient expression system in *N. benthamiana*.

In an independent approach, the NtCDPK2-binding pocket is modified to utilise a bulky ATP analogue. Background phosphorylation due to (other) kinases in plant extracts should thus be significantly reduced (because these kinases would be unable to hydrolyse the ATP analogue). The corresponding NtCDPK2 variants and controls have been generated; the modified ATP analogue can now be synthesised in our lab. We could demonstrate that only the modified CDPK but not the wild-type form of NtCDPK2 was able to hydrolyse the ATP analogue in kinase assays with a peptide substrate.

Future Developments

Biotic and abiotic stress stimuli result in the activation of parallel as well as partially overlapping signalling cascades. Multifunctional components have been identified among CDPKs as well as MAP kinases. Studying NtCDPK2, we obtained the first evidence that an interference between both protein kinase signalling cascades may exist. This potential cross-talk between different signalling pathways and its underlying molecular mechanism(s) are subject to future studies.

Scientific Publications

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Rivas, S., Romeis, T. and J.D.G. Jones: The Cf-9 disease resistance protein is present in a 420 kDa heteromultimeric membrane-associated complex at one molecule per complex. Plant Cell **14**, 689-702 (2002).

Romeis, T.: Protein kinases in the plant defence response. Curr. Opin. Plant Biol. **4**, 407-414 (2001).

Romeis, T., A. Ludwig, R. Martin and J.D.G. Jones: Calcium-dependent protein kinases play an essential role in a plant defence response. EMBO J. **20**, 5556-5567 (2001).

Structure of the group

Group leader	**Dr. Tina Romeis** since August 2001
Postdoctoral fellows	**Dr. Anne-Claire Cazalé** since March 2002 **Dr. Hiromasa Saito** since January 2003 **Dr. Anindita Seal** since March 2002 **Dr. Claus-Peter Witte** since August 2001
PhD students	**Maik Böhmer** since November 2002 **Gerald Freymark** since April 2002

Guest scientists

Raphael Demouliere, ENSAT Toulouse, France, undergraduate student

Jan Kuehling, University of Halle, undergraduate student

Oliver Batistic, University of Ulm, graduate student

Nana Zappel, University of Hohenheim, undergraduate student

Grants, external funding

Alexander von Humboldt Foundation: 1 Group Leader, 2 postdoctoral fellows

MPI Initiative Cologne/Golm: 1 Postdoctoral fellow

Collaborations

Developing plant protein chip technology using CDPK isoforms as a model system**:** MPI for Plant Physiology, Golm, Dr. Wolfram Weckwerth

Identification of protein phosphatases in *N. benthamiana*: Iwate Research Institute, Japan, Dr. Ryoshi Terauchi

Invited lectures given

Meeting "Plant Protein Phosphorylation", Vienna (13.09.01), SFB 369 Symposium, Munich (12.10.2001), Botany Institute, University of Cologne (04.11.2001), Institute of Plant Biochemistry, Halle (06.12.2001), Meeting "Molekularbiologie der Pflanzen", Dabringhausen, Germany (27.02.2002), Department of Botany, University of Göttingen (14.02.2002), MPI for Terrestrial Microbiology, Marburg (09.03.2002), Botany Institute, University of Freiburg (24.04.2002), Meeting "Signals Involved in Plant-Microbe Interactions", Gondiaz, Poland (10.05.2002), CNRS, Gif sur Yvette (14.06.2002), Gordon Conference "Salt and Water Stress in Plants", Oxford (07.2002), Deutsche Botanikertagung, Freiburg (09.2002), Keystone Meeting "Control of Cell Death in Plants", Snow bird (04.2003), Meeting "Protein Phosphorylation – Dephosphorylation", Columbia, Missouri (30.06.2003).

Insertional Mutagenesis in Barley (*Hordeum vulgare* L.) using the Maize Transposons *Activator* and *Dissociation* (*Ac* and *Ds*)

Thomas Koprek

Introduction

One of the main research objectives in our laboratory is the development of a system for insertional mutagenesis in barley using transposable elements. So far, the systematic use of insertional mutagenesis in monocots has been limited to maize and rice. Barley serves as a model of the *Triticeae* family because it is the only true diploid self-compatible species for which a large number of genetic stocks and genome tools are available and is therefore well suited for systematic insertional mutagenesis. We introduced a stabilised *AcTransposase* gene and different versions of modified *Ds*-elements into barley, activated transpositions of *Ds* and characterised the transpositional behaviour of the non-autonomous *Ds*-element. Populations of *Ds*-containing plants were developed that can be used for different gene-tagging approaches.

In a second project, we are investigating the biosynthesis and function of hordatines, defence-related secondary metabolites with strong anti-fungal activity *in vitro*. These compounds, which are homo- or heterodimers of coumaroylagmatine and feruloylagmatine, are exclusively found in representatives of the genus *Hordeum*. The populations of insertional mutants are used for the identification of hordatine deficient plants, which will help to analyse the biological function of these metabolites.

New Results

Earlier studies gave evidence of *Ds* transpositions in barley and the characteristics of *Ds* transpositions have been extensively analysed. Based on those results, two different strategies are currently being developed to use transposon tagging in barley in the most efficient way.

Establishment of a population for reverse genetics

For random forward and reverse genetics approaches approximately 20,000 plants have so far been generated. DNA-blot analysis of randomly chosen plants from the population showed that the *Ds*-elements are highly dispersed in the barley genome. Based on the results from DNA-blot analysis individual plants within this population contain on average two independent *Ds* inserts. During the establishment of the population, several mutant barley phenotypes were observed. Some of these were transmitted to the next generation in a Mendelian fashion and co-segregated with a newly transposed *Ds*-element. These mutants are currently under further investigation. Using inverse PCR and adapter-ligation – PCR, many genomic *Ds*-flanking regions have been isolated and sequenced. Sequence analysis showed for many of the *Ds* genomic borders a high homology to ESTs from public databases. The high proportion of *Ds*-elements which inserted in or near coding regions as well as the observed mutant phenotypes indicate that this approach can successfully be used for gene tagging in barley. Reverse genetics screens for sequences of known tagged sites were performed in order to test the functionality of the system. Within a collaborative effort, we are currently carrying out screens for insertions in genes for transcription factors, which are involved in seed development and grain filling in barley. Results of these screens will provide first indications of the efficiency of the system.

Generation of a system for targeted gene tagging in barley

The system of targeted gene tagging is based on plants carrying a single copy of a *Ds*-element at a genetically mapped integration site. Genes or regions of interest in close vicinity to a mapped *Ds*-element can be targeted by the activation of *Ds* through crossing with an *AcTransposase* expressing plant. Due to the preference of *Ds* to transpose over short distances, many of the resulting progeny plants will carry new *Ds* insertions which are closely linked to the original *Ds* integration site. In addition to the existing 82 single-copy *Ds* lines, the Cereal Transformation Group (ATM) at the MPIZ generated about 200 more independent single-copy *Ds*-containing as well as *AcTransposase* expressing barley lines using an *Agrobacterium*-mediated transformation system. A new binary vector carrying a modified *Ds*-element was developed that will facilitate the identification of transposition events and the determination of the physical distance of *Ds* transpositions. In turn, this will enable us to better estimate the number of plants needed to tag a specific gene.

Simultaneously, mapping of already existing single-copy *Ds*-containing lines has started. The method of choice for the mapping of independent *Ds* insertion events is RFLP mapping. RFLP mapping can easily be performed by using *Ds*-flanking genomic DNA as hybridisation probes in well-characterised mapping populations. Ten of these *Ds* flanks were tested for polymorphisms in different mapping populations. Eight *Ds*-flanking sequences proved to be polymorphic in the mapping populations Chebec X Harrington and Halcyon X Sloop and their genetic position was determined (P. Langridge, University of Adelaide, Australia). After the feasibility of this mapping procedure had successfully been tested, more *Ds*-flanking regions were isolated and analysed in the three mapping populations Igri X Franka, Steptoe X Morex and Oregon Wolfe Barley (M. Prasad, IPK Gatersleben). Overall 17 out of 25 *Ds*-flanking sequences showed polymorphisms and 15 have been mapped.

To test the targeted gene tagging strategy, mapped *Ds*-elements close to morphological markers or genes of interest have been activated by crossing with plants containing the *AcTransposase* gene under the control of the putative *AcTransposase* promoter. The resulting F_1 generation has been planted and progeny of these will be analysed for the expected phenotypes.

Analysis of the hordatine biosynthesis and function *in planta*

Since most of the published data about hordatines and their precursors is not very comprehensive, we started to repeat and expand earlier experiments. In addition to the known hordatines A, B and M, we identified a new compound which is a dimer of feruloylagmatine. This newly described compound also exhibits anti-fungal activity. In contrast to earlier reports, we detected hordatines only in *Hordeum* species which are closely related to *H. vulgare* and contain the I-genome of *Hordeum.*

To gain better insight in the biological function of these compounds, we are trying to identify hordatine deficient mutants in our transposon tagged barley populations. Together with the MALDI-TOF MS Group at the MPIZ, we developed a robust and fast method for the measurement of hordatines by using a high throughput MALDI-TOF procedure. Large-scale screens for hordatine deficient mutants will start soon.

Future Developments

An increase of the population size is the main goal in both transposon tagging strategies. The population size for reverse and forward genetic screens will be increased by 12,000 plants per year. In the targeted gene-tagging approach, the generation of further *Ds* launch pads as well as the mapping of their integration sites are the main objectives. In collaborative efforts with partners at the IPK Gatersleben and at the University of Copenhagen, we will perform several screens of the transposon mutated populations. Furthermore, we will characterise some of the identified mutant phenotypes in more detail.

To understand the biosynthesis and biological function of the hordatines better, we will start with screens for hordatine deficient mutants. Detailed analysis of these mutants will be carried out during the next reporting period.

Scientific Publications

Koprek, T.: Future breeding of cereal varieties in the post GMO era. Euphytica (in press).

Zhao, T., D. Zimmermann, P. Schulze-Lefert and T. Koprek: Systematic *Ac/Ds* transposon mutagenesis in barley. Euphytica (in press).

Structure of the group

Group leader	**Dr. Thomas Koprek**
PhD students	**Tiehan Zhao**
	since November 2001
	Anil Kumar Batchu
	since August 2002
Technical assistant	**Dominik Zimmermann**
	since November 2001

Grants, external funding

BMBF “GABI” program (0312858): 1 PhD student

DFG (1791/2-1): 1 PhD student, 1 technical assistant

Collaborations

Transcription factors involved in agronomic seed characters: IPK Gatersleben, Prof. Dr. U. Wobus

Functional characterization of peptide and amino acid transporters in barley: IPK Gatersleben, Dr. W. Weschke

Isolation of P450 CYP79A from barley: University of Copenhagen, Dr. A. K. Nielsen

Occurrence of hordatines in *H. vulgare* and *H. spontaneum*: University of Haifa, Prof.Dr. E. Nevo

Invited lectures given

Universität Giessen (07.01.2002), Plant Animal & Microbe Genomes X, San Diego (14.01.2002), Eucarpia Conference Cereal Section, Salsomaggiore, Italy (25.11.2002), University of Copenhagen (06.01.2003), IPK Gatersleben (04.04.2003)

MLO Proteins as a Model to unravel Molecular Mechanism of Defence Suppression

Ralph Panstruga

Introduction

In April 2002, I accepted a group leader position in the Department of Plant-Microbe Interactions. The primary research interest of our group is to understand molecular mechanisms of defence suppression in plant-microbe interactions. There is accumulating evidence that the establishment of compatible interactions upon attack by biotrophic pathogens depends on the successful re-programming of host cells including suppression of plant defence pathways. However, little is currently known about host targets for defence repression. One candidate is barley *Mlo*, which is essential for the establishment of compatible barley-powdery mildew interactions. The isolation of the *Mlo* wild-type gene, the biochemical characterisation of the encoded plasma membrane-resident 7 transmembrane (TM) protein, a large collection of *mlo* resistant mutant alleles, available *mlo* suppressor mutants, as well as a wealth of cytological data make MLO an attractive model to gain detailed insights into defence suppression mechanisms. Since MLO is a founder of a small family of 7-TM proteins unique to plants, our interest is also to elucidate presumed common biochemical mechanisms and biological functions of MLO isoforms. For this purpose, we utilise comprehensive genetic resources in Arabidopsis to probe functions of each family member, while transient single cell gene expression is used to assay barley MLO functions in defence suppression. The Ca^{2+}-dependent physical interaction between calmodulin and MLO proteins serves as a basis to develop non-invasive fluorescence-based single cell imaging technologies to visualise and quantify the dynamics of protein-protein interactions in living cells at subcellular resolution in real-time. These technologies are expected to replace, at least partly, technical hurdles that are inherently associated with biochemical studies of low abundance polytopic membrane proteins. Recently, we have also started a systematic genetic search for further defence suppression targets by exploiting natural genetic variation of powdery mildew resistance in Arabidopsis (see below). A separate research line was initiated after a chance observation: a large number of barley *mlo* mutants encode variant proteins containing single amino acid changes that become subject to a post-translational protein quality control. Since virtually nothing is known about this phenomenon in plants, my group is developing biochemical and genetic methods to gain insights into the underlying molecular process.

New Results

In vivo visualisation of the MLO/calmodulin interaction by fluorescence resonance energy transfer (FRET) analysis

Previously, we identified calmodulin (CaM) as an MLO interacting protein (Kim *et al.*, 2002). CaM binds in a Ca^{2+}-dependent manner to a structurally conserved CaM-binding domain (CaMBD) of MLO proteins. CaM binding to MLO enhances MLO-mediated defence suppression but is not an absolute requirement for MLO function. To study when and where MLO/CaM interaction occurs and whether this interaction is dependent on pathogen attack, we applied FRET analysis in single epidermal barley cells. We generated translational fusions of MLO and CaM with either yellow fluorescent protein (YFP) or cyan fluorescent protein (CFP). Significant FRET between fluorophore-tagged MLO and CaM was revealed by fluorescence spectral imaging microscopy (FSPIM) and acceptor photo-bleaching (Fig. 1), but was drastically reduced between MLO CaMBD mutants and CaM.

Identification of MLO-interacting proteins as a means to unravel MLO function

To identify further proteins that physically interact with MLO proteins, we used the 'yeast split ubiquitin assay'. This yeast-based method is conceptually related to the conventional yeast two-hybrid system; however, it is particularly suited for integral membrane proteins. We employed six Arabidopsis MLO proteins as 'bait' to screen Arabidopsis 'prey' libraries. To date, we have identified four candidate interactors that were recovered from 'prey' library screens with at least two distinct MLO 'baits'. From the barley EST database, we identified barley cDNAs highly homologous to the Arabidopsis 'prey' cDNAs. Currently, we are investigating whether the powdery mildew penetration success rate changes upon overexpression or dsRNAi-mediated gene silencing of these barley homologues in single barley epidermal cells.

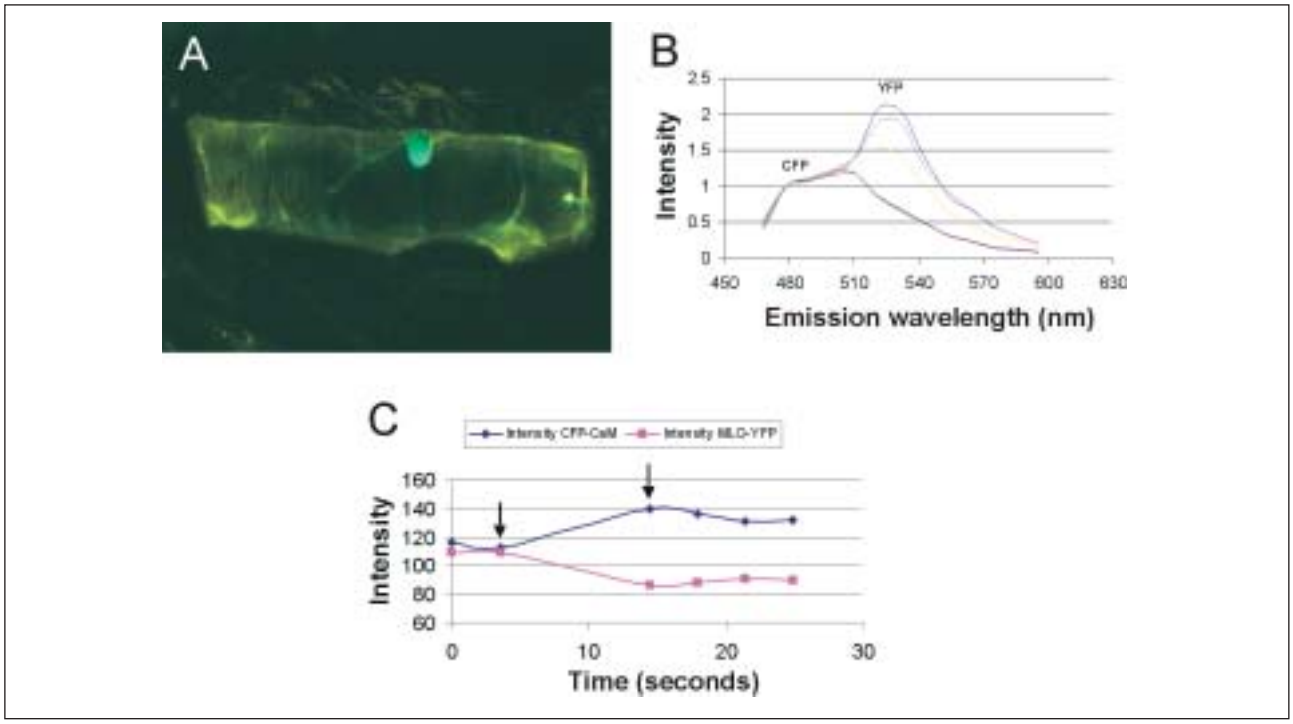

Fig. 1. *In planta* single cell fluorescence resonance energy transfer (FRET) analysis of MLO/CaM interaction. (A) 3D reconstruction based on confocal sections of a barley epidermal cell co-expressing MLO::YFP and CFP::CaM fusion proteins. (B) Fluorescence spectral imaging microscopy (FSPIM) analysis of a MLO::YFP and CFP::CaM co-bombarded barley epidermal cell. The cell was excited with a 458 nm laser line (CFP-specific) and spectra were recorded at different sites of the cell co-expressing the fusion proteins. Fluorescence energy transfer (and thus MLO/CaM interaction) occurs at different regions throughout the cell (indicated by a pronounced YFP-specific peak in fluorescence intensity). Data were normalised to CFP emission at 479 nm. (C) Acceptor photo-bleaching of MLO/CaM FRET. Photo-bleaching of YFP in the MLO::YFP fusion protein (as indicated by a decrease in YFP-specific fluorescence) results in a sudden and sharp increase of CFP-specific fluorescence resulting from the CFP::CaM fusion protein. The photo-bleaching pulse was applied between 4 and 14 sec (arrows). After photo-bleaching, when YFP becomes available for FRET again, YFP-specific fluorescence increases inversely proportionally to a decrease in CFP-specific fluorescence.

High-throughput dsRNAi-mediated gene silencing in barley epidermal cells identifies an actin-depolymerising factor in powdery mildew resistance

Previous extensive chemical mutagenesis has revealed no other broad-spectrum powdery mildew-resistant barley mutants than those affected at *Mlo*. Likewise, only two *ror* (required for *mlo* resistance) suppressor loci could be identified by mutational screens of barley *mlo* mutants. These findings suggest that further components of the *Mlo*/*mlo* pathways are either indispensable for normal plant growth or genetically redundant. To overcome this obstacle, we developed a genetic screen that is based on dsRNAi-mediated gene silencing in single barley epidermal cells. A barley epidermal unigene cDNA set forms the basis for this approach. Using Gateway® technology, we transfer about 5000 cDNAs in respective inverted repeat DNA constructs suitable for dsRNAi-based gene silencing. Pools of five dsRNAi constructs are ballistically introduced in single barley epidermal cells of either *Mlo* or *mlo* genotypes. Upon powdery mildew infection, bombarded cells are inspected for altered penetration success of fungal sporelings. So far, we have analysed about 100 pools of dsRNAi constructs. Among those, we identified one gene (encoding an actin-depolymerising factor) that upon silencing partially compromises *mlo* resistance. Subsequent detailed analysis revealed that silencing of this gene also confers super-susceptibility in *Mlo* genotypes and susceptibility to the inappropriate powdery mildew fungus, *Blumeria graminis* f. sp. *tritici*. Interestingly, race-specific resistances mediated by the *Mla1*, *Mla6* and *Mlg* resistance genes were not affected upon silencing of the actin-depolymerising factor. Identification of a component of cytoskeletal (re-)organisation suggests a crucial role for the cytoskeleton in basal defence, possibly serving as a 'highway for vesicle transport' (compare with results of the Schulze-Lefert group: Identification of barley *Ror2* encoding a syntaxin).

Post-insertional quality control of polytopic membrane proteins in plants

Western blot analysis of barley *mlo* mutants affected by single amino acid replacements surprisingly revealed that most mutant MLO versions accumulate at significantly reduced amounts (as compared to wild-type MLO) in the plasma membrane or are even undetectable. Since *Mlo* mRNA levels and membrane insertion efficiencies of these mutant variants were found to be indistinguishable from wild-type MLO, MLO mutant variants are apparently subject to post-insertional quality control. Using dual luciferase assays, we could demonstrate similar accumula-

tion of MLO variants in Arabidopsis protoplasts, yeast and a human cell line as compared to barley, suggesting conservation of MLO quality control across kingdoms. Exploiting yeast mutant strains defective in either assembly of the 26S proteasome or various components of the ubiquitination machinery, we showed that degradation of unstable MLO mutant variants in yeast requires the proteasome as well as specific E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases. Currently, we are investigating the effect of overexpressing or dsRNAi-mediated silencing of respective plant homologues of these genes on mutant MLO stability *in planta*.

Future Developments

The successful application of FRET methodology in our lab will be modified to study the dynamics of the MLO/CaM interaction upon biotic stress. In addition, we will determine changes of free cytoplasmic Ca^{2+} levels upon biotic and abiotic stress cues by expressing cameleon proteins in single epidermal barley cells. A mid-term goal is to visualise both, intermolecular MLO/CaM FRET and intramolecular cameleon FRET within the same cell (dual FRET analysis). Also, we will apply FRET analysis as a tool to test candidate interactors identified by the yeast 'split ubiquitin' system for their ability to interact with MLO *in planta*.

We will perform a genetic screen in Arabidopsis to identify components of membrane protein quality control. Transgenic plants expressing a translational fusion of an unstable MLO variant with the kanamycin resistance gene *nptII* tolerate comparatively low concentrations of kanamycin. These transgenic lines have been mutagenised with ethylmethane sulfonate. Respective M2 seedlings will be screened for elevated kanamycin tolerance, assuming that mutations in components of MLO quality control will lead to a stabilised MLO1::NPTII fusion protein.

We have recently initiated a new project to unravel the genetic diversity of powdery mildew resistance in Arabidopsis. An extensive analysis of 360 Arabidopsis ecotypes has revealed a surprising diversity of susceptibility/resistance to two tested powdery mildew species, *Erysiphe cichoracearum* and *Erysiphe cruciferarum* (Adam *et al*., 1999, MPMI **12**, 1031-1041). We will study these incompatible interactions in more detail with a particular emphasis on monogenic recessively inherited resistance. The underlying genes are candidates for compatibility factors that might be present/absent in particular accessions.

Scientific Publications

Devoto, A., H.A. Hartmann, P. Piffanelli, C. Elliott, C. Simmons, G. Tarmino, C.-S. Goh, F.E. Cohen, B.C. Emerson, P. Schulze-Lefert and R. Panstruga: Molecular phylogeny and evolution of the plant-specific seven transmembrane MLO family. J. Mol. Evol. **56**, 77-88 (2003).

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Kim M. C., R. Panstruga*, C. Elliott, J. Müller, A. Devoto, H.W. Yoon, H. Park, M.J. Cho and P. Schulze-Lefert*: Calmodulin interacts with MLO to regulate defence against mildew in barley. Nature **416**, 447-450 (2002). (*co-authors in alphabetical order)

Panstruga, R.: Establishing compatibility between plants and obligate biotrophic pathogens. Curr. Opin. Plant Biol. (in press).

Panstruga, R., M.C. Kim, M.J. Cho and P. Schulze-Lefert: Testing the efficiency of dsRNAi constructs *in vivo*: A transient expression assay based on two fluorescent proteins. Mol. Biol. Rep. (in press).

Panstruga, R., and P. Schulze-Lefert: Corruption of host seven-transmembrane proteins by pathogenic microbes: a common theme in animals and plants? Microbes and Infection **5**, 429-437 (2003).

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Structure of the group

Group leader	**Dr. Ralph Panstruga**
Postdoctoral fellows	**Dr. Riyaz Bhat**
	Dr. Judith Müller
PhD students	**Chiara Consonni**
	Katharina Göllner
	Marko Miklis
Technical assistants	**Brigitte Koop**
	Michael Decker
Collegiate assistant	**Jochen Kleemann**
	February - April 2003

Guest scientists

Nina Jaspert, Universität Tübingen, Germany, PhD student

Grants, external funding

DFG grant PA861/1-1 "Untersuchungen zur Ca^{2+}- bzw. Calmodulin-abhängigen Signaltransduktion in der *mlo*-vermittelten Mehltauresistenz von Gerste": 1 Postdoctoral fellow

Collaborations

Establishing a method for high-throughput dsRNAi-based silencing of genes in single barley epidermal cells, IPK Gatersleben, Germany, Dr. Patrick Schweizer

Systematic functional analysis of *Mlo* genes in *Arabidopsis thaliana*, University of North Carolina, Chapel Hill, USA, Dr. Alan Jones

Functional characterization of the *PPI1* gene in Arabidopsis and barley, University of Milan, Italy, Dr. Piero Morandini

Invited lectures given

Universität Halle (15.4.2003)

Genetic Engineering of Cereals: Virus Resistance, Marker Elimination, Insertional Mutagenesis and Tissue-specific Expression

Hans-Henning Steinbiss

RNA-mediated Barley Yellow Dwarf Virus Resistance

Today, it is well documented that transgenes with inverted repeats can efficiently trigger post-transcriptional gene silencing, presumably via a double strand RNA induced by complementary sequences in their transcripts. Two year ago, the cereal transformation service (ATM) designed two constructs which include inverted repeats as well as simple replicase constructs (Fig. 1). Step-by-step transformation experiments have been performed in order to obtain a representative number of transgenics per construct. A set of about 100 T_1-plants derived from transgenics harbouring inverted repeats have been infected with barley yellow dwarf virus by Dr. Habekuß at Aschersleben (Bundesanstalt für Züchtungsforschung.). This experiment will also be repeated again later this year. Complete infected plants as well as plants without virus and symptoms and also intermediates were obtained. Molecular studies will now be performed in order to correlate resistance and transgenic character of barley.

Segregational Separation of Selectable Marker Genes in Barley by *Agrobacterium tumefaciens*-mediated Transfer of Two T-DNA Regions

In a programme of plant genetic transformation, a number of arguments exist for the creation of marker-free lines. In these lines, marker genes, used in the selection process, are eliminated from the plants´s genome; thereby, leaving only the target transgene in place. This has obvious attractions in allowing the removal of antibiotic/herbicide-resistance traits from crop plants prior to field release. There are a number of alternative routes to achieving such marker-free transgenic plant lines. Of these, genetic separation of the marker and target genes in a segregating seed population seems to be the most promising strategy.
The segregational separation of transgenes requires the marker and target sequences to have been introduced into

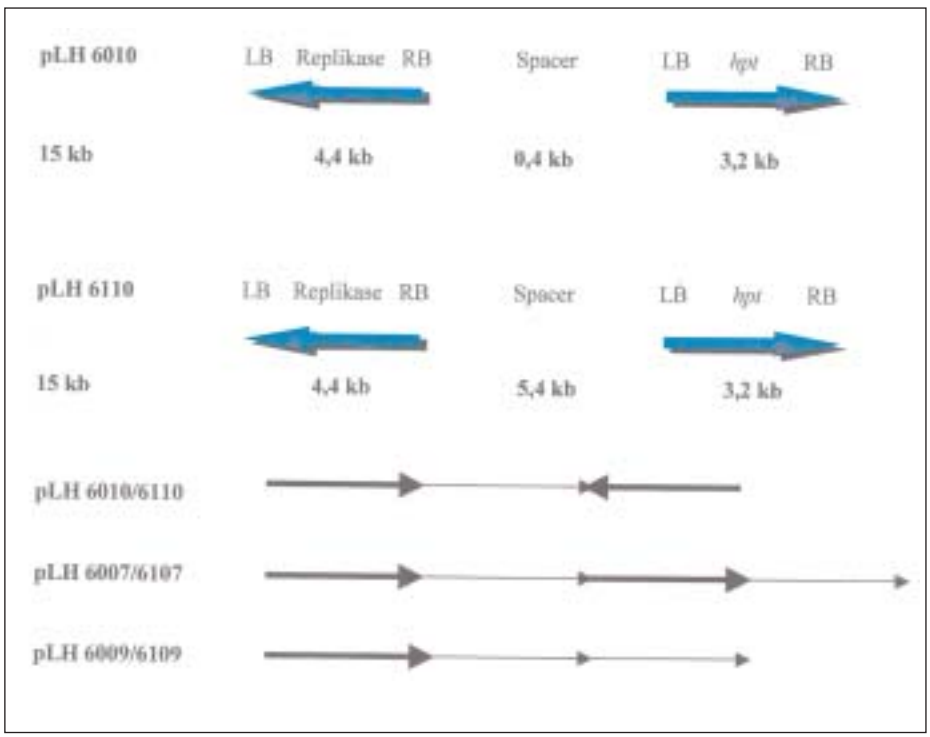

Fig. 1. Schematic representation of the plasmids pLH60 and pLH61. All plasmids contain two independent T-DNAs which are separated by a spacer of either 0.4 or 5.4 kb. The selectable marker gene *hpt* derived from the vector pWBVec8 was designed and kindly provided by Dr. Ming-Bo Wang (CSIRO, Canberra). The other T-DNA contains the expression cassette of the vector pAHC25 provided by Dr. Peter Quail, and two untranslatable replicase sequences of barley yellow dwarf luteovirus (BYDV-PAV) of different size (thick and thin arrow) and different orientations. This set of plasmids was designed and constructed by Dr. Anke Sohn and Britta Killing.

the plant genome as independent elements; thus, permitting integration at separate loci. This may be achieved by the transfer of two separate T-DNA regions located on a single binary vector (Fig. 1). The constructs differ in the size of the spacer between both T-DNAs (0.5 and 5.4 Kb). Primary transformants (T_0) have been analysed by PCR regarding the presence of *hyg* and replicase sequences. In the case of the 0.4 kb spacer, the co-transformation ratio was 64%, in contrast to the 5.4 kb, where is was only 32%. T_1-plants were planted without hygromycin selection and screened by PCR for the presence of both T-DNAs. The 0.4 kb spacer resulted in 8% loss of the replicase sequences, the larger spacer in 12.5%. These results were expected because closer linkage of the 2 T-DNAs will favour co-integration. Interestingly, a significant segregation of the selectable marker gene *hyg* did not occur. This phenomenon is now under molecular investigation.

T-DNA Insertional Mutagenesis for Functional Genomics in Barley

The use of T-DNA as a mutagen for rice was developed in 2000 by Gynheung An and coworkers (Plant J. 22, 561-570, 2000). The cereal transformation service has improved barley transformation up to such a level that T-DNA tagging is now feasible. In co-operation with Dr. Jach, a binary vector has been constructed which contains the promoterless red fluorescent protein (RFP) reporter gene. Therefore, this gene trap vector is able to detect a gene fusion between *rfp* and an endogenous gene, which is tagged by T-DNA. We have established about 170 transgenic barley plants in order to assess the usefulness of the RFP reporter gene. This is because there are still concerns about disturbing background fluorescence; a phenomenon which we are currently investigating.

Functional Analysis of Maize Transfer-cell-specific Promoters in Wheat and Barley

A number of genes expressed specifically in different tissues of the seed have been isolated and the corresponding promoter sequences have been functionally analysed. BETL-1 and BETL-2 are specifically expressed in the **B**asal **E**ndosperm **T**ransfer **L**ayer of maize. Both genes have been identified by the former MPIZ fellows Drs. R. Thompson and G. Hueros. The BETL promoters linked to the GUS reporter gene have been transformed into wheat plants. In the case of BETL-2 expression, GUS expression was localised in the endosperm histochemically. For BETL-1, all GUS assays have proven negative up to now. More experiments are in progress. In maize, BETL-1 is strongly associated with the cell wall ingrowth of the endosperm transfer layer. A microscopical investigation will be made in wheat regarding the cell wall construction of the transfer cells. Conversely, BETL-2 is not bound to the cell wall of maize transfer cells, but has a role as an antifungal protein which may explain its presence in the cytoplasm of the endosperm.

Scientific Publications

Bhat, R.A., M. Riehl, G. Santandrea, R. Velasco, S. Slocombe, G. Donn, H.-H. Steinbiss, R.D. Thompson and H.-A. Becker: Alteration of GCN5 levels in maize reveals dynamic responses to manipulating histone acetylation. Plant J. 32, 1-15 (2002).

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Structure of the group

Group leader	**Dr. Hans-Henning Steinbiß**
Postdoctoral fellow	**Dr. Ekatarina Mouradova**
Technical assistants	**Sabine Schulze**
	Christiane Horst

Department of Plant Breeding and Yield Physiology

Director: Francesco Salamini

The genetic basis of developmental traits relevant in crop production and the genetic diagnosis of crop trait variability are the two main research areas in the Department.

An example relevant to crop production concerns the genetic control of crop morphology. Patterns of shoot branching and growth characteristics of side shoots determine to a large extent the growth pattern of plants. Two branching mutants of tomato, *lateral suppressor* and *blind*, are being studied. The Ls protein shares sequence similarity with members of the family of VHIID proteins. Members of this family, SCR, GAI and RGA, show a number of features pointing to their potential role as transcriptional regulators. An *ls* mutant has also been isolated in Arabidopsis *EN* mutagenised lines, and the gene is known to restore the wild-type phenotype in tomato (Fig. 1). For the *Blind* gene, a map-based cloning approach has been used and the gene is now isolated. The gene belongs to the MYB transcription activator family. In barley, a variety of developmental mutants has been morphologically characterised and mapped to linkage groups together with their candidate genes.

Plant architecture is being studied in barley by mapping and functional analysis of homeotic gene families. Absence of recombination between putative homeotic genes and mutants has resulted in the hooded phenotype being associated with *BKn-3*, a homeobox gene. The genetics of *Hooded* has been enriched by the selection of second-site suppressor mutants. The mapping of the *K* suppressors has provided an interesting finding: out of five complementing loci, four map to chromosome 1, in a region of 5 to 10 cM spanning sublinkage groups 5, 6 and 7. Procedures have been established to isolate genes participating in BKN3 networking: two *BELL* homeobox genes have been isolated by the yeast two-hybrid system, and the intron IV duplication present in *Hooded* - when used in the one-hybrid screen - has led to the isolation of proteins that interact with its DNA sequence. The barley b recombinant (BBR) protein binds specifically to a $(GA/TC)_8$ repeat present in the 305 bp element of *Bkn3*. BBR is nuclear targeted and is characterised by the NLS sequence, a DNA-binding domain and a putative N-terminal activation domain. BBR activates $(GA/TC)_8$-containing promoters, and its over-expression in tobacco leads to a pronounced leaf shape modification. Available data suggest that expression of the barley *BKn3* is regulated, at least in part, by the binding of the transcription factor BBR to GA/TC repeats present in intron IV.

The photosynthesis and related genomics group has grown and diversified to cover the areas: “Genetics of Arabidopsis photosynthesis”, “Structural and comparative genomics”, “Chloroplast transcriptomics” and “Molecular-physiological characterisation of photosynthetic mutations”. These projects have significant overlaps, e.g. until end of 2002, the first was located mainly in the framework of ZIGIA, the second in the Department of Plant Breeding and Yield Physiology, while the third and fourth projects are/were supported by both. In the group, the present state of the projects can be summarised as follows: 67 PAM (photosynthetic) and 98 ALP (pigment-related) mutants were isolated by forward genetics and 11 mutated genes were associated to specific mutants. Alleles for 14 different photosynthetic proteins were identified by reverse genetics; based on bioinformatics, estimations have been provided concerning the number and origin of chloroplast- and

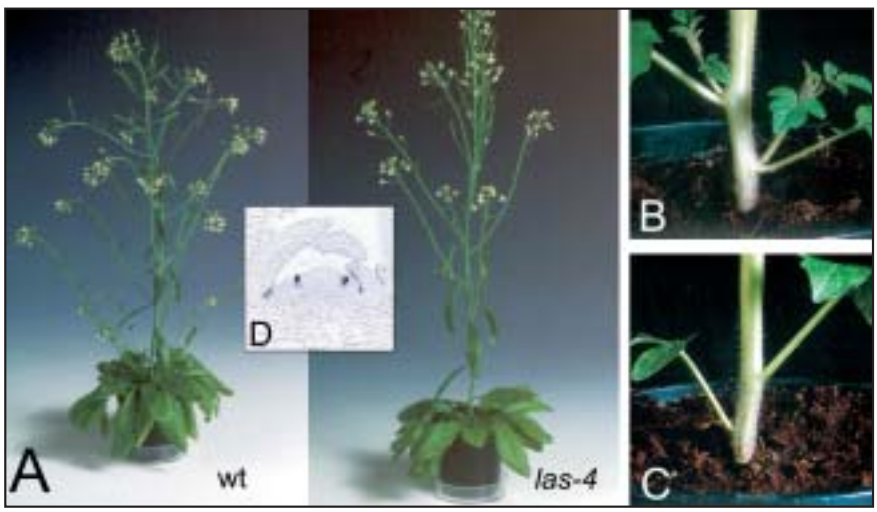

Fig. 1. The *Arabidopsis las* mutant (A) is the orthologue of *ls* of tomato (B) which conditions the absence of lateral branches. The similarity between the two genes is high enough that the wild-type *Arabidopsis LAS* gene can rescue the tomato *ls* mutant (C). In the Arabidopsis shoot apex, the distribution of *LAS* mRNA (D) is consistent with the one observed for *LS*.

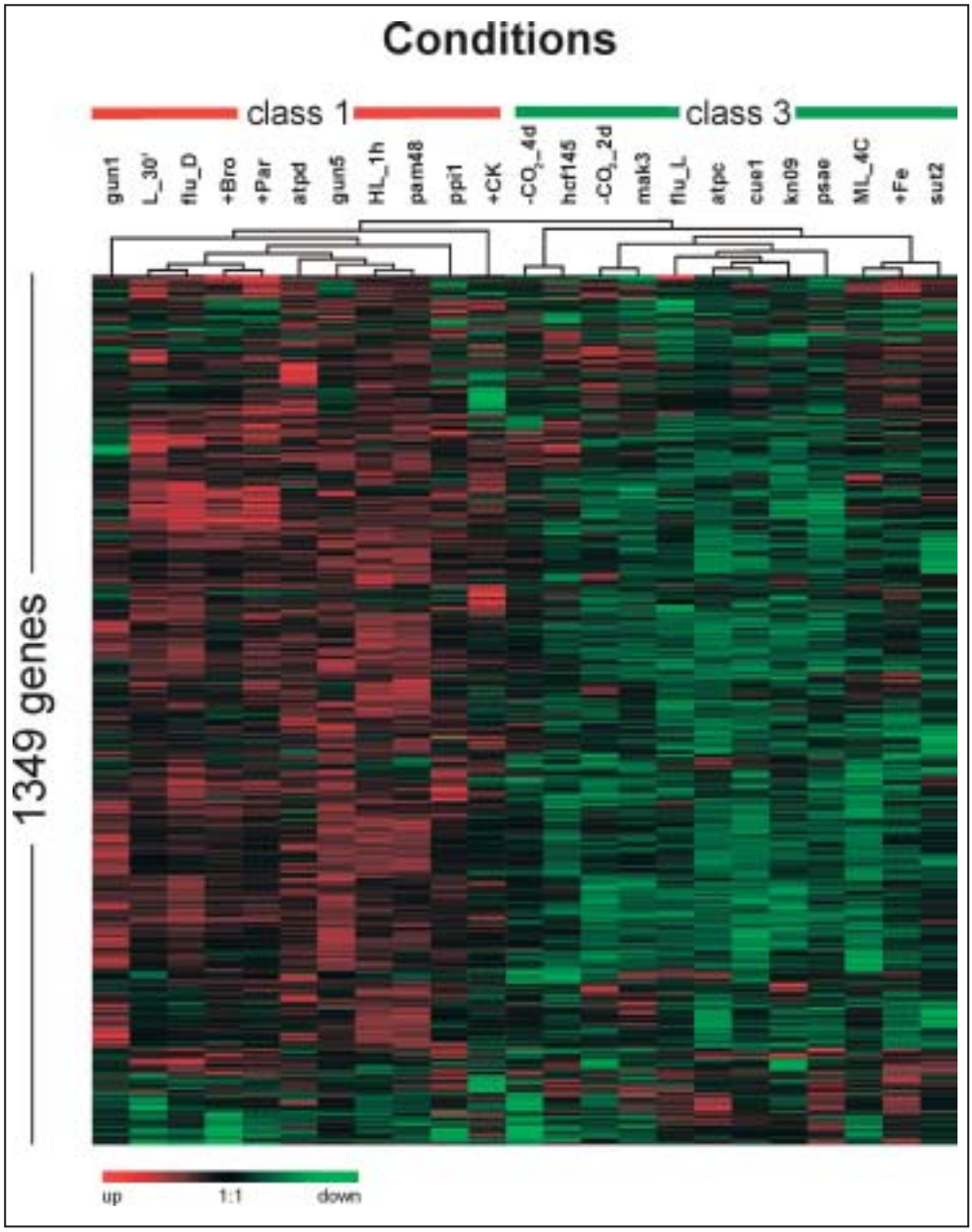

Fig. 2. Response of transcription gene to different environmental or genetic conditions is monitored by filter hybridisation of a set of 1349 Arabidopsis genes related to photosynthesis. The co-variations observed in the nuclear chloroplast transcriptome under the 35 conditions tested (red: up-regulation; green: down-regulated) indicate that large sets of genes are under a regulatory master-switch.

mitochondrion-related genes. Chloroplast transcriptomics is now well established and producing significant results (Fig. 2); the molecular characterisation of mutations has provided evidence on photosynthetic state transitions, organisation of photosystems, the essential role of plastocyanin in PSI as well as the necessity of acetylation of certain chloroplast proteins.

Genetic diagnosis of crop trait variability started 17 years ago with potato. This programme was based on RFLP, and later on AFLP, RAPD, CAPS and SNP markers. A basic genetic framework for a species of difficult genetic treatment was developed. Even today, the group still maintains a leading position in mapping qualitative and quantitative resistance factors active against viruses, fungi, bacteria and nematodes. In the last two years, the group has completed the molecular cloning and characterisation of the *R1* gene for resistance to late blight caused by *Phytophthora infestans* (Fig. 3). The group has achieved the following: (1) Completed the molecular cloning of the *Gro1* gene for resistance to the root cyst nematode *Globodera rostochiensis*; (2) sequenced a "hot spot" for pathogen resistance on potato chromosome V; (3) identified SNP markers tagging regions for pathogen resistance in the potato genome; (4) developed PCR markers for field resistance to late blight in tetraploid potatoes; and (5) analysed the molecular diversity of potato invertase genes which are candidates for cold-sweetening QTLs.

In the two last years, the molecular breeding group of **W. Rohde** has devoted particular attention to collaboration with developing countries. Accordingly, the main scientific interests of the group are: (1) The analysis of biodiversity of tropical plants; (2) coconut and palm biotechnology; and (3) the CIMBIOS activities (practical courses and lecturing). Current projects also deal with the biomonitoring of bacterial soil communities in field trials of transgenes of potato. Recent results include: (1) The development of a

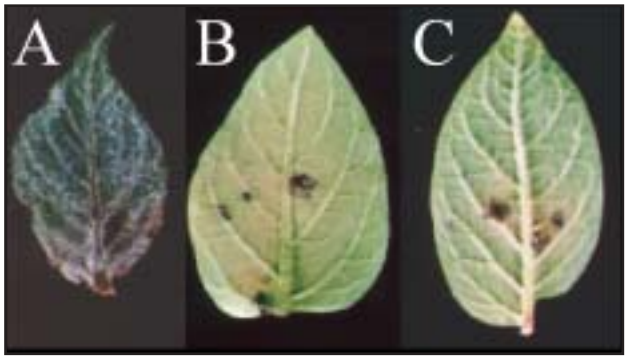

Fig. 3. Cloning of the *r1* resistance gene of potato: Complementation test. Disease symptoms are evident nine days post-inoculation on leaflets from the susceptible cultivar Desirée (A), but not on transgenic Desirée line no. 10-5-5 transformed with *R1* (B), nor the resistant parent P41 (*R1r1*) (C).

dense marker map for coconut and oil palm; (2) the development of molecular markers to measure biodiversity of tropical crops; and (3) the mapping of QTLs for vegetative traits in coconut and for productive traits in oil palm. Co-operation with developing countries is being pursued by co-ordination of an EU-funded project on molecular linkage maps in coconut and oil palm (2001-2004) with Indonesia, Malaysia and the Philippines as partners. Network formation on biodiversity analysis of economically important crops has been initiated by scientist exchange projects with Cuba and Mexico; this is being intensified by a bilateral project with Cuba (molecular linkage map in guava) funded by the DFG/BMZ (2001-2004).

