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2006

A Scientific Overview

MPIZ

Max Planck Institute for Plant Breeding Research

A Scientific Overview

Max-Planck-Institut für Züchtungsforschung

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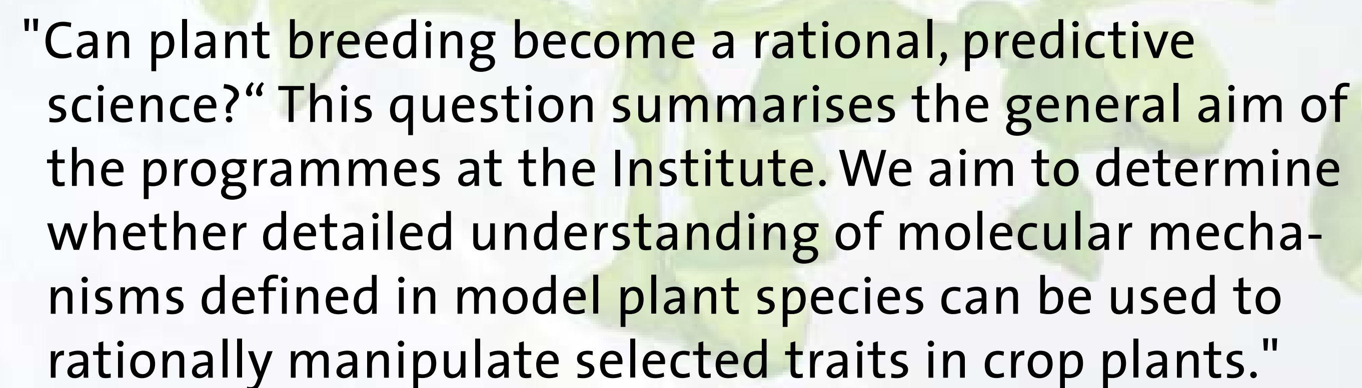


Max-Planck-Institut für Züchtungsforschung

Welcome to our Institute

We have compiled this overview to provide an introduction to the scientific work of our institute. In the following pages we summarize the organization and scientific aims of the institute as well as the projects of each research group. We have tried to present our work in a style that will appeal to a general audience as well as to scientists, and we hope that you will find the text entertaining and informative.

The compilation of the report also provides an opportunity to consider the changing face of our institute. The work of 28 research groups is described, and more than half of these groups were founded in the last five years. Moreover, the international atmosphere of the institute is underlined by the observation that around half of the scientists who lead these groups come from abroad, and from 8 different countries. In addition, the recent expansion in the number of independent research groups in the institute is represented. These three groups provide a means of establishing research areas that are not represented in the four departments, and we would like to increase the number of these groups in the future. The importance that we place in training and mentoring PhD students is also described, and we have greatly increased our activities in these areas during recent years. There are around 75 PhD students at the institute, so they represent a major fraction of the 350 staff working here. Many of these students are part of co-ordinated programmes such as the International Max Planck Research School or our EC-funded training programme, ADOPT. However, the progress of all students is followed closely by our student co-ordinator, who offers courses and advice, organizes retreats and annual student meetings.

Scientists from our institute play important roles in plant science at the national and international levels, and we are indebted to the organizations that make this possible. In particular, we have excellent connections to the University of Cologne where our PhD students are registered and with which we have many collaborative activities such as in Special Research Area grants (Sonderforschungbereiche). We are also indebted to the Max Planck Society, which provides us with an annual core grant that enables us to carry out many of our scientific activities.

We hope that whatever your background you will enjoy reading about our science in the following pages.

George Coupland
Managing Director

" A huge gap exists today between basic breeding research and practical application and the sole mission of this Institute will be to close this gap. The Institute should not compete with companies and should not develop varieties, but rather should do pioneering work and generate new technologies for breeding."

Erwin Baur (1875-1933)

Historical Background of the Max Planck Institute

The Institute was originally founded in 1928 as part of the Kaiser Willhelm Gesellschaft, and was located in Müncheberg. The founding Director, Erwin Baur, initiated breeding programmes in fruit and berries as well as basic research in *Antirrhinum majus* and on the domestication of lupins. After the Second World War, the Institute moved West to Voldagsen, and since 1955 was located in new buildings on the present site in Cologne. The modern era of the Institute began in 1978 with the appointment of Jeff Schell and the development of plant transformation technologies and plant molecular genetics. The focus on molecular genetics was extended in 1980 with the appointment of Heinz Saedler. The appointment in 1983 of Klaus Hahlbrock widened the expertise of the Institute in the area of plant biochemistry, and the arrival of Francesco Salamini in 1985 added a focus on crop genetics. During the period 1978-1990, the Institute was greatly expanded and new buildings for the Schell, Hahlbrock and Salamini Departments were constructed, as well as a new lecture hall and the Max Delbrück laboratory building that housed independent research groups over a period of 10 years.