Sugar beet molecular breeding is an important and relatively recent addition to the Department. To date 10,000 unigene cDNAs from several tissues (ADIS) have been sequenced and their use in filter-based hybridisations to monitor preferential gene expression assessed. Moreover, an automated method for multiplex SNP scoring and an approach to QTL mapping in a panel of 122 unrelated breeding lines by associating haplotypes to nitrogen-related traits have been developed. Further, 33 resistance-related genes have been mapped. Finally, the fine mapping of the *Rr1* gene on chromosome III has been completed.

Molecular markers have been used as a diagnostic tool to understand species domestication. Einkorn wheat, one of the primary domesticates of the Near East, was found to originate from the Karacadag mountains, South-east Turkey. Recent data, which concern the AFLP analysis of a collection of 4n wheats, indicate that also the domestication of Emmer and Hard wheats maps to the same area; thus, reinforcing the role of South-east Turkey as the core area for the Mesopotamic agriculture. Molecular markers are also exploited in the wheats to produce linkage maps, to describe the qualitative properties of storage proteins as well as to understand genome A variability in diploid, tetra- and hexaploid wheats.

Structure of the Department:

• Genetic and Molecular Analysis of Shoot Branching in Seed Plants	Klaus Theres
• Barley Genetics	Wolfgang Rohde + Francesco Salamini
• Potato Genetics and Genomics	Christiane Gebhardt
• Genome Analysis of Sugar Beet	Katharina Schneider
• Einkorn Wheat	Francesco Salamini
• Molecular Plant Virology and Molecular Breeding	Wolfgang Rohde
• Photosynthesis and Related Genomics	Dario Leister
• Systematic Analysis of Protein Interaction Networks with an Optimised Yeast Two-Hybrid System*	Joachim Uhrig
• Functional Analysis of Transcription Factors mediating the Formation of Flavonols in *Arabidopsis thaliana**	Bernd Weißhaar
• Engineering resistance to viral pathogens	Peter Schreier

*Groups which have recently joined the department.
The description of these groups is provided in the pages which follow.

Einkorn Wheat

Francesco Salamini

The Domestication of Emmer also maps to the Karacadag Range

Genetic identification of the natural stands from which wild crops were domesticated addresses the question of where specifically within the Fertile Crescent humans invented agriculture. The approach involves comparing wild and domesticated populations using molecular markers that give genome-wide estimates of genetic similarity. One promising technique is amplified fragment length polymorphism (AFLP), a polymerase chain reaction (PCR)-based procedure that resolves radioactively labelled electrophoretic bands (polymorphic loci) on sequencing gels. Using AFLPs, the site of domestication of einkorn – a diploid wheat was identified from the analysis of 288 AFLP marker loci. Those results indicated that wild populations from the Karacadag Mountains of southeast Turkey are more similar to domesticated einkorn than other wild populations are. The publication of the einkorn data (Heun *et al*., 1997, Science **278**, 1312) renewed the debate on the origin of Near East agriculture. Lev-Yadun *et al*. reported that the distribution of several crop progenitors intersect in a region of southeast Turkey, circumscribing a small core area that includes Karacadag (Lev-Yadun *et al*. 2000, Science **288**, 1602). Whether the core area was also the place of origin of other additional founder crops of the Fertile Crescent agriculture was tested using domesticated and wild tetraploid lines of *T. dicoccoides* (the latter being the progenitor of modern polyploid wheats). The AFLP data obtained for 204 loci localise the origin of tetraploid wheat domestication to southeast Turkey, thus lending strong support to the emerging view that agriculture originated in the small core area of southeast Turkey located near the upper course of Tigris and Euphrates rivers (Özkan *et al.*, 2002).

The A Genome Wheats

The access to a large source of genetic variability represented by the wild lines used in the experiments concerning the origin of Mesopotamic cereals, has enabled the contribution of the A genome taxa to the polyploid wheats to be reassessed. The diploid wheat *T. monococcum boeoticum*, although having an A genome, is not the A progenitor of tetraploid and hexaploid wheats. This role is supposed to have been played by *T. urartu*, a second A genome species. The situation may not be as simple as proposed. Available data indicate that: (1) when a large group of wild accessions of *T.m. boeoticum* and *T. urartu* is considered, intermediate forms between the two taxa are spotted by using molecular markers (in our collection ID lines 1529, 1231, 1122, 1528); (2) if, based on AFLP markers specific for the A genomes, 2n, 4n and 6n wheats are compared in phylogenetic analysis, the genetic distances between diploid A genomes and those of 4n and 6n genotypes depends also on the variability among accessions of a species; (3) the sterility in crosses between *T. monococcum* (wild or cultivated) and *T. urartu* is a fact. However, rare seeds can nevertheless be obtained from these F1 hybrids. By growing such seeds into plants, the recombination between *T. urartu* and *T. monococcum* chromosomes was demonstrated; it is thus predicted that such hybrid forms should be present also in natural habitats. A wild line of *T.m. boeoticum* (ID1231) was revealed to be particularly distant from the other accessions. When crossed with four different lines of *T. urartu*, ID1231 generated necrotic lethal seedlings at the ten leaf stage. Using three-way crosses, the necrotic lethal phenotype has been assigned to a single nuclear gene. Both the crossing results and the fingerprinting of ID1231 support the search of further genome A taxa deserving, as *T.m. boeoticum* and *T.m. monococcum*, the rank of subspecies. Altogether, the data on a large number of wild genome A accessions contribute to define a more precise – but also more complex – view of the wild progenitors of wheats.

The Genetics of Einkorn Domestication has relevance to Einkorn Breeding

Einkorn lines with the free-threshing character *sog* and a drastically reduced plant size are available. Crosses are being produced to integrate, in this genetic pool, superior genes for protein quality. The improvement of other agronomic traits is difficult due to a strong pleiotropic effect of the *sog* allele which conditions the appearance of very compacted spikes. An interpretation of the genetics of this character has been offered by the analysis of a *T.m. boeoticum* x *T.m. monococcum* F_3 population. Major QTL

for tough rachis maps to chromosomes 2 and 5 in syntenic position with the *Tg* and *Q* loci of 6n wheats. The chromosome 2 QTL corresponds in position to the *Sog* locus, supporting the functional orthology among the two factors, also for what concerns rachis fragility. To the same chromosome 2 region map two QTLs for spikelet fertility and seed size. QTLs for seed size have been allocated to three chromosomes. The QTL data point to chromosome 2 as the major contributor of loci affecting einkorn domestication. This offers proper molecular markers to search for Einkorn lines combining superior agronomical traits and a structure of the ear adapted to mechanical seed threshing.

Scientific Publications

Özkan, H., A. Brandolini, R. Schäfer-Pregl and F. Salamini: AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. Mol. Biol. Evol. **19**, 1797-1801 (2002).

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Structure of the group

Group leader	**Prof. Dr. Francesco Salamini**
Technical assistants	**Sieglinde Effgen**
	Jutta Hillebrand

Guest scientist

Dr. Hakan Özkan, University of Curkurova, Adana, Turkey, post-doctoral fellow, A. von Humboldt foundation

Collaborations

Genome A contribution to polyploid wheats, Istituto Sperimentale per la Cerealicoltura, Italy, G. Boggini, A Brandolini, P. Vaccino
Breeding of einkorn, Department of Chemistry & Biotechnology, Agricultural University of Norway, M. Heun

Invited lectures given

The data on domestication and genetics of einkorn have been presented by Prof. F. Salamini in several occasions, e.g. seminars at Rockefeller University, New York; EUCARPIA MEETING; Cereal Section, Salsomaggiore, Italy; and University of Düsseldorf.

Barley Genetics

Francesco Salamini and Wolfgang Rohde

Introduction

A variety of developmental mutants affecting vegetative and reproductive organs of barley (*Hordeum vulgare* L.) have been collected. Mutants can be morphologically characterised and mapped on a molecular linkage map. In some cases, attempts to map-based clone follow. In parallel, homeotic gene families are also mapped. The homeotic *Hooded* mutation has been studied in detail. The finding of absence of recombination between gene and mutant has led to the association of the *Hooded* mutant to *BKn-3*, a member of the *Knox* gene family. The mutation is caused by a 305 bp duplication in intron IV.

New Results and Future Developments

One-hybrid experiments

The *Hooded* mutant is molecularly explained by the over-expression of the *BKn-3* homeobox gene due to a duplication of 305 bp in intron IV. The 305 bp were used as bait in the yeast one-hybrid system, in order to identify proteins interacting with the 305 bp, by integrating into the yeast chromosome a *BKn-3* 305 bp/His minimal promoter/His gene construct, and screening with a barley cDNA library cloned in a GAL4-activation domain vector. The screen identified barley proteins that interacted with the sequence. Among these are a cystein-rich protein, a protein with homology to ethylene-insensitive-like proteins from Arabidopsis, a protein involved in flower development characterised by a DNA-binding domain and the BBR protein.
The barley b recombinant (BBR) protein binds specifically to a $(GA/TC)_8$ repeat present in the 305 bp element of *Bkn3*. BBR is nuclear targeted and is characterised by the NLS sequence, a DNA-binding domain and a putative terminal activation domain. BBR activates $(GA/TC)_8$ containing promoters, and its over-expression in tobacco leads to a pronounced leaf shape modification. Available data suggest that expression of the barley *Bkn3* is regulated, at least in part, by the binding of the transcription factor BBR to GA/TC repeats present in intron IV.

Playing with mutants and genes

Genetics is still proving highly productive in the analysis of the homeobox gene system in barley, particularly, when in cases of association of gene mapping and data provided by molecular analyses. The genetics of *Hooded* has been enriched by the selection of second-site suppressor mutants (*suk* mutants). The mapping of the *K* suppressors has provided an interesting finding: out of five complementing loci, four map to chromosome 1, in a region of 5 to 10 cM spanning sublinkage groups 5, 6 and 7. To the same region maps the gene locus *lk2* controlling awn length (the awn is the terminal appendage of the lemma and is homologous to a leaf blade). A morphological relation between *suk* mutants and *lk2* was evident: both types of mutations have short awns. The genetic analysis excludes that *lk2* corresponds to one of the *suk* loci; however, it is still possible that in this isophenic chromosomal segment more loci map which control the same trait.

The one-hybrid experiments provided four proteins interacting with *Bkn3* intron IV: BBR, BEIL, BAPL and BGRF. The corresponding four genes were mapped, respectively, to chromosomes 4, 1, 7 and 2. The *Beil* gene comaps with the *suk-lk2* spot on chromosome 1, sublinkage groups 5-7. The Bapl gene comaps with the mutation *lel2* (*leafy lemma*) on chromosome 7, sublinkage groups 60-63. The BBR gene maps on chromosome 4, sublinkage group 38, where also the short awn mutant lk_5 is positioned. Together, the two sets of genetic and molecular data offer new possibilities of gene to mutant association which are currently being tested.

Scientific Publications

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Structure of the group

Group leaders	**Prof. Wolfgang Rohde**
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Postdoctoral fellows	**Dr. Cristina Roig Montaner**
	Dr. Yongxiu Liu
PhD students	**Luca Santi**
	Maria Rosaria Stile

Guest scientist

Maria Rosaria Stile, University of Naples, Italy, Ph.D. student

Collaborations

Barley genetics, Dipartimento di produsione vegetale, Italy, C. Pozzi

Scientific Publications of the Department

Ballvora, A., M.R. Ercolano, J. Weiß, K. Meksem, C.A. Bormann, P. Oberhagemann, F. Salamini and C. Gebhardt: The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant J. **30**, 361-371 (2002).

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Marco Tizzano (2001) Molecular-genetic analysis of Arabidopsis mutants with *En*-transposon insertion in the *PsaG* and *PsaH2* genes coding for photosystem I subunits

Fabio Schiavon (2001) Molecular-genetic analysis of of Arabidopsis mutants with *En*-transposon insertion in the *PsbS* and *PetC* genes coding for subunits of the photosynthetic machinery

Martin Weigel (2001) Molekulargenetische Analyse von Arabidopsis-Mutanten mit beeinträchtigter Photosynthese

PhD theses

Riyaz Bhat (2002) Evidence for the biological functions of histone acetyl-transferase Gcn5 and adaptor protein Ada2 in Zea mays L

Thomas Greb (2003) Untersuchungen zur Rolle des Gens *LATERAL SUPPRESSOR* in der Seitentriebentwicklung von *Arabidopsis thaliana* H. und der Tomate (*Lycopersicon esculentum* M.)

Hannah Jaag (2001) Eine neue interne Bindungsstelle für die Translationsinitiation von Ribosomen in der Kartoffelblattrollvirus-RNA

Daniela Maiwald (2003) Molecular and physiological characterisation of the two *Arabidopsis thaliana* mutants *atpd* and *petc*

Frank Mehrtens (2002) Untersuchungen zur Funktion des Transkriptionsfaktors AtMYB12 als Regulator des Phenylpropanoidstoffwechsels

Jürgen Paal (2002*)* Molekulare Charakterisierung der *Gro1* Region in Kartoffel, die Resistenz gegen den Nematoden *Globodera rostochiensis* vermittelt

Brigitte Paap (2002) Wechselwirkungen zwischen Genprodukten des Kartoffelblattrollvirus (PLRV) und Wirtsfaktoren der Kartoffelpflanze (*Solanum tuberosum* L.)

Rosa Castillo (2002) A potato large-insert library for isolation of candidate loci for late blight resistance and studies on their genome organization

Andreas Rickert (2002) Entwicklung und Analyse von SNP-Markern (single nucleotide polymorphismes) in Pathogenresistenz-vermittelnden Regionen des Kartoffelgenoms

Marcus Riehl (2002) Charakterisierung der Histonacetyltransferase GCN5 und des Adaptorproteins Ada2 in *Zea mays* L. und *Arabidopsis thaliana*

Luca Santi(2002) Intron mediated regulation of BKn3, a plant homeobox gene, as supported by BBR (GAGA) binding factor

Bärbel Taenzler (2002) QTL-Analyse der Backqualität in Einkornweizen (*T.m. monococcum*)

Claudio Varotto (2002) Genetic and molecular dissection of Photosystem I functions in Arabidopsis and related functional genomics

Sonja Vorwerk (2003) Analysis of a novel kelch domain-containing protein from maize

Ilona Zimmermann (2003) Systemitische Untersuchungen von Proteininteraktionen der MYB und bHLH Transkriptionsfaktoren aus *Arabidopsis thaliana.*

Genome Analysis of Sugar Beet

Katharina Schneider

Introduction

Sugar beet is an important crop plant for sucrose production. Our research is focused on genomic regions with relevance to agronomic traits like disease resistance and sugar quality. Our aim is to supplement classical breeding strategies by using genomics tools. The group's key research interests and goals are as follows:

- development of SNP (single nucleotide polymorphism) markers and establishment of a semi-automated technique for genotyping
- identification of candidate genes for tap root development by cDNA macroarrays and genetic analysis
- development of an association mapping approach to sugar beet QTL for nitrogen-related traits
- identification and linkage analysis of disease resistance gene analogues and cloning of a gene conferring resistance to rhizomania

New Results

EST sequencing, development of SNP markers and semi-automated genotyping (GABI-BEET project)

A set of 10,000 unique sugar beet cDNAs derived from young and mature roots, leaves and inflorescences was sequenced by the ADIS unit at the MPIZ. ESTs were processed by the "beetbase"; a password-protected database incorporating all data generated within the project and serving as an information exchange platform for all partners.

For SNP analysis, a set of 16 breeding lines including an accession of red beet, Swiss chard and *Beta maritima* each of these has been analysed. Targets for SNP analysis are 300 markers derived from converted RFLP markers and ESTs revealing sequence similarities to genes involved in sugar and energy metabolism. In a pre-screen, PCR products amplified from the different genotypes were submitted to SSCP (single strand conformation polymorphism) analysis, a gel-based technique known to detect SNPs. To determine nucleotide variation, PCR products of 51 RFLP and 131 EST markers were sequenced in at least 8 different genotypes. The average nucleotide diversity is close to the value of $\pi = 7.6 \times 10^{-3}$ as determined in the study of Schneider *et al*. (2001).

Complementary to SSCP mapping, the SNaPshot method (Applied Biosystems) was introduced as a semi-automated method for multiplex SNP scoring. This technique is based on primer extension with fluorescently labelled nucleotides along with capillary electrophoresis. Protocols for multiplex PCR and 6-plex SNaPshot reactions were developed. SNP detection by MALDI-TOF was successfully tested by an external service.

Crosslinking functional and structural genomics with regard to sugar metabolism and root development (SWEET GABI project)

The aim of this project is to identify candidate genes with relevance to sugar metabolism and root development based on their expression pattern. Therefore, cDNA AFLP and cDNA macroarray analysis were established to compare the transcription profiles in different: (1) plant organs; (2) developmental stages; and (3) parts of the mature tap root. Using a set of 3,800 cDNA clones from young leaves and roots representing 2,048 unique sequences, a protocol was established to identify clones with preferential expression in one or two of the three organs, namely root, leaf and/or inflorescence. Based on the statistical analysis of eight replica and a threshold for expression ratios of two, 917 unique cDNAs were found to be differentially expressed. Among these, 76 preferentially root-expressed cDNAs with predicted functions in primary and secondary carbohydrate metabolism and other pathways (Fig. 1) were identified. The cDNA AFLP analy-

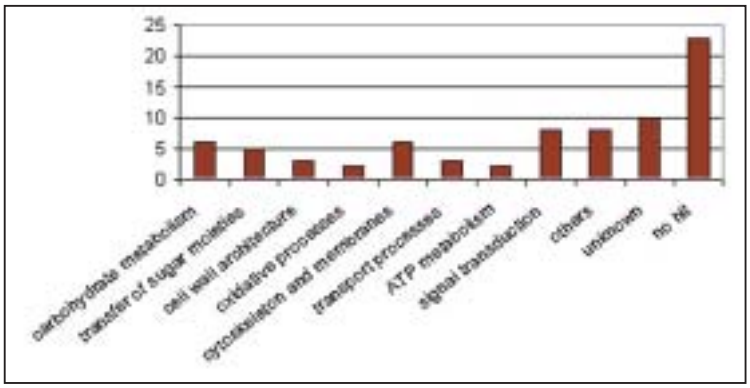

Fig. 1. Distribution of preferentially root-expressed genes in functional categories. *Y*-axis: number of ESTs.

sis yielded an additional 19 different preferentially root-expressed gene products. For 42% of the preferentially root-expressed gene products, the functions remain either unknown or no hit could be retrieved.

The genetic relevance of the identified candidate genes was assessed by genetic mapping and analysis of linkage with QTLs (Quantitative Trait Loci) for sugar quality and yield parameters (Schneider *et al.*, 2002). Loci for 50 preferentially root-expressed genes were identified, several of them in linkage with QTLs for quality traits.

Development of an association mapping approach to sugar beet QTL for nitrogen-related traits

Association mapping is based on the occurrence of a small number of haplotypes, in which several SNP alleles are inherited together due to selection. Typically, there are three to five haplotypes per gene in sugar beet (Schneider *et al.*, 2001). For candidate genes, the phenotypic performance of a plant will depend on the expression of a particular haplotype. If unrelated lines sharing the same haplotype of one gene correspond to phenotypic classes, an association is found. Sequence polymorphisms between the haplotypes are expected to explain the phenotypic variation. For sugar beet, association mapping was performed in a panel of 122 unrelated breeding lines that represent extremes for nitrogen-related traits. Based on SSCP scoring, association mapping was performed for 19 candidates and selected haplotypes were confirmed by sequencing.

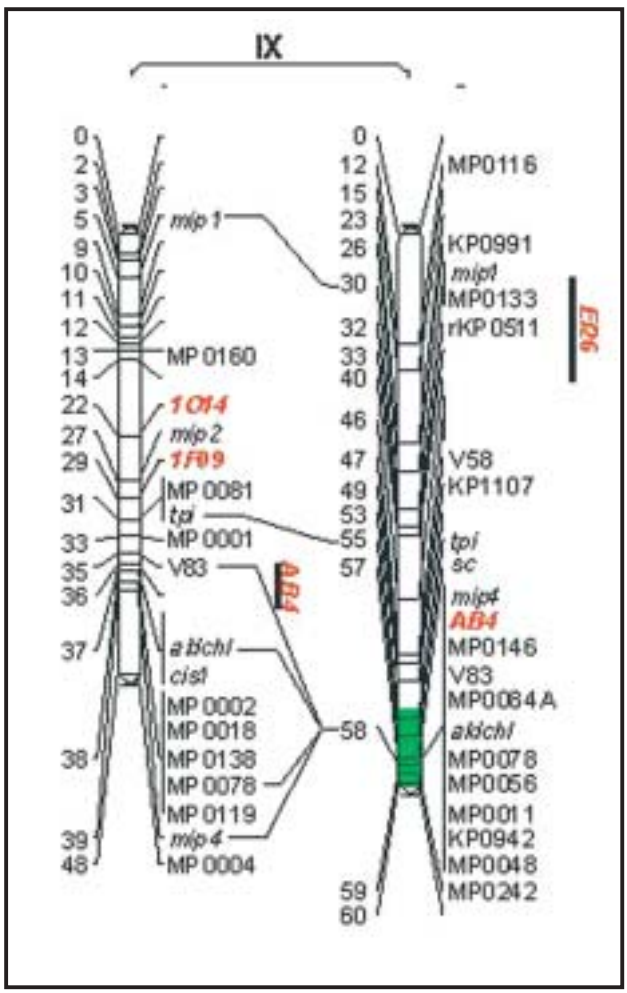

Fig. 2. Genetic map of chromosome IX from two populations. Positions of RGAs (in bold) and QTLs (grey bar) for resistance to the fungal disease *Cercospora beticola* are indicated.

Identification of disease resistance genes in sugar beet (BioBau project)

In plants, most resistance (R) genes fall into four to five categories having conserved domains. Therefore, a strategy was adopted to retrieve disease resistance gene analogues (RGAs) based on sequence similarity from sugar beet and then to assess their potential to confer resistance to either the viral disease rhizomania or the fungal disease *Cercospora beticola*. Using degenerate primers, 18 RGAs were amplified and 29 were selected from an EST sequencing programme. Twenty-one RGAs contained structures similar to the nucleotide binding site (NBS)-leucine rich repeat (LRR) domain. Among the remaining RGAs, 19 revealed similarity to the serine (threonine) protein kinase domain of R genes, four showed features related to the LRR region of the rice disease resistance gene *Xa21*, one RGA resembled the sugar beet nematode resistance gene *Hs1pro-1*, and two had homologies to other gene products associated with disease resistance. Thirty-three RGAs were spread over all nine sugar beet chromosomes, except for a cluster of nine closely linked RGAs on chromosome VII. The analysis of linkage between RGAs and loci for rhizomania and Cercospora resistance identified alleles associated with resistance. An example, the RGA AB4 linked to a Cercospora QTL on chromosome IX, is illustrated in Fig. 2.

However, no RGA was found cosegregating with the major locus *Rr1* on chromosome III conferring resistance to the viral disease rhizomania. Genetic fine mapping in a population of around 1700 F2 plants and analysis of recombinant plants narrowed the interval of the resistance locus to around 1 cM. Initial work on building a contig around the *Rr1* gene using a BAC library from a non-resistant genotype indicated major genomic rearrangements and the necessity for a BAC library from the rhizomania-resistant genotype, which is currently being constructed.

Future Developments

Within the GABI-BEET project, SNP analysis and genotyping will be continued to achieve the goal of 300 SNP markers. To increase the mapping throughput further, 72 SNP markers will be genotyped using an external service. The generation of a high density genetic map will be a requirement for the development of a physical map for sugar beet in the future.

For the identification of candidate genes involved in beet formation and sucrose accumulation, high density cDNA macroarrays with the 10,000 unique cDNA clones have recently been generated. Future analysis will include samples from a time course of developing beets, beet sectors with differing sucrose content and plants growing under

different nitrogen fertilisation regimes. Identified candidate genes will be subjected to genetic mapping and haplotype analysis. The isolation of superior alleles is intended to enable the generation of better varieties by either marker-assisted breeding programmes or transgenic approaches. The disease resistance gene project is focused on the positional cloning of the *Rr1* gene. A contig around the *Rr1* locus will be constructed using a BAC library generated from the resistant genotype. An additional F2 population providing the *Rr1* allele in a different background will be used for fine-mapping the gene. The development of markers cosegregating with the resistant phenotype will enable the breeders to select resistant plants at a very early stage for marker-assisted breeding. The isolation of the *Rr1* allele will allow the generation of rhizomania-resistant transgenic plants.

Scientific Publications

Bellin, D., M. Werber, T. Theis, B. Weisshaar and K. Schneider: EST sequencing, annotation and macroarray transcriptome analysis identify preferentially root-expressed genes in sugar beet. Plant Biol. **4**, 700-710 (2002).

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Salamini, F. and K. Schneider: Integration of new genomic technologies in variety development, Vortr. Pflanzenzüchtg Band **54**, 189-193 (2002).

Schneider, K., B. Weisshaar, D.C. Borchardt and F. Salamini: SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. Mol. Breed. **8**, 63-74 (2001).

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Structure of the group

Group leader	**Dr. Katharina Schneider**
Postdoctoral fellows	**Dr. Sandra Hunger** until August 2002
	Dr. Silke Möhring
	Dr. Elena Pestsova since January 2003
	Dr. Schäfer-Pregl until July 2002
	Dr. Jörg Wunder since November 2002
PhD student	**Diana Bellin**
Technical assistants	**Susanne Horstmann-Schwarz**
	Charlotte Kieselstein
	Antje Scherpe

Guest scientists

Gabriele DiGaspero, University of Udine, Italy, PhD student
Serena Venturi, University of Bologna, Italy, PhD student
Maria Cartolano, University of Naples, Italy, PhD student

Grants external funding

BMBF "GABI-BEET" program (grant number: 0312283B): 2 Postdoctoral fellows, 1 technician
BMBF "SWEET GABI" program (grant number: 0312283E): 1 Postdoctoral fellow, 1 PhD student, 1 technician
BMBF "Biologische Baupläne" (grant number: 0311791): 1 Postdoctoral fellow, 1 technician

Collaborations

GABI-BEET

University of Kiel, Institute for Plant Breeding Research, Prof. C. Jung
University of Halle, Institute for Plant Breeding Research, Prof. E. Weber
TraitGenetics, Gatersleben, Dr. M. Ganal
KWS SAAT AG, Einbeck, Dr. B. Schulz
Dieckmann-Heimburg AG, Sülbeck, Dr. G. Koch

SWEET GABI

KWS SAAT AG, Einbeck, Dr. B. Schulz

BioBau

KWS SAAT AG, Einbeck, Dr. D.C. Borchardt

Invited lectures given

Conference of the GPZ section for yield and stress physiology in Bonn (21.06.2001), Free University of Amsterdam (19.03.2002), University of Bonn (06.05.2002), IPK Gatersleben (19.09.2002), plantGEM conference in Berlin (30.09.2002). ■

Photosynthesis and related Genomics

Dario Leister

Introduction

From its origins as the 'Yield and Fitness' unit within the ZIGIA project, the group has grown and diversified to cover currently the areas: (1) Genetics of Arabidopsis photosynthesis; (2) Structural and comparative genomics; (3) Chloroplast transcriptomics; and (4) Molecular-physiological characterisation of photosynthetic mutations. While these projects have significant overlaps and aim to develop synergistic effects, the first project was, until end of 2002, located mainly in the framework of ZIGIA, the second in the Department for Plant Breeding and Yield Physiology, with the third and forth projects being supported by both.

New Results

Genetics of Arabidopsis photosynthesis

Photosynthesis takes place within a highly complex protein network and is thus influenced by several metabolic processes. This is why the absence or modification of any one of the gene products localised in the chloroplast may alter its photosynthetic function. Genetics for the dissection of photosynthesis-related processes involves mutant screens (forward genetics) and reverse genetics. We have continued the forward screening of Arabidopsis knock-out populations: a total of 200,000 individuals have been tested so far, resulting in the identification of 67 PAM (with alterations in the effective quantum yield of PSII) and 98 ALP (with altered pigmentation) mutants (Varotto, Pesaresi, Kurth, Richly, Weigel). We have identified 11 different mutated genes, coding for both structural and putative regulatory proteins (Fig. 1).

Forward genetics – although being the approach of choice to identify novel and unexpected gene functions relevant for photosynthesis – is, in contrast to reverse genetics, a rather inefficient tool for the large-scale analysis of gene functions. Therefore, we have focused on a saturating reverse genetics screen for genes coding for subunits of the photosynthetic apparatus, in particular of PSI. To date, we have isolated mutant alleles for 14 different photosynthetic proteins (Varotto, Dietzmann).

Structural and comparative genomics

The analysis of the completed sequence of Arabidopsis and other eukaryotic and prokaryotic species allows us to

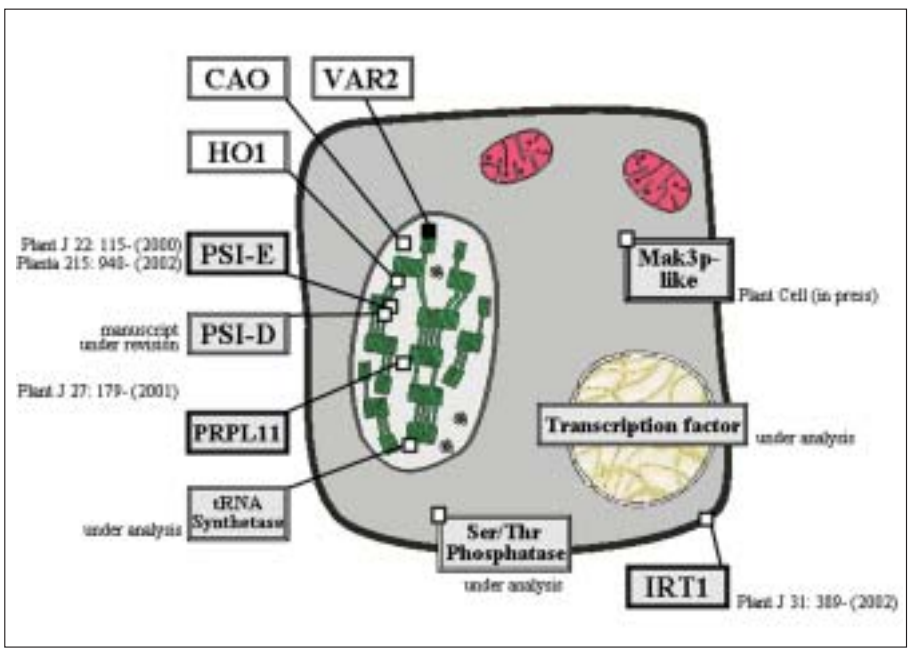

Fig. 1. Overview on subcellular locations of gene products with impact on photosynthesis which were identified by forward genetics. The most detailed characterisations have been performed for the mutation in *PsaE1* (coding for the E-subunit of photosystem I; Varotto, Pesaresi), *PsaD1* (coding for the D-subunit of photosystem I; Ihnatowicz), *Prpl11* (coding for a protein of the plastid large ribosomal subunit; Pesaresi), *AtMAK3* (encoding a cytoplasmic N-acetyl transferase; Pesaresi) and *IRT1* a gene coding for a plasma-membrane-located iron transporter (Varotto).

assess genome-wide structure, horizontal gene transfer and subcellular location of proteins.

In collaboration with William Martin (University of Düsseldorf), we compared the proteins encoded in the Arabidopsis genome to the proteins from three cyanobacterial genomes, other prokaryotic reference genomes and yeast. We found that around 4500 of Arabidopsis protein-coding genes (18% of the total) should have been acquired from the cyanobacterial ancestor of plastids. These proteins encompass all functional classes. Moreover, the majority of them are targeted to cell compartments other than the chloroplast (Richly).

Besides chloroplasts, mitochondria are also thought to descend from an endosymbiotic event. By combining comparative genomics and computational identification of protein targeting, the size and composition of the mitochondrial proteome for ten species was predicted. Functional mitochondria are predicted to harbour from a few hundred to >3000 gene products, and protein relocation from and to mitochondria occurred during evolution. While each genome studied contains lineage-specific mitochondrial proteins, conserved mitochondrial proteins also exist. Their functions mostly relate to transport and metabolism, and in humans their mutated states were found to be frequently associated with diseases (Richly).

Chloroplast transcriptomics

By scanning of the complete genome sequence of *Arabidopsis thaliana* for chloroplast transit peptides (cTPs), we identified 2661 proteins likely to be targeted to the chloroplast. Gene-sequence-tags (GSTs) for those nuclear chloroplast genes and for 631 genes encoding non-chloroplast proteins, were PCR-amplified and spotted onto nylon membranes to generate a 3292-GST array. Wild-type plants, and several mutants with defects in chloroplast function and/or plastid-to-nucleus signalling, were grown under various conditions; in all, 35 genetic or environmental conditions were tested. Most of the 35 conditions tested, including plastid signalling mutations, elicit only three main classes of response from the nuclear chloroplast transcriptome (Biehl, Dietzmann, Kurth, Richly) (Fig. 2).

We now use expression profiling routinely in the characterisation of photosynthetic mutants to assist the classification and interpretation of the effects of photosynthetic lesions on other chloroplast functions (Ihnatowicz, Maiwald, Pesaresi, Weigel).

Molecular-physiological characterisation of photosynthetic mutations

A number of the photosynthetic mutants were characterised on the physiological level. In the *psae1-1* mutant, a fraction of LHCII is stably associated with PSI, giving rise to a high molecular mass protein-pigment complex detectable in native protein gels. The formation of this abnormal LHCII-PSI complex is associated with an almost complete suppression of state transitions, a drastic increase in the levels of phosphorylated LHCII and a permanent reduction in PSII antenna size (Pesaresi).

The D-subunit of photosystem I (PSI-D) is encoded by two functional genes, *PsaD1* and *PsaD2*. The double

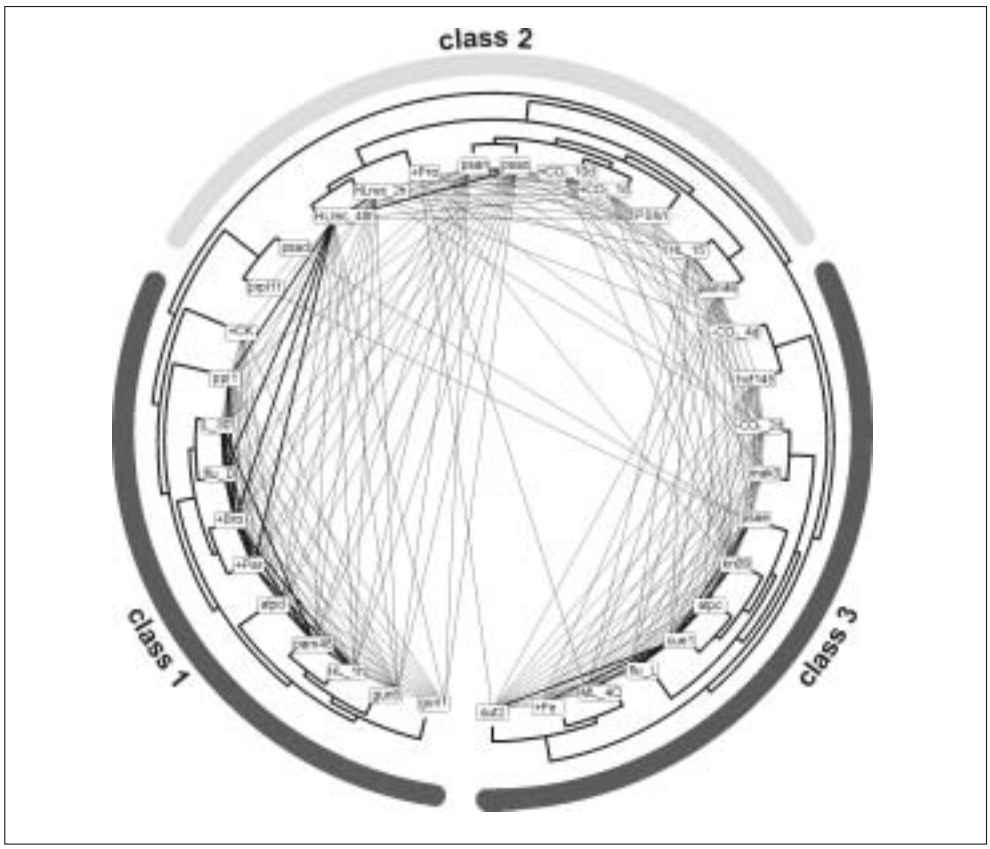

Fig. 2. The 35 nuclear chloroplast transcriptome responses fall into three main classes. The topographical arrangement of environmental and genetic conditions is derived from hierarchical clustering and the corresponding cladogram is shown at the periphery. The relationships between expression profiles are indicated inside the circle by connecting lines. Increasing line thickness indicates increasing relatedness, with the three thicknesses of lines corresponding to 65-80%, 80-90% and 90-100% of genes showing the same trend in expression in each pair of transcriptomes. Main classes of transcriptome change are indicated as class 1, 2 and 3. Two classes, probably involving GUN (genomes uncoupled)-type plastid signalling, are characterised by alterations, in opposite directions, in the expression of largely overlapping sets of genes.

mutant *psad1-1 psad2-1* displays a seedling-lethal and high chlorophyll-fluorescence phenotype, indicating that the PSI-D function is essential for photosynthesis. Of the single mutations, only *psad1-1* markedly affects the level of PSI-D expression (Ihnatowicz).

Plastocyanin, a soluble copper-containing protein present in the thylakoid lumen, transfers electrons to PSI. In the chloroplast of *A. thaliana*, a cytochrome c_6-like protein is present, which was recently suggested to function as an alternative electron carrier to plastocyanin. We showed that in Arabidopsis only plastocyanin can donate electrons to photosystem I *in vivo* (Varotto, Weigel).

The nuclear genes *PetC* and *AtpD* code for the Rieske protein of the cytochrome b_6/f complex and the δ-subunit of the chloroplast ATPase (cpATPase), respectively. Greenhouse-grown *petc-2* and *atpd-1* mutants are seedling-lethal, while heterotrophically propagated plants display a high chlorophyll-fluorescence phenotype, indicating that the products of *PetC* and *AtpD* are essential for photosynthesis. Additional physiological effects of the mutations in axenic culture were characterised (Maiwald).

In *atmak3-1*, synthesis of the plastome-encoded PSII core proteins D1 and CP47 is affected, resulting in a drop in the abundance of thylakoid multiprotein complexes. The mutation responsible was localised to *AtMAK3*, encoding a homologue of the yeast protein Mak3p. In yeast, Mak3p together with Mak10p and Mak31p, forms the N-terminal acetyl transferase complex C (NatC). The cytoplasmic AtMAK3 protein can functionally replace both Mak3p and Mak10p of NatC in acetylating N-termini of endogenous proteins and the L-A virus Gag protein. This, together with the finding that knock-out of the Arabidopsis *MAK10* homologue does not result in obvious physiological effects, indicates that AtMAK3 function does not require NatC complex formation as in yeast. We suggest that N-acetylation of certain chloroplast precursor protein(s) is necessary for efficient accumulation of the mature protein(s) in chloroplasts (Pesaresi).

Future Developments

Identification and characterisation of novel photosynthesis-related gene functions by forward genetics will continue. Additionally, saturation of mutant alleles of nuclear genes coding for subunits of the photosynthetic machinery will be attempted. We will also shift reverse genetics efforts to the systematic mutational analysis of chloroplast protein kinases to investigate the regulation of organelle function based on protein phosphorylation.

Scientific Publications

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Diploma theses

Marco Tizzano (2001) Molecular-genetic analysis of Arabidopsis mutants with *En*-transposon insertions in the *PsaG* and *PsaH2* genes coding for photosystem I subunits.

Fabio Schiavon (2001) Molecular-genetic analysis of Arabidopsis mutants with *En*-transposon insertions in the *PsbS* and *PetC* genes coding for subunits of the photosynthetic machinery.

Martin Weigel (2001) Molekulargenetische Analyse von Arabidopsis-Mutanten mit beeinträchtigter Photosynthese.

Dissertations

Claudio Varotto (2001) Genetic and molecular dissection of photosystem I functions in Arabidopsis and related functional genomics.

Paolo Pesaresi (2002) Molecular and physiological characterisation of the photosynthetic mutants *prpl11-1*, *psae1-1*, and *mak3-1*.

Daniela Maiwald (2003) Molecular and physiological characterisation of the photosynthetic mutants *petc* and *atpd* in *Arabidopsis thaliana.*

Structure of the group

Group leader	**Dr. Dario Leister**
Postdoctoral fellows	**Dr. Claudio Varotto** until December 2002 (PhD thesis until 12/2001) **Dr. Paolo Pesaresi** since April 2002 (PhD thesis until 3/2002) **Dr. Joachim Kurth** since November 1999
PhD students	**Daniela Maiwald** until April 2003 **Erik Richly** **Alexander Biehl** **Martin Weigel** **Ania Ihnatowicz**
Technical assistant	**Angela Dietzmann**

Grants, external funding

BMBF "ZIGIA" program (03117519): 3 PhD students, 1 postdoctoral fellow (until 12/2002)

DFG Habilitation (until 6/2003) and Heisenberg (since 7/2003) stipend for D.L. (LE 1265/1 and 18)

DFG-Graduiertenkolleg *Molekulare Analyse von Entwicklungsprozessen*: 1 PhD student

DFG: Arabidopsis Functional Genomics Network: 1 PhD student

DFG Sachbeihilfe: 1 PhD student (LE 1265/1)

DFG Sachbeihilfe: 1 Postdoc (LE 1265/8)

EU Research Training Network: 2 PhD students

Collaborations

Chloroplast physiology, University of Milano, Prof. Dr. Carlo Soave

Pigment profiling, Heinrich-Heine-Universität Düsseldorf, Dr. Peter Jahns

Chloroplast phylogeny, Heinrich-Heine-Universität Düsseldorf, Prof. Dr. William Martin

Chloroplast proteins, Ludwig-Maximilians-Universität München, Dr. Lutz Eichacker, Dr. Jörg Meurer

Photosystem I, The Royal Veterinary and Agricultural University, Copenhagen, Prof. Dr. Henrik Scheller

Oxidative Stress, ETH Zürich, Prof. Dr. Klaus Apel

N-acetylation in yeast, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Prof. Dr. Reed Wickner

Invited lectures given

Department of Plant Biology, The Royal Veterinary and Agricultural University, Copenhagen (24.01.2001), Gesellschaft für Pflanzenzüchtung e.V. AG Ertrags- und Streßphysiologie, Bonn (21.6.2001), Institut für Pflanzenbau & Pflanzenzüchtung, Justus-Liebig-Universität Giessen (03.12.2001), DFG-Forschergruppentreffen Redox Signals as Key Regulators in Photosynthesis, Universität Bielefeld (26.03.2002), Institut für landwirtschaftliche Botanik, Universität Bonn (06.06.2002), Institut für Biologie und Pflanzenphysiologie der Humboldt Universität Berlin (11.06.2002), Institute of Plant Sciences, ETH, Zürich (11.09.2002), Friedrich Miescher Institute, Basel (12.09.2002), Botanikertagung, Freiburg (25.09.2002), The 6th Nordic Congress on Photosynthesis, Umea (26.10.2002), Shinshu University, Ueda (25.02.2003), The Rice Genome Forum, Tsukuba (27.02.03), International Symposium "Molecular Plant Physiology – Combining Levels of Function", Max-Planck-Institut für molekulare Pflanzenphysiologie, Golm (13.05.2003). ■

Potato Genome Analysis

Christiane Gebhardt

Introduction

The research activities of the group concentrate on the potato (*Solanum tuberosum*), which is the most important crop species of the Solanaceae family worldwide. The potato is closely related to about 200 tuber-bearing and non-tuber-bearing Solanum species; hence, it represents a rich source of biodiversity. Our long-term goals are to contribute: (1) to the improvement of the cultivated potato by developing molecular diagnostic tools to assist the breeding of new cultivars; and (2) to the understanding of structure, function and natural diversity of crop plant genomes. Our outputs are: (a) DNA-based markers that can be used for marker-assisted selection of superior cultivars; (b) knowledge of genomic positions and identity of genes controlling qualitative or quantitative agronomic characters; and (c) cloned genes and superior alleles of agronomic characters, which may be transferred into cultivars by genetic engineering.