A new generation of Directors was appointed from 2000 with the approaching retirements of Klaus Hahlbrock and Jeff Schell. Paul Schulze-Lefert and George Coupland were appointed in 2000 and 2001, respectively, and Maarten Koornneef arrived three years later with the retirement of Francesco Salamini. The new scientific departments brought a strong focus on utilising model species to understand regulatory principles and the molecular mechanisms underlying selected traits. The longer-term aim is to translate these discoveries to breeding programmes through the development of rational breeding concepts. The appointment of a new generation of Directors also required modernisation of the infrastructure. So far, this has involved complete refurbishment of the Plant Developmental Biology laboratory building (2004), construction of a new guesthouse and library (2005), planning of new buildings for the administration and technical workshops (planned completion 2007) as well as a new laboratory building for the Koornneef Department (planned completion 2009). The new laboratory building will include a communal building that will link all four scientific departments and house meeting rooms, offices and the Bioinformatics Research Group.

Aerial view - Max Planck Institute for Plant Breeding Research, Cologne

Directors at the Institute for Plant Breeding Research in Cologne since 1955

1936 - 1961	Wilhelm Rudorf
1961 - 1979	Josef Straub
1967 - 1978	Wilhelm Menke
1978 - 2000	Jeff Schell
1980 - present	Heinz Saedler
1983 - 2002	Klaus Hahlbrock
1985 - 2004	Francesco Salamini
2000 - present	Paul Schulze-Lefert
2001 - present	George Coupland
2004 - present	Maarten Koornneef

Organisation and Governance of the Institute

The Institute comprises four scientific departments, two independent research groups, four scientific service groups, the greenhouse service group, an outreach department that presents plant science to the public and press as well as the administration, which includes the technical workshops and library.
The Board of Directors is responsible for the management of the Institute. The four Directors and the Head of Administration sit on this Board which meets monthly and is chaired by the Managing Director. The Board of Directors makes decisions on matters such as how the budget of the Institute should be allocated, recruitments, promotions and the purchase of major equipment. The Board frequently invites senior scientists and/or service managers to these meetings for consultation on these issues and to obtain a broader view for decision making.
Each Director is also head of a scientific department and is responsible for the scientific programme, budget and personnel within the department. Each department contains research groups that are led by research scientists who are responsible for the scientific programme, personnel and budget of their research groups.
The service groups provide support in technical areas that underpin the work of the scientific departments. Each service group is managed by a service facility leader who is responsible for the services provided and the management of staff within the group. Each service group consults with a users committee comprising a scientist from each scientific department and the head of the service group. Each of these groups is chaired by a Director.

The Head of Administration is responsible for managing the administration department, workshops, library and security. The administration department manages issues such as appointment contracts, the budget of the Institute, the building programme, the Institute canteen, Institute housing and maintenance of the Institute grounds.
The student co-ordinator, who manages the Graduate Schools and is responsible for student welfare, is formally also part of the Administration Department.
The Senior Scientists Research Council (SSRC) is a newly founded committee that comprises the Directors of the Institute, a scientist from each department, heads of service groups and independent research group leaders. This committee does not have a Chair, but meets biweekly to discuss issues raised by its members. Major issues discussed have been future scientific strategy, purchase of large pieces of equipment and new recruitments. This committee is an important means of channelling the views of research scientists to the board of Directors, and creates a more horizontal management structure within the Institute.
The scientific programme of the Institute is assessed biannually by the Scientific Advisory Board, which reports to the President of the Max Planck Society.

The Board of Trustees meets annually and oversees the management of the Institute. The Board also provides important connections with local and national organisations within the sphere of activity of the Institute.

MPIZ Departments

Department of Plant Developmental Biology

Department of Plant Microbe Interactions

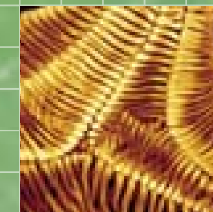
Department of Plant Breeding and Genetics

Independent Research Groups

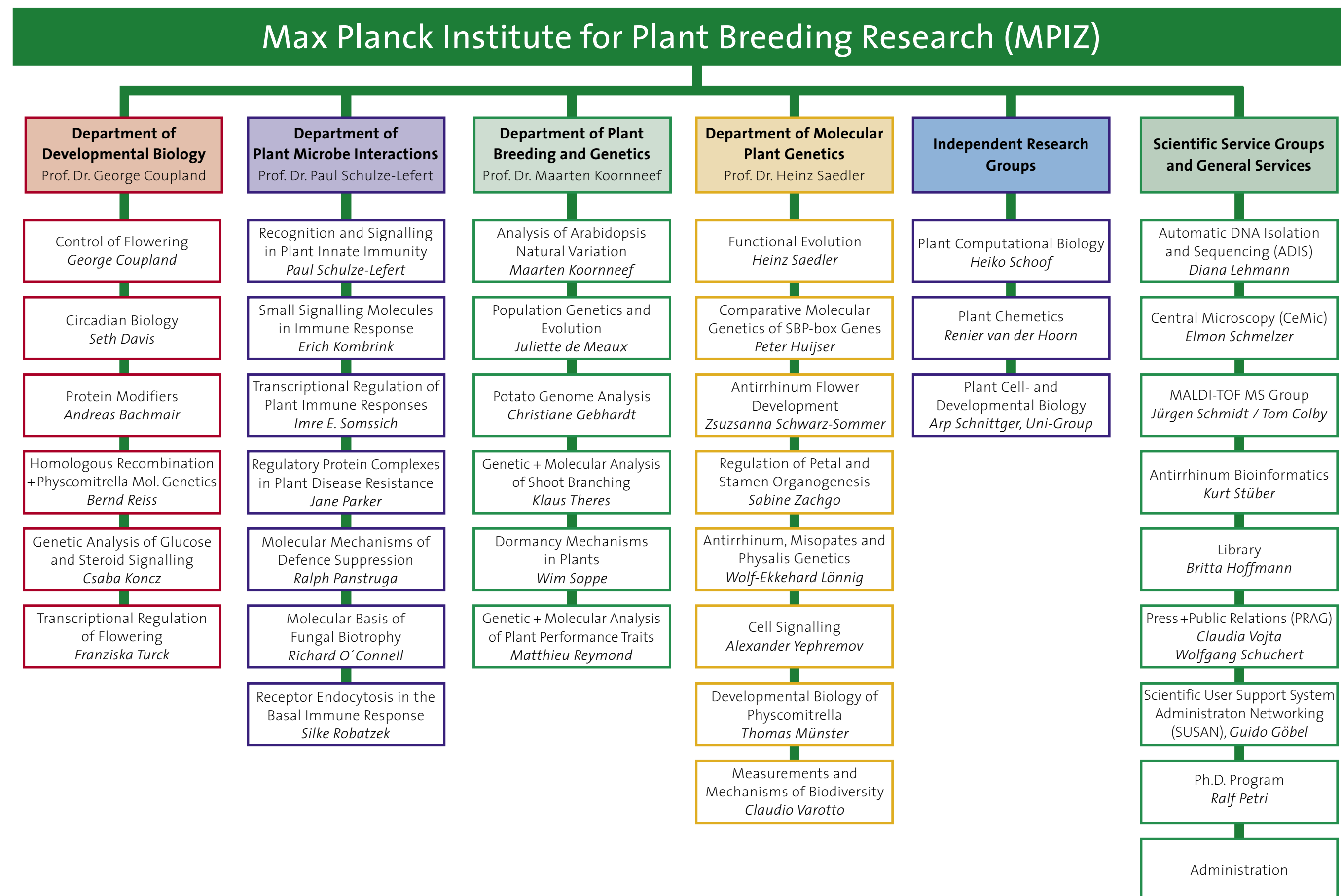
Department of Molecular Plant Genetics

Service Groups

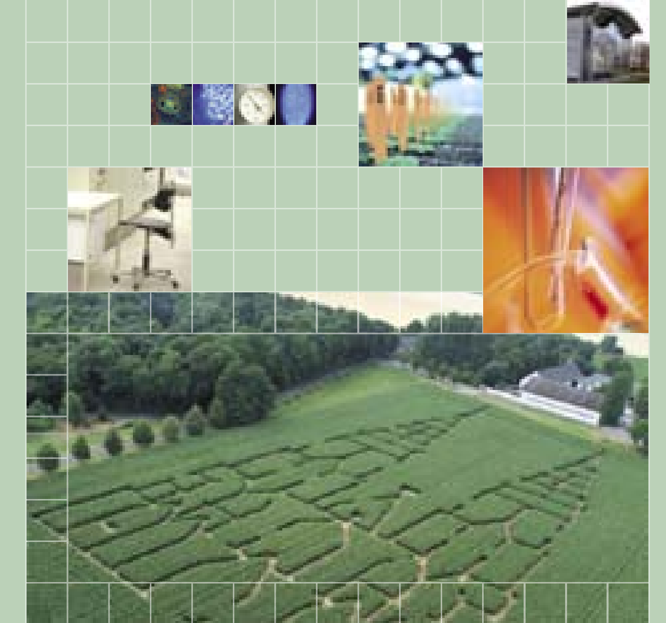
Future discovery oriented plant biology at the MPIZ will utilise integrated approaches to elucidate networks of fundamental biological processes in plants.