Over the last two years, the group has worked on the following projects:

- Completion of the molecular cloning and characterisation of the *R1* gene for resistance to late blight, caused by *Phytophthora infestans*.
- Completion of the molecular cloning of the *Gro1* gene for resistance to the root cyst nematode *Globodera rostochiensis*.
- Genomic sequence analysis of a "hot spot" for pathogen resistance on potato chromosome V.
- Identification of SNP markers tagging regions for pathogen resistance in the potato genome.
- Development of PCR markers for field resistance to late blight in tetraploid potato.
- Analysis of molecular diversity of potato invertase genes, which are candidates for cold-sweetening QTL.

New Results

Cloning and characterisation of the *R1* gene for resistance to *Phytophthora infestans* (late blight)

Ballistic transformation of *R1* susceptible cv Desireé with the DNA of a candidate BAC plasmid (ca. 120 kbp) resulted in transgenic plants that expressed the *R1*-hypersensitive resistance phenotype. Agrobacterium-mediated transformation with a 10 kbp subclone of the same BAC plasmid was sufficient to transfer the *R1* hypersensitive resistance phenotype to *R1* susceptible cv Desireé. *R1* transgenes were inherited as single, dominant factors, although with distorted segregation ratios in some crosses. The 10 kbp genomic sequence contained a single, leucine zipper/NBS/LRR-type gene encoding a protein of 1293 amino acids. The *R1* resistance gene is expressed and is one of at least two members of a gene family.

Cloning and characterization of the *Gro1* gene for resistance to *Globodera rostochiensis* (root cyst nematode) based on a candidate gene approach

Eight members of the candidate gene family, which co-segregated with the *Gro1* resistance phenotype (see last report) were sequenced, subcloned in binary transformation vectors and tested for complementation of nematode resistance by Agrobacterium-mediated stable transformation of susceptible cv Desireé. All candidate genes were members of the TIR/NBS/LRR-type class of plant resistance genes. Based on sequence analysis, only three members encoded functional TIR/NBS/LRR proteins. One of these three candidate genes transferred the nematode resistance phenotype to the susceptible cv Desireé.

Physical mapping and genomic sequencing of a resistance "hot spot" on potato chromosome V

Two partial contigs were constructed within the genetic interval bordered by markers GP21 and GP179 based on 14 overlapping BAC insertions (Fig. 1). Six BACs were fully sequenced, covering ca. 400 kbp potato genomic sequence. As the BAC clones originate from a heterozygous genotype, we can compare ca. 50 kbp of allelic sequence originating from the homologous chromosomes. A total of 35 putative genes were identified, among those 8 retrotransposon-like elements and 4 resistance-gene-like sequences, one of which encodes the *R1* resistance gene (see above).

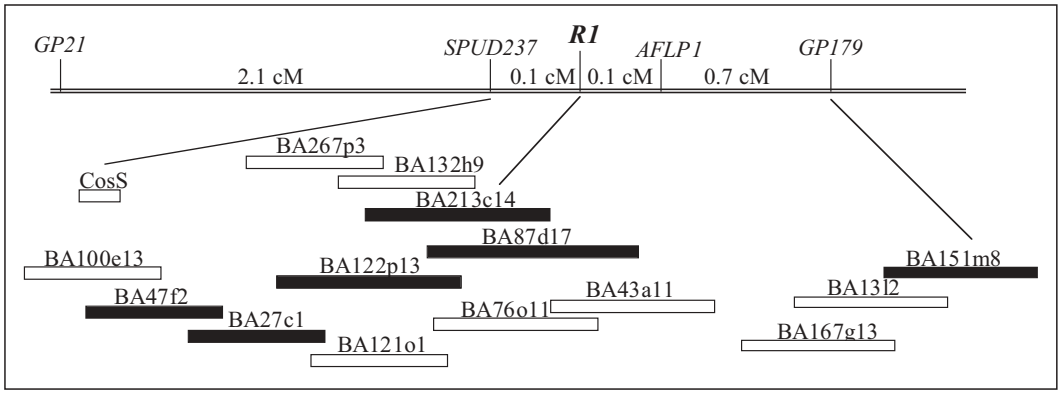

Fig. 1. Genetic and physical map of the interval GP21-GP179 on potato chromosome V. Fully sequenced BAC clones are shown in black.

SNP/InDel markers tagging regions for pathogen resistance in potato

A panel of 17 tetraploid and 11 diploid potato genotypes was screened by comparative sequence analysis of PCR products for single nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (InDels) in regions of the potato genome where genes for qualitative and/or quantitative resistance to different pathogens have been localised. Most of these regions were tagged with BAC clones containing sequences similar to the NBS-LRR class of plant genes for resistance. Few resistance QTL not linked to resistance-gene-like sequences were tagged with other markers. In total, 79 DNA fragments were comparatively sequenced in the panel of 28 genotypes, resulting in 31 kbp genomic sequence. From this, 1498 SNPs and 127 InDels were identified, which corresponded, on average, to one SNP every 20 bp and one InDel every 242 bp. The nucleotide diversity of the tetraploid genotypes ($\pi = 0.72 \times 10^{-3}$) was lower when compared with diploid genotypes ($\pi = 2.31 \times 10^{-3}$). A set of selected SNP/InDel markers were scored by sequencing the amplicons in 96 tetraploid genotypes, half of which were highly resistant (cases) to the root cyst nematode *Globodera pallida*; whereas, the other half was highly susceptible (controls). Cases and control plants were selected and provided by Nordkartoffel Zuchtgesellschaft mbH and Saka-Ragis Pflanzenzucht GbR. SNP/Indels linked to the nematode resistance were converted in haplotype specific, diagnostic PCR assays (Fig. 2).

Development of PCR markers for marker-assisted-selection (MAS) for field resistance to late blight in tetraploid potato

DNA markers known to be linked to QTL for resistance to late blight (LB) in diploid potato, were tested for linkage to LB field resistance in two tetraploid potato populations. The two populations originated from crossing the same LB susceptible parent with two different parents having good field resistance to late blight. Quantitative LB resistance and plant maturity of the populations were evaluated over two years in the field (Saka-Ragis Pflanzenzucht GbR). Increased resistance to late blight is normally correlated with late plant maturity. The goal is to find markers for resistance QTL alleles that do not affect plant maturity. The plants were genotyped with 29 PCR markers (SCAR, CAPS, SSCP). Marker genotypic classes were tested for having significant effects on quantitative resistance and plant maturity. PCR markers still significantly correlated with resistance after correction for maturity were identified and used for a first marker-assisted selection experiment, which will be evaluated in the field in the season in 2003 by Saka-Ragis.

Sequence diversity at invertase loci and association with tuber traits

Based on a potato function map for tuber traits, which includes QTL for cold-sweetening and loci for genes functional in carbohydrate metabolism, invertase genes are among the candidates for controlling cold-sweetening and therefore, chipping quality. Tetraploid breeding clones were analysed for sequence polymorphism at invertase loci and several invertase alleles were identified in the population. Specific invertase alleles were associated with chipping quality of the breeding clones.

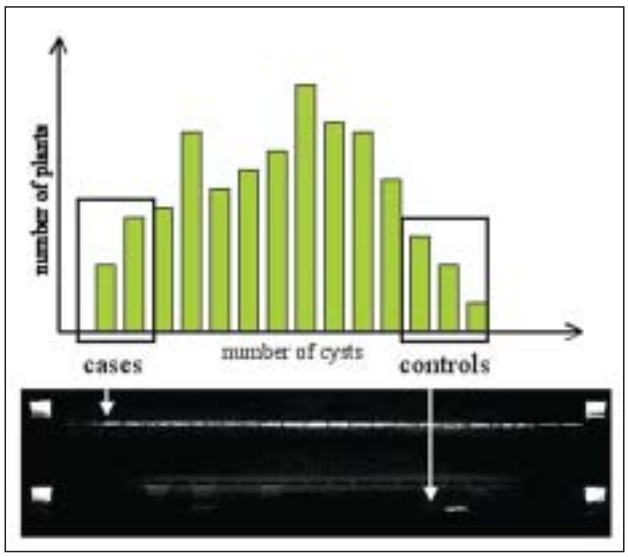

Fig. 2. Haplotype-specific marker linked to quantitative resistance to the root cyst nematode *Globodera pallida*. From two F1 hybrid families with various levels of nematode resistance, 25 highly resistant (cases) and 25 highly susceptible (controls) plants were selected per family (Saka-Ragis GbR and Böhm-Nordkartoffel Agrarproduktion) and scored for SNP and InDel markers (MPIZ). The marker shown is linked with high significance to nematode resistance.

Future Developments

We have started to analyse the sequence diversity of the *R1* gene family and to clone *R1* homologous genes from *Solanum nigrum*, a wild species that is highly resistant to late blight.

We have started to search for the potato homologues of genes from *Arabidopsis thaliana*, which play a role in the signal transduction between pathogen recognition and defence response. We want to study their genome organisation in potato and their potential role as candidates for quantitative resistance to pathogens.

In collaboration with the potato breeding industry, we will further develop association mapping of quantitative resistance and tuber quality traits based on candidate genes and our strong marker resource base.

We will continue with physical mapping and functional analysis of the resistance "hot spot" on potato chromosome V.

We are looking for the opportunity to establish potato "proteomics" in the context of genetic and phenotypic diversity, using the technology platform for protein identification available in the institute (MALDI-TOF MS).

Scientific Publications

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Heibges, A., Ballvora, A., Salamini, F. and C. Gebhardt: Functional comparison of homologous members of three groups of Kunitz-type inhibitors from potato tubers (*Solanum tuberosum* L.). Mol. Gen. Genomics (in press).

Marczewski, W., B. Flis, J. Syller, R. Schäfer-Pregl and C. Gebhardt: A major QTL for resistance to *Potato leafroll virus* (PLRV) is located in a resistance hotspot on potato chromosome XI and is tightly linked to *N*-gene-like markers. Mol. Plant-Microbe Interact. **14,** 1420-1425 (2001).

Marczewski, W., J. Hennig and C. Gebhardt: The potato virus S gene *Ns* maps to potato chromosome VIII. Theor. Appl. Genet. **105**, 564-567 (2002).

Menendez, C.M., E. Ritter, R. Schäfer-Pregl, B. Walkemeier, A. Kalde, F. Salamini and C. Gebhardt: Cold-sweetening in diploid potato. Mapping QTL and candidate genes. Genetics **162,** 1423-1434 (2002).

Rickert, A.M., A. Premstaller, C. Gebhardt and P.J. Oefner: Genotyping of SNPs in a polyploid genome by pyrosequencing. Biotechniques **32**(3), 592-593, 596-598, 600 (2002).

Dissertations

Jürgen Paal (2002) Molekulare Charakterisierung der *Gro1* Region in Kartoffel, die Resistenz gegen den Nematoden *Globodera rostochiensis* vermittelt.

Rosa Castillo (2002) A potato large-insert library for isolation of candidate loci for late blight resistance and studies on their genome organization.

Andreas Rickert (2002) Entwicklung und Analyse von SNP-Markern (single nucleotide polymorphisms) in Pathogenresistenz-vermittelnden Regionen des Kartoffelgenoms.

Structure of the group

Group leader	**Dr. PD Christiane Gebhardt**
Postdoctoral fellows	**Dr. Agim Ballvora**
	since February 1999
	Dr. Andreas Heibges
	since March 2001
	Dr. Li Li
	since December 2000
	Dr. Jürgen Paal
	April 2002-December 2002
	Dr. Andreas Rickert
	December 2002-March 2003
PhD students	**Christina Bormann**
	since January 2001
	Evgenia Ilarionova
	since September 2002
	Karolina M. Pajerowska
	since September 2002
	Rosa Castillo
	until February 2002

	Jürgen Paal until March 2002
	Andreas Rickert until November 2002
Diploma student	**Amirali Sattarzadeh** since April 2002
Technical assistants	**Birgit Walkemeier**
	Heike Henselewski since August 1998
	Werner Lehmann until July 2002
	Monique Winkler until June 2001
	Iris Schmitz since July 2001
	Tamara Rotshteyn since April 2002
	Thi Ha Nguyen since April 2003

Guest scientists

Jadwiga Sliwka, MS, Plant Breeding and Acclimatization Institute (IHAR), Mlochow Research Center, Poland.

Grants, external funding

BMBF: GABI-CONQUEST (0312290): 1 Postdoctoral fellow, 1 PhD, 1 technician

BMBF: Biologische Baupläne (0311791): 1 postdoctoral fellow, 1 PhD, 1 technician

GIF (G-566-269.12/97): 1 PhD

CIP/BMZ: 1 PhD

IMPRS: 1 PhD

Collaborations

Ballistic transformation with whole BAC DNA: AG Dr. H.H. Steinbiss, MPIZ.

Simulation of synteny between potato and Arabidopsis: Dr. K. Stüber, MPIZ.

Whole BAC sequencing and SNP identification by sequencing: ADIS, Dr. B. Weisshaar.

Identification and characterization of genes controlling quantitative agronomic characters in potato by a candidate gene approach (GABI-CONQUEST): Nordkartoffel Zuchtgesellschaft mbH, Dr. H.-R. Hofferbert, Dr. E. Tacke; Saka-Ragis Pflanzenzucht GbR, Dr. J. Lübeck.

Entschlüsselung von Resistenzgenen und Nutzung bei Kartoffel und Zuckerrübe (Biologische Baupläne): Saka-Ragis Pflanzenzucht GbR, Dr. J. Lübeck; KWS Saat AG.

Mapping of genes for resistance to potato viruses PLRV and PVM: Plant Breeding and Acclimatization Institute (IHAR), Mlochow Research Center, Poland, Dr. W. Marczewski.

DNA-based markers for resistance to late blight: Plant Breeding and Acclimatization Institute (IHAR), Mlochow Research Center, Poland, Dr. E. Zimnoch-Guzowska, J. Sliwka.

Potato database construction: RZPD German Resource Center for Genome Research, Berlin, Germany; Dr. S. Meyer, Dr. A. Nagel.

Potato BAC library "BC": Southern Illinois University, Carbondale, USA, Dr. K. Meksem.

FISH-mapping of *R1* transgenes: University of Wisconsin, Madison, USA, Dr. J. Jiang.

Invited lectures given

Weizmann Institute of Science, Rehovot, Israel (06.2001), Centro International de la Papa (CIP), Lima, Peru (11.2001), Centro Internacional Agricultura Tropical (CIAT), Cali, Colombia (12.2001), 15. Dreijahreskonferenz der Europäischen Gesellschaft für Kartoffelforschung (EAPR), Hamburg (07.2002), Symposium "Genetic Mechanisms of Phenotypic Variation in Plants" at the MPI for Plant Breeding Research, Köln (11.2002), MPI for Molecular Plant Physiology, Golm (26. 02.2003) ■

Molecular Genetics of Crop Plants

Wolfgang Rohde

Introduction

Our current scientific interests (http://www.mpiz-koeln.mpg.de/~rohde/) are directed towards five areas:
Molecular breeding in potato
Biodiversity analysis of tropical crop plants by DNA marker technology
Application of DNA markers for the improvement of the perennial tropical oil crops coconut and oil palm
CIMbios: Transfer of biotechnology and bioinformatics "in the region for the region"
DAAD lectureship in plant molecular biology, biochemistry and biotechnology in Cuba and Mexico

Molecular Breeding in Potato

The current project deals with the development of technologies (T-RFLP, reverse line hybridisation) and their application to the biomonitoring of bacterial communities of the soil in field trials with the potato starch variety "Tomensa" (transgenic and non-transgenic), in order to monitor changes in the rhizosphere as a result of transgene expression and agrochemical application. With the non-transgenic potato variety, the statistical analysis of results from field trials over 2 years indicated a significant change in the bacterial community after the application of the herbicide "Roundup". In contrast, the application of other chemicals (fungicides, insecticides) did not affect the bacterial community. A possible effect of the transgenes (amylose-free and virus-resistant potato lines) can be studied later this year at the earliest, since permission for a field trial was only granted in autumn 2002.

Biodiversity Analysis of Tropical Plants

Within these projects, DNA markers are being applied to the analysis of accessions of economically important tropical fruit trees in Cuba (mango, avocado, coconut, guave) and of henequén in Yucatán (Mexico). A third project aims at the establishment of molecular linkage maps for guave (*Psidium guajava* L.). Three mapping populations were established by our Cuban partner. Currently, some 200 segregating AFLP markers have been identified for the first population and phenotypes have been recorded (leaf shape, dwarfism, flowering time). It is expected that the first linkage maps for each of the two parents will be available by mid-2003 and include first QTLs.

Coconut and Oil Palm Biotechnology

The general objectives of this research project are:

- to provide the methodological basis and molecular tools for improving the breeding efficiency in the two perennial tropical oil crops coconut (*Cocos nucifera L.*) and oil palm (*Elaeis guineensis*)
- to develop these DNA marker-based breeding strategies in collaboration with the most important countries in coconut and oil palm production in Asia
- to directly transfer to developing countries small-scale technological solutions for the genetic improvement of these tropical oil crops by marker-assisted breeding

As a basis to achieve these objectives, high-density (HD) molecular linkage reference maps are being developed using different DNA marker techniques (AFLP, SSR, SNP) for standard mapping populations in coconut and oil palm. Within the first 18 months, the project constructed molecular maps based mainly on AFLP and SSR markers. Microsatellite (SSR) and single nucleotide polymorphism (SNP) markers were developed by screening genomic libraries enriched for $(GA)_n$ microsatellite sequences (SSRs) and by sequencing individual cosmid (COS) clones (SNP). From some 310 and 420 screened SSR sequences, 260 and 364 functional SSR primer pairs were developed for coconut and oil palm and in part mapped as anchor markers to the corresponding linkage maps. Cross-taxa transferability of these SSRs corresponded to 32 to 34% and thus, forms the basis for future synteny studies in these two palm species. A total of 120,000 COS clones were individualised in triplicates from genomic libraries of oil palm and coconut and multiple filter sets prepared for genomic studies, gene isolation and sequencing (with primary emphasis on resistance gene-like [RGL] sequences, genes for plant development [homeobox, MADS-box, MYB genes] and the gene for Δ-9-stearyl-ACP desaturase which is involved in oil biosynthesis).
With respect to the construction of HD reference maps, for coconut the following maps were established on the basis

of the two mapping populations: East African Tall (EAT_{0707}) x Pemba Red Dwarf (PRD) and Malayan Yellow Dwarf (MYD) x Laguna Tall (LAGT): EAT_{0707}: 707 markers on 16 linkage groups, map length 2083 cM; $LAGT_{07}$: 235 markers on 16 linkage groups, map length 2478 cM; and MYD_{20}: 181 markers on 16 linkage groups, map length 1394 cM.

A total of 1108 AFLP and of 282 SSR markers were produced and scored for the oil palm HD map of a LM2T x DA10D cross. This HD map (in progress) already has a total of 682 markers (475 AFLP, 207 SSR) mapped on 16 linkage groups representing 1532 cM. This is the first linkage map of oil palm with 16 groups representing the homologous chromosomes on the plant. Mapped co-dominant markers, such as SSRs and SNPs, will help in the integration of the five different coconut maps for the genotypes MYD, LAGT, EAT (0707, 1011) and RLT into a general coconut reference map. It is expected that the project will finally deliver some 1800-2200 mapped markers for each of the reference linkage maps of coconut and oil palm.

QTL analyses were performed for coconut and oil palm. For the EAT coconut linkage map, QTLs for vegetative traits were mapped. Future DNA marker and QTL mapping will be directed to a second EAT mapping population (EAT_{1011} x RLT_{0710}) for which a larger progeny size is available. For oil palm, QTL analyses of vegetative and production characters are undertaken using an original factorial genetic design of nine different crosses connected by common parents. Three QTLs involved in stem height were already detected in the LM2T parent, while two QTLs (leaf F17 length) are located in the DA10D parent. Currently, QTLs are being mapped for wax and palm oil biosynthesis. These studies are complemented by the physical mapping of at least one important gene in oil biosynthesis, namely Δ-9-stearyl-ACP desaturase (ACPD) which catalyses the conversion of stearyl-ACP (C18:0) into oleate-ACP (C18:1), with oleic acid being the main constituent of palm oil. Such physical mapping is another general aspect of the project in a genomics approach: some 28,800 individualised COS clones have been assembled into thirty 8-dimensional pools which are currently used in associating cloned genomic DNA to mapped AFLP markers. Another strategy follows the development of SNPs from terminal sequences of COS clones and their application in mapping.

Due to the structure of the available mapping populations, the detected QTLs are more likely to be responsible for the 'within' population variations. The project includes the set-up of field experiments (field trial systems, FTSs) based on a second generation of inter-population crosses involving more and selected parents. These activities have been initiated within the first year by the project partners in the Philippines (coconut) and in Indonesia (oil palm). In the future, these trials will allow us to apply the project results in breeding programmes by marker-assisted selection (MAS) and to identify and compare effects of additional QTL alleles accounting for 'between' population variations. In this way, the efficiency of breeding programmes can be optimised.

For dissemination and training purposes, this project includes holding a symposium and practical course on "Biotechnology in Coconut and Oil Palm" that will take place in April 2004 in the Philippines.

CIMbios and DAAD Lectureship

The CIMbios programme as well as a personal DAAD lectureship granted to Wolfgang Rohde aim at transferring expertise and latest results in the areas of biotechnology and bioinformatics. The areas covered include:

Plant biotechnologies (tissue culture, biopesticides, biofertilizer, pathogen diagnosis, DNA marker technology, gene technology)

Environmental biotechnology (water and waste treatment, bio- and phytoremediation, biomonitoring)

Biopharmaceuticals (molecular pharming, edible vaccines, plants as bioreactors)

High-throughput technologies and genomics, proteomics, metabolomics

Industrial biotechnology (microbial biotechnology, engineering and process development)

The transfer is being achieved by a five-week programme (CIMbios) at the Mexican partner's institution consisting of lectures and a symposium along with practical courses in biotechnology and bioinformatics. The DAAD lectureship in Cuba and Mexico provides a basic introduction into these areas by theoretical courses and training of students in areas of biotechnology (pathogen diagnosis, biodiversity analysis) relevant to the mandates of the partner institutes in scientific projects. Current efforts are being devoted to the development of a joint Mexican-German study course in biotechnology at the CICY (Mexico) together with IME (Fraunhofer Society) and the RWTH Aachen. The maestria awarded to Mexican and Latin American students will be an accepted qualification for entry into a PhD programme at the RWTH Aachen.

Scientific Publications

Billotte, N., N. Marseillac, A.-M. Risterucci, Asmady, A. Herrán, R. Singh, P. Amblard T. Durand-Gasselin, P. Brottier, B. Courtois, S.C. Cheah, W. Rohde and E. Ritter: Reference and SSR multi-parent linkage maps for molecular breeding in oil palm (*Elaeis guineensis* Jacq.). In: Sustainable Forestry, Wood Products & Biotechnology (E. Ritter, S. Espinel and Y. Barredoin, eds.). Diputacion Foral de Alava, Vitoria, Spain (in press).

Capote, M., D. Becker, J. Cueto, and W. Rohde: Development and application of DNA markers for the characterization of genetic diversity within commercial mango varieties in Cuba. J. Genet. Breed. (in press).

Commandeur, U., W. Rohde, R. Fischer and D. Prüfer: Gene expression strategies of plant RNA viruses. In: Plant Viruses as Molecular Pathogens (J.A. Khan and J. Dijkstra, eds.). Haworth Press Inc., New York, pp. 175-202 (2002).

Herrán, A., L. Estioko, M.J.B. Rodríguez, D. Becker, V. Sniady, N. Billotte, A. Kullaya, W. Rohde and E. Ritter: Exploitation of high-density DNA marker maps in coconut. In: Sustainable Forestry, Wood Products & Biotechnology (E. Ritter, S. Espinel and Y. Barredoin, eds.). Diputacion Foral de Alava, Vitoria, Spain (in press).

Jaag, H.M., L. Kawchuk, W. Rohde, R. Fischer, N. Emans and D. Prüfer. An unusual internal ribosomal entry site (IRES) with a sequence-specific motif of inverted symmetry directs the *in vitro* and *in vivo* expression of the replication-associated protein 1 (RAP1) of potato leafroll polerovirus (PLRV). Proc. Natl. Acad. Sci. USA (in press).

Kawchuk, L., H.M. Jaag, K. Toohey, R. Martin, W. Rohde and D. Prüfer: *In planta* agroinfection by Canadian and German potato leafroll polerovirus full-length cDNAs. Can. J. Plant Pathol. **24**, 239-243 (2002).

Llauger, R., D. Becker, J. Cueto, E. Peralta, V. González, M. Rodríguez and W. Rohde: Detection and molecular characterization of phytoplasma associated with Lethal Yellowing disease of coconut palms in Cuba. J. Phytopathol. **150**, 390-395 (2002).

Müller, J., Y. Wang, R. Franzen, L. Santi, F. Salamini and W. Rohde: Interactions between barley TALE homeodomain proteins suggest a role for protein-protein interactions in the regulation of *Knox* gene function. Plant J. **27**, 13-23 (2001).

Ramírez, I.M., J.L. Fuentes, N.N. Rodriguez, J. Cueto, D. Becker and W. Rohde: DNA polymorphisms in Cuban varieties of avocado (*Persea americana* Mill.) as detected by inverse sequence-tagged repeat (ISTR) analysis. Cultivos Tropicales **23**, 85-88 (2002).

Rohde, W., V. Sniady, A. Herrán, L. Estioko, S. Sinje, N. Marseillac, A. Berger, P. Lebrun, D. Becker, A. Kullaya, J. Rodriguez, N. Billotte and E. Ritter: Construction and exploitation of high-density DNA marker and physical maps in the perennial tropical oil crops coconut and oil palm: from biotechnology towards marker-assisted breeding. http://www.wiz.uni-kassel.de/dtt2002/abstracts/full/44.pdf, pp. 1-8 (2002).

Santi, L., Y. Wang, M.R. Stile, K. Berendzen, D. Wanke, C. Roig, C. Pozzi, K. Müller, J. Müller, W. Rohde and F. Salamini: The GA octadinucleotide repeat binding factor BBR participates to the transcriptional regulation of the homeobox gene *Bkn3*. Plant. J. **34**, 8133-816 (2003).

Dissertation

Brigitte Paap (2002) Wechselwirkungen zwischen Genprodukten des Kartoffelblattrollvirus (PLRV) und Wirtsfaktoren der Kartoffelpflanze (*Solanum tuberosum* L.)

Structure of the group

Group leader	**Prof. Dr. Wolfgang Rohde**
PhD student	**Volker Sniady**
Technical assistants	**Dieter Becker**

Guest scientists

Dr. Nerdo Rodriguez, IIFT, Cuba, Visiting scientist
Maricela Capote, IIFT, Cuba, Visiting scientist
Juliette Valdes-Infante, IIFT, Cuba, Ph.D. student
I. Perez, CEADEN, Cuba, Maestria student
Ales Vokurka, University of Zagreb, Croatia, PhD student
Dr. Tomas Gonzales, CICY, Mexico, Visiting scientist
Dr. Enrique Ritter, NEIKER, Spain, Visiting scientist
Konstanze Freiesleben, University of Cologne, Germany, Ph.D. student
Dr. Diogenes Infante, CICY, Mexico, Visiting scientist

Grants, external funding

EU: "INCO-DEV": 1 PhD student
DLR-IB "Scientific and Technological Cooperation"
DFG/BMZ "Research Cooperation with Developing Countries"
BMBF "Biotechnology 2000": 1 Technical assistant
DAAD "Study, Research and Teaching Abroad"

Collaborations

Biodiversity analysis of tropical fruit trees by PCR-based DNA marker technology (2000-2002)

Instituto de Investigaciones en Fruticultura Tropical (IIFT), La Habana, Cuba, Dr. J. Cueto

Biodiversity analysis of important crop plants in Mexico by DNA markers (2000-2003)

Centro de Investigación Científica de Yucatán (CICY), Mérida, Mexico, Dr. C. Oropeza

Establishment of different DNA marker types for biodiversity analysis and construction of a molecular linkage map in guava (*Psidium guajava L.*) (2001-2004)

Instituto de Investigaciones en Fruticultura Tropical (IIFT), La Habana, Cuba, Dr. N. N. Rodriguez

Construction and exploitation of high density DNA marker and physical maps in the perennial tropical oil crops coconut and oil palm: from biotechnology towards marker-assisted breeding (2001-2004)

NEIKER, Granja Modelo – CIMA, Vitoria-Gasteiz, Spain, Dr. E. Ritter

Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD), Montpellier, France, Mr. N. Billotte

PCA Albay Research Center, Banao, Guinobatan, Philippines, Dr. J. Rodriguez

Indonesian Oil Palm Research Institute (IOPRI), Medan, Indonesia, Mr. D. Asmono

Malaysia Palm Oil Board (MPOB), Kajang Selangor, Malaysia, Dr. S.-C. Cheah

P.T. SOCFIN INDONESIA (SOCFINDO), Medan, Indonesia, Mr. Asmady

Development of biosensors for application in diagnosis of the microbial environment (2000-2003)

MPI für terrestrische Mikrobiologie (MPITM), Marburg, Germany, Dr. W. Liesack

BNA Böhm Nordkartoffel, Lüneburg, Germany, Dr. H. Böhm

CIMbios – Transfer of biotechnology and bioinformatics "in the region for the region" (2001-2003)

Fraunhofer-Institut für Molekularbiologie und Angewandte Ökologie (IME), Aachen, Germany, Dr. D. Prüfer

Centro de Investigación Científica de Yucatán (CICY), Mérida, Mexico, Dr. C. Oropeza ■

Genetic and Molecular Analysis of Shoot Branching in Seed Plants

Klaus Theres

Introduction

In seed plants, shoot branching is initiated by the formation of new meristems in the axils of leaves. Axillary meristems function like the SAM of the primary shoot initiating the development of lateral organs, a process that results in the formation of an axillary bud. In many plant species, further development of axillary buds into shoots is blocked to a different extent by the influence of the primary shoot. This phenomenon is known as apical dominance and seems to be mediated by plant hormones.

The origin of axillary meristems is presently unclear. For several plant species, it has been suggested that axillary meristems are initiated from cell groups detached from the primary SAM and retain their meristematic identity. Alternatively, axillary meristems may originate *de novo* later in development from partially or fully differentiated cells.

Despite the importance of shoot branching for plant development, very little is known about the molecular mechanisms controlling the formation of axillary meristems in higher plants. The aim of our work is to understand the mechanisms controlling the process of axillary meristem formation at the molecular level.

New Results

Characterisation of Arabidopsis *las* mutants

Six independent mutants harbouring mutations at different positions of the *LATERAL SUPPRESSOR* open reading frame were characterised. In comparison to wild-type plants, *las* mutants showed an almost complete suppression of lateral shoot formation during vegetative development. However, side-shoot development from the axils of cauline leaves was not inhibited. Different from the tomato *lateral suppressor* mutant, the Arabidopsis *las* mutants did not show a defect in petal formation or fertility.

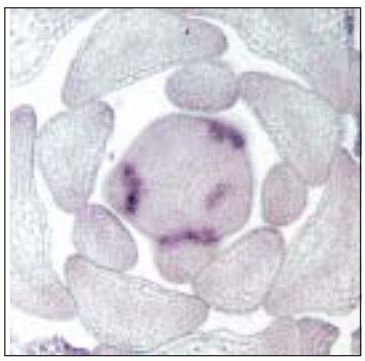

Fig. 1. Pattern of *LAS* mRNA accumulation in the vegetative shoot apex. Transverse sections through Landsberg *erecta* plants were hybridised with a probe from the *LAS* gene.

Expression pattern of the *LATERAL SUPPRESSOR* gene in *Arabidopsis thaliana*

In situ hybridisation analysis revealed that *LAS* transcripts accumulated in the axils of all primordia derived from the SAM. During the vegetative phase, *LAS* mRNA was found in a band shaped domain at the adaxial side of leaf primordia and leaves from P1 to P20/22 (Fig. 1). The *LAS* expression domain was initially about 3-5 cell layers deep, including the L1-L3 layers of the SAM, and extended 1 or 2 cell layers in the adaxial-abaxial dimension. Different from Columbia, in Landsberg *erecta* plants, a down-regulation of the *LAS* signal was observed in a small oval area in the centre of the expression domain. After the transition to flowering, *LAS* transcripts were detected in the axils of all leaf primordia originating from the elongating stem and at the adaxial base of flower primordia. During flower development, *LAS* mRNA was found in the axils of sepal primordia.

las is epistatic to *axr1* with respect to axillary shoot development during the vegetative phase

Besides other defects, the recessive Arabidopsis *axr1* mutant shows an increased branching at maturity. To test whether the increased shoot-branching potential conferred by the *axr1* mutation is able to override the defect caused by *las*, we have constructed an *axr1-12 las-4* double mutant. Phenotypic analysis revealed that, with respect to shoot branching, the double mutant was similar *las-4*: lateral shoot development was blocked in the older rosette leaf axils. This finding demonstrates that *las-4* is epistatic to *axr1-12*. Comparing *axr1-12* with *axr1-12 las-4* plants, we also observed a strong reduction in the development of accessory side-shoots from the axils of cauline leaves in the double mutant. This finding indicates that, despite the formation of lateral shoots observed in the cauline leaf axils of the *las-4* mutant, the *LAS* gene has an influence on the shoot branching potential during reproductive development.

The *Blind* gene encodes a MYB transcription factor controlling axillary meristem formation

The *Blind* gene was isolated using a map-based cloning approach. We found that the *blind* and *torosa* mutant phe-

notypes are caused by a loss of function of an R2R3 class Myb gene. RNAi induced inhition of *Blind* activity led to a partial suppression of lateral meristem formation, confirming the identity of the isolated gene. Experiments have been initiated to study the function of *Blind*-homologous genes in *Arabidopsis thaliana*. Toward this goal, we have screened insertion line populations for knock-out mutations in *Blind*-related Myb genes. Knock-out mutants, which have been identified for several *Blind*-related genes, are presently under investigation. In addition, dominant negative and overexpression constructs will be introduced into Arabidopsis plants to study the functions of these genes.

Analysis of tillering in barley

In barley, a large collection of mutants exhibiting different tillering phenotypes is available. With the aim of studying the mechanisms controlling tiller formation in barley, we have isolated two *LAS*-homologous genes from this species. Mapping of one of the candidate genes revealed that it does not cosegregate with one of the known mutations; mapping of the second gene is in progress. Experiments have been initiated to suppress the activity of these genes in transgenic barley plants.

Future Developments

Future work will focus on the further elucidation of the mechanisms controlling shoot branching in higher plants. Toward this goal, we will identify new regulators of shoot branching in Arabidopsis. Using the *las-4* mutant, we have performed an enhancer/suppressor screen, which resulted in the identification of new mutants. Some of the respective genes will be isolated and characterised. In addition, we will use the knowledge gained to modify shoot architecture in tomato. The aim of this work is to obtain a fertile tomato line developing no or very few axillary shoots.

Scientific Publications

Burbidge, A., P. Lindhout, T.M. Grieve, K. Schumacher, K. Theres, A.W. van Heusden, A.B. Bonnema, K.J. Woodman and I. B. Taylor: Re-orientation and integration of the classical and interspecific linkage maps of the long arm of tomato chromosome 7. Theor. Appl. Genet. **103**, 443-454 (2001).

Gidoni, D., E. Fuss, A. Burbidge, G.-J. Speckmann, S. James, D. Nijkamp, A. Mett, J. Feiler, M. Smoker, M.J. de Vroomen, D. Leader, T. Liharska, J. Groenendijk, E. Coppoolse, J.J.M. Smit, I. Levin, M. de Both, W. Schuch, J.D.G. Jones, I.B. Taylor, K. Theres and M.J.J. van Haaren: Multi-functional T-DNA/Ds tomato lines designed for gene cloning and molecular and physical dissection of the tomato genome. Plant Mol. Biol. **51**, 83-98 (2003).

Greb, T., O. Clarenz, E. Schäfer, D. Müller, R. Herrero, G. Schmitz G and K. Theres: Molecular analysis of the *LATERAL SUPPRESSOR* gene in *Arabidopsis* reveals a conserved control mechanism for axillary meristem formation. Genes Dev. (accepted).

Greb, T., G. Schmitz and K. Theres: Isolation and characterization of the *Spindly* homologue from tomato. J. Exp. Bot. **53**, 1829-1830 (2002).

Mishra, S.K., J. Tripp, S. Winkelhaus, B. Tschiersch, K. Theres, L. Nover and K.D. Scharf: In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. Genes Dev. **16**, 1555-1567 (2002).

Rossberg, M., K. Theres*, A. Acarkan, R. Herrero, T. Schmitt, K. Schumacher, G. Schmitz and R. Schmidt*: Comparative sequence analysis reveals extensive microcolinearity in the *Lateral suppressor* regions of the tomato, Arabidopsis, and Capsella genomes. Plant Cell **13**, 979-988 (2001).*Equal contribution authorship

Schmitz, G., E. Tillmann, F. Carriero, C. Fiore, F. Cellini and K. Theres: The tomato *Blind* gene encodes a MYB transcription factor that controls the formation of lateral meristems. Proc. Natl. Acad. Sci. USA 99, 1064-1069 (2002).

Suzuki, Y., S. Uemura, Y. Saito, N. Murofushi, G. Schmitz, K. Theres and I. Yamaguchi: A novel transposon tagging element for obtaining gain-of-function mutants based on a self-stabilizing *Ac* derivative. Plant Mol. Biol. **45**, 123-131 (2001).

Diploma thesis

Dörte Müller (2001) Untersuchungen zur Struktur und Regulation des *Lateral suppressor*-Gens in Arabidopsis thaliana.

Dissertation

Thomas Greb (2003) Untersuchungen zur Rolle des Gens *LATERAL SUPPRESSOR* in der Seitentriebentwicklung von *Arabidopsis thaliana* H. und der Tomate (*Lycopersicon esculentum* M.).

Structure of the group

Group leader	**Dr. Klaus Theres**
Postdoctoral fellow	**Dr. Gregor Schmitz**
PhD students	**Oliver Clarenz**
	Andrea Eicker
	Thomas Greb
	until December 2002
	Dörte Müller
	Smita Raman
	since March 2003
Technical assistants	**Ursula Pfordt**
	part time since Sept. 2002
	Elisabeth Schäfer
	Edith Tillmann
	part time

Grants, external funding

European Commission, RD programme „Quality of Life and Management of Living Resources“, Project: “Architecture engineering in the tomato”: 1 Postdoctoral fellow

Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 572: “Festlegung von Zellverbänden und Zelltypspezifizierung”: 2 PhD Students

Deutsche Forschungsgemeinschaft, Schwerpunkt: “Genetische und molekulare Aufklärung von Prozessen der Merkmalsausprägung bei Nutzpflanzen”: 1 PhD Student, 1 Technical assistant (until December 2002)

Invited lectures given

Center for Plant Molecular Biology, Universität Tübingen, Tübingen (06.06.2002), 1st International Symposium of the SFB 572 on “Pattern formation and cell type specification”, Universität zu Köln, Köln; Institut für Spezielle Botanik, Mainz (16.01.2003) ■

Comprehensive Analysis of Protein-interaction Networks: A Basis for Functional Proteomics and Engineering

Joachim Uhrig

Introduction

The rapid accumulation of complete genomic sequence data directs research in molecular biology towards attempting to comprehensively understand protein functions on a whole genome basis. As functional predictions from sequence data alone are often not possible, new technologies have been – and still have to be – developed to derive hypotheses on protein functions on a large scale. So far, these approaches mostly rely on correlations (expression profiling, 2D-PAGE proteomics, metabolomics) and only few large-scale techniques aim directly at the systematic characterisation of functional properties of proteins (structural genomics, protein arrays). The transient or stable formation of protein complexes is fundamental to almost all cellular processes; therefore, comprehensive knowledge of protein interactions will provide valuable information to functionally categorise the vast number of unknown proteins and to find new functional links of "known" proteins. The yeast two-hybrid system (Y2H) has proven sufficiently simple, fast and robust to be widely and successfully used in genome research. We have developed a large-scale technology allowing multiparallel Y2H library screenings. Currently, we are applying this technology to systematically investigate protein interaction networks of plant transcription factors and regulatory interactions of small GTPases. The functional significance of identified protein interactions is followed up with biochemical and cell biological approaches as well as by phenotypic and physiological characterisation Arabidopsis mutants. Furthermore, the structural basis of protein interactions is being investigated by the identification of single amino-acids constituting interaction-surfaces. This information is used to predict protein interactions from genomic sequence data and then to engineer dominant interfering molecules to target and modulate protein interactions *in vivo*.

New Results

High-throughput yeast two-hybrid (Y2H) screening technology

In plants – as in all higher eucaryotic organisms – the open reading frames predicted from genomic sequences are not as easily available as in bacteria or yeast; therefore, one-by-one, all-against-all approaches can not be applied to plant genomes. However, the throughput of the alternative approach, i.e. the screening of random cDNA or genomic libraries, is limited by the experimental effort, predetermined by parameters immanent to the system (large numbers of clones, conditions for yeast growth etc.). We have developed a Y2H library screening technology that significantly simplifies the whole process. This was achieved by developing: (1) a highly efficient liquid-mating procedure to scale down the double-transformation process; and (2) a three-dimensional liquid-immobilisation method to select yeast colonies, thus omitting laborious plating of the cells. These new developments allow the handling of up to 100 screenings in parallel without the need of robots or expensive equipment. A pilot experiment of 200 screenings demonstrated the reliability of the technology and resulted in the isolation of >2000 positive colonies. The screening process has been integrated into a largely standardised protocol including all steps between "bait" cloning and confirmation of the specificity of interactions in networks.

Transcription factor networks

The *A. thaliana* genome encodes more than 1500 transcription factors; however, most of them as yet have no assigned function. Combinatorial protein interactions are considered an important means to regulate activity and specificity of transcription factors. Our work in this project focuses on the proteins of the TALE homeodomain, Myb and bHLH.

Despite a rather unspecific DNA-binding capacity, homeodomain proteins have been shown to very specifically regulate fundamental developmental programmes. This specificity is supposed to be provided by the assembly of protein complexes formed from different homeodomain subfamilies, including additional accessory factors. We have systematically analysed the mutual interactions within the Arabidopsis TALE family and have identified several new TALE-interacting proteins (Fig. 1A). To support functional implications from the interaction networks, we investigated knock-out and overexpressing Arabidopsis mutants. As the knock-out of homeotic genes mostly does not lead to visible phenotypes, we are focussing on the analysis of those interaction partners exhibiting a high connectivity within the network; thereby, indicating a central, general regulatory function (Fig. 1B).

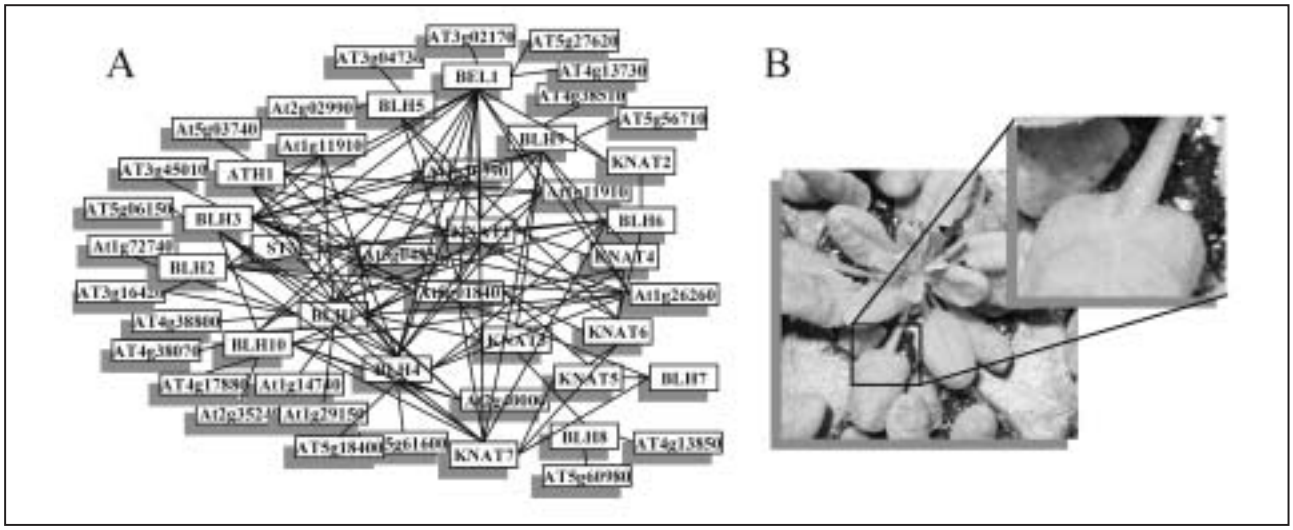

Fig. 1. TALE homeobox and interacting proteins. (A) Highly connected interaction network of TALE homeobox proteins and newly identified TALE-interacting proteins. (B) Overexpression of TALE-interacting protein 1 (TIP1) leads to changes in leaf morphology.