Organigram



Research Objectives and Major Emphasis



Background and present status

“Can plant breeding become a rational, predictive science?” This question summarises the general aim of the programmes at the Institute. We aim to determine whether detailed understanding of molecular mechanisms defined in model plant species can be used to rationally manipulate selected traits in crop plants. At present, creation of new cultivars by plant breeding is based on carefully planned genetic experiments that create new combinations of genetic information and thereby generate phenotypic variation. However, the last 10 years have seen a tremendous increase in our knowledge of the molecular mechanisms underlying plant biology. This progress is largely based on studying model species, principally *Arabidopsis*. However, the deeper knowledge of the regulatory components and mechanisms controlling plant traits that have resulted from these studies have not made a sustained impact on plant breeding. The work of the Institute aims to test whether a deeper understanding of regulatory mechanisms obtained in model species will allow rational approaches to making desirable changes in selected traits in crop plants.
Even in *Arabidopsis* our understanding of the regulatory mechanisms that control plant traits is limited to a patchwork of individual genes, and the connections between the encoded proteins are often poorly understood. Therefore, focussed programmes have been established within the Institute to understand the molecular mechanisms controlling traits of agronomic importance. These programmes are on seed dormancy, plant growth and plant architecture (Koornneef), plant pathogen interactions (Schulze-Lefert), flowering time control (Coupland) and floral development (Saedler). All of these traits are studied within a genetic framework. In addition to intensive studies based on induced mutations and reverse genetics, natural genetic variation is exploited to provide understanding of natural plasticity of complex traits through quantitative genetic variation. To study this variation in more detail, molecular-population genetics has been initiated as a field of research in the Koornneef Department. Comparative biology is also exploited to understand how genetic variation between species can alter conserved regulatory networks to create new structures or responses to the environment. Model plants are carefully chosen to provide meaningful comparisons with *Arabidopsis* or because of their specific genetic complexities (or properties) such as the tetraploid potato. Better understanding of this Darwinian variation may be the first step in allowing known regulatory networks to be manipulated in order to create traits not normally found in a particular species. Furthermore, we have increasingly complemented our genetic approaches with biochemistry and cell biology. These methods both identify proteins that are refractory to genetic approaches and reveal further layers of regulation beyond transcriptional control. We believe that integrated approaches bridging traditional research disciplines like genetics, molecular biology, biochemistry, cell biology and computational biology are key towards developing a multidimensional understanding of selected plant traits. These approaches allow detailed 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? Which of these components vary in nature and how many of them can be changed without pleiotropic effects? Can directed genetic alterations be made in crop plants to create desirable phenotypic changes in selected traits?
The Institute has developed an extensive technological infrastructure to reach these goals. In recent years, we have greatly improved our technology platforms in protein mass spectrometry and confocal microscopy. In addition, the Institute has recently improved its infrastructure for bioinformatics, annotation of genome sequences and comparisons of sequences between species with the appointment of an independent research group in this area and heavy investment in new computer infrastructure. Furthermore, the infrastructure for DNA sequencing,

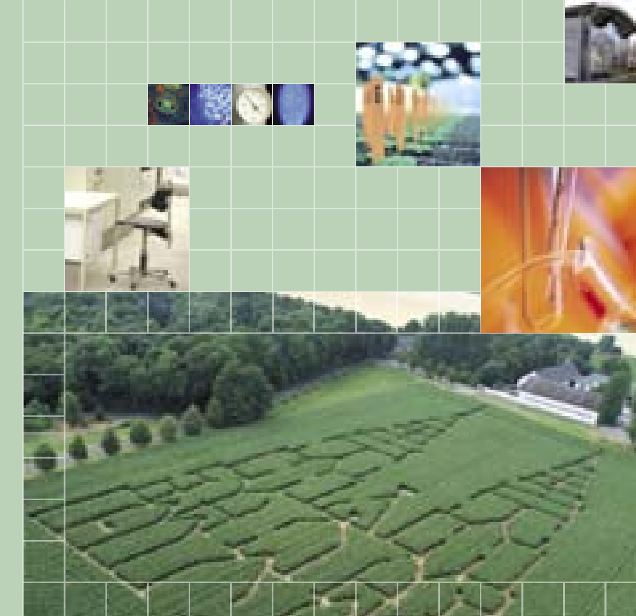
Research Objectives and Major Emphasis

arraying clone libraries and polymorphism detection has been constantly modernised. Continuous development of this complex technological infrastructure is critical for a rational understanding of plant traits in each of the four departments and provides attractive training opportunities for students that in universities are still mainly educated in a single research discipline such as genetics or biochemistry.