Two of the largest transcription factor families in Arabidopsis are the Myb (~130 members) and bHLH (133 members) proteins. In collaboration with Bernd Weisshaar's group, the functional significance of myb-bHLH interactions is being investigated. Using our large-scale Y2H technology, we have systematically analysed the combinatorial interactions between selected subfamilies of the R2R3-Myb and bHLH proteins. A detailed deletion analysis in combination with random- and site-directed mutagenesis allowed us to predict a four amino acid motif common to R/B-bHLH interacting Myb proteins. Searching the Arabidopsis genome with this motif revealed additional R2R3- and one-repeat Myb proteins which in turn could be shown to interact with R/B-bHLH proteins.

Functional characterisation of the ARF family of small GTPases

Co-ordination of cell and tissue polarity is dependent on the directional transport of proteins from and to the ER/golgi. Proteins are transported in vesicles, which fuse at their place of destination with the target membrane. ADP-ribosylation factors (ARF), i.e. small GTP-binding proteins, are supposed to be involved in many different steps of intracellular vesicle transport and trafficking pathways. Although considerable information is available about ARF proteins of animals and yeast, almost nothing is known about the plant vesicle transport system. The Arabidopsis genome encodes 19 putative ARF proteins which can be clustered into three major groups: ARF, ARF-like (ARL) and ARF-related proteins (ARP). In a first step towards functionally characterising the ARF proteins, putative downstream effectors (i.e. proteins interacting selectively with the activated form of ARF) were identified using the Y2H system. Our research is currently focussing on a potential regulatory role of ARF proteins in blue-light signalling suggested by the specific interaction with Phototropins (blue light photoreceptors) and supported by physiological experiments using Brefeldin A, a specific inhibitor of ARF activation.

Yeast-based strategies to develop concepts to control plant pathogens

In two collaborative projects with Peter Schreier's group and the Bayer AG, the potential of large-scale yeast-based screening technologies is being exploited to develop new concepts to control plant pathogens. In the framework of the *U. maydis* genome project, we aim to pinpoint new fungicidal targets by a combination of bioinformatics and the "yeast signal sequence trap" to systematically identify secreted proteins. In the second project, we have developed a novel, knowledge-based strategy to engineer virus resistant plants. Tospoviruses have a significant impact on agronomic losses worldwide. The type member tomato spotted wilt virus (TSWV) causes losses of more than $ 1 billion per year alone. Using the Y2H system, we have dissected the molecular basis of TSWV N protein multimerisation in detail. From a random N-protein fragment library, the

Fig. 2. TSWV-infected tobacco plants evaluated 23 days after inoculation. A: Wild type; B: β-Glucuronidase expressing plants; C: plants expressing β-glucuronidase fused to a 29 aa peptide derived from TSWV N protein.

smallest length peptide containing the interaction domain was selected. This 29 amino acid long peptide strongly interacts with TSWV N protein and, heterologously with N proteins of several different Tospovirus species. *Nicotiana benthamiana* lines expressing the peptide were strongly resistant to TSWV (Fig. 2). Additionally, correlating with the interaction data, significant resistance to five other Tospoviral species was observed.

This strategy exemplifies a promising new concept that uses target-specific peptides ("aptamers") selected with the Y2H system to modulate protein functions *in vivo*.

Future Developments

A Y2H technology suitable for high-throughput approaches has been established and can now be used on a broader basis to eventually build up a genome-wide interaction map of *A. thaliana*. Functional implications from the interaction data will be complemented with phenotypic data of knock-out and overexpressing *A. thaliana* lines (T-DNA insertion lines for several TALE and ARF genes are available). Furthermore, we are planning to express constant active and dominant negative forms of ARF proteins, in order to study the effects on vesicle trafficking, cell polarisation and blue-light signalling.

Scientific Publications

Rudolph C., P.H. Schreier and J.F. Uhrig: Peptide-mediated broad-spectrum plant resistance to Tospoviruses. Proc. Natl. Acad. Sci. USA **100**, 4429-4434 (2003).

Soellick T.-R. and J.F. Uhrig: Development of an optimized interaction-mating protocol for large-scale yeast two-hybrid analyses. Genome Biol. **2,** 521-527 (2001).

Uhrig, J.F., T. Canto, D. Marshall and S.A. MacFarlane: The silencing suppressor P19 protein of *Tomato bushy stunt virus* interacts with and relocates the RNA export factor ALY/REF. EMBO Journal (submitted).

Zimmermann I.M., V. Spies, W. Schmalenbach, C. Phillipp and J.F. Uhrig: High-throughput Yeast Two-Hybrid library screenings using a novel liquid-immobilization technique of yeast colonies. Plant Journal (submitted).

Uhrig, J. F.: (2003) Peptide-mediated resistance to plant vruses. Trends in Biotechnology (in press).

Diploma thesis

Anja Boettcher (2002) Systematische Charakterisierung der BELL-Homöobox-Proteine in *Arabidopsis thaliana*

Structure of the group

Group leader	**Joachim Uhrig**
Postdoctoral fellows	**Ulrike Unte**
	since January 2003
	Tim Soellick
	until July 2002
PhD students	**Olaf Müller**
	Cooperation with AG Schreier
	Jana Hackbusch
	Klaus Richter
	Ilona Zimmermann
	until June 2003
Diploma student	**Anja Boettcher**
	July 2001-June 2002
Technical assistants	**Wolfgang Schmalenbach**
	Marc Hallstein

Guest scientists

Ute Vothknecht, Ludwig-Maximilians-Universität München Department Biologie I, Germany, Group Leader

Stuart MacFarlane, Scottish Crop Research Institute, UK, Group Leader

Alan Jones, University of North Carolina at Chapel Hill, USA, Professor

Ullrich Herrmann, Universität zu Köln, Germany, PhD student

Grants, external funding

DFG "AFGN": 1 Postdoctoral fellow

BMWi "ProInno" program: 1 PhD student

British Royal Society "ESEP"

Bayer AG: 1 PhD student

Collaborations

Plant targets of viral silencing suppressors, Scottish Crop Research Institute, UK, Dr. Stuart MacFarlane

Regulatory mechanisms of plant heterotrimeric G-proteins, University of North Carolina at Chapel Hill, USA, Prof. Dr. Alan Jones

Molecular and functional profiling of vesicle trafficking pathways, Institut für Biologie II Universität Freiburg, Prof. Dr. Klaus Palme

Invited lectures given

Scottish Crop Research Institute, Dundee (24.05.2002) ■

Molecular Biology of Tomato Spotted Wilt Virus

Peter Schreier

Introduction

Tomato spotted wilt tospovirus (TSWV) is the type member of the tospovirus genus, the only plant-infecting genus of the arthropod-borne family of Bunyaviridae. Tospoviruses have a very broad host range encompassing more than 650 plant species belonging to 70 families, including many crops and ornamentals. Losses to world agriculture are estimated to be more than 1 billion US $ per year. Molecular biological analysis has confirmed that TSWV is a typical member of the Bunyaviridae. The tospoviral particle consists of a core of nucleocapsids in which the genomic single-stranded RNA molecules are tightly associated with N proteins and a few copies of the L protein.

A lipid membrane carrying two glycoproteins surrounds these nucleocapsids. TSWV has a tripartite genome consisting of three RNA species called L, M and S, encoding structural and non-structural proteins in a negative or ambisense orientation. The very ends of the genomic ssRNA molecules contain complementary sequences, which are thought to form a double-stranded secondary structure ("panhandle") that may be important for binding specificity and packaging. Despite the fact that the TSWV genome was sequenced several years ago, knowledge of the biological function of the viral proteins is still very poor. One of the reasons for this lack of information is the limited availability of reverse genetics for segmented negative-strand RNA viruses, which so far has been developed for Bunyamwera virus only. The TSWV L RNA encodes a polypeptide of 2875 amino acids proposed to be the viral RNA-dependent RNA polymerase. The M RNA codes for the putative movement protein NSm and for a glycoprotein precursor that is processed to the spike proteins G1 and G2 involved in thrips transmission. The S RNA encodes the non-structural protein NSs with unknown function and the nucleocapsid protein N, the main constituent of the TSWV nucleocapsid.

Data on nucleocapsid proteins of related viruses suggest multiple functions of the TSWV N protein: nucleic acid binding capacities to package and protect the RNA genome, regulation of the viral RNA polymerases, and binding to cell membranes.

We have studied homotypic interaction of the N-Protein and investigated the interaction of the protein with viral RNA-by-RNA gel retardation assays.

New Results and Future Developments

Peptide-mediated broad-spectrum plant resistance to tospoviruses

We had developed a transgenic approach resulting in a broad transgenic resistance towards tospoviruses (Schreier et al. US patent 5, 569, 823). However the molecular basis of this protein-mediated resistance is still not understood. This is why a rational and reliable construction of resistance is still not predictable.

Thus, a new strategy for engineering virus resistant plants by transgenic expression of a dominant interfering peptide was established in collaboration with Joachim Uhrig. This 29 amino acid long peptide strongly interacts with the nucleocapsid proteins (N) of different Tospoviruses. On the basis of previous work, the target specific peptide was selected with the yeast two-hybrid system, in order to inhibit protein function *in vivo*.

Transgenic *Nicotiana benthamiana* lines expressing the peptide fused to a carrier protein were challenged with five different tospoviruses, which have a nucleocapsid protein interacting with the peptide. In the transgenic plants, strong resistance to TSWV, TCSV, GRSV and CSNV was observed. Hence, for the first time, the feasibility of using peptide „aptamers" as an *in vivo* tool to control viral infection in higher plants was demonstrated.

TSWV N protein for RNA interactions

To investigated the interaction of viral RNA and the N protein, the composition of the nucleocapsid mentioned above was studied in detail. Gel filtration and "Blue Native" gels revealed that wt N protein is mostly a high molecular complex with decreasing amounts of tetramer, trimer, dimer and monomer. These low molecular complexes might account for stable intermediates during the encapsidation process.

Biochemical analysis of point mutations or small deletions suggests a head-to-tail interaction of N- and C- terminal interaction domains. Secondary structure analysis with CD-spectroscopy suggests that α-helices are involved in the interaction.

Protein RNA complexes were investigated in gel-retardation experiments and with filter binding assays. Mutants

with reduced capacity to oligomerise showed an increase in RNA-binding capacity, suggesting that monomeric N protein is the preferred binding partner of viral RNA molecules. This is further enhanced by the detection of co-operativity in binding. The 5'-end of the TSWV S RNA has a higher degree of binding affinity than the 3'end or no viral RNA molecules. A specific sequence of 39 nucleotides at the 5' end could be identified for binding with N protein. A model for encapsidation and for the N protein concentration depending switch from transcription to replication has been elucidated.

Identification of interacting partners of proteins involved in pathogenesis in *Ustilago maydis*

The phytopathogenic fungus *Ustilago maydis* is the causal agent of smooth disease on corn. This basidiomycete is well characterised and accessible for genetic and molecular methods as well as for cytological studies. Since pathogenesis is one of our key research areas, we are highly interested to identify possible interacting partners of single components of the cytoskeleton and proteins of the outer cellular membrane. We are currently using a large-scale yeast two-hybrid approach, which was first established in the group to examine interactions of TSWV proteins and was further improved in Joachim Uhrig's group. The project was started in the framework of the Ustilago genome project and is being carried out in collaboration with the group of Joachim Uhrig.

Scientific Publications

Leuthner B., C. Aichinger, E. Oehmen, R. Kahmann, M. Bölker, P.H. Schreier: A H_2O_2 producing glyoxal oxidase is required for filamentous growth and pathogenicity in *Ustilago maydis.* Proc. Natl. Acad. Sci. USA (submitted).

Rudolph C., P.H. Schreier and J.F. Uhrig: Peptide-mediated broad-spectrum plant resistance to Tospoviruses. Proc. Natl. Acad. Sci. USA **100**, 8 4429-4434 (2003).

Diploma thesis

Verena Spies (2002) Funktionelle Analyse kleiner GTPasen aus *Ustilago maydis* mit Hilfe des Zwei-Hybrid Systems

Dissertations

Christoph Rudolph (2002) Protein vermittelte Tospoviren-Resistenz

Gregor Bucher (2002) Molekulare Charakterisierung des Nukleokapsidaufbaus von TSWV (Tomato Spotted Wilt Virus)

Structure of the group

Group leader	**Prof. Dr. Peter H. Schreier**
Postdoctoral fellow	**Dr. Peter Porschewski**
PhD students	**Christoph Rudolph**
	Gregor Bucher
	Olaf Müller
Diploma student	**Verena Spies**
Technical assistant	**Christina Philipp**

Invited lectures given

University of Giessen, Giessen (June 2002)

Functional Analysis of Transcription Factor Gene Families involved in the Formation of Flavonols in *Arabidopsis thaliana*

Bernd Weisshaar

Introduction

Plants are exposed to sunlight which contains significant amounts of potentially harmful high-energy photons. A potential protective mechanism against this deleterious radiation is the accumulation of pigments, and it has long been proposed that flavonoids are such pigments.

Flavonols absorb UV-B light. They accumulate after an inductive light treatment in their glycosylated form in the vacuoles of epidermal cells. Four enzyme-encoding genes are required for the formation of the C-15 basic flavonol aglycon from central biosynthesis intermediates. 4-coumaroyl-CoA is the main substrate of the first enzyme specific for flavonoid biosynthesis, chalcone synthase (CHS). Chalcone-flavanone isomerase (CFI) and flavanone 3-hydroxylase (F3H) are required for synthesis of 3-hydroxy-flavanone, which serves as a substrate for either dihydroflavonol reductase (DFR) or flavonol synthase (FLS). DFR reduces the heterocyclic C-ring of dihydroflavonols and leads to the formation of pigments absorbing in the visible range of the spectrum, such as anthocyanins, phlobaphenes or condensed tannins. In contrast, FLS oxidises the C-ring and results in the formation of colourless flavonols which predominantly absorb UV light. UV and blue light stimulate the transcription of the *CHS*, *CFI*, *F3H* and *FLS* genes; however, these genes are also activated at certain developmental stages and in specific tissues. The various signals are integrated and transmitted to the nucleus, and the set of genes required for flavonol accumulation is activated in a co-ordinated way. Our studies concentrate on the co-ordinated activation of a number of genes, and include the analysis of the biochemistry and physiology of flavonoid biosynthesis. Since the transcription factors (TFs) involved are encoded by complex gene families, systematic reverse genetic approaches were applied to study TF function in *A. thaliana*.

New Results

The *Arabidopsis thaliana* seed coat is brown due to the accumulation of condensed tannins in the testa. Mutants of *A. thaliana* with defects in tannin pigment biosynthesis often produce seeds that are yellow or light-brown in appearance; the genetic loci responsible for this, are referred to as *TRANSPARENT TESTA* (*TT*). Since tannins are derived from flavonoid biosynthesis, many *tt* mutants are affected in flavonoid biosynthesis genes (FBGs) or at loci that regulate these genes. The *TT1* gene was isolated by reverse genetics using an *En-1* transposon mutagenized *A. thaliana* population. *TT1* gene expression was detected in developing ovules and young seeds only, and the gene was shown to encode a nuclear protein. Mutant seeds displayed altered morphology of the seed endothelium in which brown tannin pigments accumulate in wild-type plants, indicating that TT1 is involved in the differentiation of this cell layer. When overexpressed in transgenic *A. thaliana* plants, TT1 caused aberrant development and organ morphology. The protein contains a novel combination of two TFIIIA-type zinc finger motifs. Closely related motifs were detected in a number of putative proteins deduced from plant genomic and EST sequences. The new protein domain containing this type of zinc finger motifs was designated WIP, according to three strictly conserved amino acid residues. Our data indicate the existence of a small gene family in *A. thaliana*, which was defined by the occurrence of the WIP domain. *WIP* genes may play important roles in regulating developmental processes, including the control of endothelium differentiation. We are now addressing the question how TT1/WIP1 affects FBG promoter activity in the endothelium by using quantitative RT-PCR and *in situ* hybridisation.

To be able to analyse the effects of (potential) TFs on our set of target gene promoters, an assay system was established on the basis of protoplasts prepared from dark-grown cultured cells and transient gene expression. Using this system, three TF gene families, namely those encoding bZIP, bHLH and R2R3-MYB proteins, have been identified as being involved in controlling FBG expression. MYB factors represent a family of proteins which include a conserved domain, i.e. the MYB DNA-binding domain. In contrast to animals, plants contain a MYB protein subfamily which is characterised by the R2R3-type MYB domain. The 'classical' MYB factors, which are related to c-Myb, seem to be involved in the control of the cell cycle in animals, plants and other higher eukaryotes. Systematic screens for knock-out mutations in MYB genes followed by phenotypic analyses along with the analysis of mutants with interesting phenotypes, have begun to unravel the functions of the 128 R2R3-MYB genes in *Arabidopsis thaliana*. R2R3-type *MYB* genes control many aspects of plant secondary metabolism as

well as the identity and fate of plant cells. Also, the bZIP gene family has been analysed in collaboration with partners from the REGIA consortium (F. Parcy and others, see references).

Recently, a genome-wide survey of bHLH factors in *A. thaliana* was completed (in co-operation with the group of C. Martin). Basic-helix-loop-helix (bHLH) TFs belong to a family of transcriptional regulators present in three eukaryotic kingdoms. Many different functions have been identified for these proteins in animals, including the control of cell proliferation and development of specific cell lineages, and that their mechanism for controlling gene transcription often involves homo- or hetero-dimerization. In plants, little is known about the bHLH family; however, we have determined that there are 133 *bHLH* genes in *Arabidopsis thaliana* and have confirmed that of these at least 113 are expressed. The *AtbHLH* genes constitute one of the largest families of transcription factors in *A. thaliana*, with significantly more members than are found in most animal species and about an equivalent number to those in vertebrates. Comparisons with animal sequences suggest that the majority of plant *bHLH* genes have evolved from the ancestral group B class of *bHLH* genes. By studying the *AtbHLH* genes collectively, twelve subfamilies have been identified. Within each of these main groups, there are conserved amino acid sequence motifs outside the DNA binding domain. Potential gene redundancy amongst members of smaller subgroups has been analysed, and the resulting information is presented to provide a simplified visual interpretation of the gene family identifying related genes that are likely to share similar functions. Based on the current characterisation of a limited number of plant bHLH proteins, we predict that this family of TFs has a range of different roles in plant cell and tissue development as well as plant metabolism.

After 2003, this research work will be continued at Bielefeld University.

Scientific Publications

Bellin D., M. Werber, T. Theis, B. Schulz, B. Weißhaar and K. Schneider: EST Sequencing, Annotation and Macroarray Transcriptome Analysis Identify Preferentially Root-Expressed Genes in Sugar Beet. Plant Biol. **6**, 700-710 (2002).

Heim M.A., M. Jakoby, M. Werber, C. Martin, B. Weißhaar and P.C. Bailey: The Basic Helix-Loop-Helix Transcription Factor Family in Plants: A Genome-wide Study of Protein Structure and Functional Diversity. Mol. Biol. Evol. **20**, 735-747 (2003).

Herwig R., B. Schulz, B. Weißhaar, S. Hennig, M. Steinfath, M. Drungowski, D. Stahl, W. Wruck, A. Menze, J. O'Brien, H. Lehrach and U. Radelof: Construction of a "unigene" cDNA clone set by oligonucleotide fingerprinting allows access to 25,000 potential sugar beet genes. Plant J. **32**, 845-857 (2002).

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Koch M.A., B. Weißhaar, J. Kroymann, B Haubold and T. Mitchell-Olds: Comparative genomics and regulatory evolution: conservation and function of the *Chs* and *Apetala3* promoters. Mol. Biol. Evol. **18**, 1882-1891 (2001).

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Schneider K., B. Weißhaar, D.C. Borchardt and F. Salamini: SNP frequency and allelic haplotype structure of Beta vulgaris expressed genes. Mol. Breed. **8**, 63-74 (2001).

Stracke R., M. Werber and B. Weißhaar: The R2R3-MYB gene family in *Arabidopsis thaliana*. Curr. Opin. Plant Biol. **4**, 447-456 (2001).

Dissertations

Frank Mehrtens (2003) Untersuchungen zur Funktion des Transkriptionsfaktors AtMYB12 als Regulator des Phenylpropanoidstoffwechsels in Arabidosis thaliana

Structure of the group

Group leader	**Prof. Dr. Bernd Weisshaar**
Postdoctoral fellows	**Dr. Ralf Stracke**
	Dr. Marc Jakoby
	Dr. Gieta Dewal
	Dr. Frank Mehrtens
PhD students	**Marc Heim**
	Martin Werber
	Birgitta Holtrup
	(with Prof. Weissenböck, Uni Köln)
	Ilona Zimmermann
	(with Joachim Uhrig)
Technical assistants	**Ute Tartler**
	Doris Falkenhan
	Sabine Pfaul

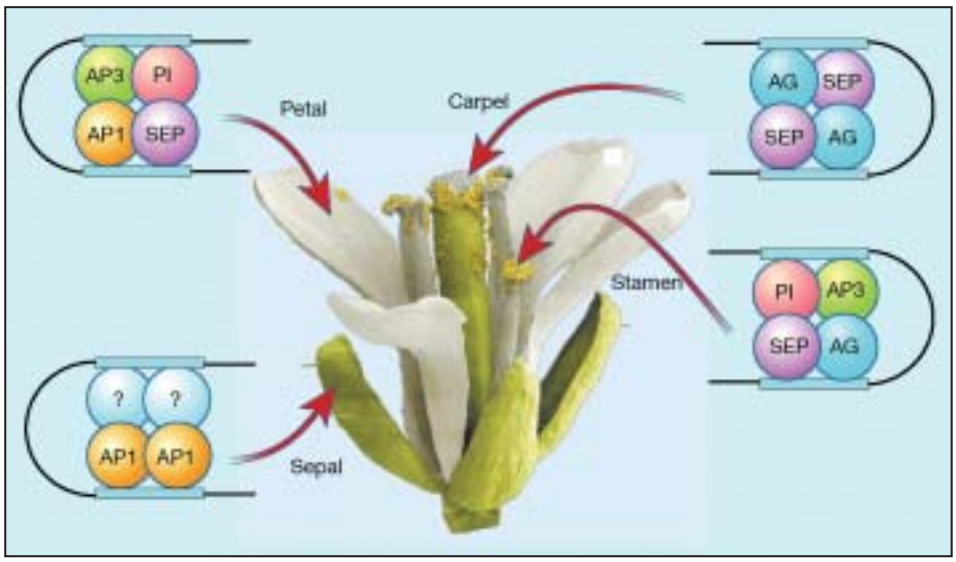
Grants, external funding

BMBF GABI program (TF genes): 1 Postdoctoral fellow, 1 PhD student, 1 technican
BMBF GABI program (SNPs): 1 Postdoctoral fellow, 1 technican
DFG Graduiertenkolleg: 1 PhD student
NPZ: 1 PhD student
EC FW5 program REGIA: 1 Postdoctoral fellow

Department of Molecular Plant Genetics

Director: Heinz Saedler

MADS-box proteins are encoded by multigene families in the genome of all higher plants thus far studied. They control many developmental processes. For example, mutation in a MADS-box gene can result in a phenotype featuring homeotic transitions of floral organs. Detailed molecular analysis of such mutations led to the ABC-model of flower development and to a concept called "Floral Quartets", featuring combinatorial interactions between different MADS-box proteins, which determine organ identity (Fig. 1, taken from Nature 409, 469 (2001)).
In the past two years, research in the Department has dealt with phase transition from vegetative to generative growth with a still ongoing focus on floral development. More recently, we have applied the materials obtained, the tools generated and the knowledge acquired on questions concerning evolution of MADS-box proteins and the organs specified by them especially in monocot and non-seed plants.
Transition to flowering is an important part in the life cycle of higher plants and for many years was of prime interest to the group of **Peter Huijser**. Besides studying MADS-box genes involved in this transition in Antirrhinum as well as in Arabidopsis, the group has also discovered a novel class of transcription factors within the last few years. This novel class of transcription factors was termed SBP-domain proteins, since they did bind to the promoter of the MADS-box gene *SQUAMOSA* of *A. majus* controlling floral meristems. In *A. thaliana*, 16 members of SPL genes (**S**QUA-**P**romoter-Binding-Protein-**L**ike genes) were described by the group. A major breakthrough was when a mutant of *SPL8* was obtained featuring reduced fertility of both sexes. Molecular characterisation of this family of transcription factors is now at the centre of interest.
The networks of interactions of MADS-box proteins in the control of floral development are of particular interest to **Hans Sommer**´s group. On top of this, in cooperation with the group of Zs. Schwarz-Sommer and K. Stüber, about 12,000 unique ESTs were sequenced and are now exploited for mapping and arrays in various projects.
Kurt Stüber has annotated the above isolated ESTs and he has contributed to a linkage map set-up for *Antirrhinum majus*. Some of the results are part of the www.anthirrhinum.org. web site project.
The main scientific interest of **Zsuzsanna Schwarz-Sommer** is in cell-cell communication, which seems to be an important component in the control of concerted expression of the B-function during development. In addition, the definition and control of the whorl boundaries is being studied. Mutants and genes affecting this process have been isolated and are currently being characterised.
Sabine Zachgo´s scientific interest is primarily in petal-specific target genes of the B-function genes *Deficiens* and *Globosa* in *Antirrhinum majus*. This is being achieved by using macroarrays of 12,000 unique ESTs followed by xCHIP. The first positive candidates have been identified and are currently being studied in greater detail.



Alexander Yephremov´s research is centred around epidermal cell differentiation and on the biosynthesis and the function of cutin. He succeeded in the molecular characterisation of a novel pathway leading to the biosynthesis of fatty-acid-derived cell signalling molecules.
The ZIGIA project was terminated at the end of 2002 and hence, the Groups of Koen Dekker and Anna Sorensen have finished their work. In addition, Günter

Theißen also left the laboratory and accepted a professorship at the University of Jena.
Due to this our work in the field of evolution has become re-oriented.

Wolf-Ekkehard Lönnig has isolated a number of mutants in *Misopates orontium*, phenotypically resembling known hometic mutants of the close relative *Antirrhinum majus,* yet displaying particular features in Misopates. These mutants are currently being characterised molecularly. Of particular interest is a molecular comparison of a trait distinguishing Misopates from Antirrhinum, i.e. the extended long versus the short sepals, respectively. Unravelling the molecular basis for the extended sepal syndrome might reveal some mechanisms involved in diversification and speciation.

Thomas Münster is heading a group focusing on the function of MADS-box genes in the moss Physcomitrella. Initial results clearly show that *PPM2* is somehow involved in the control of the moss life cycle. The primary goal is to isolate knock-out mutants from all MADS-box genes to study their role in moss development. In addition, this system is well suited to study the function of MADS-domain proteins from differently evolved genera.
Heinz Saedler has initiated a new group to study the origin of morphological novelties. While most solanaceous species feature small sepals with respect to the size of their petals, Physalis, as well as a few other genera within the Solanaceae have inflated calyces or "Chinese lantern". To date, we have isolated several MADS-box genes, which seem to be involved in the generation of this syndrome. For verification, we are currently trying to isolate knock-out mutants within Physalis.

In the past two years, the following groups have been working in the Department:

Developmental Biology

Comparative Genetics of SBP-box Genes	Peter Huijser
MADS-box Protein Networking	Hans Sommer
Bioinformatics: Genomics	Kurt Stüber
Cell communication and Flower Organ Identity	Zsuzsanna Schwarz-Sommer
Petal and Stamen Development	Sabine Zachgo
Cell Signalling	Alexander Yephremov
Central Facility of ZIGIA	Koen Dekker (terminated 2002)
Sexuality in *A. thaliana* (ZIGIA)	Anna Sorensen (left 2002)

Functional Evolution

Genetics of Antirrhinum and of Misopates	Wolf-Ekkehard Lönnig
Macroevolution of Plant Reproductive Organs	Günter Theißen (left 2001)
Development and Evolution of Non-Seed Plants	Thomas Münster
Origin of Morphological Novelties	Heinz Saedler

Origin of Morphological Novelties

Heinz Saedler

Introduction

In 2002, we have initiated a new project dealing with the origin of morphological novelties. The system chosen to study this is Physalis, a member of the Solanaceae. The structure of the calyx within the Solanaceae varies considerably. From deeply cleft sepals through fused tubular structures to inflated calyces. The majority of species have small sepals with respect to the size of their petals. These sepals are deeply cleft, as in Lycopersicon or dented as in *Solanum tuberosum*. However, in several genera of this family, a new trait emerged during evolution, featuring an inflated calyx (Fig. 1). Among the 85 genera of Solanaceae the inflated-calyx-syndrome (ICS) is observed in Nicandra, Przewalskia, Physalis, Physochlaina and Withania. The question arising is: how did ICS evolve?

New Results

Based on our studies with the Tunicate mutant of *Zea mays* (see Thomas Münster), in which the MADS-box gene *ZMM19* is ectopically expressed and extended glumes surrounding each kernel are observed, we assumed that members of this clade of MADS-box genes might also be responsible for ICS. This assumption was supported by the observation that *ZMM19* transgenic Arabidopsis plants featured large sepals.

The flowers of all Physalis species are characterised by a perianth consisting of dented sepals, which are fused at their base followed by sympetalous 2. whorl organs. After pollination, the sepals resume growth and ultimately encompass the mature fruit; thus, leading to an inflated calyx or the "Chinese lantern" (see Fig. 1). We have isolated several MADS-box genes from tetraploid *Physalis peruviana* and diploid *Physalis floridana* belonging to the *StMADS 11*, *StMADS16* (*ZMM19*) and *SQUA* sub-clades as revealed by phylogenetic reconstructions. Northern experiments showed that *MPF2* of *P. floridana* is expressed predominantly in the leaf and to a lower extent also in sepals. This is in contrast to the expression of its orthologue StMADS16 in *Solanum tuberosum* (Fig. 2), which is not expressed in the sepals.

Emasculation as well as removal of developing fruits lead to abortion of ICS, suggesting that signals elicited by pol-

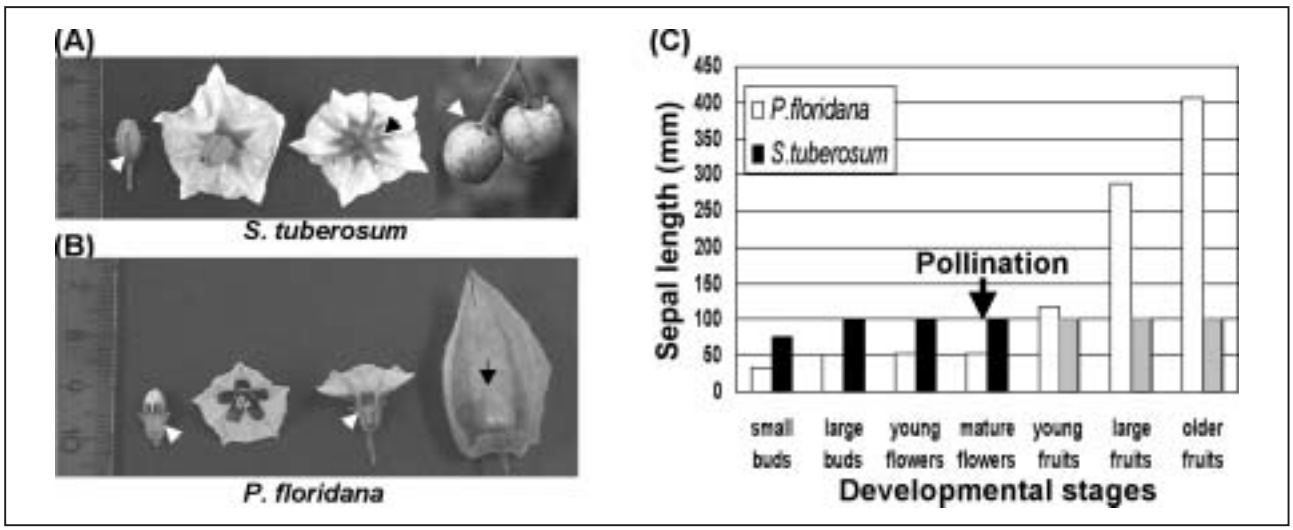


Fig. 1. Developmental variations of calyx morphology in *Solanum tuberosum* and *Physalis floridana*. (A) The morphology of buds, flowers and berries from *S. tuberosum*. The arrows indicate the small sepals on bud, flower and berry. (B) Developmental changes in calyx morphology of *P. floridana* featuring the "Chinese lantern". Part of the ICS has been removed to reveal the mature fruit marked by the arrow. (C) Comparison of developing calyces in *S. tuberosum* and *P. floridana*. In contrast to the sepals of *S. tuberosum (*black and grey colums*)*, the calyx of *P. floridana* (white colums) expands rapidly upon pollination (marked by the arrow).

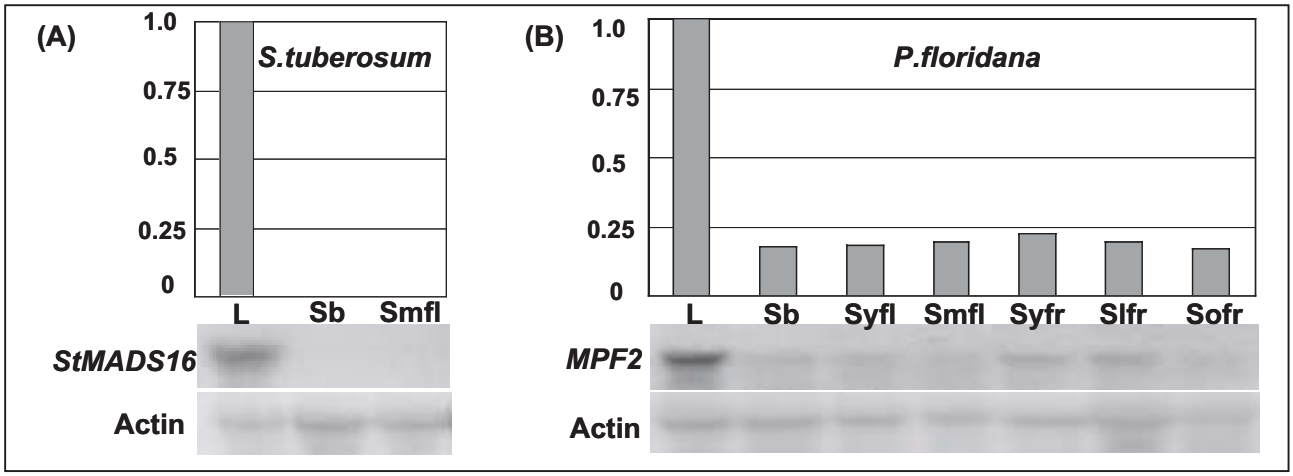


Fig. 2. Expression pattern of *StMADS16* of *Solanum tuberosum* and its ortholog *MPF2* from *Physalis floridana*. Samples were taken from leaf (L), from buds (Sb), from young flowers (Syfl), from mature flowers (Smfl), from young fruits (Syfr), from larger fruits (Slfr) and from old fruits (Sofr). In Northern blotting analysis, 25 ug of total RNA was used. cDNAs containing the C-domains of *StMADS16* and *MPF2*, respectively, were used for hybridisation. For normalisation, an actin cDNA probe was used. Signals were quantified and the ratio of (MADS-box gene/actin gene) expression was determined. Values for leaves are arbitrarily set as 1.0 and all other values are expressed accordingly.

lination and/or the developing fruit are important for the onset of secondary sepal growth and thus, for the formation of the "Chinese lantern".

These results seem to indicate that *StMADS16*-like genes of Physalis trigger target genes to respond to endogenous signals and thus, ICS. The orthologues from tomato and potato behave differently and do not promote further development of their Calyx.

ICS could be interpreted as an extension of the vegetative into the floral programme. If this is true, then MPF2 might interact with various MADS-box proteins involved in flower organ identity, especially with orthologues of A-function proteins like AP1.

Future Developments

To verify the above hypothesis that ectopic expression of *MPF2* of *Ph. floridana* results in ICS formation, phenotypes of knock out and over expressing mutants might be revealing. A transformation protocol for Physalis species was established and first transformants are growing. In addition, *MPF2* and other MADS-box genes from Physalis were transformed into Arabidopsis to corroborate the observation that genes from the *StMADS16* sub-clade generate transgenic plants featuring large sepals. Moreover, yeast two-hybrid tests were initiated to substantiate the above interpretation.

Future goals are to search for the signal emitted by the developing fruit, the target genes affected and receptor sites within these genes to better understand the mechanism underplaying ICS formation.

Scientific Publications

(only publications not listed in other projects)

Becker, A., K. Kaufmann, A. Freialdenhoven, C. Vincent, M.A. Li, H. Saedler and G. Theißen: A novel MADS-box gene subfamily with sistergroup relationship to class B floral homeotic genes. Mol Genet Genomics **266**, 942-950 (2001).

Kim, J.T., H. Schwöbbermeyer, G. Theißen and H. Saedler: Biodiversitätsmessung bei Pflanzen anhand molekularer Daten: Ein Beitrag zur wissenschaftlichen Definition von Biodiversität. In: Wissenschaftsethik und Technikfolgenbeurteilung, Biodiversität, Wissenschaftliche Grundlagen und gesellschaftliche Relevanz (P. Janich, M.Gutmann, K. Prieß, Hrsg.). Springer Verlag, Berlin, Band **10**, S. pp. 181-234 (2001).

Theißen, G., A. Becker, Ch. Kirchner, T. Münster, K.-U. Winter and H. Saedler: How land plants learned their floral ABCs: the role of MADS-box genes in the evolutionary origin of flowers. In: Developmental Genetics and Plant Evolution (Q.C.B. Cronk, R.M. Bateman, J.A. Hawkins, Hrsg.). Taylor & Francis, London, pp. 173-205 (2002).

Theißen, G. and H. Saedler: Floral quartets. Nature, **409**, 469-471 (2001).

Becker, A., M. Bey, T.R. Bürglin, H. Saedler and G. Theißen: Ancestry and diversity of *BEL1*-like homeobox genes revealed by gymnosperm (*Gnetum gnemon*) homologs. Dev. Genes Evol. **212**(9), 452-457 (2002).

Winter, K.-U., H. Saedler and G. Theißen: On the origin of class B floral homeotic genes: functional substitution and dominant inhibition in *Arabidopsis* by expression of an ortholog from the gymnosperm *Gnetum*. Plant J. **31**, 457-475 (2002)

Winter, K.-U., Ch. Weiser, K. Kaufmann, A. Bohne, Ch. Kirchner, A. Kanno, H. Saedler and G. Theißen: Evolution of class B floral homeotic proteins: Obligate heterodimerization orginated from homodimerization. Mol. Biol. Evol. **19**, 587-596 (2002).

Dissertations

Annette Becker (2001) Charakterisierung *GGM13*- u. *STMADS11*-ähnlicher MADS-Box-Gene der Gymnosperme *Gnetum gnemon.*

Charlotte Kirchner (2001) Untersuchungen zum Funktionswandel der *AGAMOUS*-ähnlichen MADS-Box-Gene aus Samenpflanzen im Verlauf der Evolution.

Luzie Wingen (2001) Charakterisierung der *STMADS11*-ähnlichen MADS-Box-Gene in Mais.

Wim Deleu (2002) Molecular and Functional Analysis of AGL2-like MADS-box Genes in Maize (*Zea mays ssp.mays*) – Indications for their involvement in grass inflorescence architecture.

Katrin Henschel (2002) Strukturelle und funktionelle Chrakterisierung von MADS-Box-Genen aus dem Laubmoos *Physcomitrella patens* (hedw.) B.S.G.

Structure of the group

Group leader: **Prof. Dr. Heinz Saedler**
Postdoctoral fellows: **Dr. Chaoying He**
Dr. Jong-Hee Kim
Technical assistant: **Britta Grosardt**

Grants, external funding

BMBF "ZIGIA-programm", DFG "SFB 572", AvH, Volkswagenstiftung "Collaboration India", FCI "Fond der Chemischen Industrie"

Collaborations

India, University of Hyderabad (since 2000)
Italy, Istituto Agrario di S. Michele all'adige, Trento (2003)

Invited lectures given

Symposium der Universität Hohenheim (24.05.01), Symposium der Gesellschaft für Genetik in Halle (24.11.01), Aachener Textiltagung (28.11.01), Universität Konstanz, Studium Generale (17.12.01), Dabringhausen/SFB Bochum (24.05.02), China/Chin. Akademie und Korea, Kooperation DFG/KOSEF (22.06.02), Kunming Zoology (24.06.02), Kunming Botany (25.06.02), Peking Genetics (03.07.02), Peking Botany (04.07.02), Chinju/Korea (08.07.02), Europäische Akademie, Marburg (09.09.02), Botanikertagung Freiburg: (24.09.02), EPSO-Konferenz, Brunnen/Schweiz: (27.10.02), CIMbios Symposium Merida, Mexico (25.11.02), Universität Freiburg (27.01.03), Walberberg, SFB Symposium (20.03.03). ■

Comparative Genetics of SBP-Box Genes: A Family of Plant-Specific Transcription Factors

Peter Huijser

Introduction

As regulators of co-ordinated gene expression, the function of transcription factors is central to the development of plants. For many years, the scientific interest of the group concerned the role that MADS-box transcription factors played in the process of flowering, e.g. in controlling the floral transition and the determination of floral meristem identity (see previous reports). During the course of this research, a novel family of plant-specific transcription factors, the SBP-box genes, emerged. The first members of the family were identified by means of their binding capability to a defined sequence motif within the promoter region of the Antirrhinum MADS-box gene *SQUAMOSA*; hence their name: SQUAMOSA PROMOTER-BINDING PROTEINS.

Since their discovery, SBP-box genes have been found to represent moderately sized families in all higher plants. Despite this general distribution, the role of SBP-box genes in plant development remains largely unclear. From the perspective of (comparative) molecular plant genetics and the general belief that transcription factors play key roles in development, this situation is highly unsatisfactory. Therefore, we decided to bring the SBP-box genes into the focus of our research. It is our aim to contribute to a better understanding of the role that SBP-box genes play in plant development as well as to the elucidation of their origin and evolution in the plant kingdom.

To uncover SBP-box gene functions in higher plants, we selected Arabidopsis as our major model system. The SBP-box genes in Arabidopsis comprise a family of 16 members known as *SPL* genes. In the scientific report 2001, we described the search and isolation of the first mutant alleles for these genes.

New Results

The Arabidopsis SBP-box gene *SPL8* affects sporogenesis

SPL8 represents the first Arabidopsis SBP-box gene with a known loss-of-function phenotype. Three independent transposon-tagged *spl8* mutant alleles all cause a strong reduction in fertility when present homozygously. Detailed microscopical analysis revealed this is primarily due to abnormal differentiation of anther tissues. Firstly, the proper initiation of all four microsporangia within the anther may fail, resulting in anthers with less or no pollen sacs at all. Secondly, if the formation of a microsporangium is initiated, it is followed by an aberrant differentiation of sporogenous and parietal cell layers. The outcome is either a failure of the microsporocytes to enter meiosis followed by a complete degeneration of the pollen sac or the formation of less pollen per pollen sac. (Fig. 1) *In situ* hybridisation revealed *SPL8* to be transcriptional active in both sporogenous cells and parietal layers, but to what extent the developmental fates of these tissues are interdependent remains unclear. Additional histological analysis suggests that also megasporocytes may encounter difficulties to undergo meiosis in the absence of *SPL8* and, as a consequence, degenerate.

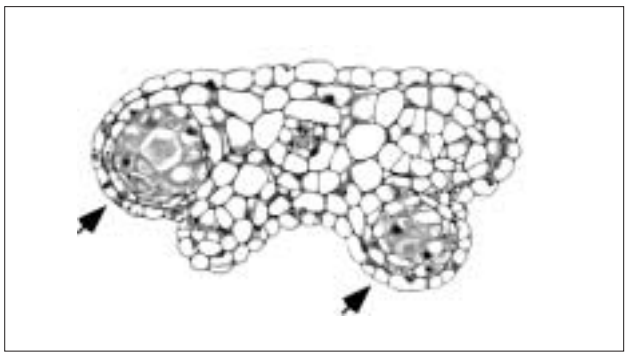


Fig. 1. Cross-section through *spl8*-1 mutant anther with two instead of four pollen sacs (arrows).

Biochemical characterisation of the SBP-domain

The SBP-box encodes a protein domain, the SBP-domain, previously shown to be necessary and sufficient for specific DNA binding. Alignment of known SBP-domain sequences reveals conserved cysteine and histidine residues at positions, suggesting an involvement in the formation of "zinc-finger" motifs. The actual requirement for Zn^{++}-ions in the binding of the SBP-domain to DNA could be demonstrated in gel retardation assays. In addition, mutagenesis of selected cysteine and histidine residues showed that not all are required for stable DNA binding.

The SBP-domain could be shown to be involved in nuclear import with the help of GFP translational fusions transiently expressed in protoplasts. Mutagenesis of a conserved serine residue in the vicinity of a bipartite nuclear localisation signal within the SBP-domain results in subcellular redistribution, suggesting a role for phosphorylation in nuclear import control.

SBP-box gene family phylogeny

To facilitate a phylogenetic reconstruction of SPB-box gene evolution in the plant kingdom, we started to clone SBP-box gene family members from lower plants. So far, SBP-box genes from the moss Physcomitrella seem to fit in some of the sub-groups formed by higher plant SBP-box genes, whereas family members identified in the alga Chlamydomonas represent sister groups to these.

Future Developments

To gain further insight into the functions of SBP-box genes in higher plant development, there is an obvious need for more mutant phenotypes. We will continue to analyse already identified T-DNA and transposon mutagenised *SPL* gene alleles and to apply dsRNAi in Arabidopsis. Furthermore, we will study the possibility of post-transcriptional control of *SPL* gene expression due to the action of recently identified micro-RNAs.

To obtain a better understanding of the role *SPL8* plays in sporogenesis, we will search for possible interacting proteins using the yeast two-hybrid system. In addition, we intend to identify putative target genes *in planta* either by micro-array analysis following exogenous controlled induction of an *SPL8* transgene or by chromatin immunoprecipitation (X-ChIP). More detailed information concerning the recognition sequence as determined by random target site selection will help to validate the targets.

Besides the model system Arabidopsis, we have chosen the moss Physcomitrella for comparative studies that may allow inferences about plant phenotypic evolution. Physcomitrella enables a systematic knock-out of SBP-box genes through homologous recombination. In addition, moss SBP-box genes will be expressed in transgenic Arabidopsis plants and *vice versa*.

SBP-domain proteins represent a new class of proteins and we aim to determine their structure. As a first step towards this goal, there will be an attempt to crystallise a selected SBP-domain interacting with DNA.

Scientific Publications

Cremer, F., W.-E. Lönnig, H. Saedler and P. Huijser: The Delayed terminal flower phenotype is caused by a conditional mutation in the *CENTRORADIALIS* gene of snapdragon. Plant Physiol. **126**, 1031-1041 (2001).

Cremer, F., H. Saedler and P. Huijser: *Matura*, an early-flowering mutant of *Antirrhinum majus*. Flowering Newsletter **31**, 49-56 (2001).

Sorensen, A.-M., S. Kröber, U.S. Unte, P. Huijser, K. Dekker and H. Saedler: The Arabidopsis *ABORTED MICROSPORES* (*AMS*) gene encodes a MYC class transcription factor. Plant J. **33**, 413-423 (2003).

Unte, U.S., A.-M. Sorensen, P. Pesaresi, M. Gandikota, D. Leister, H. Saedler and P. Huijser: *SPL8*, an SBP-Box Gene Affecting Pollen Sac Development in Arabidopsis. Plant Cell **15**, 1009-1019 (2003).

Dissertations

Ulrike S. Unte (2001) Funktionelle Charakterisierung von SBP-Box-Genen mit Hilfe der Modellpflanze *Arabidopsis thaliana*

Structure of the group

Group leader	**Dr. Peter Huijser**
Postdoctoral fellows	**Dr. Ulrike Hartmann**
	Dr. Madhuri Gandikota since September 2001
	Dr. Ulrike Unte December 2001 - December 2002
	Dr. Rainer Birkenbihl since January 2002
	Dr. Mariola Pislewska-Bedmarek since November 2002
PhD students	**Ulrike Unte** until December 2001
	Yan Zhang since September 2002
Technical assistant	**Susanne Höhmann**

Grants, external funding

Sonderforschungsbereich 572: 1 Postdoctoral fellow
Arabidopsis Functional Genomics Network (DFG 458): 1 Postdoctoral fellow
IMPRS: 1 PhD student

Collaborations

Spl8 mutant analysis, ZIGIA (Center for functional genomics in Arabidopsis), Dr. Anna-Marie Sorensen, Dr. Dario Leister, Dr. Paolo Pesaresi
In silico SBP target-site recognition, Max Planck Institute for Plant Breeding Research, Kurt Stueber

Invited lectures given

University of Louvain, Louvain (28.03.2001), University of Cologne, Cologne (27.04.2001), EMBO course, John Innes Centre, Norwich (16.06.2001), University of Cologne, Cologne (17.05.2002), University of Liège, Liège (15.10.02), University of Oxford, Oxford (26.02.2003) ■

Genetic and Molecular Mechanisms Controlling Antirrhinum Floral Organogenesis

Zsuzsanna Schwarz-Sommer

Introduction

Floral organ primordia become determined to a particular organ type shortly after their appearance on the floral meristem. This process is regulated by the combinatorial action of three sets of organ identity genes, termed Class A, Class B and Class C, controlling the A-, B- and C-functions, respectively. In the current model, the A-function controls the identity of the first and second whorl organs, the B-function controls the second and third whorls, and Class C controls the third and fourth whorls. Misexpression of Class B or Class C genes confers ectopic organ identity changes within the flower; thus, indicating that the spatial control of their expression patterns is crucial for wild-type flower development. The molecular mechanism, which governs the spatially correct expression of Class B and Class C organ identity genes, is not well understood. Our current studies focus on the control of the outer boundary of the C-function to whorl three.
According to the generally accepted concept, floral meristems are organised in layers, and the three meristem layers termed L1, L2 and L3 contribute differentially to the formation of various tissues and to the development of different floral organs. Organ identity becomes manifest in the number, shape, size and function of the cells that make it up. It follows that communication between neighbouring cells and cell layers is necessary to co-ordinate the pattern and rate of cell divisions and cell enlargement during organ development. Our interest is to elucidate the role of class B floral homeotic genes in this process, with special focus on the developmental role of the epidermis in Antirrhinum and Arabidopsis.

New Results

Genetic and molecular mechanisms controlling homeotic gene expression domains

In Antirrhinum, at least four mutants display phenotypes revealing the loss of control over the establishment or maintenance of boundaries of the B- or C domains. Genetic

analyses uncovered some important common features of these mutants. One of these is their (partial) epistasis over the *fimbriata* (*fim*) mutant. FIM is an F-box protein, most likely involved in the degradation of repressors of the B- and C-functions in the wild-type flower. Epistasis thus indicates that in the four mutants the function of a repressor is impaired, hence alleviating the function of FIM. Unexpectedly, in most of the mutants, a role of the impaired genes in controlling B or C cannot be separated, suggesting that the repressive control mechanism is more complex than affecting just a single gene. Genetic interactions between the four mutants furthermore suggest that their protein products interact *in vivo*.

One of these genes, *STYLOSA* (*STY*), has been cloned. It represents the orthologue of *LEUNIG* in Arabidopsis and encodes a GRO/TUP1-like co-repressor, intensively studied in Drosophila and yeast. Models of GRO/TUP1 function suggest that these proteins associate with DNA-binding repressors or activators and recruit histone deacetylases to their targets. Accordingly, isolation of protein partners of STY in a yeast two-hybrid screen is a promising approach to obtain information on proteins that directly control target genes of STY. Based on their genetic interaction with *sty*, *cho* or *fis* could be such a protein partner.

Epidermal control of petal organ identity by Class B homeotic genes in Antirrhinum and Arabidopsis

We generated transgenic Antirrhinum plants that, in a b- mutant background, express the Class B genes *DEFICIENS (DEF)* or *GLOBOSA (GLO)* under the control of the epidermis-specific promoter of the *ANTIRRHINUM FIDDLEHEAD* (*AFI*) gene (in collaboration with A. Yephremov, MPIZ). The phenotype of these transgenic chimeras is similar to somatically induced periclinal chimeras that express *DEF* in L1 under the control of its own promoter in Antirrhinum. Hence, this experimental approach is a reliable method to study the epidermal contribution of genes; it can be used to conveniently produce epidermal chimeras in Antirrhinum and, for comparative purposes, in Arabidopsis.

Epidermal B-function autonomously controls the differentiation of Antirrhinum petal epidermal cell-types, but cannot fully control the pattern of cell divisions and the specification of sub-epidermal petal cell identity by epidermal signalling. As a consequence, petal shape, size and overall morphology differ in epidermal chimeras from that in the wild type. In Arabidopsis, in contrast, epidermal B-function can control most if not all epidermal and sub-epidermal differentiation events in petals, without any contribution from the endogenous class B genes. It seems, therefore, that the mechanisms involved in sending/receiving a B-function dependent 'signal' between layers differ in Antirrhinum and Arabidopsis. Possibly, factors are present in Arabidopsis that reinforce the ability of the DEF/GLO (and AP3/PI) proteins to control epidermal production and transmission, or sub-epidermal reception of the currently unknown 'signal'.

The limited efficiency of epidermal B-function to control sub-epidermal developmental events can be due to limitations in the epidermal production or transmission of a 'signal'. To test this assumption, an EMS mutagenesis programme of Antirrhinum epidermal chimeras to detect mutants with improved or impaired petal morphology in the M2 generation has been initiated. *def* null mutants carrying the epidermal AFI::DEF transgene are male sterile and cannot be used to produce seed for mutagenesis by selfing. To circumvent this limitation, the AFI::DEF transgene has been introduced to the background of the temperature sensitive *def*-101 mutant, which is wild-type-like and male fertile when grown under permissive conditions (thus permitting seed production); however, it is mutant when grown under non-permissive conditions (thus allowing to screen for mutants with enhanced or reduced *DEF*-dependent signalling). To this end, we grew ~500 mutagenised M1 plants in the cold and selfed them. Fifty M2 populations (each consisting of 27 plants) were then cultivated at 26°C and yielded three segregating mutants with improved petal morphology. The mutants are now under genetic investigation to corroborate their heritability and independence from changes in the function of the transgene as well as to determine allelic relations between them. The long-term goal will be to isolate the respective genes and to elucidate the mechanism involved in *DEF*-controlled cell-cell communication.

Future Developments

Molecular mechanisms underlying STYLOSA-mediated control of genes

Screening for proteins that interact with STY in yeast promises access to transcription factors that directly associate with genes controlled by *STY*. Using STY as bait, several groups of proteins including four transcription factors could be detected in a yeast two-hybrid screen. Confirmation of the relevance of these partners for the *in vivo* function of STY is being attempted by isolating their mutants and testing for genetic interactions. Additionally, biochemical methods, such as co-immunoprecipitation of the respective proteins with STY using plant protein extracts are being employed.

Map-based cloning approaches in Antirrhinum

In spite of its overall success, transposon tagging in Antirrhinum failed in some instances, thus, necessitating the establishment of other approaches. Map-based cloning has now become a feasible alternative, after establishment of a detailed physical linkage map and a BAC library for Antirrhinum (Brendan Davies, University of Leeds). For cloning of *FISTULATA* by a map-based strategy, we developed large populations segregating for the *fistulata* mutant along with CAPS markers, in order to map the gene more precisely.

Scientific Publications

Efremova, N., M.C. Perbal, A. Yephremov, W.A. Hofmann, H. Saedler and Z. Schwarz-Sommer: Epidermal control of floral organ identity by class B homeotic genes in *Antirrhinum* and *Arabidopsis*. Development **128**, 2661-2671 (2001).

Müller, I., W. Wagner, A. Völker, S. Schellmann, P. Nacry, F. Küttner, Z. Schwarz-Sommer and G. Jürgens: Syntaxin specificity in *Arabidopsis* cytokinesis. Nature Cell Biol. **5**, 531-534 (2003).

Schwarz-Sommer, Z., E. de Andrade Silva, R. Berndtgen, W.-E. Lönnig, A. Müller, I. Nindl, K. Stüber, J. Wunder, H. Saedler, T. Gübitz, A. Borking, J.F. Golz, E. Ritter and A. Hudson: A linkage map of an F2 hybrid population of *Antirrhinum majus* and *A. molle*. Genetics **163**, 699-710 (2003).

Schwarz-Sommer, Z., Davies, B. and A. Hudson: An everlasting pioneer: the story of *Antirrhinum* research. Nature Reviews Genetics, **4** (in press 2003).

Structure of the group

Group leader	**Dr. Zsuzsanna Schwarz Sommer**
Postdoctoral fellows	**Dr. Rosa Castillo**
	since June 2002
	Dr. Nadia Efremova
	Dr. Cristina Navarro
	since December 2001
	Dr. Roger Rubiera
Technical assistants	**Rita Berndtgen**
	Markus Kuckenberg
Gardeners	**Manfred Pohe**
	Sybille Richter

Guest scientists

Dr. Brendan Davies, University of Leeds, UK, Senior Lecturer
Dr. John Golz, University of Edinburgh, UK, Postdoctoral fellow
Dr. Richard Ingram, University of Leeds, UK, Postdoctoral fellow
Dr. Mark Wilkinson, NCBI Saskatoon, Canada, Postdoctoral fellow

Grants, funding from sources other than MPG

Sonderforschungsbereich 572: 1 Postdoctoral fellow
Marie Curie Fellowship to Dr. Cristina Navarro

Collaborations

Map-based cloning of Antirrhinum genes, University of Leeds, Dr. Brendan Davies
RFLP linkage map of *Antirrhinum majus*, University of Edinburgh, Dr. Andrew Hudson
Snapdragon database, NCBI Saskatoon, Dr. Mark Wilkinson
Functional analysis of an Antirrhinum syntaxin in Arabidopsis, University of Tübingen, Dr. Gerd Jürgens

Invited lectures given

Tagung der Gesellschaft für Entwicklungsbiologie, Ulm (2001), Tagung der Deutschen Botanischen Gesellschaft, Freiburg (2002), Martin-Luther-Universität Halle; SFB 363 (2003), Universität Tübingen; SFB 446 (2003) ■

Networks of interacting Regulatory Factors in Floral Morphogenesis of Antirrhinum and Arabidopsis

Hans Sommer

Introduction

Work in our laboratory is focussing on understanding molecular aspects of regulatory processes in flower development. We are trying to gain insight into the molecular regulatory mechanisms which govern establishment of flower architecture and floral organ development in *Antirrhinum majus*. Genetic and molecular analyses in different plant species have identified key regulators of floral organogenesis, several of which belong to the MADS-box gene family which comprises, in Arabidopsis, more than 100 members, ca. 40 of which belong to the MIKC-type of MADS genes. The genes encode transcription factors which apparently can homo- and/or heterodimerise and likely are able to form higher oligomers (tetramers), as might be deduce from the properties of other transcription factor(s) and families. This ability potentially allows formation of numerous combinations of regulators of gene expression, which control (activate or repress) specific expression of distinct sets of target genes in time and space. Since the beginning of 2002, we have included *Arabidopsis thaliana* in these studies, hoping that a comparison of the mechanisms in both species might lead to deeper insight into the control of floral morphogenesis.
Using different yeast-based screening systems, such as one-hybrid (for protein-DNA interactions), two-hybrid

and a newly developed 'ternary factor trap' system for investigating protein-protein interactions, we have taken a first step towards identifying and analysing such regulatory interactions involved in the control of floral development. The molecular results are being corroborated by genetic analyses. We have begun to unravel a rather complex network of interactions between the known homeotic regulators, which control floral organogenesis, and newly discovered MADS factors, which may have implications for models of organ and meristem identity determination and development.

New Results

Several new yeast libraries, i.e. oligo-dT and random primed as well as normalised, have been made from various stages and organs of *A. majus*. These libraries allow rapid screening for protein-DNA and protein-protein interaction, in one-, two- and three-hybrid systems, by yeast mating.

In an attempt to extend the network of possible interactions of floral MADS-box factors in Antirrhinum, ternary factor screens (three-hybrid) were carried out using PLENA (PLE) and DEFH200 as baits. PLE and DEFH200 are the orthologs of AGAMOUS and SEP1 in Arabidopsis. Among others, the MADS-box protein DEFH70 was isolated as a ternary interaction partner of the PLE/DEFH200 bait.

DEFH70 shows strongest similarity to SVP (short vegetative phase) of Arabidopsis and JOINTLESS of tomato. Reverse genetic screens revealed that *DEFH70* is identical to the *INCOMPOSITA (INCO)* locus of *A. majus*, of which several mutant alleles are known or have been isolated recently. The mutant phenotypes of these alleles indicate that *INCO (DEFH70)* has a function in establishing floral architecture and control of organ number. This is quite different from the function of *SVP* and *JOINTLESS*. Molecular and morphological characterisation of the gene and the mutant alleles have been carried out. In addition, genetic analysis by double mutant combinations with various other floral mutant alleles is underway.

To investigate the spatial and temporal regulation of the Antirrhinum B-function gene, *DEFICIENS*, yeast one-hybrid screens have been undertaken with a 200 bp upstream region of the promoter as bait, which previously led to the isolation of a b-ZIP like protein, ROSINA (RSI). The gene has been further analysed, revealing the presence of several copies in the genome of Antirrhinum, the number of which varies with the genetic background. RSI binds to the CArG motif in the DEF-promoter, a regulatory element which is also bound by the MADS-box protein heterodimer DEF/GLO in an auto-regulatory fashion. *DEF* and *GLO* control development of petals and stamens in *Antirrhinum majus*. The identical DNA-binding motif and the complementary expression pattern of *RSI* and *DEF* suggests that RSI could act as a repressor of *DEF* expression.

Several additional factors have been isolated in recent one-hybrid screens that are currently being investigated. One very interesting candidate belongs to the TCP family of plant-specific transcription factors. Members of this family are *TB* from maize and *CYC* from Antirrhinum. The possible function of this TCP-like gene is currently being analysed.

As part of a project of the DFG-supported Arabidopsis Functional Genomics Network (AFGN), normalised yeast mating libraries from Arabidopsis organs and tissues at different developmental stages have been prepared and successfully used to discover ternary interactions among floral MADS-box proteins, and of these with non-MADS factors, using, for instance, AGAMOUS/SEP1 as bait. The candidate clones isolated are currently being analysed. The libraries are freely available. Several groups of the MPIZ have used the libraries for one- and two-hybrid screening for interactors with their baits of interest, and in addition, research groups in England, Spain and Italy have obtained them.

As a service for research groups of the DFG-SFB 572, normalised yeast mating libraries from dissected apical meristems of Arabidopsis have been made to facilitate isolation of rare interactors not found in normal libraries. These are also freely available upon request.

Future Developments

We shall further characterise the *INCO* mutant alleles, morphologically and genetically, in order to gain insight into its function in floral development. Overexpression in the heterologous Arabidopsis system should provide additional information. The TCP-like factor will be characterised further, molecularly, and possibly mutants will be obtained by reverse genetic screens. This holds also for RSI.

Concerning the Arabidopsis floral factors isolated in the ternary factor screen, additional molecular experiments will be conducted to substantiate the yeast results. In addition, we shall try to obtain genetic evidence for these interactions, by screening for mutants and constructing double/triple mutants.

Dissertations

Ming Ai Li (2002) Molecular and Genetic Characterisation of New MADS-box Genes in *Antirrhinum majus*

Ok Ran Lee (2002) AmGRAS, a GAI and RGA-like protein, interacts with ROSINA (RSI), a transcription factor which may control expression of the B-function gene *DEFICIENS*

Structure of the group

Group leader	**Dr. Hans Sommer**
Postdoctoral fellows:	**Dr. Simona Masiero**
	Dr. Mario Roccaro
	Dr. Yubin Li
Graduate student/	**Ming Ai Li**
Post-graduate	**Ok Ran Lee**
Graduate students	**Farnusch Kaschani**
Technical assistant	**Isa Will**

Guest scientists

Dr. Maria Penas, University of Navarra, Pamplona, Spain
Dr. Ana Berbel, University of Valencia, Valencia , Spain

Grants, external funding

DFG (Arabidopsis Functional Genomics Network, AFGN): 2 PhD students

Collaborations

The network of regulatory interactions of MADS-box proteins during floral organ development in *Arabidopsis thaliana*. MPIZ, Dr. Sabine Zachgo.
Characterisation of *INCOMPOSITA* of *Antirrhinum majus*. MPIZ, Dr. Zs. Schwarz-Sommer.

Invited lectures given

Technische Universität München, Munich (16.01.2002). ■

Regulation of Petal and Stamen Organogenesis

Sabine Zachgo

Introduction

Analysis of informative homeotic flower mutants in model organisms, such as Antirrhinum and Arabidopsis, has led to the establishment of the floral ABC model. The model explains how the single or combined activity of the regulatory class A, B and C genes controls the organogenesis of the four different floral whorls. Except for one, all of the ABC genes studied in different species are MADS-box transcription factors.
Although floral MADS-box genes have been intensively studied during the last decade in different plant model species, little is known about the target genes that realise their regulatory potential. We are presently conducting an expression profiling approach using a conditional Antirrhinum mutant, in order to isolate petal target genes of the class B genes *DEFICIENS* and *GLOBOSA*, which reveal identical mutant phenotypes. In the *DEF* null-mutant, the second and third whorl organs are homeotically transformed. Sepals replace petals in the second whorl and instead of stamens, a fused central carpeloid structure is formed in the third whorl. After initiation of the floral primordia, the *DEF* and *GLO* expression is maintained by an autoregulatory mechanism, as the two proteins heterodimerise and bind together *in vitro* to specific DNA elements, so called CArG boxes, located in their own promoters.
The large size of the Antirrhinum flower allows harvesting stage- and organ-specific material. Hence, highly specific probes can be prepared for expression profiling experiments, circumventing the problem that differences in expression levels become blurred due to a highly heterogeneous tissue representation. To discriminate between directly and indirectly regulated genes, a chromatin-immunoprecipitation protocol was established to deliver proof for *in vivo* binding of the DEF protein to regulatory sequences of target genes. The combination of the two techniques aims at the isolation of a comprehensive set of target genes that act downstream of the MADS-box gene in establishing petal organogenesis.

New Results

Characterisation of Antirrhinum petal development

To form a mature Antirrhinum corolla, petal development progresses through different developmental stages. The production of macroarrays representing more than 11,000 unique partially sequenced cDNAs from Antirrhinum (collaboration with Zs. Schwarz-Sommer, H. Sommer and K. Stüber, MPIZ) has allowed us to conduct expression profiling experiments. By this approach, gene clusters are identified that are regulated in a synchronised fashion during the respective petal stages and contribute to different developmental processes.

Def-101: A conditional mutant to identify *DEF* petal target genes

After identification of the stage-specific petal transcriptome, the temperature-inducibility of the *def*-101 mutant (Fig. 1) was exploited to investigate which of the petal genes respond to a reduction of the *DEF* function. The *def*-101 plants were cultivated at the permissive temperature

and then transferred to the non-permissive temperature for increasing time spans. Hybridisation of the macroarray allowed us to identify target genes that become activated or repressed by reduced *DEF* activity. The promptness with which the target genes respond to an altered *DEF* function in the profiling experiments is used as an indicator to position the genes in the cascade downstream of *DEF*. Additional semi-quantitative RT-PCR experiments are currently conducted with further decreased shifting times, in order to select a group of putative direct target genes for detailed analysis.

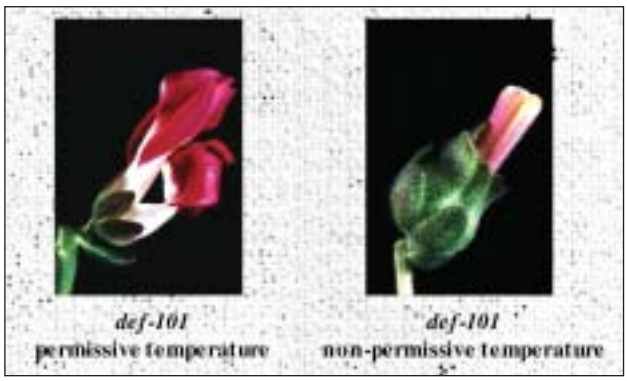


Fig. 1. Phenotype of *def*-101 flowers used for the profiling experiments grown at the permissive (15ºC) and non-permissive temperature (26ºC).

X-ChIP experiments to prove direct protein/DNA interactions

To deliver the proof of direct *in vivo* interaction of the DEF protein at the regulatory DNA sequences of a target gene, a cross-linked chromatin-immunoprecipitation procedure (X-ChIP) was established. Cross-linking of protein/DNA complexes is conducted with formaldehyde that allows the reversal of the fixation. Cross-linked complexes are immunoprecipitated with an anti-DEF antiserum and with pre-immune serum as a mock control. To optimise the protocol, we took advantage of the *DEF* and *GLO* autoregulatory control mechanism maintaining their expression at later stages. PCR reactions were conducted on the immunoprecipitated genomic DNA fragments with primers spanning the CArG box motives in the *DEF* and *GLO* promoters. The strong amplification of *DEF* and *GLO* promoter sequences compared to a weak amplification in the mock control was achieved by enriching *DEF* and *GLO* promoter sequences during the immunoprecipitation procedure. These data deliver the first proof of direct *in vivo* binding of the DEF protein at the *DEF* and *GLO* promoters.

Future Developments

Besides using the X-ChIP technique for delivering evidence of direct binding of target genes that were isolated by profiling experiments, it also bears the potential to isolate new *in vivo* target genes by subcloning and further analysis of the precipitated genomic DNA fragments.

The aim of combing profiling and chromatin-immunoprecipitation experiments is to isolate and analyse direct and indirect target genes that are crucial for our understanding of how key regulatory genes control organogenesis. As we expect that many aspects of these processes are conserved, we want to extend the acquired knowledge to other plant systems, such as Arabidopsis.

Recently, persuasive evidence has been generated that the formation of higher order complexes between MADS-box proteins contributes to the specification of the floral organs. To analyse these interactions that apparently provide a mechanism by which specificity of target binding can be conferred, yeast two- and three-hybrid screens with floral transcription factors will be carried out.

Expression profiling experiments will be extended to test whether morphological variations displayed by different species within the Scrophulariaceae can be detected and measured at the transcriptomics level.

Analysis of *apo*, a mutant with an aberrant petal organogenesis

To understand the regulatory processes that control the initiation and positioning of petal primorida, we are currently analysing an Arabidopsis T-DNA mutant named *apo*, which was isolated from the GABI-KAT collection. Instead of four, only one to two or more rarely, three petals are formed. The number of petals is reduced and petals are abnormally positioned and reveal strong bending during further differentiation. Currently, the cloning of the gene involved is being undertaken. We aspire to position *APO* into the floral regulatory network by double mutant analysis and we will study its expression in wild-type and mutant flowers.

Scientific Publications

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Structure of the group

Group leader	**Dr. Sabine Zachgo**
Postdoctoral fellows	**Dr. Shuping Xing**
	Dr. Mariola Pislewska-Bedmarek
	September and October 2002
PhD students	**Melanie Bey**
	Bettina M. Müller
	Andrea Lauri
	Andrea G. Busch
Technical assistant	**Anja Hörold**

Grants, external funding

DFG (ZA-259/3-1): 1 PhD student
DFG, Arabidopsis Functional Genomics Network: 1 PhD student
DFG, Graduierten Kolleg "Molekulare Analyse von Entwicklungsprozessen": 2 PhD students
Ministerium für Schule und Weiterbildung, Wissenschaft und Forschung, NRW; Lise Meitner scholarship: Sabine Zachgo (2001)

Collaborations

Processing of DNA array hybridisation data: DKZF Heidelberg, Dr. Kurt Fellenberg; http://www.dkfz-heidelberg.de/tbi/mchips/
Monitoring biodiversity using macroarray technology: Instituto Agrario di S. Michele all'Adige, Italy, Dr. Claudio Varotto

Invited lectures given

15. Tagung Molekularbiologie der Pflanzen, Dabringhausen (01.03.2002), XII International *Antirrhinum* Meeting, Korsika (18.04.2002), XIII International Conference on *Arabidopsis* Research, Sevilla (29.06.2002), SFB 480, Ruhr-Universität Bochum (18.11.2002), Center for Applied Plant Molecular Biology, Universität Hamburg (04.12.2002), AFGN Symposium Wittenberg, Germany (10.03.2003). ■

Biodiversity: Antirrhinum and Misopates – What are the Genetic Differences?

Wolf-Ekkehard Lönnig

Introduction

The celebrated Human Genome Project has been the basis for another larger sequencing project, the Chimpanzee Genome Project. This was inaugurated to approach, *inter alia*, the question of the genetic basis for the fundamental anatomical and other differences between man and chimp, especially in the face of the strong sequence similarities found so far on the DNA and protein levels.

A similar, if smaller project focussing on the main genetical differences between closely related genera in the plant world was started with our Misopates project. In relation to Antirrhinum, Misopates is its nearest relative being distinguished from the former essentially by two morphological features, i.e. extremely elongated sepals (see Fig. 1) and cup-like seeds. Additionally, there are differences in the life cycle (annual in Misopates versus perennial in Antirrhinum) and the tendency to autogamy in Misopates. With special focus on the question whether the differences between Antirrhinum and Misopates can be overcome by mutant genes, the following points are being investigated:

(1) Can the long Misopates sepals be reduced in perhaps one mutant step to those of the length of Antirrhinum sepals? In the case of ectopic expression of leaf programmes in the sepals by an activator, loss of activator function could perhaps lead to shorter sepals (Antirrhinum example: *Macho* back to wild-type – although, this was TE-based). If, however, a repressor is lost, the chances to restore that repressor appear to be nearly zero.

(2) To what extent are mutant features of leaves, bracts and sepals correlated in Misopates?

(3) Can the (annual) Misopates life cycle be extended to that of Antirrhinum (perennial)?

(4) What do the corresponding homeotic flower and inflorescence mutants of Misopates and Antirrhinum reveal about genetical differences between the two closely allied genera?

(5) Does Misopates display features of regressive evolution (losses of functions) in comparison to Antirrhinum or the Antirrhineae?

Fig. 1. Antirrhinum (left) and Misopates (right) flowers. Note the strongly elongated sepals in Misopates.

New Results

Mutagenesis

For the first time, the following homeotic mutants of the wild species *Misopates orontium* (Vogelsang) have been detected in field trials in 2002: Two *deficiens*-like phenotypes (one has so far been sequenced and proved to be a deletion of 21 bp in the *Deficiens* gene), a *plena* mutant and an extraordinarily strange-looking *squamosa* candidate. Moreover, a *fistulata*-like mutant was isolated, but another mutant displaying a very unusual phenotype, now referred to as *bizarre*, has not as yet been classified with any of the phenotypes known from Antirrhinum (for most of the phenotypes mentioned, see Fig. 2). Additionally, a series of multiple alleles for flower colour has been isolated as well as many dwarfs and other mutants not to be enumerated here. Also, several phenotypes with larger than normal flowers have been detected, isolated and documented as well as a variegated line, which is probably due to transposable element activation (see also Fig. 2). Concerning the five points enumerated above, the following summary can be given:

(1) Except for one mutant, where all the leaves were somewhat distorted and shortened, to date, we could not essentially reduce the length of the sepals in an otherwise normal Misopates, although up to now more than 310,000 plants (about 10,000 M2-families) have been investigated.

(2) Up to now in Misopates, all mutants affecting leaf structure also affect bract- and sepal-structure and *vice versa*; in contrast, there are absolutely clear-cut exceptions from this rule in Antirrhinum. The strict correlation in Misopates so far found may point to a loss of function of a regulator in the sense mentioned above. Perhaps in agreement with this hypothesis, confirmed data show that in Antirrhinum, *AmM1* is strongly expressed in the leaves, weakly in bracts, but there is no expression in the sepals. In Misopates, however, expression is weak in the leaves, strong in the bracts and moderate in the sepals.

(3) In three homeotic Misopates mutants, the life-time is strongly extended (the *deficiens*-mutant, the *plena*-mutant and one *floricaula*-like mutant), i.e. from about 3 months in the wild type to roughly 12 months in the mutants. And in all three cases, the cuttings continue flourishing so luxuriantly, that there is good reason to assume that all three mutants can indefinitely be propagated purely vegetatively. Most importantly, the *deficiens*-mutant is femally fully fertile, yet even after seed-set the longevity does not seem to be reduced. One hypothesis could be that some target genes necessary for the short wild-type cycle are not addressed anymore in the homeotic mutants.

(4) A comparison of the homeotic mutants appears to point to absence of gene-functions in all cases for Misopates as compared to Antirrhinum. The *deficiens*-mutant of Misopates displays only four loculi (in *A. majus* there are five), and this is also the case in the second similar phenotype, which might be a *globosa*-mutant. The *Misopates*

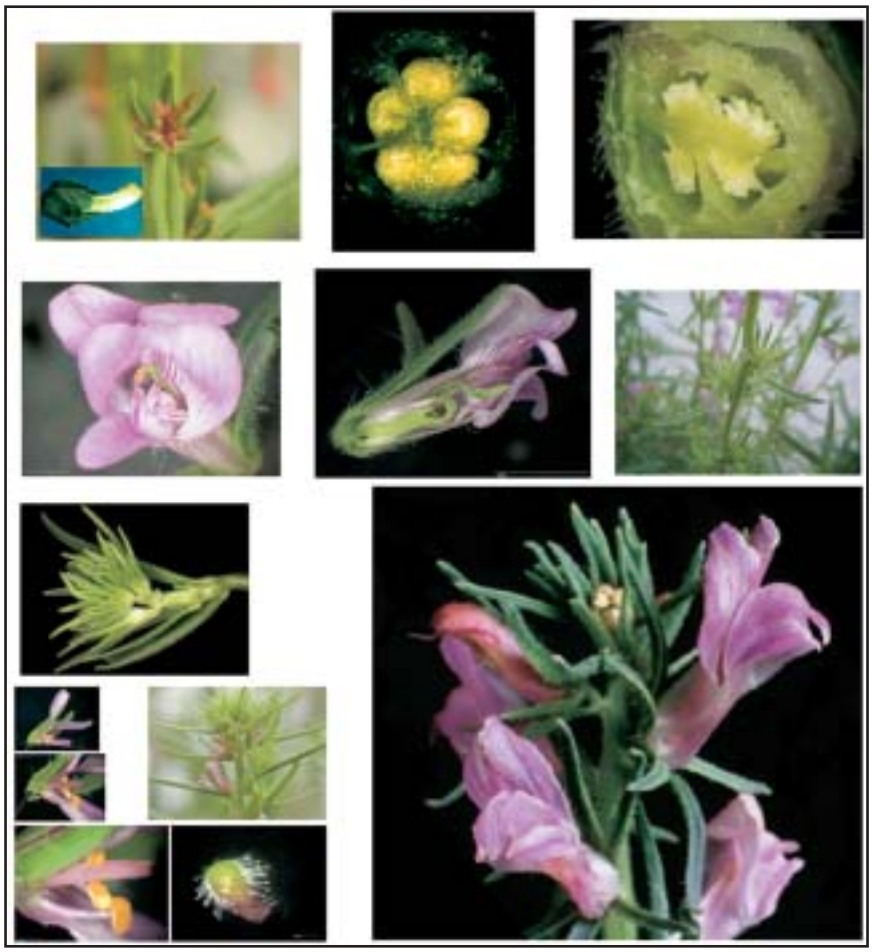

Fig. 2. Misopates mutants.
From left to right. First row. New *deficiens* mutant of Misopates in comparison to a *deficiens* mutant of *Antirrhinum* (small insert). Middle: Five loculi in the *deficiens* of Antirrhinum (photo by Zs. Schwarz-Sommer) in comparison to right: Four loculi in the *deficiens* mutant of Misopates. Second row: Left: The *plena* mutant of Misopates forming sterile anthers. Middle: Longitudinal section through that *plena* mutant: Note the strongly elongated sepals *within* the flower. Right: A *squamosa* candidate of Misopates. Third row: Left: Longitudinal section through the *squamosa* candidate: Note the strongly compressed internodes. Left, below the *squamosa* candidate: A series of five small photos of the *bizarre* mutant. Right: A newly arisen variegated phenotype of Misopates probably due to the activation of transposable elements.

plena mutant – although producing anthers like a corresponding fertile Antirrhinum *plena* mutant – appears to be totally sterile (only a hitherto unknown *plena* allele in Antirrhinum also producing sterile anthers, could cover up the difference). The *squamosa* candidate displays strongly reduced internodes pointing to the absence of some gene function for normal internode development in Misopates – a loss of function that is revealed only when the basic function for flower formation due to another mutation is missing. The *floricula*-like mutants (three independently arisen cases) all display the missing differentiation of bracts and sepals by repeating leaf-like structures in the inflorescence part of the plants. Moreover, in Antirrhinum, the *squamosa*- and *squamata*-mutants can produce fertile flowers which has never been the case in the four candidates with similar phenotypes in Misopates; hence, the second pathway for flower-formation of Antirrhinum could be absent in Misopates. Also, the flowers of the Coimbra-line of Misopates are nearly white indicating some loss (or temporary switching off) of function in the anthocyanine pathway.

(5) Thus, many features in Misopates appear to point to the absence of gene functions in comparison with Antirrhinum. Since most of the distinguishing characteristics of Misopates are apomorphic (derived) features in comparison to the overwhelming majority of the species of the tribus Antirrhineae in general and in Antirrhinum in particular, losses of gene functions in an Antirrhinum-like ancestor displaying at least the plesiomorphous (original) characters of the tribus is a hypothesis which appears to be worth while to be further investigated.

Moreover, since a range of recent investigations have corroborated and greatly enlarged the number of earlier cases of TE-target site selection (hot spots for TE integration, see Lönnig and Saedler, 2002) thereby, implying preestablished rather than accidental gene inactivations (and larger chromosome rearrangements), the possibility of a predetermined origin of Misopates should be considered.

To sum up what appears to be one of the most important points of our investigations so far: By carefully comparing all the mutants of Misopates with the corresponding phenotypes of Antirrhinum, the most dominating morphological feature distinguishing Misopates from the latter, i.e. the strongly elongated sepals, appears to be not only a nearly invariable character (except in correlation with changes in overall flower or general leaf size), but also dominates the phenotypes of all homeotic mutants found as yet (see Figs.), i.e. any effects of mutant genes found to date do not significantly reduce/affect sepal size individually. Constant features are the basis of any systems in biological systematics.

Future Developments

Some of the work ahead and questions to be solved are as follows:

(1) What is the anatomical and genetic basis for the infertility of the Misopates *plena* mutant with anthers? What is different in the fertile Antirrhinum mutant?

(2) Why is the fifth locule absent in the Misopates *def* and *glo* mutants?

(3) What is the genetic basis of

a) the four *squamosa*-like mutants (at least two different phenotypes)? (Which factors are missing so that there is no [or hardly any] flower formation in these mutants? Are the expression patterns of *squa* and *flo* different from the patterns in Antirrhinum and if so, are they relevant for the question of regressive evolution?)

b) the second *globosa*-like mutant: is it really *globosa*?

c) the *cycloidea*-like mutants (would the double mutant *cyc*/*squa* produce flowers?)

(4) What does the phenotype of the double mutant of *def/ple* look like and is it relevant to help explain the genetic differences between Misopates and Antirrhinum?

(5) What is the genetic basis of the colour reversion in the Coimbra Misopates wild type (white to violet)?

(6) Comparison of the different classes and families of TEs in Misopates and Antirrhinum (numbers and locations).

(7) Comparison of the genetic maps (gene linkages on chromosomes) of Antirrhinum and Misopates (no map as yet).

(8) Perhaps F1 by cell fusion.

Scientific Publications

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Schwarz-Sommer, Z., E. de Andrade Silva, R. Berndtgen, W.-E. Lönnig, A. Müller, I. Nindl, K. Stüber, J. Wunder, H. Saedler, T. Gübitz, A. Borking, J.F. Golz; E. Ritte and A. Hudson: A linkage map of an F2 hybrid population of Antirrhinum majus and A. molle. Genetics **163**, 699-710 (2003).

And 14 non-refereed publications (comprising about 800 pages of re-edited or newly written material on basic biological problems which are also clearly relevant for our experimental work).

Structure of the group

Group leader	**Dr. Wolf-Ekkehard Lönnig**
Postdoctoral fellow	**Dr. Jeong Hee Kim**
	March 2001 – May 2003
Gardeners	**Manfred Pohé**
	Sybille Richter
	Franz Heister
Student help	**Katja Deborah Lönnig**
	November 2000 -March 2003

Collaboration

Molecular methods: MPIZ, Department of Molecular Plant Genetics, Dr. Thomas Münster and Britta Grosardt (technical assistant)

Invited lectures given

Institute of Genetics, University of Cologne (08.10.2003) ■

Cell Signalling: Dissecting Lipid Metabolic and Signalling Pathways in the Epidermis

Alexander Yephremov

General Introduction

The regulation of the contact response of epidermal cells plays a crucial role in the fusion of carpels and the formation of the gynoecium. Carpel development involves the co-ordination of cell growth and differentiation in response to contact with other carpels. On the other hand, plants demonstrate a complexity of forms that can only be achieved through the proper isolation of organs that regularly are in close contact during development.

There are, in principle, two possibilities to regulate the contact response of epidermal cells: (1) By controlling properties of an extracellular barrier; and (2) through signalling molecules or their receptors.

We have used a combined genetical and biochemical approach to study a class of Arabidopsis mutants that are characterised by ectopic epidermal organ fusions.

Using a transposon tagging approach, we have cloned three genes: *FIDDLEHEAD (FDH)*, *LACERATA (LCR)* and *BODYGUARD (BDG)*. The characterisation of these genes and the finding that repression of trichome differentiation occurs in the *fdh*, *lcr* and *bdg* mutants pointed towards a role in a novel lipid metabolism pathway that might be involved in the biosynthesis of cuticle and cell differentiation.

New Results

The fourth mutant showing ectopic organ fusions has been identified in the ABP24 family of the ZIGIA *En/Spm* transposon population. Genetic analysis of reversions, which occurred in some mutant plants, provided evidence that they appear due to transposon excision events. We combined transposon insertion display with denaturing high performance liquid chromatography (DHPLC) in order to perform a co-segregation analysis and to identify the mutant allele bearing a transposon insertion of interest. The ABP24 gene, identified by transposon tagging as described above, appears to encode a protein with unknown function. Sequence homology searches in databases retrieved 17 proteins sharing the same structural domain composition. Distinct functions of only a few of these proteins have been proposed; therefore, the mode of action of the APB24 protein was rather difficult to infer from these data. Nevertheless, our hypothesis appeared to be supported by the instrumental analysis of the APB24 mutant plants that has been performed in Dr. Klaus Tietjen's lab (Bayer CropScience). A joint patent application based on these studies has been filed.

Using GUS and GFP reporter genes, we performed promoter expression analysis of the *BDG*, *LCR* and APB24 genes. We found that the BDG and APB24 promoters show epidermis-specific expression, which could be detected in young organs including roots. This pattern correlates with the epidermal organ fusion phenotype of corresponding mutants.

Based on sequence similarity, the *FDH* gene is believed to belong to a gene family of fatty acid elongases created by *FAE1* of Arabidopsis. To establish the biochemical function of FDH, two kinds of experiments have been performed. Firstly, we expressed *FDH* cDNA from the seed-

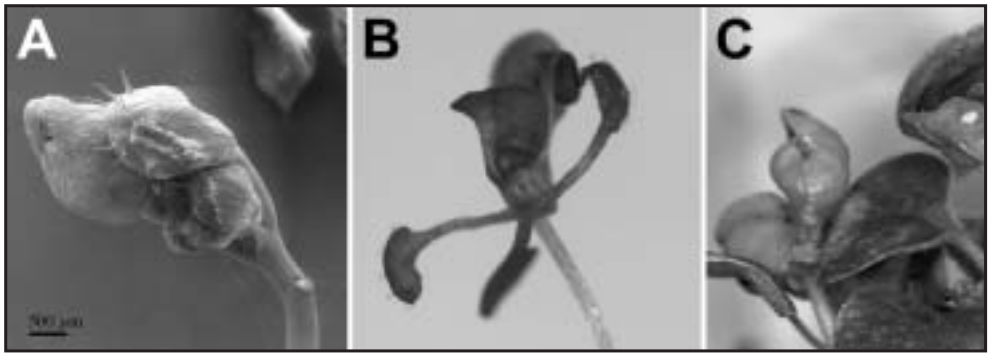

Fig. 1. Genetically and chemically induced organ fusions in Arabidopsis and Antirrhinum. (A) Fusion of floral organs in a mutant. Scale bar is shown in the scanning electron micrograph. Similar organ fusions may be caused by application of some chemicals to Arabidopsis (B) and Antirrhinum (C). The use of the chemical approach may allow identifying new genes involved in the pathway.

specific *FAE1* promoter in transgenic Arabidopsis plants and subjected the seed oil composition to analysis (Prof. Dr. Lukas Schreiber's lab, Universität Bonn). Secondly, fatty acid composition of total lipids has been determined in the tissues of the *fdh* mutant and wild types (Dr. Klaus Tietjen's lab, Bayer CropScience). Results of both experiments support the idea that the FDH protein is a fatty acid elongase involved in biosynthesis of cutin monomers.

The *ANTIRRHINUM FIDDLEHEAD* (*AFI*) gene was examined for its ability to complement *fdh*. Because the *fdh* mutant is sterile, complementation analysis was performed using segregating progeny of *FDH*/*fdh* heterozygotes (T_0) transformed with a *FDH*::*AFI* construct. T_1 transformants have been genotyped using DHPLC. Among T_2 wild types, *fdh*/*fdh FDH*::*AFI* plants have been found. They stably transmitted the wild-type phenotype to the successive generation, corroborating that full complementation of the *fdh* mutation could be achieved with the *AFI* gene driven by the *FDH* promoter. This result will allow us to determine features and structural elements of the class of FDH-like proteins.

Using collections of Arabidopsis mutants in collaboration with ZIGIA and GABI, we performed a reverse genetic screening for mutants in the families defined by four genes cloned in our lab: *FDH*, *LCR*, *BDG* and APB24. A number of mutants have been isolated in the course of these experiments. One mutant in the BDG family is particularly interesting with regard to our project as it shows a lethal phenotype.

We were interested to know whether any chemicals - inhibitors of various enzymes that have been described - could inhibit the pathway of interest. In the course of screening, we have found several compounds that mimic mutations resulting in the organ fusion phenotype in Arabidopsis. Interestingly, with these compounds, organ fusions can be induced in Antirrhinum, suggesting that the pathways appear to be similar in both species (Fig. 1).

The *cer10* mutation is one of a few mutations in Arabidopsis that reduces wax load and causes organ fusions - likely due to a defect in cuticle formation. As it is a component of the organ fusion pathway, we aim to identify *CER10* by a map-based cloning strategy. Using DHPLC for heteroduplex analysis and SNP detection, we mapped the *cer10* locus to a narrow region of 0.2 cM on chromosome 3.

In some organ fusion mutants, defective cuticle may impose stress on epidermal cells and therefore, inhibit cell differentiation, in particular, trichome differentiation. However, using genetic analysis, we obtained evidence that overlapping but distinct lipid pathways control cuticle biosynthesis and regulate differentiation of trichomes (Fig. 2).

Future Developments

We are en route to establishing biochemical functions for all genes that are components of the organ fusion pathway and have already been molecularly isolated in our lab. New genes implicated in the pathway will be identified using forward and reverse genetic screening in ZIGIA and GABI collections of Arabidopsis mutants. Our long-term goals are to depict the pathway of interest, to study its regulation, to identify natural substrates for all enzymes involved and to characterise chemical inhibitors. We will also continue to work on issues related to the role of organ fusion pathway in cell differentiation in the epidermis.

Scientific Publications

Efremova N., M-C. Perbal, A. Yephremov, W.A. Hofmann, H. Saedler and Z. Schwarz-Sommer: Epidermal control of floral organ identity by class B homeotic genes in Antirrhinum and Arabidopsis. Development **128**, 2661-2671 (2001).

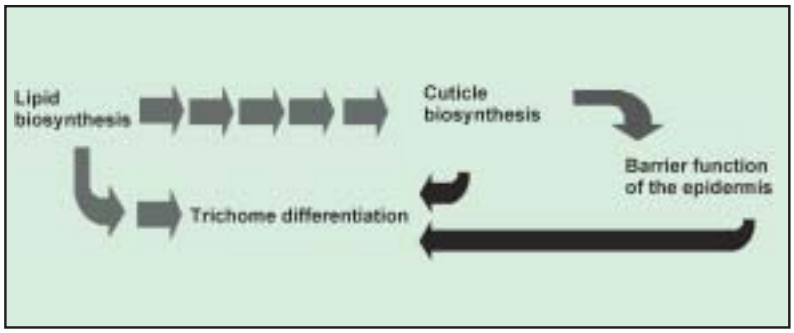

Fig. 2. Interrelations between the cuticle biosynthesis pathway and trichome differentiation in the epidermis. It appears that a part of the lipid biosynthesis pathway is directly connected to a pathway controlling trichome differentiation. In some mutants, cell differentiation may also be suppressed due to the poor barrier function. Probably, imbalanced composition of cutin monomers may also affect cell differentiation; however, this has to be experimentally verified.

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Yephremov, A., and H. Saedler: Display and isolation of transposon-flanking sequences starting from genomic DNA or RNA. Plant J. **21**, 495-505 (2000).

Structure of the group

Group leader	**Dr. Alexander Yephremov**
Postdoctoral fellow	**Dr. Sergey Kurdyukov**
Technical assistant	**Sascha Bär**
Laboratory assistant	**Aldona Ratajek-Kuhn**

Guest scientists

Prof. Dr. P. B. Kirti and Mr R. Kumar, Department of Plant Sciences, University of Hyderabad, India

Collaborations

Fatty acid composition analysis, chemical analysis of cuticle constituents, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany, Prof. Dr. Lukas Schreiber

Metabolic analysis, fatty acid composition analysis, Bayer CropScience, Germany, Dr. Klaus Tietjen

Cytochrome P450s, Dr. Franck Pinot, Dr. Irene Benveniste, Dr. Daniele Werck-Reichhart, IBMP-CNRS, France

Structural analysis of cuticle, Dr. Christiane Nawrath, Université de Fribourg, Switzerland

Antirrhinum molecular genetics, Max-Planck-Institut für Züchtungsforschung, Dr. Zsuzsanna Schwarz-Sommer, Dr. Nadia Efremova

Invited lectures given

Institut de biologie moléculaire des plantes-CNRS, Strasbourg (06.02.2001), Rheinische Friedrich-Wilhelms-Universität, Bonn (21.06.2002), Bayer CropScience, Frankfurt/M (22.01.2003), Universiteit Maastricht, Maastricht (19.06.2003) ■

The Diversity of MADS-box Gene Functions and their Roles in Plant Development and Evolution

Thomas Münster

Introduction

MADS-box genes encode a family of highly conserved transcription factors, which play important roles in developmental processes and signal transduction in higher eukaryotes. They are well-known for the roles they exert in the formation of flowers, where they control, for example, the identity of the different floral organs. The interaction of the floral-specific MADS-box genes has been summarised in the ABC-model of flower development. However, MADS-box genes involved in a variety of other processes have been reported in the last years, suggesting that these genes may participate in nearly all aspects of plant development.

For a better understanding of the roles MADS-box genes play in plant development and evolution, we are currently examining various aspects of this gene family in the monocotyledonous crop maize, in ferns and in the moss *Physcomitrella patens*.

New Results

MADS-box gene diversity in the crop plant *Zea mays*

The basic idea of this project was to establish MADS-box genes from an economical important crop plant as tools for future transgenic plant design approaches. Hence, knowledge about structure, expression domains and functions of these genes is an inevitable prerequisite.

Using an extensive cloning approach employing various techniques, we targeted MIKC-type MADS-box gene cDNAs in maize and isolated 29 different genes. All of these belong to the well-defined MADS-box gene-subfamilies found in dicotyledonous plants. However, it is remarkable that the number of maize MADS-box genes in many subfamilies is higher than that of the dicotyledonous model plant *Arabidopsis thaliana*. Phylogeny reconstruction revealed that the increase in gene number might have had two different origins: (1) Independent gene duplications in the lineage that led to the grasses; or (2) the ancient segmental allotetraploid nature of the maize genome.

Fig. 1. The distinctive phenotype of a *Tunicate* cob (left) compared to a wild-type maize cob (right). Especially of note are the enlarged glumes, which encase the kernels in the *Tunicate* cob.

Determination of the mapping positions of the maize MADS-box genes has been used to find out whether they coincide with known mutant loci. One of the most interesting candidates emerging is the gene *ZMM19*, which belongs to the *STMADS11*-like subclade of MADS-box genes. We have collected a number of independent evidences that *ZMM19* may cause the *Tunicate1* (*Tu1*) phenotype of maize. *Tunicate1* is a well-known mutant, which was first described by Saint Hilaire at the beginning of the 19th century. The most impressive feature of the *Tunicate* phenotype is the elongation of glumes in male and female inflorescences, which have given rise to its popular name "pod-corn" (Fig. 1).

The molecular events which generated the *Tunicate ZMM19* locus are complex and very likely unique in the maize history. We have demonstrated that different accessions of *Tunicate* maize from collections all over the world possess the same molecular rearrangement at the *Tunicate ZMM19* locus. This is in agreement with the fact, that no independent *Tunicate* mutant line could be discovered in the past. Anthropologists have speculated that Indian shamans used *Tunicate* cobs for religious purposes and by that way distributed the mutant kernels across South and Central America in prehistoric times.

Two rearrangements of the maize genome seem to be involved in the formation of the *Tunicate ZMM19* locus: A disruption or loss of large parts of the *ZMM19* promoter and a subsequent duplication of the gene locus. These alterations have led to a change in the expression pattern of *ZMM19* transcripts in mutant plants from *ZMM19* being usually only expressed in leaves and husk leaves of wild type, to a strong ectopic expression in the male and female inflorescences of *Tunicate* maize. Meanwhile, to elucidate the *Tunicate* mutation in detail, we are analysing the structure of a collection of wild-type and mutant *ZMM19* loci.

To find out more about gene function, *ZMM19* has been over-expressed in the heterologous background of *Arabidopsis thaliana*. The majority of the resulting transgenic plants produced flowers with strongly enlarged sepals, which has led to the "inflated-calyx-syndrome" (ICS) hypothesis (see page report: Heinz Saedler).

The function of MADS-box genes in non-seed plants

In the past, we have characterised a number of MADS-box genes in the leptosporangiate ferns *Ceratopteris richardii* (Fig. 2a) and *Ceratopteris pteridoides*. In phylogeny reconstructions, all isolated genes came out as homologues but not orthologues of the seed plant MIKC-type MADS-box genes. The total number of MADS-box genes in the Ceratopteris genome could be estimated as more than 30. The analysis of the expression profiles of these genes using Northern and *in situ* data revealed that they are ubiquitously expressed in the gametophytic as well as in the sporophytic phase of the fern life cycle. This is in clear contrast to seed plant MADS-box genes, which usually have defined tissue or organ-specific expression patterns.

To determine whether these features are common to fern MADS-box genes in general or unique in the lineage of highly derived ferns like Ceratopteris, we initiated the analysis of MADS-box genes in the ancestral eusporangiate fern *Ophioglossum pedunculosum* ("Adder's tongue", see Fig. 2b), which is only distantly related to Ceratopteris. cDNAs for four different genes (*OPM1*, *3–5*) have been isolated, of which *OPM1* and *OPM5* show a relative close phylogenetic relationship to the *CRM6*-like genes of Ceratopteris. In contrast, *OPM3* and *OPM4* seem to have no close relatives. While most of the Ophioglossum MADS-

Fig. 2. MADS-box genes are currently under investigation from a variety of non-seed plants. (a) *Ceratopteris richardii*, a derived fern; (b) *Ophioglossum pedunculosum*, an ancestral fern; and the moss model system, (c) *Physcomitrella patens*.

box genes show a widely spread expression profile, the expression of *OPM4* seems to be restricted to the generative part of the Ophioglossum leaves. This may suggest that reproduction-specific MADS-box genes evolved not only in higher plants, but independently also within ferns. However, more detailed studies are required to confirm these results.
Bryophytes are the earliest land plants; they appeared on earth around 450 MYA, 50 million years before the ferns. Among the mosses, a new plant model species has been established over the last years: *Physcomitrella patens* (Fig. 2c) is the only land plant, which offers the possibility to efficiently knock out genes via homologous recombination. In collaboration with a Japanese group, we isolated seven different MADS-box genes from Physcomitrella. The structural analysis revealed the presence of a new class of MIKC-type MADS-box genes in the moss due to distinct differences: (1) in the length of the I-domain; (2) in the conservation of the hydrophobic amino acids in the K-domain; and (3) in the exon/intron organisation of the genomic loci. This new gene type has been designated as "MIKC*"-type MADS-box genes. So far, only one further MIKC*-type gene has been described from the club moss *Lycopodium annotinum*. However, using phylogenetic reconstructions, five putative MIKC*-type MADS-box genes could be identified in the genome of *Arabidopsis thaliana*. Just as it has been demonstrated for the fern genes, the expression analysis of the Physcomitrella MADS-box genes revealed ubiquitous expression pattern in all tested moss tissues.
Some MADS-box genes from Physcomitrella have been knocked out and the mutant phenotypes analysed. Up to now, only *PPM2* disrupted lines show some divergence in the control of the timing of the moss life cycle.

Future Developments

MADS-box genes and maize mutants

The detailed characterisation of the *Tunicate ZMM19* locus will be completed soon. The mapping of the maize MADS-box genes resulted in some additional candidates for known mutants. We would like to screen these candidate genes, especially members of not well-analysed MADS-box gene subfamilies, for their involvement in the generation of the mutant phenotypes.

MADS-box gene function in lower plants

Unfortunately, there is currently no effective method to obtain stable transgenic ferns. As a consequence, functional analysis of the fern MADS-box genes in Ceratopteris or Ophioglossum is strongly limited. Therefore, we decided to concentrate on our functional studies of MADS-box genes in Physcomitrella as the moss offers the complete spectrum of molecular tools we need for our future work. We would like to knock out all MADS-box genes systematically in Physcomitrella and create double or triple knock-out strains in cases of partial redundancy of closely related genes. Moreover, we are highly interested in the evolution of MADS-domain protein functions. For this reason, we are in the process of setting up mutant-backgrounds to test seed plant MADS-box genes in Physcomitrella and the moss genes in higher plants.

Scientific Publications

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Diploma thesis

Dörthe Ahlbory (2001) Studien zur Expression von *ZMM19*, einem Kandidaten-Gen für den *Tunicate* Locus

Dissertations

Katrin Henschel (2002) Strukturelle und funktionelle Charakterisierung von MADS-Box-Genen aus dem Laubmoos *Physcomitrella patens* (Hedw.) B.S.G.

Structure of the group

Group leader	**Prof. Dr. Günter Theißen**
	until April 2001
	Dr. Thomas Münster
Postdoctoral fellow	**Dr. Katrin Henschel**
	until December 2002
PhD student	**Katrin Henschel**
	until July 2002
Diploma student	**Dörthe Ahlbory**
	until November 2001
Technical assistant	**Wolfram Faigl**

Collaborations

Physcomitrella MADS-box gene functions: Institute of Basic Biology, Okazaki, Prof. Mitsuyasu Hasebe
Physcomitrella MADS-box gene functions: Faculty of Science, Kanazawa University, Dr. Rumiko Kofuji

Invited lectures

Jagiellonian University, Krakow (12-13.11.2002)

Five years ZIGIA (Zentrum zur Identifikation von Genfunktionen durch Insertions mutagenese bei *Arabidopsis thaliana*) – a Centre for Functional Genomics in *A. thaliana*

Koen Dekker

Introduction

ZIGIA was set up in 1998 with the aim of establishing and exploiting two types of insert-tagged mutant populations of Arabidopsis Col-0 for the discovery of new gene-phenotype relationships. Either T-DNA or the autonomous maize transposon *En-1/Spm* (hereafter referred to as En) were used to disrupt the genes and in addition, provided a tag for the characterisation of the mutation. Throughout this project, this artificial genetic diversity was successfully used by four screening groups at the MPIZ (supervised by Drs. Dario Leister, Bernd Reiss, Heinz Saedler and Imre Somssich) and a technology centre that provided an array of services, most notably the knock-out screening facility.

During the first three years of ZIGIA (1998 - 2000), a total of 13 new gene-phenotype relationships were discovered and proposals about development for commercial application were discussed with the industrial partners in the project, namely, BayerCropScience, Deutsche Saatveredelung, KWS SAAT AG and Norddeutsche Pflanzenzucht. Given this proof-of-principle, it was decided to continue the project in 2001 and 2002, with the expectation that due to the gained experience and expertise, the efficiency should increase. By the end of 2000, the activation-tagged T-DNA-line approach (Dr. B. Reiss) along with the insert-flanking-sequence amplification technique from the En-approach, were used by Dr. B. Weisshaar as the starting point for the separate GABI-Kat project (see report Bernd Weisshaar). GABI-Kat provides the scientific community with access to sequence-characterised T-DNA-lines that are currently the material of first choice for reverse genetics studies. In 2003, the established resources from ZIGIA were transferred into the German plant genomics initiative by integration of GABI-Kat and ZIGIA.

Here, we present an overview of the ZIGIA milestones and its main contributions in the years from 1998 to 2002.

Project Highlights

The final ZIGIA populations consisted of 16,000 En-lines, each carrying 5-20 copies of the transposon, representing on average six independent insertions per plant or ca. 96,000 integration sites for the whole En-population, and an additional 7,000 T-DNA lines with on average 1.5 insertions per plant. The En-lines were used to set up a reverse genetics screening service in which the identification of En-tagged knock-out mutants for 3465 genes was attempted, either by PCR on pools or since July 2000 by hybridisation to En-flanking-filters (Fig. 1). These results are currently available through a public database (www.mpiz-koeln.mpg.de/zigia/publicdb/) where identified candidates for a particular gene are described.

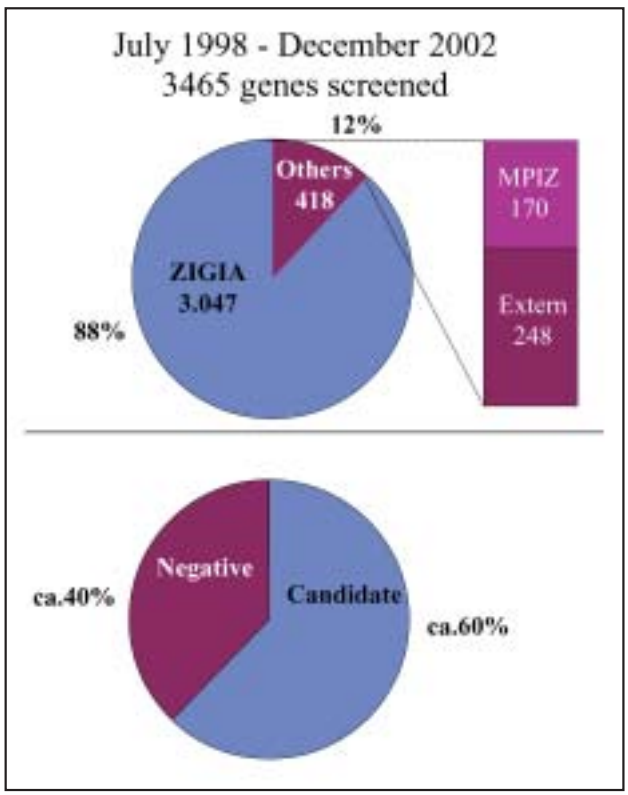

Fig. 1. Overview of the ZIGIA reverse genetics screening service. The ZIGIA service attempted to identify En-tagged knock-out mutants for 3465 genes by PCR on pools or since July 2000 by hybridisation to En-flanking-filters. Requests from outside the project were received for 418 genes, which resulted in ca. 250 candidates delivered worldwide.

The ZIGIA screening groups also used both populations for forward screening. By combining forward screening and reverse genetics a total of 30 gene-phenotype relationships were discovered, of which 21 relationships were not described before (Fig. 2). Upon request from the industrial partners, a patent application was filed for six of these relationships.

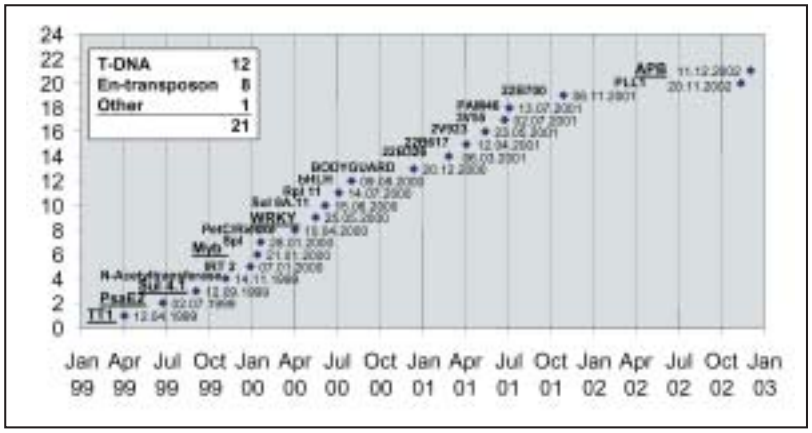

Fig. 2. New gene-phenotype relationships discovered in ZIGIA. Of these discoveries, 12 were made with T-DNA lines and 8 with En-lines either by forward screens or by reverse genetics. Six discoveries (underlined) were filed for patent application upon request from the industrial partners concerned.

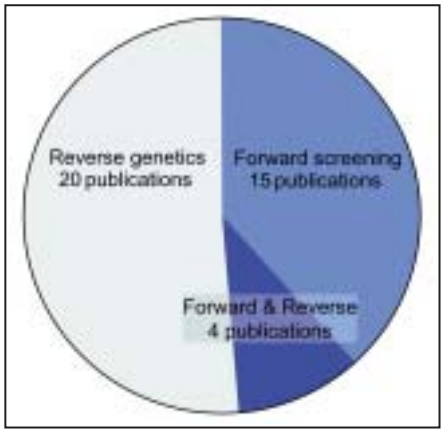

Fig. 3. Publications using the ZIGIA populations. This overview also includes collaborations that started in the pre-ZIGIA period (1996-1998) as part of DFG and EU projects.

Part of the material that was exploited in the ZIGIA project derived from pre-dating DFG and EU projects at the MPIZ. Now that databases of sequence-characterised T-DNA lines have taken over the role of these projects, the importance and scientific contribution of these earlier projects is best underscored by the number of publications in which insert tagged lines from the ZIGIA populations have featured (Fig. 3).

Scientific Publications (by ZIGIA co-workers)

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Kalde, M., M. Barth, I.E. Somssich and B. Lippok: Members of the Arabidopsis WRKY group III transcription factors are part of different plant defense signaling pathways. Mol. Plant-Microbe Interact. **16**, 295-305 (2003).

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Structure of the group

Group leader	**Dr. Koen Dekker** until December 2002
Postdoctoral fellows	**Dr. Barbara Blumbach** until December 2002 **Dr. Luca Eckstein** until December 2002 **Dr. Alexander Efremov** until December 2002 **Dr. Sirak Kifle** until October 2001 **Dr. Joachim Kurth** until December 2002 **Dr. Bernadette Lippok** until August 2002 **Dr. Anna Sorensen** until December 2002 **Dr. Sabine Steiner-Lange** until December 2002 **Dr. Ulrike Unte** December 2001 - December 2002 **Dr. Luzie Wingen** until December 2002
PhD students	**Daniela Maiwald** until December 2002 **Ernesto Olmos** until December 2002 **Paolo Pesaresi** until December 2002 **Claudio Varotto** until December 2002
Technical assistants	**Sascha Bär** until December 2002 **Angela Dietzman** until December 2002 **Heidrun Häweker** until June 2002 **Sandra Heppelmann** until June 2001 **Anne Holstein** until December 2002 **Christiane Horst** until November 2002 **Nicole Kamphaus** August 2001 - December 2002 **Sandra Kröber** until November 2002 **Ellen Mahlow** until July 2002 **Tia Ngyen** August 2002 - December 2002 **Eva Schlösser** until June 2002 **Valentina Strizhova** until December 2002 **Marc Wolf** until December 2002
Other support	**Guido Schmidt** until December 2002 **Aldona Ratajek-Kuhn** until December 2002 **Suzanne Dorweiler** until December 2002 **Regina Gentges** until December 2002 **Martina Kania** until December 2002 **Angelika Krull** until September 2002

Grants, external funding

BMBF "Biotechnologie 2000" program (0311751)
BayerCropScience GmbH, Frankfurt
Deutsche Saatveredelung Lippstadt-Bremen GmbH, Lippstadt
KWS SAAT AG, Einbeck
Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Holtsee

Invited lectures given

GPZ Tagung, Bonn (20.06.2001), University of Amsterdam, Amsterdam (05.10.2001), University of Arizona, Tucson (28.06.2001), Deutsche Botaniker Tagung, Freiburg (24.09.2002), CIMBios, Merida (27.11.2002), PAG XI, San Diego (12.01.2003) ■

Service Groups

ELMON SCHMELZER

Central Microscopy (CeMic)

Elmon Schmelzer

Introduction

To satisfy the growing demand to understand gene function and networking, cytological approaches play an important part. The service group Central Microscopy (CeMic) meets the increasing requests for cell biological investigations within the Institute. This central facility provides comprehensive background knowledge concerning cytology along with practical experience and technical skills as well as appropriate instrumentation and equipment; thereby, enabling optimal cytological/microscopical work to be carried out. The group is involved in studies of a wide range of living or fixed specimens and employs conventional light or fluorescence microscopy, confocal laser scanning microscopy and scanning or transmission electron microscopy. Besides performing experiments, we give advice on cytological questions and instruct researchers in cytological methods, operation and usage of microscopes and their accessories as well as introducing them to new techniques and equipment. Additionally, we are also carrying out our own research, concentrating on dynamics and function of actin filaments and actin filament-binding proteins.

New Results

Achievements of the service

The usage of confocal laser microscopy in connection with digital image processing and analysis in biology has led to an extraordinary rise in cell biological knowledge and to a rapid development of the technology and methods for microscopical live examination. To keep pace with this ongoing technological progress and the ensuing new research possibilities, a new confocal laser scanning microscope (CLSM) equipped with the latest technology was purchased at the end of year 2002 (Fig. 1). The major, novel features of the instrument are the following. (1) The system contains a special detector (META-detector) which allows the collection of spectral information in each image point. Thus, in addition to the normal image of a fluorescent specimen, a spectral fingerprint of each image point is obtained. This function is essential for investigations employing the various variants of the fluorescent protein tags (GFP, CFP, YFP, dsRED) whose emission spectra largely overlap. (2) A further module integrated into the microscopical system (ConfoCor 2) serves the performance of fluorescence correlation spectroscopy (FCS). This relatively new technology has a high potential for answering questions about parameters such as local concentrations, translational and rotational diffusion and transport coefficients of molecules, rates of fast intramolecular and slow intermolecular dynamics, molecule or particle mobil-

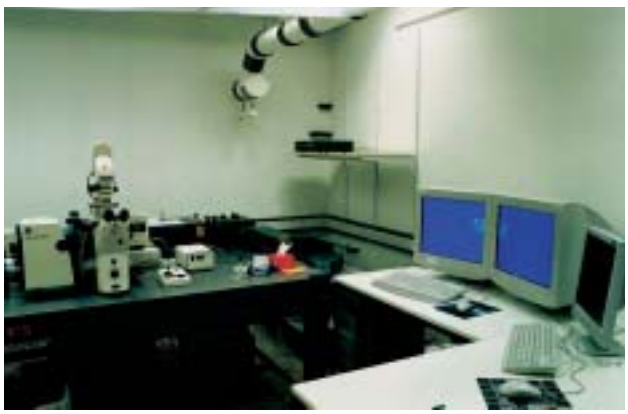

Fig. 1. The new confocal scanning microscope (CLSM).

ity and aggregation. FCS represents a powerful method to study thermodynamic and kinetic features of molecular interactions and enables the biophysical analysis of molecular processes within living cells. (3) An additional ultrafast oscillating near infrared laser system for multiphoton excitation is highly advantageous for live examinations. The high energy of the beam of conventional lasers penetrating into the tissue or into cells causes damage by photooxidation processes. The novel multiphoton excitation lasers yield ultra short pulses of near infrared light. This low energy light normally does not harm living specimens. Furthermore, infrared light reaches far deeper into tissue compared to normal visible light. These physical properties allow long-time experiments in deep tissue layers causing minimal damage. (44) A further module provides fluorescence lifetime imaging (FLIM) where instead of the spectral characteristics, the lifetime of the fluorescence signal is measured. This is a signature of a fluorophore which is only weakly affected by scattering in tissue, making it possible for measurements to be made through greater tissue depths. Fluorescence lifetime is also a function of fluorophore environment. Thus, FLIM can be used to map chemical or physical changes within a sample.
In the institute many research projects, have been started applying the different capabilities of the new CLSM, with special focus on spectral fingerprinting and fluorescence resonance energy transfer analysis to visualise protein-protein interactions in living cells.

Results of our own research

Actin filament functions are absolutely necessary for papilla formation and defence of fungal invasion. The two major activities of the cytoskeleton, dynamic dis- and reassembly and mediation of directed transport, apparently drive the changes in cytoplasmic organisation. This results in the establishment of a specific cytoplasmic domain at the penetration site, which has the major task of local reinforcement of the cell wall. Recently, we have started investigations on actin-binding proteins and their potential functions in cell polarisation and defence. As a representative of the proteins involved in polymerisation/depolymerisation of actin filaments and, potentially, signalling towards the cytoskeleton, we have chosen profilin. We have identified and characterised cDNAs from parsley and Arabidopsis, and have produced affinity purified antibodies against cloned profilin. Immunocytochemical staining of infected cells with these antibodies showed accumulation of profilin at the penetration site, indicating a high turnover of actin filaments at this site. Experiments for testing the involvement of profilin in papilla formation and fungal defence are in progress. Antibodies against small GTPases from plants were also employed for cytochemical stainings of infected cells. Preliminary experiments display weak labelling around the penetration site; thus, it might be possible that small GTPases play a role in regulation of cytoskeletal polarisation in plant cells as already known from animal systems. To further study actin-based transport processes and their role in cell polarization and papilla formation in detail, we are investigating plant myosins. We have cloned and characterised a representative of the two classes of higher plant myosins (class VIII and XI). By investigating their subcellular targeting in protoplasts from cultured cells of parsley and Arabidopsis overexpressing the respective GFP-fusion proteins, we have obtained initial indications of their functions. The results of these experiments revealed differential localisation of the two myosins and clearly indicated a role of the myosin XI in mediating intracellular transport of organelles, perhaps preferentially ER vesicles.

Future Developments

Service

The development of new genetically encoded fluorescent labels and sensors together with advances in laser scanning microscopy have pushed and certainly will further push the boundaries of fluorescent protein imaging; thereby, enabling researchers to work with living samples. Hence, we expect that our service activities will increasingly deal with projects applying such imaging technologies. Such projects will essentially concern analysis of dynamic processes in living cells, such as growth and development as well as responses to external stimuli and environmental changes, using fluorescence marker proteins (GFP, YFP, CFP, dsRed). Therefore, the following will be analysed: (1) Alterations in shape or dimension of whole cells; (2) structural and spatial changes; and (3) movements of cellular components such as cytoskeleton, organelles, membrane systems or individual proteins. Furthermore, physiological processes in tissue or single cells will be analysed and monitored, e.g. gene activation, synthesis of proteins or metabolites, and spatial changes in ion concentrations (Ca). Finally, taking advantage of FLIM and FCS technology will provide new insight into intermolecular dynamics and kinetics as well as physiological changes. Besides using this newly and rapidly developing technology for in *vivo* examinations, electron microscopical analysis is of high value for morphological analysis. We have noticed an increasing demand for scanning and also transmission electron microscopy to characterise mutant phenotypes in detail. In particular, the resolutional power of transmission electron microscopy represents the ultimate tool to identify fine structural, subtle changes in intracellular morphology of mutants.

Own future research

Our own future research will focus on the intracellular targeting and function of plant myosins. Selected myosins will be transgenically expressed as GFP-tagged molecules in *Arabidopsis thaliana* and *Hordeum vulgare*. We will take advantage of the recently developed transient assay system for barley epidermal leaf cells. This involves biolistic transformation of epidermal cells with a gene of choice followed by inoculation with barley powdery mildew (*Blumeria graminis*). The dynamics of intracellular distribution of these myosins during cell polarisation will be monitored. The function regarding pathogen

response will be tested by "gene silencing" or/and by "knock out" lines. We will make constructs to specifically silence myosin genes by post-transcriptional silencing (dsRNAi) in the single cell assay system with barley and *Arabidopsis* plants. A particularly valuable tool for the assignment of individual myosin gene functions are knock-out mutants. Respective mutant collections are publicly available. A search in several T-DNA insertion mutant collections revealed hits in almost all of the 17 Arabidopsis myosin genes. Such lines will be employed for characterisation of their phenotype regarding fungal resistance or other morphogenetic or developmental traits. Furthermore, expression of GFP-tagged wild-type myosins (or their domains) in the respective knock-out background would not be biased by the presence of the endogenous gene product. Thus, the intracellular targeting of specific myosins would probably more precisely reflect the wild-type situation. Again, these targeting studies can be performed with the single cell assay system or in transgenic plants expressing GFP-tagged myosins. These experiments will directly show whether or not specific myosin genes are functionally involved in cell polarisation and fungal defence. Finally, we plan to identify proteins interacting with tail regions of specific myosins with the yeast two-hybrid screening or split ubiquitin analysis. CFP- and YFP-fusion constructs of some selected pairs of myosin/partner proteins will be generated and used for co-expression in the single cell assay system. The actual interaction in living cells can then be visualised via fluorescence energy transfer technology (FRET) and fluorescence correlation spectroscopy (FCS).

Scientific Publications

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Structure of the Group

Group leader	**Dr. Elmon Schmelzer**
PhD student	**Amirali Sattarzadeh**
Technical assistants	**Hans Ullrich Martin,**
	Ila Rouhara
	Rainer Franzen
Engineer	**Rolf-Dieter Hirtz**

Grants, external funding

DFG priority program (Molecular Motors): 1 PhD student

DFG program collaboration with South Korea: 1 Postdoctoral fellow, shared with Dr. Ralph Panstruga

Collaborations

Investigations of the role of profilin in cell polarization and pathogen defence: MPIZ, Cologne, Dr. Paul Schulze-Lefert

Ca^{2+}- and calmodulin-dependent signal transduction in the *mlo*-mediated resistance against powdery mildew: MPIZ, Cologne, Dr. Ralph Panstruga

Structure and function of plant myosins: Department of Botany, University of Bonn, Prof. Dr. Diedrik Menzel; Institute of Botany, University of Freiburg, Dr. Peter Nick

Expression studies on Mykorrhiza-specific genes: Department of Botany, University of Cologne, Prof. Hermann Bothe

Invited lectures given

Elsa-Brandström Conference Center, Hamburg (1.12.2002) ■


Protein Identification and Analysis of Protein Modifications by Mass Spectrometric Methods at the MPIZ

Horst Röhrig and Jürgen Schmidt

General Introduction

The availability of plant genome sequences (e.g. Arabidopsis, rice, etc.) provides opportunities for systematic approaches towards understanding plant processes. One of the most important post-genomic approaches is proteomics, which can be defined as a large-scale analysis of proteins, protein-protein interactions and post-translational modifications (Tyers and Mann, 2003, Nature **422**, 193-197).

Since spring 2002, our group is involved within the Institute in an integrated approach to protein identification by MALDI-TOF mass spectrometry, which has become a key technique in proteomics (Aebersold and Mann, 2003, Nature **422**, 198-207). The great accuracy of mass determination and the outstanding sensitivity achieved by the MALDI technique represents enormous progress in biological mass spectrometry, which together with bioinformatics enables the rapid identification of proteins in the fmol to pmol range. For this purpose, a MALDI-TOF mass spectrometer (Bruker REFLEX IV) is run by our group.

In addition, a Q-TOF mass spectrometer (Micromass, *Q-tof2*) has been purchased by the Department of Molecular Plant Pathology not only for the identification of proteins based on their tryptic peptide mass and partial amino acid sequence, but also for the identification of post-translational modification sites. Between the responsible scientists handling these instruments, an effective collaboration has developed.

In order to give an example for the successful application of the MALDI technique for protein identification we refer to our recent data concerning the molecular analysis of the *ENOD40* gene, which appears to play an important role in root nodule organogenesis. Using peptide mass fingerprinting, we showed that a subunit of sucrose synthase binds specifically to *ENOD40* gene peptides (Röhrig *et al.*, 2002).

New Results

MALDI-TOF technology

MALDI-fingerprinting should always be considered as a first screen in protein identification prior to further biochemical functional analysis. We have established MALDI here at the Institute and provide this as a service to scientists, who can come to us with their 1- or 2-D-gels. Separated protein spots of interest are cut from the gel and proteolytically digested. For this purpose, a recently installed robot for automated in-gel digestion and target plate preparation for subsequent MALDI-TOF analysis is now in operation (Bruker Proteineer dp). Since the requests for MALDI analyses are constantly increasing, this machine is intended to avoid bottlenecks in high throughput proteomics, although the robot can only process coomassie–stained spots containing proteins at a minimal concentration of 200 fmol. Therefore, in other cases, we encourage our colleagues to carry out the tryptic digests themselves. Interested groups are supplied with the corresponding digestion protocols and receive support upon request.

Mass spectra of the extracted peptide mixtures are acquired by MALDI-TOF, and the resulting peptide mass fingerprints of proteins under investigation are compared to peptide fingerprints of all known proteins in the database. For the database search, we mainly use the Mascot protein database search software from Matrix Science Ltd., which is used by many proteomics laboratories. Matching proteins are scored and ranked by the number of matching peptide masses. As more full-length genes become available in the database, the success rate of identification by MALDI will increase.

In collaboration with the group of Thomas Koprek, MALDI-TOF mass spectrometry is presently not only used for protein identification, but also in a large-scale screening programme for the analysis of hordatines, which are phenolic compounds with antifungal activity (Roepenack *et al.*, 1998 JBC **273**, 9013- 9022).

Functional analysis of the peptide-encoding gene *ENOD40*

Scientifically, our group is attached to the Department of Plant Developmental Biology and is working on the functional analyses of biological processes, which occur during nodule organogenesis in symbiotic plant-microbe interactions. Recently, we have focused on the biological role of the *ENOD40* gene (Röhrig *et al.*, 2002), which is expressed at early stages during root nodule organogenesis in legumes. Identification of *ENOD40* homologues in non-leguminous plants suggests that this gene may have a more general biological function.

In vitro translation of soybean *ENOD40* mRNA in wheat germ extracts revealed that the conserved nucleotide sequence at the 5´ end (region I) encodes two peptides of 12 and 24 aa residues (peptides A and B). These peptides are synthesised *de novo* from very short overlapping open reading frames. Appropriate open reading frames are present in all legume *ENOD40*s studied thus far. To our knowledge, this is the first case in which small peptides are directly translated from polycistronic eukaryotic mRNA.
The 24 amino acid peptide B was detected in nodules by Western blotting. Proteolytic degradation could be one of the reasons why we could not detect ENOD40 peptide A in mature soybean nodules using a combination of immunoprecipitation, HPLC and MALDI MS analyses. However, in a recent study, peptide A was detected in nanogram amounts in extracts from 15-day-old soybean nodules by a competitive ELISA (van de Sande *et al.,* 1996 Science **273**, 370-373).
To identify the molecular targets of these unusual short translation products, we have used biotinylated peptides A and B for affinity purification of potential binding proteins from soybean nodule extracts. Using this approach, we could show that both peptides specifically bind to a target protein of 93 kDa. To identify this protein, tryptic peptides were generated and subjected to MALDI-MS analyses. Searching of the database with the resulting peptide mass fingerprints identified the 93 kDa protein as soybean Nodulin100, which is a subunit of sucrose synthase. The enzyme acts as a homotetramer and is one of the major nodule-enhanced proteins (Thummler and Verma, 1987 J. Biol. Chem. **262**, 14730-14736). This is consistent with our finding that the level of the 93 kDa protein is strongly enhanced in nodules compared to uninfected roots.
Further investigations concerning the biochemical nature of ENOD40 peptide binding to sucrose synthase are in progress.

Future Developments

Sucrose synthase is a key enzyme of carbohydrate utilisation in plants and catalyses the reversible conversion of sucrose and UDP into UDP-glucose and D-fructose. It is believed that this enzyme is essential for the cleavage of sucrose translocated from the shoots to nodules to provide nutrients for the bacteroids, energy for the nitrogenase and carbon skeletons for the assimilation of fixed NH_4^+ (Morell and Copeland, 1985 Plant Physiol. **78**, 149-154; Thummler and Verma, 1987). However, relatively little is known about the regulation of this important enzyme. Since the cysteine residue of peptide A seems to be essential for binding of this peptide to sucrose synthase (Röhrig *et al.*, 2002), the biochemical nature of this interaction is being studied in more detail. We found that DTT treatment inhibits binding of peptide A to this enzyme, indicating that sucrose synthase may be regulated by enzyme S-thiolation. Based on this hypothesis, we want to find out the biological role of ENOD40 peptides in plants.

Due to the constantly increasing interest of various groups at the MPIZ for mass spectrometric analyses, a future bottleneck of high throughput proteomics can be expected concerning the acquisition of samples from 1D or 2D gels. Therefore, the group has asked for financial support to purchase a spot picker at the beginning of 2004. This robot enables gel imaging, spot detection and selection from gels into 96 microwell plates and allows the workflow from gel separation to mass spectrometric identification and characterisation to become fully automated.

Scientific Publications

Miklashevichs, E., H. Röhrig, J. Schell and J. Schmidt: Perception and signal transduction of rhizobial Nod factors. Crit. Rev.Plant Sci. **20**, 373-394 (2001).

Miklashevichs, E., and J. Schmidt: Small peptides and their roles in plant cells. Int. Rev. Cytol. (in press).

Röhrig, H., J. Schmidt, E. Miklashevichs, J. Schell and M. John: Soybean ENOD40 encodes two peptides which bind to sucrose synthase. Proc. Natl. Acad. Sci. USA **99**, 1915-1920 (2002).

Stoll, M. et al.: Activation T-DNA tagging using *Nicotiana tabacum* mesophyll protoplasts. (submitted).

Dissertation

Marion Stoll (2002) Aktivierende T-DNA–Mutagenese in *Nicotiana tabacum* Mesophyll-Protoplasten

Structure of the group

Group leaders	**Dr. Horst Röhrig**
	Dr. Jürgen Schmidt
Postdoctoral fellow	**Dr. Michael John** (50%)
PhD student	**Marion Stoll**
	until November 2002
Technical assistant	**Ursula Wieneke** (50%)

Guest scientist

Dr. Edvins Miklashevichs, A. Kirchenstein Institute of Microbiology and Virology, Riga Latvia, postdoctoral fellow (until December 2001)

Grants, funding from sources other than MPG

TMR network, grant No. FMRX-CT 98-0243, 1.5.98-30.04.03: 1 Postdoctoral fellow

Collaborations

Studies on the biological function of ENOD40 peptides: A. Kirchenstein Institute of Microbiology and Virology, Riga,, Dr. Edvins Miklashevichs; Clusius Laboratory, Leiden, Dr. Mette Gronlund

Invited lectures given

Meeting of the Green Max Planck Institutes, Harnack-Haus, Berlin (26.11.2002), 38th Winter Seminar: Biophysical Chemistry, Molecular Biology and Cybernetics of Cell Functions, Klosters, Switzerland (17.01.2003). ■

ADIS: A core facility for DNA-related technological service at the MPIZ

Bernd Weisshaar

General Introduction

The increasing automation of modern molecular biology techniques increasingly requires complex and expensive equipment. Competitive research, as well as education of students, demands direct and easy access to (at least) the central technologies. This is especially true for DNA isolation, DNA sequencing, quantitative PCR, clone and library handling, and the generation and analysis of arrays of defined DNA sequences (DNA arrays or DNA chips) in combination with the respective bioinformatics tools. In November 1995 and in accordance with the program ”Zukunftsperspektiven des MPIZ“, a core facility for DNA-related technology was started to provide a service for highly requested and automatable modern techniques for all departments at the MPIZ. The unit was designated ADIS for ”automated DNA isolation and sequencing“.

New Results

ADIS is running the ”GABI-Kat“ knock-out (KO) service for access to sequence-characterised T-DNA insertions in a given gene. To build up resources for efficient progress in plant genomics, a set of T-DNA mutagenised lines with insertion sites identified by DNA sequencing is being generated. The output is a database describing, for the lines in the collection, which gene(s) has been disrupted, so that users can select KO alleles for their needs. Finally, mutant seeds are delivered to researchers for physiological and gene-function relationship studies. This project has profited from the previous experience gained in ZIGIA (Zentrum zur Identifikation von Genfunktionen durch Insertionsmutagenese bei *Arabidopsis thaliana*) and the know-how related to DNA sequencing accumulated by ADIS. The actual start of the GABI-Kat project was June 2000. At present, about 60,000 lines have been analysed and the coverage of the genome with ”gene hits“ is 45% (see http://www.mpiz-koeln.mpg.de/GABI-Kat/).

ADIS Services

DNA synthesis

ADIS provides a html/cgi-based oligonucleotide ordering software to purchase oligos. The program, designated OligoOrderDB, was developed in-house and has the following features (current version in 04/2003: v3.7):

- password-protected user identification (based on UserDB)
- control of cost centre (”Kostenstellen“) accessibility
- supplier-specific price information
- options to order specialised ”TaqMan“ oligos
- plausibility check of order
- evaluation of each order (”4-Augen-Prinzip“)
- storage of billing information
- usage and financial statistics
- storage of all oligonucleotide-related information for subsequent user access
- download of user-specific lists of ordered oligos
- generation of an order E-mail in the respective supplier-dependent format

At present, the MPIZ orders about 260 oligonucleotides on average per week, mainly for PCR-related experiments. Without this eCommerce system, the fast and reliable access to oligos would create an high additional workload for the purchase department (”Zentraler Einkauf“).

DNA sequencing

In 2002, ADIS processed about 30,000 DNA single sequencing probes, and about 200,000 reactions in 96- and 384-well microtiter plate format. Templates vary from small PCR products to complete BACs. The complete workflow is handled by a laboratory information management system (LIMS) which was developed in-house under the designation SeqOrder. The resulting sequence data (GCG-formatted sequence text file) are written into the UNIX account of the user. SeqOrder has the following features (current version in 04/2003: 2.0):

- user and ”Kostenstellen“ identification (based on UserDB)
- data collection with an improved user interface
- linking of oligo (primer) information to sequence reactions
- generation of coherent sample (= file) names
- generation of orders with 96 or 384 reactions
- option to write reaction-specific information into trace files
- generation of sample sheets to operate sequencers
- database of all orders and sequencing reactions including results

- user access to information on queuing requests (”request tracking“)
- generation of ”result reports“
- automation of the accounting (”Umbuchungsbelege“)
- user access to the data back-up system (via ”getadisdata“)

SeqOrder has increased productivity significantly as it reduces the work required to keep track of the high number of requests and the related administrative paperwork. The data delivery system works virtually without human interaction. At present, the goal is to replace the data prints (sequence ”spherograms“) with electronic distribution of pdf files.
In parallel to DNA sequencing, the instrumentation is also used for DNA fragment analysis in AFLP and related genetic mapping experiments by using the same fluorescence-based detection technique.

Clone handling and arraying (”picking & spotting“)

ADIS's activities now include clone handling, automated colony picking, and gridding of colonies and DNAs to filters. Picking & spotting is now offered as a ”full service“. However, printing to glass was not followed up.

Other technologies

ADIS provides access to a 7700 ”TaqMan“ instrument (Abi) for quantitative PCR as well as to a BioAnalyzer (Agilent).

Future Developments

The next goals are further cost reductions and productivity improvements while keeping the ”response time“ as short as possible. Sequencing costs will be further reduced by setting up ”paper-free reporting“ (automatised generation of pdf-files of the spherograms, automatic generation of quality scores for each read, training of users to use the trace files). In the area of picking & spotting, the increasing clone handling requires the set-up of a system (database) to keep track of all the generated clone libraries and microtiter plates.

Scientific Publications

Li, Y., M. Rosso, N. Strizhov, P. Viehoever and B. Weißhaar: GABI-Kat SimpleSearch: a flanking sequence tags (FST) database for the identification of T-DNA insertion mutants in *Arabidopsis thaliana.* Bioinformatics (in press)

In addition, ADIS has contributed to many publications from the MPIZ. In several cases, this has been mentioned in the ”acknowledgements“.

Structure of the group

Group leader	**Prof. Dr. Bernd Weisshaar**
Postdoctoral fellows	**Dr. Mario Rosso**
	Dr. Yong Li
	Dr. Sabine Steiner-Lange
Technical assistants	**Prisca Viehoever**
	Diana Lehmann
	Tanja Theis
	Iris Bürst
	Daniel Roth
	Elena Schmidtke
	Andrea Brüggen
Gardener, scientific software, etc.	
	Thomas Rosleff-Soerensen
	Monika Bürgel
	Heike Eichler

Grants, external funding

BMBF GABI program (GABI-Kat): 3 Postdoctoral fellows, 3 technical assistants, 1 gardener

BMBF GABI program (FST exchange): 1 Technician

Cereal Transformation Service (ATM)

Hans-Henning Steinbiss

Introduction

The Cereal Transformation Service (ATM) provides plant material, protocols, know-how and practical assistance to research groups of the MPIZ within a framework of allocated personnel, consumables and facilities. At present, this service is provided by one technician and group leader.

Technical support

Particle bombardment

Particle bombardment represents a transformation method which is applicable to all important crops. It is related to the group of the so-called "direct gene transfer methods" which competes with the Agrobacterium–mediated transformation. Particle bombardment was established at the MPIZ by Dr. Steinbiß in 1994 . At present, particle bombardment is frequently used for transformation of wheat (ATM) and potato (Salamini Department) as well as for essential transient gene expression studies by different MPIZ groups in a variety of plant species (tobacco, potato, maize, barley, coconut, Arabidopsis).

BY2-tobacco-suspension culture

The tobacco suspension culture BY2, originally introduced to the MPIZ by Prof. T. Nagata, is frequently used by several groups for transient gene expression assays and even transformation studies, although the culture has lost its regeneration capacity. The homogeneity and the remarkable dividing rate of this line seems to be still very attractive. The line is cultivated by ATM as a back-up source. The culture medium has to be changed weekly. Therefore, fresh suspension culture batches can be ordered by MPIZ users every week.

Transformation service

Maize

At present, there is no call for a maize transformation service at the MPIZ. In the past, the cereal transformation service has produced 1597 transgenic maize plants mainly for the. Salamini (671) and Saedler (700) Departments. ATM has used 226 transgenic plants to study coat protein-mediated virus resistance (MDMV). Precise protocols are summarised on the ATM homepage.

Wheat

Although wheat was successfully transformed by Agrobacterium for the first time in 1996, the method itself seems to be still far away from routine. Therefore, particle bombardment of immature embryo-derived callus has been established. ATM frequently transforms wheat in order to study maize endosperm promoter function in wheat as part of a co-operation with three European groups. At present, there is no other demand at the MPIZ.

Barley

Barley transformation via *Agrobacterium tumefaciens* has been remarkably improved at the MPIZ since the last report in 2001. The most objective mode to measure the current barley transformation efficiency is simply to summarise the number of transgenic barley plantlets which has been given/offered to users at the MPIZ in a period of about one year. Precise details as well as a couple of photos of the transformation procedure are presented on the ATM homepage.

User	Department	Plasmids	Plantlets
S. Bieri	Schulze-Lefert	7	1058
D. Leister	Salamini	2	292
A. Freialdenhoven	Schulze-Lefert	6	1156
R. Panstruga	Schulze-Lefert	1	216
J. Müller	Schulze-Lefert	2	400
T. Zhao	Schulze-Lefert	2	804
H.-H. Steinbiß	ATM	2	394
Total		**22 plasmids**	**4320 plantlets**

Future plans

Depending on the needs of the Institute, ATM will change its service areas in the future. However, the transformation of barley has already reached an outstanding level with no other institute seeming to have a more advanced output of barley plantlets per person and per year. We predict that T-DNA insertional mutagenesis for functional genomics in barley will also become feasible.

At present, the main goal of the cereal transformation service is to improve the Agrobacterium-mediated transformation of wheat in order to obtain a transformation frequency comparable to barley in the near future. Today, the bottleneck seems to be the poor quality of the donor plants which must be overcome before we can successfully reach our goal.

Scientific Publications

Bhat, R.A., M. Riehl, G. Santandrea, R. Velasco, S. Slocombe, G. Donn, H.-H. Steinbiss, R.D. Thompson and H.-A. Becker: Alteration of GCN5 levels in maize reveals dynamic responses to manipulating histone acetylation. Plant J. **32**, 1-15 (2002).

Ercolano, M.R., A. Ballvora, J. Paal, H.-H. Steinbiss, F. Salamini and C. Gebhardt: Functional complementation analysis in potato via biolistic transformation with BAC large DNA fragments. Mol. Breed. (in press).

Structure of the group

Group leader	**Dr. Hans-Henning Steinbiß**
Technical assistance	**Sabine Schulze**

Fungal pathogens, infestation and effects

Werner Josef Gieffers

Introduction

Infestation by fungal pathogens is not only important for studies in epidemics but also suitable for the characterisation of the effects of genes in plants. We are able to observe and determine these phenomena by using our methodology of quantitative resistance examination. This method comprises natural infection in field trials and artificial infection tests in the laboratory. Infection tests in the lab take place on leaf discs from detached leaves. In most cases, the results of such artificial infection are highly correlated with infestation in the field. The methodology was described in the framework of the project "Investigations into the hormone and carbohydrate metabolism in *rol*C transgenic aspen clones (*Populus tremula* and *P. tremula* x *P. tremuloides*) and their effects on phytopathological properties". With the help of this method, reproducible results were obtained (*Gieffers and Fladung,* 1999a,b, *Gieffers* 2000).

This method is now used in the project for biosafety: "Infestation of oilseed rape hybrids with fungal pathogens". The objective of which is to study the outbreeding effects of oilseed rape in other species and especially, resistance in the hybrids.

In the lab, artificial infection is carried out with spores of pathogenic fungi. Under artificial conditions several fungi produce no spores or only a very low number. We observed that some fungal species produce spores only in the presence of ionised oxygen, some of them dramatically. Therefore, under lab conditions, the presence of ionised oxygen is a highly reliable method to obtain a very high number of fungal spores (*Gieffers* 2002 and 2003).

New Results

Project

Investigations into the hormone and carbohydrate metabolism in *rol*C transgenic aspen clones (*Populus tremula* and *P. tremula* x *P. tremuloides*) and their effects on phytopathological properties

Here, a new method for quantitative resistance examination of disease infection on aspen (*Populus tremula*) is described. The necessary equipment is a transparent chamber with a continuous and uniform water supply for the plant material. Comparable green plant parts are taken from the field or the greenhouse, in most cases leaves or

leaf disks. This material is inoculated in the laboratory and then incubated under defined conditions. Generally infection symptoms appear after 6-14 days and are assessed with a new iterative method of estimation. The results of this procedure correspond well with those in the field. Lab-based resistance tests have also shown a high correlation with the natural infestation in the field with potato, tobacco, petunia, wheat, corn and four grass species. The test duration is short and allows repetition of the tests on the same plant. An analysis of resistance (distribution of resistance factors in the plant, resistance behaviour of single plant organs, influence of the resistance through plant stage and exogenous conditions and others) is possible. The methodology is suitable for recording latent infection as well as for the detection of pathogens and their isolation.

By this method, the first laboratory infection was carried out on aspen leaves by *Pollaccia radiosa*, and infection differences were detectable. By application of the iterative method of estimation, the natural infestation of *P. radiosa*, a leaf spot disease, and of *Melampsora* sp., a rust disease, were determined.

In the interim, the method was further developed. Now the artificial infection with fungal spores of pods or pieces of stems is possible and leads to reliable infection results.

Project
Infestation of oilseed rape hybrids with fungal pathogens

The aim of this study is to investigate the influence of outbreeding resistance factors, as found in oilseed rape, on the infestation of plant diseases of related species. Outbreeding was examined in bird rape. For that purpose, hybrids were made between bird rape and oilseed rape. Infection tests were carried out in one variety of bird rape (Nokonava), in four varieties of oilseed rape (Star, Tiger, Jumbo, Lambada) and their hybrids. In the field trial, the natural infestation of the phyllosphere was determined. Green material from the field without symptoms (leaves, stems, pods) was tested in the lab by artificial infection with five fungal pathogens (*Alternaria brassicae*, *Botrytis cinerea*, *Phoma lingam*, *Sclerotinia sclerotiorum* and *Verticillium longisporum*). In most cases, both the natural and the artificial infection of hybrids was significantly lower compared with the infection of the varieties. This project is currently ongoing.

Influence of oxygen on the production of fungal spores

The influence of air oxygen, oxygen from hydrogen peroxide (35%), of ionised oxygen, and of UV light radiation (350 nm, OsramL18W) was examined. The amount of ionised oxygen is generally low under normal conditions (between 250 and 750 ions/cm^3). However, UV light ionises molecular gases and thereby, increases the amount of ionised oxygen. Hydrogen peroxide releases molecular oxygen, which is ionised under UV light. An electric air ioniser (model Greenair from Konovit, 250.000 ions/cm^3/sec) produces a constant source of ionised oxygen by high voltage. The amount of ionised oxygen is also increased in the presence of UV light, hydrogen peroxide or an electric air ioniser. The experiments took place in an incubator at 12°C and with liquid water on a filter paper. The plant material is lying on this filter paper. The results demonstrated (Fig. 1) that pathogenic fungi produced a high number of spores in the presence of ionised oxygen. The genera Alternaria, Botrytis, Phytophthora, Pseudocercosporella and Verticillium increased spore numbers significantly under these conditions.

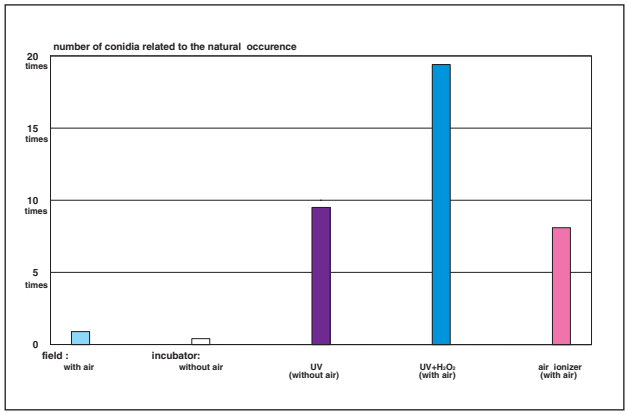

Fig. 1. Influence of oxygen and UV light on the conidia production of *Pseudocercosporella capsellae* on leaves from oilseed rape for six days and at 12°C.

Scientific Publications

Gieffers, W.: Methodik der quantitativen Resistenzprüfung. 52. Deutsche Pflanzenschutztagung, Freising-Weihenstephan **376**, pp. 383-384 (2000).

Gieffers, W. J.: Sauerstoffeinfluß auf die Bildung von Pilzsporen. 53. Deutsche Pflanzenschutztagung, Bonn **390**, pp. 512-513 (2002).

Gieffers, W.J.: Sauerstoffeinfluß auf die Bildung von Pilzsporen. Gesunde Pflanze **55(4)**, pp. 111-117 (2003).

Gieffers, W. and M. Fladung: Methodology of infection examination of pathogenic aspen fungi. Workshop: Release of transgenic trees – present achievements, problems, future prospects. Humboldt University, Berlin, Proceedings, pp. 87-92 (1999b).

Dissertations

Olaf Brinkmann (2003) Wirkung verschiedener Abwehrproteine auf phytopathogene Pilze *in vitro* und ihre Resistenzwirkung in transgenen Kartoffel- und Tabakpflanzen

Structure of the group

Group leader	**Dr. Werner Josef Gieffers**
Technical assistants	**Walter Halfenberg**

Grants, external funding

BMBF "Biosafety research and monitoring" FKZ: 0312628D, 1 Technical assistant

Collaborations

Oilseed rape diseases and outbreeding

BBA Braunschweig, Dr. A. Dietz-Pfeilstetter; NPZ, Hohenlieth, Dr. G. Leckband; Göttingen University, Prof. A. v. Tiedemann; Paderborn-Soest University, Prof. V. Paul; Pflanzenschutzamt Schwerin, Amtsleiter Dr. E. Erichsen

Pathogens and resistance in transgenic aspen

BBA für Forst- und Holzwirtschaft – Forstgenetik, Großhansdorf, Dr. M. Fladung

Biology of pathogenic fungi

Bonn University, Dr. H. Hindorf; BBA, Braunschweig, Dr. C. Smalla; Göttingen University, Dr. B. Koopmann

Methodology of artificial infection

Bionik, Bonn University, W. Roden

Central Scientific Computing (ZWDV)

Kurt Stüber

Simulation of Genomic Evolution

(in collaboration with Christiane Gebhardt, MPIZ)

The genomes of Solanum and Arabidopsis were experimentally compared and the positions of 190 markers common to both organisms were recorded. The level of synteny is assessed by counting the number of homologous genes occurring close to each other in both organisms. Since such closeness can also result from a statistical distribution of the genes in otherwise unrelated genomes, a program was developed to simulate randomly distributed genomes. The amount of syntenic relations in these random data sets is compared with the experimentally derived values. Experimentally, 95 syntenic blocks were found where at least three markers are within a short range in the genome (10 cM in potato genome and 2 Mbyte in Arabidopsis genomic sequence).

The program simulated the number and multiplicity of the potato markers exactly and the recorded number of markers in the Arabidopsis genome was randomly shuffled. After shuffling, the number of resulting syntenic blocks were counted. A total of 120 shuffling experiments revealed that 22.3 (standard deviation: 4.85) syntenic blocks containing three markers are expected on the average (Table 1). Four markers in a block are expected with a frequency of only 0.24 (standard deviation: 0.43). Therefore, the experimentally derived number of 95 blocks shows that the microsynteny between these very distantly related plants is about four-times higher than expected.

Bin size		Slot size	Average number (standard deviation) of random syntenic blocks with N hits			
S. tub.	*A. tha.*		N = 2	N = 3	N = 4	N = 5
10 cM	2 mb	20	177,15 (10,23)	22,88 (4,85)	0,24 (0,43)	0.01 (0.09)
20 cM	1 mb	20	87,75 (8,24)	6,32 (2,47)	0,41 (0,58)	0,00 (0,09)
15 cM	2 mb	30	175,33 (10,06)	22,55 (4,75)	2,39 (1,65)	0,18 (0,41)

Table 1. Simulation of random syntenic blocks between *S. tuberosum* (potato) and *A. thaliana*. (120 independent runs).

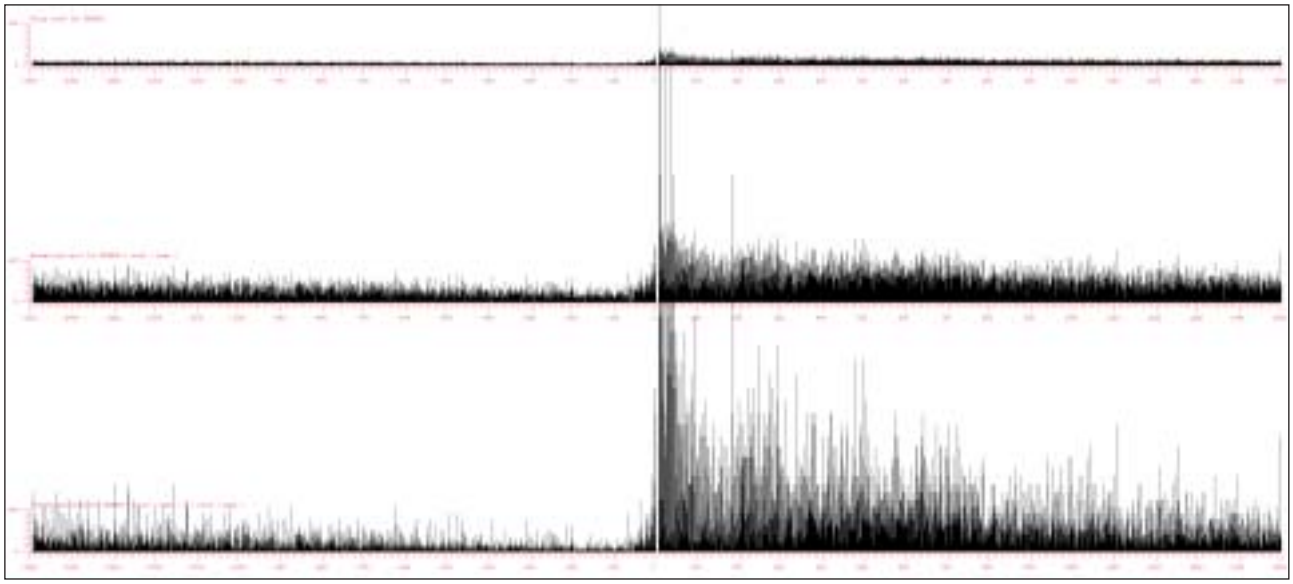

Fig. 1. Lane 1: Count of the oligomer GGAGGA in the promotor regions of all described *Arabidopsis thaliana* reading frames. Position 1 is the start of the AUG codon. Lanes 2 and 3 shows transformations to increase signal to noise ratio.

Statistical Analysis and Prediction of Transcription Factor Binding Sites

(in collaboration with Dierk Wanke and Kenneth Berendzen, MPIZ)

Many genes are regulated by *cis*-regulatory elements, which constitute mainly binding sites for transcription factors. In most identified genes, the regulatory elements are unknown. Only in a small subset of elements, can expression data be correlated with specific sequence patterns. A program was developed to extract all regulatory regions from GenBank entries and statistically analyse the occurrence of any given oligomeric pattern.

Using this program, all described reading frames in *Arabidopsis thaliana*, yeast, Drosophila and rice were investigated (Fig. 1). The resulting distribution patterns allow the identification of conspicuous regulatory patterns and the determination of conserved patterns between the investigated organisms.

Further Activities

We also provide user support with regular seminars each Friday. The seminars are practical workshops. Programs are first introduced, demonstrated, and in a hands-on approach the participants exercise them directly using a set of eight laptop PCs dedicated to this teaching activity.

Additionally, we have developed a database program package to organise a large internet virtual library of historical biological documents (see: http://www.biolib.de). Furthermore, we take care of a genetical database of *Antirrhinum majus* (snapdragon) (see: http://www.antirrhinum.org) and are currently analysing a set of 11,000 transcripts from *Antirrhinum majus*.

Scientific Publications

Gebhardt, C., B. Walkemeier, H. Heselewski, A. Barakat, M. Delseny, and K. Stüber: Comparative mapping between potato (Solanum tuberosum) and Arabidopsis thaliana reveals structurally conserved and ancient duplications in the potato genome. Plant J. **34**, 529-541 (2003).

Schwarz-Sommer, Z., E.D. Silva, R. Berndtgen, W.E. Lönnig, A. Muller, I. Nindl, K. Stüber, J. Wunder, H. Saedler, T. Gubitz, A. Borking, J.F. Golz, E. Ritter and A. Hudson: A linkage map of an F-2 hybrid population of *Antirrhinum majus* and *A. molle*. Genetics **163**, 699-710 (2003).

Structure of the group

Group leader	**Dr. Kurt Stüber**
PhD student	**Dr. Jörg Wunder**
	January 2001 - August 2002
Technical assistant	**Frank Al-Dabbagh**
	from July 2002

Collaborations

Analysis of Antirrhinum EST collection: MPIZ, Zsuzsanna Schwarz-Sommer

Simulation of genome evolution: MPIZ, Dr. Christiane Gebhardt

Analysis of TF binding sites: MPIZ, Dierk Wanke, Kenneth Berendzen.

Scientific documentation: University Library Cologne, Dr. Beßelmann.

Scientific documentation: Library of the Herbarium Haussknecht, Jena, Dr. Wissemann ■

Guest Scientists

Cross Talk between Cell Cycle and Development

Arp Schnittger • Universtity of Cologne

Introduction

In September 2001, the new university group moved into the ground floor of the former MDL laboratory. After an initial period of setting up the lab, the group resumed its work in December 2001.

The aim of the group is to explore the connection between cell cycle control and development. For proper functioning of a multicellular organism, cell-cycle progression needs to be co-ordinated with developmental rulings. For instance, as cells adopt a certain fate and differentiate, they most often stop dividing. The controlled shift from mitosis to differentiation is very important for the regulation of cell form and number, and thus, is crucial for the development of proper organ size and function.

The analysis of cell-cycle control in development bears three major problems. First, interfering with cell-cycle regulation is often embryo-lethal. Second, cell-cycle control appears to contain a number of "back-up" systems and often mutants in even major cell-cycle regulators display no obvious phenotypes. Third, the decision-making process for cell-cycle progression involves many components and it is often the relative concentration of two or several components that trigger a decision. For these reasons, we were trying to develop a single-cell test system to study cell-cycle control in development. Two cells of the model plant *Arabidopsis thaliana* appear to be suitable for this purpose: root hairs (trichoblasts) and leave hairs (trichomes).

Trichomes and trichoblasts offer several advantages as a single cell test system. First, both cell types are easily accessible being epidermal outgrowth structures (Fig. 1). Second, both cell types are not necessary for plant survival and plants without any trichomes or root hairs have no growth disadvantage in comparison to wild-type plants under laboratory conditions. Most important, both cell types are molecularly addressable since several promotor fragments have been isolated allowing a cell-specific (mis)expression of regulator genes.

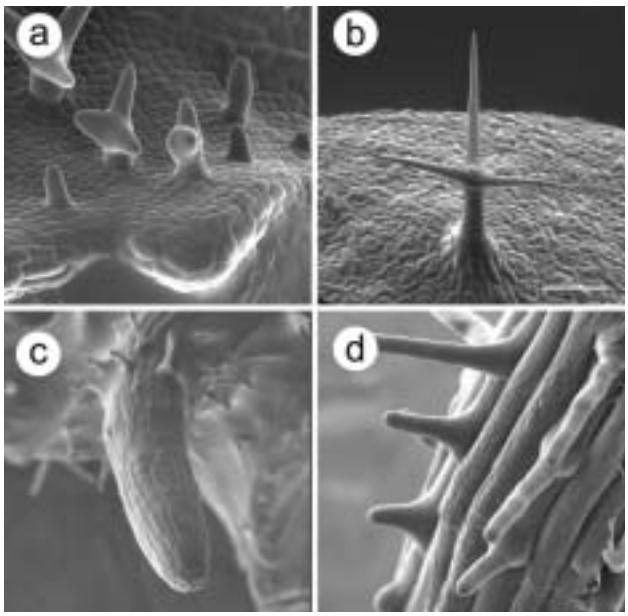

Fig. 1. Trichome (leaf hair) and trichoblast (root hair) development. (a) Trichomes are initiated at the base of young leaves. (b) Mature trichomes are large single cells, reaching up to 500 µm in length and display a characteristic three-branched shaped. (c) Trichoblast (root hair) development in the differentiation zone of Arabidopsis roots. (d) Files of trichoblast alternate with non-root-hair cells in the Arabidopsis epidermis (rhizodermis).

New Results

In a pilot experiment, 12 cell-cycle control genes were misexpressed in trichomes to explore the usability of this system. It turned out that the generated phenotypes were

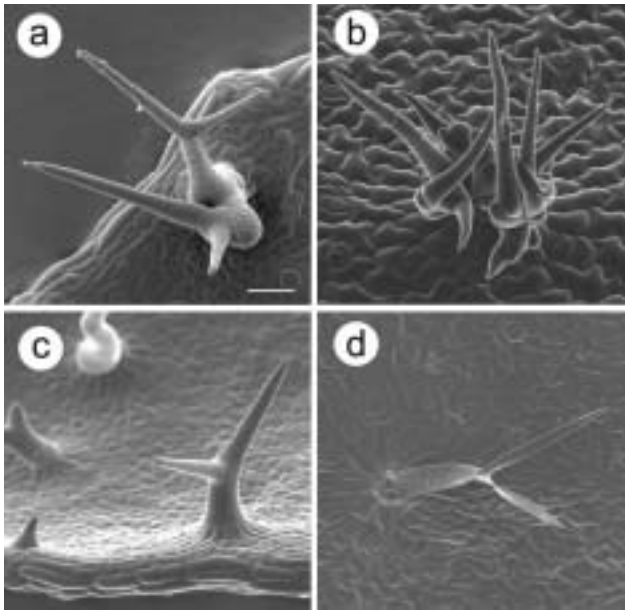

Fig. 2. Phenotypes of trichome specific misexpression lines (a) Trichome misexpressing *CYCLIN B1;2* develop into multicellular structures. (b) Trichomes misexpressing both *CYCLIN B1;2* and *CYCLIN D3;1* undergo many cell divisions leading to leaf hair clusters with up to 80 cells. (c) Small and under-branched trichomes upon misexpression of an CDK inhibitor, *ICK1/KRP1*. (d) *ICK1/KRP1*-misexpressing trichomes die on mature leaves.

instructing. Moreover, these lines circumvented problems resulting from an ubiquitous misexpression. E.g. downregulation of RBX1, a major component of the SCF complex via an trichome-specific RNAi construct resulted in a trichome phenotype but not – as observed with the ubiquitous *35S* promotor – in sterility and strongly reduced plant growth.

Among the 12 regulator genes tested were two B1-type cyclins, proteins known to have a function for entering mitosis. The misexpression of one of them, *CYCLIN B1;2*, was found to induce mitotic divisions in endoreduplicating trichome cells, giving rise to multicellular trichomes (Fig. 2a). Thus, one B-type cyclin is sufficient to turn on the complete cell division machinery. This also demonstrated that a downregulation of this B-type cyclin is an important step for entering an endoreduplication programme. Interestingly, another misexpressed and on the sequence-level highly homologous B1 cyclin, *CYCLIN B1;1*, was not able to induce mitosis. This is the first indication that the different members of the large cyclin families found in plants have specific functions in cell-cycle control.

To have a single cell test system has also proven to be valuable for the analysis of D-type cyclins. Earlier studies indicated a dual role of one D-type cyclin, CYCLIN D3;1, for the entry into S-phase and progression into mitosis. Misexpression of *CYCLIN D3;1* in trichomes promoted cell divisions and DNA replication and thus, brought functional evidence for this dual role. Interestingly, the expression of *CYCLIN B1;2* was found to be induced in the *CYCLIN D3;1*-misexpressing trichomes. However, a genetic combination of the two misexpression lines, *CYCLIN B1;2* and *CYCLIN D3;1*, led to a tremendous increase in the number of cells per trichome (up to 80 cells, Fig. 2b). Thus, even in a *CYCLIN D3;1*-misexpression line, CYCLIN B1;2 is rate-limiting; thus, indicating the existence of other regulators repressing mitosis in endoreduplicating trichomes.

In another set of experiments, we could dissect different cell-growth processes by misexpressing an inhibitor of CDKs, *ICK1/KRP1*. In previous experiments, other groups had found by misexpression of CDK inhibitors that DNA content and cell size could be uncoupled, which throws doubt on the long-standing dogma of a correlation between cell size and nuclear size. However, all these studies were carried out with ubiquitous expression systems, and especially from studies in animals, it is known that cell size and cell number can compensate each other in order to maintain organ size. The expression in trichomes, which do not contribute much to overall leaf size and thus, are presumably independent from this organ size checkpoint, allowed us to finely dissect DNA-dependent growth processes. Our results showed that there is an upper limit of cell growth defined by DNA content but that below this limit, a cell volume can expand without a concomitant increase in DNA content (Fig. 2c). Interestingly, we found that *ICK1/KRP1*-misexpressing trichomes died on older leaves, opening a potential parallel to cell-cycle control in animals, where the homologous CDK inhibitor, p27, is also involved in control of programmed cell death (Fig. 2d).

In the last year, we have further developed and explored the trichome and root-hair expression systems. There are now four trichome- and root–hair-/non-root-hair-specific promoters with different expression strength and timing. Up to now, we have generated about 100 misexpression lines, which are currently under investigation.

Future Developments

In the future, we would like to establish a third model cell type to study cell-cycle control: stomata. Stomata do not endoreduplicate and could help to investigate the transition from mitosis to endoreduplication. Several expression constructs with two different promoters have been created and transformed. The transgenic plants will be investigated in the near future.

Scientific Publications

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Structure of the group

Group leader	**Dr. Arp Schnittger**
PhD student	**Christina Weinl**

Guest scientists

Xiaoguo Zhang, University of Alabama, USA, postdoctoral fellow

Jeanette Nadeau, University of Ohio, USA, postdoctoral fellow

John Larkin, University of Louisana, USA, associate professor

Grants, external funding

DFG Sachbeihilfe grant SCHN 736: 1 PhD student

Collaborations

Plant-antibodies against CDKA;1: VIB, Gent, Dr. Geert de Jaeger

Function of A-type cyclins: John Innes Centre, Norwich, Dr. John Doonan

Function of B-type cyclins: Royal Holloway, University of London, Dr. Laszlo Boegre

Cyclin mutants: University of Edinburgh, Dr. Peter Doerner,

Function of KRP proteins: VIB, Gent, Prof. Dr. Dirk Inzé

Stomata development: University of Ohio, Dr. Janeatte Nadeau

Isolation of B-type cyclin mutants: MPIZ, Cologne, Dr. Csaba Koncz

Invited lectures given

University of Agricultural Sciences Vienna (18.01.2002), VIB, Gent (14.02.2002), University of Wageningen (28.02.2002), Royal Holloway, University of London (22.03.2002), CNRS, Gif sur Yvette (05.04.2002), 13th Arabidopsis Conference, Sevilla (30.06.2002), University of Strasbourg (12.02.2003), CNRS, Gif sur Yvette (26.02.2003), University of Montepellier (05.05.2003), University of Utrecht (03.06.2003)

Analysis of the Molecular Basis of Auxin Transport and Cell Polarity

Klaus Palme • University of Freiburg

Introduction

The phytohormone auxin is involved in the regulation of virtually all aspects of plant growth and development. Synthesised predominantly in young leaves and the shoot apex, it is transported throughout the plant body, thereby affecting embryo and vascular tissue development, apical dominance, fruit setting and ripening, root development, and tropisms. Auxin transport occurs in part in an undirected way through the phloem; however, an active and polar auxin transport stream is maintained over the plant body. This polar auxin transport is required for tropistic responses, and most probably accounts for the pleiotropic effects of the hormone, which has recently been discussed to function as a morphogen in several developmental processes. Moreover, it provides positional and directio-

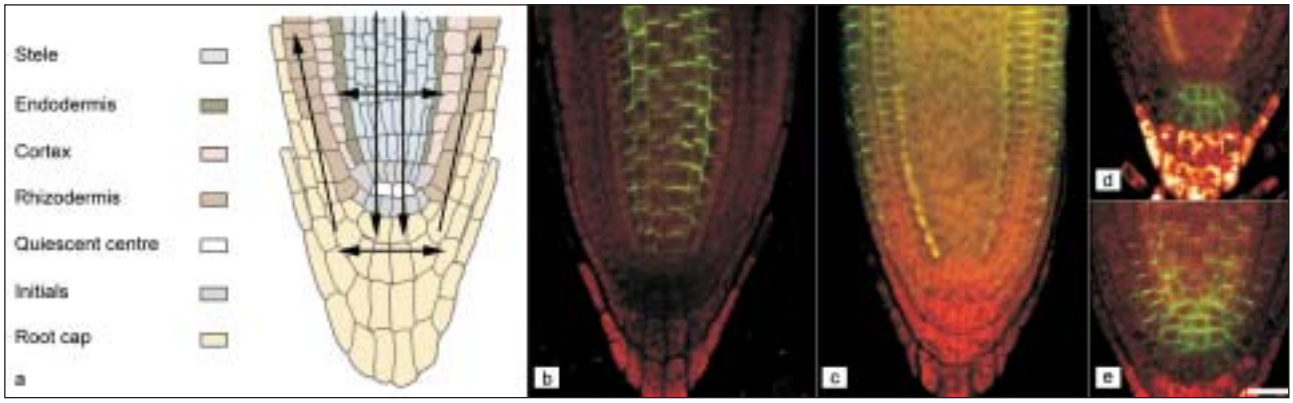

Fig. 1. PIN proteins on the tracks of polar auxin transport in the Arabidopsis root tip. a: Schematic representation of the Arabidopsis seedling root tip (arrows indicate directions of polar auxin transport); b, c, d, e: Whole-mount immunolocalisations with specific antibodies for PIN1 (b), PIN2 (c), PIN3 (d) and PIN4 (e) in seedling root tips (modified from Palme *et al.*, 2002).

nal information, an important determinant of cell polarity. Our recent research focuses on the mechanisms by which plant cells and tissues develop and maintain polarity. We basically study two groups of Arabidopsis proteins: (1) the PIN proteins, which are presumed regulators of the auxin efflux machinery; and (2) Rop GTPases, which play a key role in vesicle trafficking and polarisation. Both types of proteins show polar distribution within plant cells and therefore, represent suitable molecular tools to study cell polarity regulation.

New Results

PIN proteins, polar auxin transport, and cell polarity

The Arabidopsis *PIN* gene family consists of eight members, coding for putative membrane proteins with homology to the major facilitator superfamily of transporters. Genetic, molecular and cell biological evidence points to the PIN proteins as important regulators of polar auxin transport and thus, plant development. They have been advocated as components of the auxin efflux carrier machinery since they meet the criteria proposed for the auxin efflux carrier by the chemiosmotic hypothesis. According to this model, polar auxin transport occurs from cell to cell, whereby auxin uptake takes place via diffusion of the protonated molecule (facilitated by specific influx carriers). Auxin efflux requires specific carriers as a main portion of the molecule is deprotonated in the cytoplasm and therefore, trapped in the cell.

As one of the first plant proteins, PIN1 was shown to have a polar localisation within the cell, corresponding to the direction of polar auxin transport. *Pin1* mutants express severe developmental defects due to an altered polar auxin transport. Immunolocalisation studies with specific antibodies in WT seedlings revealed PIN1 to be situated at the plasma membrane of the distal anticlinal walls in root stele cells (Fig. 1b), where auxin is transported from the root base towards the tip (acropetal polar auxin transport). In roots, however, different directions of polar auxin transport have been described (Fig. 1a), and we could assign other PIN proteins to play a role in their regulation. A basipetal auxin transport from the tip towards the base takes place in the outer root tissues; it has been demonstrated that this basipetal transport is essential for root gravitropism. Root gravitropism is abolished in the *pin2* mutant. We demonstrated that in WT seedling roots the PIN2 protein localises to membranes of lateral root cap (LRC), cortex and rhizodermis cells in a polar fashion (Fig. 1c), corresponding to the direction of basipetal auxin transport. Two other PIN proteins, PIN3 and PIN4, are also expressed in the root tip, partially displaying polar cellular localisation (Fig. 1d,e).

To address the question how the polarity of these proteins is achieved and regulated, we took a pharmacological approach, analysing the effects of a variety of cellular drugs on PIN protein localisation as detected by whole mount immunolocalisation. By treating root tissue with latrunculin B, we could demonstrate that correct plasma membrane targeting of PIN1 requires an intact actin cytoskeleton. Furthermore, the substance brefeldin A (BFA), which inhibits guanine nucleotide exchange factors (GEFs) on small GTPases (see below), abolishes PIN1 plasma membrane localisation and leads to an accumulation of the protein in cellular vesicles. Most strikingly, the PIN1 polar plasma membrane localisation can be restored by washing out BFA from the tissue, even in the presence of the protein synthesis inhibitor cycloheximide. This finding surprisingly revealed that a relatively stable PIN1 pool is cycling between the plasma membrane and intracellular compartments. Mutant analysis depicted the Arabidopsis ARF-GEF GNOM protein to be regulating this process. We further observed that substances like 1-naphtylphtalamic acid (NPA) and triiodobenzoic acid (TIBA), which have been used as specific inhibitors of polar auxin transport for decades, affect PIN1 protein cycling.

Assuming that PIN proteins are regulating polar auxin transport, the PIN cycling provides a means to quickly adjust auxin efflux to the developmental requirements. The PIN3 protein, which is amongst other PIN proteins expressed in columella cells, which represent the site of gravity perception in the root cap, shows cycling between the plasma membrane and endosomes. In columella cells,

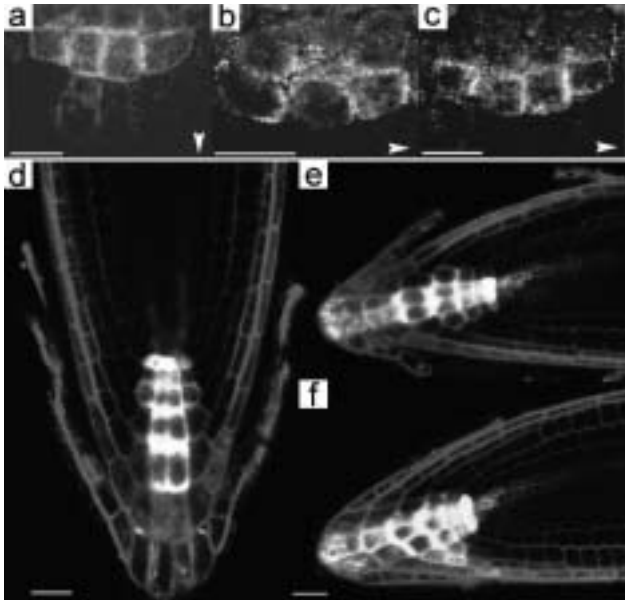

Fig. 2. PIN3 re-location and asymmetric auxin redistribution in the Arabidopsis root tip. a, b, c: Whole-mount immunolocalisation of PIN3 in root cap columella cells of vertically grown (a), and gravistimulated seedling roots after rotation to 90° for 2 min (b) and 10 min (c); d, f: Auxin-induced DR5-GFP signal in Arabidopsis seedling root tips of vertically grown (d) and gravistimulated roots after rotation to 135° for 1.5 h (e) and 3 h (f).

PIN3 generally appears to be distributed evenly over the plasma membrane in a non-polar fashion. However, if roots are gravistimulated laterally by rotation to an angle of 90-135° from the vertical, PIN3 is rapidly accumulating at the periclinal cell walls facing the centre of the earth (Fig. 2a). By using a synthetic auxin responsive promoter driving GFP expression (DR5-GFP), we were able to infer auxin fluxes from root cap columella cells to the neighbouring lateral root cap and further towards the elongation zone. We could demonstrate that the auxin transport towards the lower side of gravistimulated root tips increases (Fig. 2b); thereby, giving first visual evidence for the long proposed ”fountain model“ of polar auxin transport and its role in root gravitropism. The apparent gravity-dependent PIN3 re-localisation, which might be the snapshot of a continuously gravity-controlled PIN3-regulated lateral auxin transport, emphasises the importance of fined-tuned vesicle trafficking.

Rop GTPases

Cycling between GTP-bound ”on“ and GDP-bound ”off“ forms, G-proteins are pivotal switches that control a wide spectrum of signalling pathways in all eukaryotes. Lower and higher eukaryotes possess a large number of small GTPases. Plants are unique as they lack Ras, Rac/CDC42 and Rho GTPases. Instead, they possess a unique plant-specific class of small GTPases belonging to the RHO family of the superfamily of Ras GTPases, termed Rop (for RHO of plant). Rop GTPases take a central position in many protein interaction networks involved in cell signalling and cell polarity maintenance. The GTPase cycle of RHOs is regulated by GTP/GDP exchange factors (GEF, see above), which activate the GTPase protein by catalysing exchange of GDP for GTP, and GTPase-activating proteins (GAP), which switch off the small GTPase by stimulating its intrinsic GTPase activity.

We have characterised several Rop GTPases from Arabidopsis and showed that polar localisation of AtRops in trichoblasts specifies the growth sites for emerging root hairs. Expression of constitutively active AtRop4 and AtRop6 mutant proteins in root hairs of transgenic Arabidopsis plants abolished polarised growth, and delocalised the tip-focused Ca^{2+} gradient. Our results demonstrated a general function of AtRop GTPases in tip growth as well as polar diffuse growth. This project aims to study the molecular basis of the role of the Rop GTPases in plant signalling pathways controlling cell polarity.

Future Developments

PIN proteins, polar auxin transport and cell polarity

To further analyse the regulation of polar protein targeting, we are currently identifying genes involved in the regulation of PIN protein localisation using pharmacological approaches as well as genetic screens. In addition, gene chips are being used to study the PIN-related signalling pathways and the kinetics of root gravitropism.

Rop GTPases

To find interaction partners, we will perform two-hybrid screens and also use the recently developed yeast split ubiquitin system. Rop GTPases and interacting proteins will be expressed, ultimately crystallised, and the crystal structures will be determined. We will isolate and characterise Rop GTPase containing protein complexes directly from plants, identify copurified proteins by mass spectrometry and immunological methods, and in the longer term, visualise interactions between Rop GTPases and interacting proteins *in planta* by using GFP-based FRET microscopy. This will allow us to study the Rop nanomachine and visualise *in vivo* dynamic interactions of Rops and their partners during root hair formation or upon stimulation with phytohormones.

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Dissertations

Iris Ottenschläger (2002) Gravity induced differential transport in *A. thaliana* roots and the search for interaction partners of AtPIN1

Alexander Szyroki(2002) Charakterisierung der Kaliumkanal-α-Untereinheiten AtKC1 und KAT2 aus *Arabidopsis thaliana*

Olaf Tietz (2002) Funktionelle Charakterisierung des AtPIN1 Proteins aus *Arabidopsis thaliana*

Structure of the group

Group leader	**Dr. Klaus Palme**
Postdoctoral fellows	**Dr. Ines Brüntrup**
	Dr. Tinka Eneva
	Dr. Arthur Molendijk
	Dr. Iris Ottenschläger
	Dr. Alexander Szyroki
	Dr. Olaf Tietz
	Dr. Patricia Wolff
PhD students	**Maria Piques**
	Filipa Santos
Technical assistant	**Claudia Gilles**

Collaborations

University of Nottingham, Prof. Malcolm Bennett

University of Alberta, Prof. Mark Estelle

Universität Würzburg, Prof. Rainer Hedrich

Universität Tübingen, Prof. Gerd Jürgens

Umeå University, Prof. Göran Sandberg

Utrecht University, Prof. Ben Scheres ■

Supporting Activities

International Max Planck Research School (IMPRS) on the Molecular Basis of Plant Development and Environmental Interactions

The IMPRS was created in 2001 and is organised by the MPIZ together with its partners from the Institutes of Botany (Prof. U.I. Flügge, Prof. M. Hülskamp) and Biochemistry (Prof. R. Krämer, Prof. D. Schomburg) of the University of Cologne. In contrast to other research schools, the IMPRS Cologne breaks new ground by cooperating with three international partner institutions, which provide additional expertise in the field of molecular plant biology and biological chemistry. The list of cooperating partners is completed by the Institute of Bioorganic Chemistry (Prof. A. Legocki, Prof. M Jaskolski; Poznan, Poland), the Institute for Plant Sciences (Prof. A. Kondorosi; Gif sur Yvette, France) and the Biological Research Centre (Prof. F. Nagy; Szeged, Hungary). Through the addition of international partners the IMPRS therefore provides a link to existing European networks of scientific cooperation and emphasises collaboration with scientific institutions of accession countries of the European Union. The scope of the IMPRS at the MPIZ is that students finish their doctoral degree within three years. This is achieved by replacing the classical semester structure by an intensive and structured training program that emphasises an interdisciplinary approach towards understanding of molecular mechanisms in plant development. The program includes lectures, seminar series, and laboratory courses. At the end of the program, a written thesis and a successful thesis defence ("Disputation") are required to obtain the Ph.D. (Dr. rer. nat.) degree. However, IMPRS students also have the choice to receive their doctoral degree from a university of their home country.

An essential characteristic of the IMPRS is that students from all countries can apply, and that the entire program including lectures and training courses is taught in English. Therefore, a high proficiency in English as a second language is required to enrol in the program. The constellation of participating institutions provides excellent conditions and expertise in plant genetics, structural biology/biochemistry, molecular plant biology/biochemistry, and cell biology. The students will be able to carry out their experimental work on topics such as structural and functional aspects of plant enzymes and regulatory proteins, intra- and intercellular signalling, exogenous signals for cell division and differentiation, determinants of plant-microbe development, molecular genetics of plant evolution, symbiotic and pathogenic plant/microbe interactions, resistance-tolerance to abiotic stress factors. Besides the experimental work done by the Ph.D. students, the program is complemented by a strong training module, providing the students with up-to-date scientific skills (Table 1). The training rests on three main pillars representing method courses on specific subjects, complementary training and a transfer of knowledge component. For the entire duration of the training, there is a set of indicators available that serve students, faculty members and outside evaluators as benchmarks for monitoring the program implementation and each fellow's progress and achievements. The Ph.D. program includes one-week block courses at the participating institutions in Cologne, Poznan, Szeged and Gif-sur-Yvette. During the courses, students attend lectures and are trained in a range of specific techniques. Student training however is not restricted to increasing their experimental knowledge. An entire set of courses providing 'soft skill' expertise is also offered. Among these units are courses on scientific presentation, scientific writing, and database organisation and statistics.

Student Recruitment

In response to our international recruitment efforts in 2002 and 2003, we received a total of 203 applications from 46 different countries. For both years, the country list is lead by India (36% of all applications) followed by China (11%), Pakistan (7%) and Germany (6%). A similar picture is evident at a continental level with 67% of all applications being from Asia followed by Europe (15%) and Africa (13%), while only 3% were from the Americas. The first 13 students took up their training in September 2002 and we offered 9 fellowships again in 2003. A key feature of the IMPRS is the presence of a scientific coordinator who also acts as a personal counsellor for the fellows. Besides their thesis supervisors, students have an additional focal point to help them overcome hurdles in their day-to-day life. The coherent class character is further implemented through regular meetings and activities of the student group. This way of student counselling at the MPIZ was further improved by the initiation of the MPIZ "International Office" in December 2002. The goal of the International Office is to act as an early contact point for new students and post-docs from foreign countries, before they depart to start their new position.

Research training			Benchmark
Method courses	**Complementary skills**	**TOK**	
Functional analysis of plant disease (Dr. P. Schulze-Lefert)	Performing an oral scientific presentation	Work group progress report	Written progress report every six months
Protein control of plant development (Dr. G. Coupland)	Database organisation	Departmental seminar at host organisation	Annual progress workshop
Genetic variation in molecular plant breeding (Prof. F. Salamini)	Statistics for plant biologists	Seminar presentation within ADOPT network	Contribution to international conference
New microscopy techniques (Prof. H. Saedler)	Scientific writing, Part a	Contributions to international conferences	Written Ph.D. thesis
Analysis of plant cytoskeleton (Prof. M. Hülskamp)	Scientific writing, Part b		
Molecular characterisation of Arabidopsis mutants (Prof. U. Flügge)	Career perspectives workshop for women		
Batch and continuous cell culture (Prof. R. Krämer)	Language training at host organisation		
Mass-spectrometry based protein analysis (Prof. D. Schomburg)	Science communication for the general public		
Structural biochemistry (Prof. A. Legocki)			
In vivo imaging of gene expression (Prof. F. Nagy)			
Plant symbiosis (Prof. A. Kondorosi)			

Table 1. Breakdown of all training components and benchmarks for the IMPRS. Methods courses are taught on location, while complementary skills are taught in Cologne and transmitted simultaneously to the other host institutions. All transfer of knowledge (TOK) components will consist of oral presentations, while benchmarks consist of written contributions.

Current IMPRS student group

Name (Nationality)	Affiliation
Marcella Santaella-Tenorio (Colombia)	University of Cologne, Institute of Botany
Vijayarangakannan Parthiban (India)	University of Cologne, Institute of Biochemistry
Malgorzata Domagalska (Poland)	MPIZ, Dept. of Plant Developmental Biology
Karolina Pajerowska (Poland)	MPIZ, Dept. of Plant Breeding and Yield Physiology
Henrike Gebhardt (Germany)	University of Cologne, Institute of Biochemistry
Hugo Konijn (The Netherlands)	MPIZ, Dept. of Plant Developmental Biology
M. Shahid Mukhtar (Pakistan)	MPIZ, Dept. of Plant Microbe Interactions
Rainer Saedler (Germany)	University of Cologne, Institute of Botany
Ruchika Budhiraja (India)	MPIZ, Dept. of Plant Developmental Biology
Stefan Mauch (Germany)	MPIZ, Dept. of Plant Microbe Interactions
Tim Müller (Germany)	University of Cologne, Institute of Biochemistry
Yan Zhang (China)	MPIZ, Dept. of Molecular Plant Genetics
Stephan Wenkel (Germany)	MPIZ, Dept. of Plant Developmental Biology
Nina Möker (Germany)	University of Cologne, Institute of Biochemistry
Evgeniya Ilarionova (Bulgaria)	MPIZ, Dept. of Plant Breeding and Yield Physiology

Communicating Science at the MPIZ

Press and public relations activities at the MPIZ aim to fulfil two main criteria. One is to foster science at the Institute by using appropriate forms of lobbying, such as the Institute´s representation in the media and the public. The other task is to support the Institute's infrastructure with several administrational and organisational services. The press and public relations unit therefore comprises a broad spectrum of different internal and external activities. In additional to the press and media work, the Institute is also putting great effort into realising its communication concept based on an active and open dialogue with the general public. This includes a variety of measures directed at different target groups.

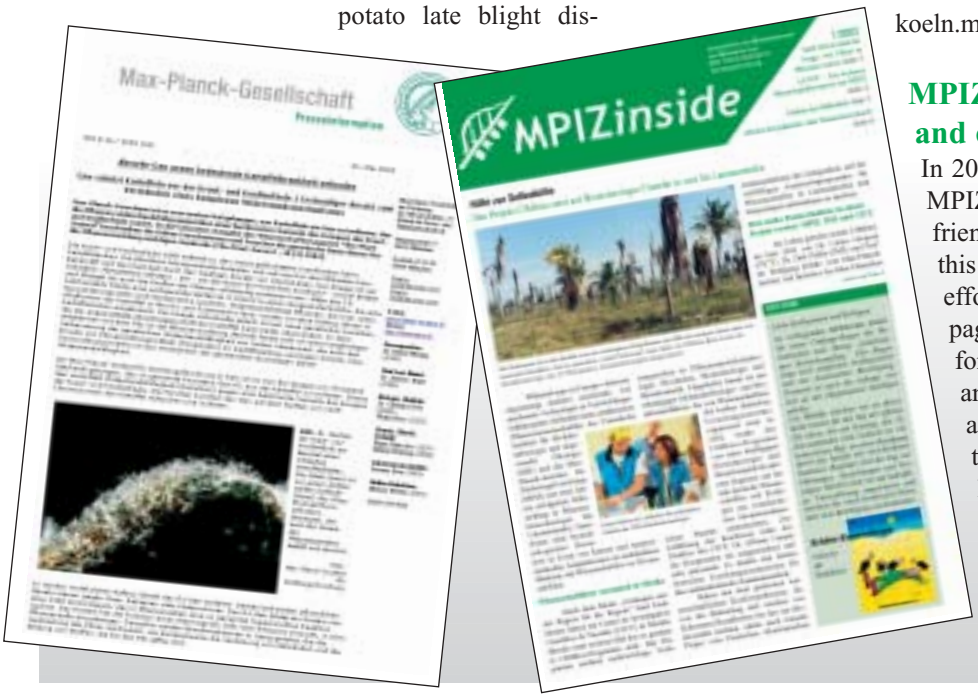
Communication activities are structured as follows:
1. Press and media work
2. Public relations work directed at the
   Scientific public, General public, Internal personnel
3. Further activities (e.g., event management)
4. Administrational and organisational tasks
   (e.g., Internet)

Science and Media

To highlight the research at the MPIZ, we regularly issue press releases in parallel with the publication of scientific papers in high ranking journals whose results are of interest to the media and public. In 2002, we issued six press releases out of which the article from Christiane Gebhardt´s group on the discovery of a resistance gene for potato late blight disease provoked the greatest response from the media.
Press information is published in co-ordination with the PR headquarters of the Max Planck Society as well as on a local basis. Requests from the media on biotech information, statements, experts, pictures and graphics are handled individually. In contrast, student´s requests on biotech information are answered with the help of published MPIZ statements and brochures along with references to other sources of information. However, as biotechnology in plant breeding has became a part of the German school curriculum over the past few years, we now receive an ever increasing number of requests in this area.

Internet versus hardcopies - MPIZ publications

Although the demand on Internet-accessible information is rapidly increasing, hardcopy publications such as "MPIZ inside" and "MPIZ aktuell" are still in high demand. While "MPIZ inside" is a newspaper for the MPIZ staff which focuses on current research topics and scientific groups as well as internal activities and social events, "MPIZ Aktuell" is targeted at science teachers and presents in-depth overview on selected topics.
Besides the biannual scientific report of the Institute that is targeted at the international scientific community, the image brochure "Welcome" provides concise information about the key research interests and services at the MPIZ. All printed MPIZ documents are also available from the Institute´s website (http://www.mpiz-koeln.mpg.de).



MPIZ website and corporate design

In 2000, we started to restructure the MPIZ website to provide a user-friendly layout and structure. While this process is still ongoing, a lot of effort has been put into internal web pages which are the main sources for staff information in German and English. Thus, sections such as "Safety and Health", "Whom to contact" and "New colleagues" have been integrated.
In addition, templates for letters and presentations using the corporate design guidelines of the Max Planck Society and the

new MPIZ logo are now accessible on the internal web pages. The Max Planck Society is in the process of relaunching a new website including a content management system which will allow all institutes to update daily. A future task will be to merge the MPIZ website into the MPG site: retaining all data base accessibilities but presenting the Institute as part of the framework of the MPG.

Third prize for MPIZ presentation within the Year of Life Sciences

To stimulate public interest in molecular plant science research conducted at the Institute, we presented the exhibition "Petri heil – or how to fish for genes within the gene pool of plants" in 2001. The presentation was part of the so-called Science Street within the "Year of Life Sciences" organised by the Ministry for Education and Research in different German cities. Research institutions were invited and financially supported to present their research with the help of exhibitions. For ten days, the Cologne public was invited to fish for genes, to talk to scientists or look at plant mutants at the MPIZ exhibit.

The idea was to develop an exhibition that not only attracts attention and provokes curiosity, but which also demonstrates "real" scientific research. Attracted by a huge fish pool, visitors analysed fished gene sequences online with registered ones in Arabidopsis genome databases. While scientists demonstrated the DNA extraction and sequencing, guests could precipitate and stain plant DNA. Finally, visitors could compare Arabidopsis mutants containing the tagged DNA sequence with wild-type plants in a real greenhouse.

The third prize (5000 Euro) was awarded to the Institute for its presentation "Petri heil" with the general public acting as the jury.

More than 100 crops in the Institute´s demonstration garden

Guided tours, open days and lectures at research institutions are the best way to promote a positive attitude towards science. This is because visitors get the opportunity to meet scientists in person and to see the work they are doing for themselves. In 2002, about 1400 people took part in guided tours at the MPIZ including 31 school classes and 24 special interest groups.

The motivation of our Institute to provide a special service for visitors is based on the growing demand to discuss research topics and questions in molecular plant breeding including the GMO debate. In general, the visits start with a theoretical introduction including an overview of the research. They are followed by a tour through the Institute, the greenhouses and the demonstration garden.

At present, about 100 agricultural and horticultural plants are growing in small plots in our exhibition on crop plants. Several topics of plant breeding research in an agricultural and environmental context are included, such as:

- Evolution of plants (wheat and kale)
- Biodiversity (potato, tomato, aubergine)
- Mendelian laws (inheritance of intermediate and dominant traits Cosmea and Anthirrhinum)
- Hybrid breeding (maize and sunflower)
- Transgenic plants (Bt-maize, herbicide-tolerant maize, virus-resistant sugar beet)
- Plant diseases (different susceptible and resistant varieties)
- Renewable resources (fibre, oil, starch)
- Old forgotten species and varieties

As part of the celebrations for the Institute's 75 anniversary, we are currently enlarging the demonstration garden with ancient fruit varieties bred by MPIZ staff members in the 1950s. This was possible with the help of the gene bank for fruit trees at Dresden Pillnitz.

Oil mill at the MPIZ farm starts operating – providing a new alternative energy supply

The project "Recycling Economy with Plant Oils" completes the work of the farm "Gut Vogelsang" at the MPIZ in terms of sustainable development, and demonstrates how modern technology can be used synergetically with nature. Renewable resources, such as rape seed and other oil plants, can be used as an energy source in an eco-friendly way.

To this end, the MPIZ farm has recently invested in a new oil mill and has started to grow oil plants in its fields. It aims to use chemically untreated plant

oil as fuel for its farm vehicles. At first, in order to gain some initial experience, one transport van at the Institute's farm has been equipped before introducing it to other vehicles. In 2002, the farm harvested rape seed from five hectares. An increase in the numbers of oil plants cultivated is planned for the coming years.
The Institute´s approach to realise a model of sustainable and natural friendly agriculture and environment is also highlighted by projects such as promoting biodiversity. We therefore established several new biotopes on the Institute´s and farm's grounds. This project was funded by the Federal foundation on environment (Deutsche Umweltbundesstiftung). In addition, a co-operation with the regional water supply organisation (GEW) could be realised to minimise the leaching of nutrients and fertilisers into groundwater.

Scientific expertise for the Cologne Science Centre and Bundesgartenschau

The Cologne Science Centre is an ambitious project of the Stadtsparkasse Köln to set up a science centre focusing on modern technologies and their implications on future life. Being known for its communication activities in biotechnology, members of the Institute (Heinz Saedler and PRAG) were selected to be on the scientific advisory board of the Cologne Science Centre in 2001. Together with a science journalist, we developed a concept on genetic engineering in agriculture. To date, the topics have been converted into a scenography for the exhibition. In addition, the PRAG was asked to advise and participate in the Bundesgartenschau (Federal garden show) in Munich in 2005 and in the Landesgartenschau Nordrhein-Westfalen in Leverkusen 2005.

1928 – 2003: 75th anniversary of the MPIZ

This year, the MPIZ is celebrating its 75th anniversary with a range of activities with an open day, a special week for schools, a maize maze, and of course, a science week including the international symposium "Genetic Systems and Plant Breeding" along with its traditional celebration ceremony. The anniversary is accompanied by a festschrift comprising various scientific periods of the Institute and giving a glimpse into its future.
The implementation of all these celebratory activities demands enormous effort and input from all Institute members, who in addition to their daily work, have this extra workload which is subject to tight financial restraints.

Further activities of the MPIZ

Being known as a leading research institution in plant molecular biology, the Institute is an active member of various committees and organisations, such as the European Plant Science Organisation (EPSO) and the European Federation of Biotechnology (EFB). On a national level, it is represented in the relevant working groups like "BioGenTec NRW", "Wissenschaftlerkreis Grüne Gentechnik" and "Kölner Transferrunde der IHK". Furthermore, the Institute is consulted, for instance, in preparing the mode of operation of the "Gentechnikrecht" (requirements for greenhouses to cultivate transgenic plants, field trials and statements with regard to amendment of the Gentechnikgesetz).

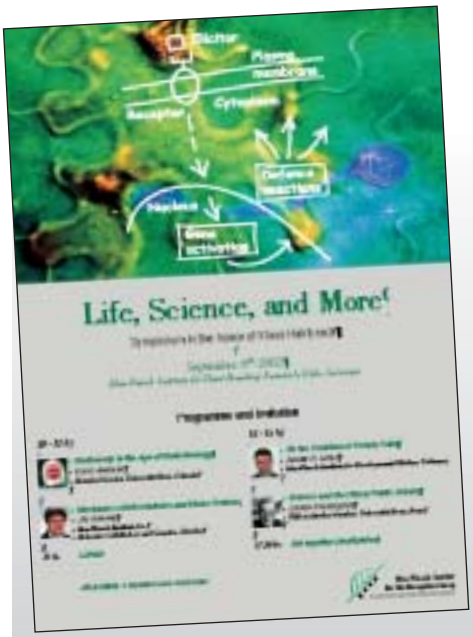
Minister of the Basque Country visits the Institute

The Institute´s reputation as an international leading research centre on plant molecular biology and plant breeding involves several requests from organisations, such as ministries and offices from abroad to visit the MPIZ. In general, the Institute serves as a contact partner to discuss topics like networks between research institutions, industry and the government in plant breeding. In this respect, the visit of the minister of the Euskadi

(Basque Country), Spain, was targeted to intensify relations between research groups in the Basque Country and the Institute.

Life, sciences, and more - Scientific meetings and seminars at the MPIZ

Seminars, symposia and conferences are without doubt excellent occasions to deepen knowledge in current scientific research areas. They also promote personal contact and act as source for new research ideas and co-operations. Besides hundreds of high ranking invited speakers (see seminar list) the Institute regularly organises symposia and conferences. One highlight was the symposium "Life, Sciences, and More" which was organised in September 2002 to honour Prof. Klaus Hahlbrock´s retirement. A few months later, internationally leading scientists in Plant Molecular Biology met at the Institute on the occasion of the symposium "Genetic Mechanisms of Phenotypic Variation in Plants" which was organised to identify a possibly successor for Prof. Franceso Salamini.



Publications directed at the general public:

Saedler, H., and W. Schuchert: Gentechnisch veränderte Pflanzen Erwartete Vorteile-denkbare Risiken. 28. Aachener Textiltagung „Zukunftsweisende Konzepte für Fertigung und Produktion" Herausgeber: Deutsches Wollforschungsinstitut an der RWTH Aachen e.V., Veltmannplatz 8, 52062 Aachen. Redaktion: Dr. Brigitte Küppers (2002).

Saedler, H., and W. Schuchert: Denkbare Risiken durch den Anbau gentechnisch veränderter Pflanzen. In: Biotechnologie als interdisziplinäre Herausforderung Deutsche Bundesstiftung Umwelt. Herausgeber: S. Heiden, C. Hertel und R. Erb, Spektrum Akademischer Verlag, S. 244-258, ISBN 3-8274-0893-8 (2001).

Deutsche Forschungsgemeinschaft: Gentechnik und Lebensmittel. Mitteilung 3. Herausgeber: Senatskommission für Grundsatzfragen der Genforschung. WILEY-VCH Verlag GmbH, ISBN 3-527-27217-8 (2001).

Benner S.: Germany. In: Biotechnology, Educating the European Public, European Project, Final Report, http://www.boku.ac.at/iam/ebe/finalreport.htm

IT facilities at the MPIZ

The Service Unit SUSAN (scientific user support - system administration - networking) is responsible for the maintenance of all computing facilities of the MPIZ. To manage user requests, SUSAN provides a request service containing a database. This database is frequently used and generally, we have between 100 and 200 inquiries per month. Currently, we are working on a tender in co-operation with the general administration of the Max Planck Society and other Max Planck Institutes located in the region. This will not only accelerate the ordering of PCs but also decrease the hardware variety within and between the institutes.

The current situation concerning the local area network and central storage facilities corresponds to the standard as set out by the Max Planck Society. Hence, we have structured cabling, i.e. GBit Backbone (fibre optic) and 100 MBit (twisted pair), to the labs and offices, along with a wireless local area network in the greenhouses and foyer of the lecture hall. A Cisco 7206 functions as the gateway. The band width is 34 Mbit per second and the transfer rate to the Institue is 1380 Gbyte per month.

The availability of data and network services not only from within the Institute but also from outside is becoming increasingly important for researchers. Therefore, to keep up with this demand, a dial-in service via ISDN cards or analogue modems within Germany will soon be established.

We have about 10 TByte of central storage for the whole Institute. Each department has about one TByte and the rest is shared by service units like ADIS, CeMic or SUSAN as well as the administration. An increasing amount of disk space is also required and is being provided for scientific program packages like GCG or Staden and the corresponding databases like GenBank, EMBL etc.

For our servers, we use Alpha and Intel hardware architecture running true64, Linux and Windows (for our Windows 2000 domain). Our most powerful computer for sequence analysis is a 4 processor ES40 DEC alpha with one GByte memory. In the near future, we will be replacing this computer with a more powerful one.

Last year, for the first time, SUSAN engaged a trainee and the work experienced gained will contribute as part of 3 years' training towards a qualification in IT. This year SUSAN will also take on a second trainee. ■

Library Service

The library services are being increasingly shaped by the standards of the electronic world. The number of electronic journals available has steadily increased over the years and currently, we retrieve about 2600 articles a month. This translates into about 80 articles per day being downloaded from the Internet. If articles are not present on the web nor directly available from the library, we ensure to obtain them as quickly as possible. Scientists and researchers rely on this just-in-time availability of scientific information and this is why the library is spending vast sums of money on improving electronic access.

This year, the library has undergone extensive reorganisation and renovation with a large part of the printed issues being put in storage to make room for computer work stations. The library now provides five PC work stations in addition to the traditional reading and working areas. Also, there are a number of workspaces equipped with internet access for those who wish to use their own laptop.

Although the time has not yet come for our library to do without printed issues of journals, we are clearly focussed on the electronic supply of articles (and databases) as the major source of information for our researchers and scientists. The implementation of these changes calls for a high degree of co-operation between the Max Planck libraries, so that improved and optimal terms and conditions can be negotiated with the information providers.

Furthermore, all libraries within the Max Planck Society are currently participating in a project to bring together their information supplies into a joint virtual catalogue and to optimise access to all kinds of information, be it conventional or electronic. This is and will be the objective of all Max Planck libraries in the future. ■

Seminars

at the MPIZ, April 2001-June 2003

2001			
04-04	**Martin Hülskamp**	**Pattern formation and cell morphogenesis: trichomes in Arabidopsis as a model system**	Universität of Cologne, Cologne, Germany
04-09	**Hanjo Hennemann**	**Studying protein-protein interactions with the Ras-Recruitment System**	University of Essen, Essen, Germany
04-11	**William Martin**	**Gene transfer from organelles to the nucleus: How much what happens and why?**	University of Düsseldorf, Düsseldorf, Germany
04-18	**Sacco de Vries**	**Functional and biochemical characterization of the Arabidopsis LRR-RLK AtSERK1**	Agricultural University Wageningen, Wageningen, The Netherlands
04-24	**Graham Moore**	**Polyploidy, chromosome pairing and evolution in the cereals**	John Innes Centre, Norwich, U.K.
04-25	**Christiane Gatz**	**Salicylic acid inducible transcription in tobacco: the role of transcription factors TGA2.1 and TGA2.2**	Georg August University, Göttingen, Germany
04-27	**Jane Glazebrook**	**Combining genetics and genomic analysis to understand disease resistance in plants**	Novartis Agricultural Discovery Institute, San Diego, USA
05-02	**Mike Bevan**	**Sequence and analysis of the Arabidopsis genome**	The Cambridge Laboratory, Norwich, U.K.
05-09	**Thomas Mitchell-Olds**	**Functional evolutionary genetics of Arabidopsis and Arabis**	Max Planck Institute for Chemical Ecology, Jena, Germany
05-21	**Ottoline Leyser**	**Hormonal control in Arabidopsis thaliana**	Department of Biology, University of York, Heslington, U.K.
05-22	**Anne-Claire Cazale**	**Molecular analysis of plant heavy metal tolerance and accumulation: Phytochelatin synthase genes in Arabidopsis**	Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany
05-22	**Laurent Noel**	**Identification of virulence factors secreted by Xanthomonas campestris pv. vesicatoria type III secretion system using a cDNA-AFLP approach**	Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany
05-23	**Wilhelm Gruissem**	**Arabidopsis RBR and FAS proteins – Integration of cell cycle regulation and development**	Swiss Federal Institute of Technology, Zürich, Switzerland
05-25	**Xinnian Dong**	**Regulation of systemic acquired resistance in Arabidopsis**	Duke University,Durham, USA
06-06	**Wolf Frommer**	**Intercellular transport of sucrose transporters**	University of Tübingen, Tübingen ermany
06-12	**Guido Van den Ackerveken**	**Genetic analysis of disease susceptibility in the Arabidopsis thaliana - Peronospora parasitica interaction**	University of Utrecht, Utrecht, The Netherlands
06-13	**Eberhard Schäfer**	**Analysis of phytochrome signal transduction**	University of Freiburg, Freiburg, Germany
06-18	**Wendy Gerber**	**A novel strategy to identify proteins required for enhancer function in Drosophila**	Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
06-18	**Claus-Peter Witte**	**Urease: a housekeeper with many surprises**	Scottish Crop Research Institute, Invergowrie, U.K.
06-20	**Thomas Boller**	**PPP – peptide perception in plants**	University of Basel, Basel, Switzerland
06-25	**Elliot Meyerowitz**	**The difference between plant and animal development: a view from the Arabidopsis genome**	California Institute of Technology, Pasadena, USA
06-29	**Christine Rusterucci**	**Genetic Interactions between EDS1, PAD4, NDR1, NPR1 and SA with LSD1, a negative modulator of plant cell death**	John Innes Centre, Norwich, U.K.
06-29	**Lucia Jorda Miro**	**Investigating the functions of EDS1 and PAD4 in plant systemic immunity**	John Innes Centre, Norwich, U.K.
07-04	**Dani Zamir**	**QTL Genes in Plants**	The Hebrew University of Jerusalem, Jerusalem, Israel
07-11	**Elisabeth Knust**	**Protein scaffolds and cell polarity in Drosophila**	University of Düsseldorf, Düsseldorf, Germany
07-24	**Julia Kehr**	**From plants to cells: celltype specific analysis of intact plants under different environmental conditions**	Max Planck Institute of Molecular Plant Physiology, Golm, Germany
07-25	**Steven Spiker**	**The nuclear matrix, MARs and transgene expression in plants**	North Carolina State University, Raleigh, USA
08-22	**Michael Snyder**	**Large-scale analysis of the yeast genome and proteome: a tale of two chips**	Yale University, New Haven, USA
09-11	**Manfred Heinlein**	**TMV as a model to study RNA transport in plants**	Friedrich Miescher-Institute, Basel, Switzerland
09-12	**Edward Farmer**	**Cyclopentenone jasmonates as signals in plant defense**	University of Lausanne, Lausanne, Switzerland
09-18	**Karl Schmid**	**Evolutionary genomics in Arabidopsis: Investigating the roles of mutation, selection and drift**	Max Planck Institute for Chemical Ecology, Jena, Germany
09-19	**Ueli Grossniklaus**	**The role of genomic imprinting in seed development**	University of Zürich, Zürich, Switzerland

09-24	**Mark Estelle**	**SCF-TIR1, a ubiquitin-protein ligase required for auxin response in Arabidopsis**	The University of Texas at Austin, Austin, USA
09-27	**Peter Oefner**	**Ways and means of dissecting genetic traits**	Stanford Genome Technology Center, Palo Alto, USA
10-08	**Thomas Merkle**	**Nucleo-cytoplasmic partitioning of regulatory proteins: a novel approach to plant signal transduction**	University of Freiburg, Freiburg, Germany
10-10	**Ben J.G. Scheres**	**Control in cell division and cell fate in the Arabidopsis root**	University of Utrecht, Utrecht, The Netherlands
10-23	**Toshiyuki Nagata**	**The presence of a signalling pathway that perceives pH change of plant cells**	University of Tokyo, Tokyo, Japan
10-25	**Maria C. Albani**	**Seasonal flowering in the perennial cycle**	The University of Reading, Reading, U.K.
10-26	**Morten Petersen**	**Suicide in plants: characterization of the Arabidopsis acd11 mutant**	University of Kopenhagen, Kopenhagen, Denmark
10-31	**Rudi Grosschedl**	**Wnt and SUMO in LEF1-mediated transcription and organogenesis**	University of Munich, München, Germany
11-06	**Klaus Harter**	**Plant response Regulators: signal transduction elements with versatile functions**	University of Freiburg,Freiburg, Germany
11-07	**Ralf Reski**	**Physcomitrella: development, cell biology, functional genomics**	University of Freiburg,Freiburg, Germany
11-12	**Thomas Kretsch**	**Spotlights on phytochrome A signal transduction**	University of Freiburg,Freiburg, Germany
11-13	**Thomas Debener**	**Gene for gene interactions in rose pathosystems and their utilization for molecular breeding**	Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Ahrensburg, Germany
11-13	**Seth Jon Davis**	**Integration of plant signaling systems: Light, the clock, hormones, and flowering**	University of Warwick, Coventry, U.K.
11-14	**Anthony Trewavas**	**Aspects of plant intelligence**	University of Edinburgh, Edinburgh, U.K.
11-20	**Andreas Bachmair**	**Ubiquitin-dependent proteolysis in Arabidopsis**	University of Vienna, Vienna, Austria
11-21	**Peter Uetz**	**Systematic two-hybrid screens in yeast: from proteomes to protein domains**	Forschungszentrum Karlsruhe, Karlsruhe, Germany
11-22	**Fred Rook**	**Integration of carbohydrate and plant developmental signalling pathways**	John Innes Centre, Norwich NR4 7UH, U.K.
11-28	**Catherine Curie**	**Iron transport in Arabidopsis**	Institut National de la Recherche Agronomique, Montpellier, France
11-29	**Thomas Pfannschmidt**	**Redox-control of plant gene expression**	University of Jena, Jena, Germany
12-04	**Kim Hammond-Kosack**	**Beware: Your bread and beer are under attack from fusarium**	Monsanto UK Ltd. The Maris Centre, Trumpington, Cambridge, CB2 2LQ, U.K.
12-05	**Witold Filipowicz**	**Protein and ribonucleoprotein factors involved in ribosome biogenesis in eukaryotic cells**	Friedrich Miescher Institute, Basel, Switzerland
12-11	**Janine Gielbert**	**Monolithic supports for protein immobilization and on-line mass spectrometric analysis**	University of Manchester, Manchester, U.K.
12-12	**John Turner**	**Defence signalling in Arabidopsis**	School of Biological Sciences, Norwich, U.K.
12-19	**Göran Sandberg**	**Auxin-cytokinin interactions in plants**	The Swedish University of Agricultural Sciences, Umea, Sweden
2002			
01-09	**Brent D. Mishler**	**Green plant phylogeny and prospects for comparative genomics: an example from desiccation-tolerance**	University of California, Berkeley, USA
01-15	**Adriana Cabral**	**SPRP4, a novel human cornified envelope precursor: UV dependent epidermal expression and selective incorporation into fragile envelopes**	Laboratory of Molecular Genetics, Leiden University, Leiden, The Netherlands
01-16	**Anna Haldrup**	**The function of individual PSI subunits detected in transgenic plants**	The Royal Veterinary and Agricultural University, Copenhagen, Denmark
01-23	**Teemu Teeri**	**Flower development in Gerbera - what can we learn from the sunflower family?**	Vikki Biocenter, Helsinki, Norway
01-25	**Markus Pauly**	**Plant cell walls: biosynthesis, structure, and function**	MPI for Molecular Plant Physiology, Golm, Germany
01-30	**Kurt Fellenberg**	**Storage and analysis of microarray data**	DKFZ, Heidelberg, Germany
01-31	**Mike Thomashow**	**Molecular switches that regulate environmental stress tolerance in plants**	Michigan State University, East Lansing, USA
02-05	**Helle Ulrich**	**The role of the ubiquitin system in DNA damage repair**	MPI Terrestrial Microbiology, Marburg/Lahn, Germany
02-06	**Christiane Nawrath**	**Salicylic acid-induction deficient (sid) mutants of Arabidopsis and the role of cutin in the cuticle**	University of Fribourg, Fribourg, Switzerland
02-20	**Jürgen Soll**	**Protein transport into chloroplasts**	Ludwig Maximilians University, Munich, Germany
02-26	**Robbie Waugh**	**Physical mapping technologies for large genome crop plants**	Scottish Crop Research Institute, Invergowrie, U.K.
03-06	**Ferenc Nagy**	**The role of nucleo/cytoplasmic partitioning of photoreceptors in phototransduction**	Agricultural Biotechnology Centre, Gödöllö, Hungary
03-12	**Frank van Breusegem**	**Catalase deficient tobacco plants : tools for in planta studies on the role of hydrogen peroxide in plant cell death**	University of Gent, Gent, Belgien
03-12	**Katrien Devos**	**Comparative genome analysis across the grass family**	John Innes Centre, Norwich, U.K.
03-12	**Jeff Bennetzen**	**The evolution of genome structure and gene function in plants**	Purdue University, West Lafayette, USA
03-18	**Daniel Klessig**	**Nitric oxide- and salicylic acid-mediated Signal transduction in defense responses to pathogens**	Boyce Thompson Institute for Plant Research, Ithaca, USA

03-20	**Alan Jones**	**Multiple signal coupling by Arabidopsis heterotrimeric G protein**	The University of North Carolina at Chapel Hill, Chapel Hill, USA
03-21	**Waltraud Schulze**	**Interactions between plant sucrose transporters in the plasma membrane detected by the split-ubiquitin system**	Universität Tübingen, Tübingen, Germany
03-27	**Salome Prat**	**Function of the U-box/arm-repeat protein PHOR1 in GA signalling**	Centro de Investigacion y Desarrollo-CSIC, Barcelona, Spain
04-03	**Jerôme Giraudat**	**Abscisic acid signalling in Arabidopsis seeds and stomatal guard cells**	Institut des Sciences Végétales, Gif-sur-Yvette, France
04-24	**Mark Stitt**	**Sense from Antisense? What do we learn after systematically decreasing the expression of most of the enzymes in primary photosynthetic metabolism?**	Max Planck Institute of Molecular Plant Physiology, Golm, Germany
05-08	**John Walker**	**Functional analysis receptor protein kinase signalling pathways in Arabidopsis thaliana**	University of Missouri, Columbia, USA
05-10	**Rod King**	**The search for "florigen(s)" in plants: gibberellins and flowering**	Commonwelth Scientific & Industrial Research Organisation, Canberra, Australia
05-15	**Mohamed A. Marahiel**	**Multimodular biocatalysts for natural products assembly**	Philipps Universität Marburg, Marburg, Germany
05-22	**Matthias Wilm**	**functional proteomics in molecular biology**	European Molecular Biology Laboratory, Heidelberg, Germany
05-27	**Chris Somerville**	**Genetic dissection of cell wall structure and function**	Carnegie Institution of Washington, Stanford, USA
05-29	**Toshiya Muranaka**	**Toward understanding comprehensive regulation of the mevalonate pathway based on the function of HMG-CoA reductase**	RIKEN Institute, Saitama, Japan
05-31	**Mohammed Bendahmane**	**Beyond petal identity genes: Identification of petal specific genes and characterization of their involvement in petal development and senescence**	Institut National de la Recherche Agronomique, Lyon, France
06-05	**Ortrun Mittelsten-Scheid**	**Genes and genetics of transcriptional silencing**	Friedrich-Miescher-Institut for Biomedical Research, Basel, Switzerland
06-13	**Ute Vothknecht**	**Biogenesis and evolution of thylakoid membranes**	Ludwig-Maximilian Universität München, Munich, Germany
06-14	**Michael Lenhard**	**Regulation of cell identity in the shoot meristem by the homeobox genes WUSCHEL and SHOOTMERISTEMLESS**	Universität Freiburg, Freiburg, Germany
06-17	**Volker Wollscheid**	**Ciphergen ProteinChip® Technology - Accelerating the discovery and characterisation of proteins**	CIPHERGEN Biosystems Ltd., Guildford, U.K.
06-17	**David Laurie**	**Genes regulating flowering in barley**	John Innes Centre, Norwich, U.K.
06-18	**Mark Wilkinson**	**Defragmenting biological information through BioMOBY**	Plant Biology Institute, Saskatoon, Canada
06-19	**Albert Heck**	**The use of proteomics in studying protein machineries and cell proliferation**	Utrecht University, Utrecht, The Netherlands
06-21	**Farhah Assaad**	**Polarised secretion and the regulation of plant growth**	Carnegie Institution, Department of Plant Biology at Stanford, Stanford, USA
06-26	**Christian Schwabe**	**Evolution and the biological invariance principle**	University of South Carolina, Charleston, USA
07-03	**Christine Foyer**	**The role of Vitamin C contents in the modulatation of plant defence and development**	IACR-Rothamsted, Rothamsted, U.K.
07-04	**Koji Goto**	**Molecular functions of two TERMINAL FLOWER genes of Arabidopsis**	Research Institute for Biological Sciences, Okayama, Japan
07-09	**Jian-Kang Zhu**	**Cell signalling under salt, drought and cold stress**	University of Arizona, Tucson, USA
07-10	**Sophien Kamoun**	**Functional genomics of phytophtora-plant interactions**	Ohio State University, Wooster, USA
07-12	**Carl J. Douglas**	**A journey from phenylpropanoid metabolism to root develpment**	University of British Columbia, Vancouver, Canada
07-12	**Carl Ramage**	**Plant regeneration and genome stability in tissue culture: surmountable challenges for plant biotechnology?**	University of Queensland-Gatton, Queensland, Australia
07-17	**Thomas Laux**	**"Pattern moving over matter": maintaining the shoot meristem organization**	Universität Freiburg, Freiburg, Germany
07-24	**Martin Müller**	**Cyclopentenone isoprostanoids induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation**	Julius-von-Sachs-Institut für Biowissenschaften, Würzburg, Germany
08-01	**John Golz**	**The ins and outs of petals and the ups and downs of leaves**	University of Edinburgh, Edinburgh, U.K.
08-30	**Viviane Jaenicke**	**Investigation of key genes in maize domestication from archaeological samples**	Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
09-18	**Martin Parniske**	**Genetics and evolution of plant root symbiosis**	The Sainsbury Laboratory, Norwich, U.K.
09-24	**Manuel Pineiro**	**The role of EBS in the repression of flowering in Arabidopsis**	Universidad Autonoma de Madrid, Madrid, Spain
10-01	**Ed Buckler**	**Candidate gene association approaches: bridging genomics and breeding**	North Carolina State University, Raleigh, USA
10-09	**Majorie Matzke**	**Epigenetic regulation of the genome by double stranded RNA**	Austrian Academy of Sciences, Salzburg, Austria
10-16	**Ralf R. Mendel**	**The molybdenum cofactor pathway and its essential role for ABA-mediated stress response and nitrogen assimilation in Arabidopsis**	Technical University Braunschweig, Germany,
10-21	**Mark Estelle**	**How auxin works and other degrading tales in plant biology**	Indiana University, Bloomington, USA
10-30	**Jonathan Howard**	**Cell-autonomous immunity in animals**	University of Cologne, Köln, Germany

11-06	**Klaus Pillen**	**Localization of favorable QTL alleles from exotic barley germplasm and their introgression into elite varieties**	University of Bonn, Bonn, Germany
11-13	**Heribert Hirt**	**From signal to form: Role of stress-induced MAP kinase in root hair formation**	University of Vienna, Vienna, Austria
11-27	**Carlos F. Barbas**	**Designed polydactyl zinc finger transcription factors for the control of endogenous genes**	The Scripps Research Institute, La Jolla, USA
12-04	**Petra Schwille**	**FCS: New concepts to probe molecular trafficking and interactions in the live cell**	Technical University Dresden, Dresden, Germany
12-18	**Christian Fankhauser**	**Signaling components implicated in phytochrome mediated light perception in Arabidopsis**	Université de Genève, Genève, Switzerland
2003			
01-08	**Francis-André Wollman**	**In vivo studies of chloroplast protein expression**	Institut de Biologie Physico-Chimique/UPR-CNRS, Paris, France
01-15	**Maria Leptin**	**Control of morphogenesis in the Drosophila embryo**	University of Cologne, Köln, Germany
01-23	**Werner Klotzbücher**	**DRITTMITTEL With a Finger in the Honeypot**	MPI for Radiation Chemistry, Mülheim an der Ruhr, Germany
02-05	**Ute Höcker**	**Fine-tuning of phytochrome A signal transduction in Arabidopsis**	Heinrich-Heine-University, Düsseldorf, Germany
02-12	**Catherine Feuillet**	**Genome organisation and evolution at disease resistance loci in wheat and barley**	University of Zürich, Zürich, Switzerland
02-19	**Holger Puchta**	**DNA recombination in plants: mechanisms, mechanics and evolutionary consequences**	University of Karlsruhe, Karlsruhe, Germany
03-05	**Rod J.Scott**	**Epigender - the epigenetic basis of gender in flowering plants**	University of Bath, Bath, U.K.
03-10	**Jen Sheen**	**Signal Transduction from Genome to Cell to Plant**	Harvard University, Boston, USA
03-19	**Karl J. Oparka**	**The search for novel proteins that interact with plasmodesmata - a novel approach using viral vectors**	Scottish Crop Research Institute, Invergowrie, Dundee, U.K.
03-26	**Michael Udvardi**	**Functional genomics of plant nitrogen acquisition**	Max Planck Institute of Molecular Plant Physiology, Golm, Germany
04-02	**Jean Christophe Glaszmann**	**A genomics-based revision of sugarcane genetics**	Université Montpellier, Montpellier, France
04-04	**Dr. Susan Kalisz**	**Evolutionary transitions in plant mating systems: Floral development & natural selection**	University of Pittsburgh, Pittsburgh, USA
04-09	**Nam Hai Chua**	**Roles of ubiquitination and sumoylation in plant signaling**	Rockefeller University, New York, USA
04-16	**Bernd Weisshaar**	**From the parsley CHS promoter to transcription factor genomics in Arabidopsis thaliana**	Universität Bielefeld, Bielefeld, Germany
04-23	**Peter Geigenberger**	**Regulation of storage starch metabolism - signals and mechanisms**	Max Planck Institute of Molecular Plant Physiology, Golm, Germany
04-30	**Nick Harberd**	**Release from restraint: DELLA protein destabilization and the growth of plants**	John Innes Centre, Norwich, U.K.
05-12	**Jeff Dangl**	**Many rivers to cross: genetic dissection and biochemical description of disease resistance gene function**	University of North Carolina, Chapel Hill, USA
05-14	**Ulrich Schurr**	**Dynamics of leaf and root growth - new insights in an old topic by digital image processing**	Juelich Research Center, Jülich, Germany
05-21	**Andrej Shevchenko**	**Functional proteomics in organism with unsequenced genomes**	Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany
05-28	**John F. Allen**	**The function of genomes in bioenergetic organelles**	Center for Chemistry and Chemical Engineering, Lund, Sweden
06-06	**Jiming Jiang**	**Structure and function of centromeres in higher eukaryotes: A rice model**	University of Madison-Wisconsin, Madison, USA
03-06	**Claus Wasternack**	**Jasmonates in plant stress responses and development - a new function for12-hydroxyjas-monic acid in flower development**	Institute of Plant Biochemistry, Halle, Germany
06-06	**Jiming Jiang**	**Structure and function of centromeres in higher eukaryotes: A rice model**	University of Madison-Wisconsin, Madison, USA
06-11	**Mathilde Causse**	**Genetic and genomic analysis of organoleptic quality components in tomato fruit**	Institut National de la Recherche Agronomique, Montfavet/Avignon, France
06-16	**Hong Ma**	**Arabidopsis microsporogenesis: the role of a receptor-linked protein kinase and a novel cyclin**	Pennsylvania State University, Pennsylvania, USA
06-18	**Sandra Knapp**	**More than just potatoes: flower and fruit diversity in the Solanaceae**	The Natural History Museum, London, U.K.