Future orientation

The Institute's mission requires a more concentrated and co-ordinated effort to balance research in model and crop plants in addition to the ongoing work in potato and tomato. Such a focus would allow hypotheses based on analysis of *Arabidopsis* and other models to be tested in a crop. In addition, by initiating genetic analysis and by creating characterised genetic resources directly in the crop species, this is expected to enable isolation of crop loci important for control of selected traits and to obtain insights into domestication events. To reach these goals, we intend to initiate cross-departmental programmes in barley. These would be timely, as they would be based on the improving genomic information that will soon become available for barley, and would build on expertise available in the Institute on barley transformation, genetics and plant pathogen interactions. During 2006, we intend to appoint an independent group leader that will help generate genetic material in barley. This genetic material will be used to address the selected traits presently analysed in *Arabidopsis* and to sustain a dialogue between studies in model species and crops that are being carried out in the Institute.
The Institute will also develop new approaches to better understand regulatory mechanisms in *Arabidopsis*. In the future, this will include broadening and deepening our approaches in chemical genetics. In short, this approach involves using libraries of chemical substances to impair or enhance biological processes, and subsequently utilising the compound to identify the target protein. Within the context of plant breeding, such an approach could have the advantage that the function of the chemical might be conserved in crop plants providing the opportunity to translate findings directly to studies/applications in crops. The Institute is already a participant in the Max Planck Society Chemical Genomics Centre where collaborations with other Max Planck Institutes will provide expertise in chemistry and access to chemical libraries. The appointment of the independent group of Renier van der Hoorn provides a bridge with the Chemical Genomics Centre in Dortmund, as he will also maintain a small research group there.

Co-operation, Communication and Education



Interactions within the Institute

The Departments of Plant Microbe Interactions and Plant Developmental Biology co-operated to establish the approaches of chemical genetics within the Institute. Both departments took part in collaborations within the Max Planck Society's Chemical Genomics Centre and worked closely with Professor Herbert Waldmann whose department has synthesised a number of chemical libraries. Furthermore, both departments collaborated with an industrial partner.

Co-operation with the University

Groups from the Institute take part in two Special Research Area (SFB) programmes with the University that are funded through the DFG (Deutsche Forschungsgemeinschaft). One of these programmes focuses on cellular specification (SFB 572) and the other on post-translational control of protein function (SFB 635). In total, seven research grants are funded in the Institute within these programmes. In addition, during the last year, two new SFB applications have been submitted that involve groups from the Institute. These applications focus on evolutionary biology and innate immunity to pathogens in plants and animals. The graduate schools of the institute are run as close collaborations with the University. The International Max Planck Research School and the DFG-funded Research Training Group (Graduiertenkolleg) both include faculty from the Institute and the University. These provide a forum for scientific communication as meetings between the students and faculty are held each week.

Ph.D. Program and education

The excellent education of young researchers is an important asset for the MPIZ. We support the future of plant science through well-educated young scientists with new ideas, novel concepts, uncommon approaches, creativity, and scientific curiosity that promote multilateral collaborations in an increasingly complex scientific network. Young scientists from all over the world with diverse scientific backgrounds find a research environment that supports their development as a researcher. The institute's research would not be thinkable without the contribution of the students. Moreover, the internationality of the institute offers deep insight into intercultural differences that allow the students to reconsider their position in the globalised world and to prepare for the international challenges to come.

The Ph.D. education and especially the graduate programmes IMPRS and ADOPT also promote scientific collaboration among European institutions. Training in modern plant sciences bears a component of continental importance. This is explicitly acknowledged by the European Commission among the key priorities for an anticipated European Research Area.

The research school in Cologne helps to constrain the loss of talented young scientists and the decreasing interest in plant science as a career path. This interdisciplinary approach ensures that the students not only pass a Ph.D. degree but that they also have the opportunity to learn complimentary skills that will be recognized as a true career qualification. During the entire graduate program each student receives scientific support from a Ph.D. advisory board but also general support from fellow students and scientists from neighboring disciplines. The demands of the modern scientific community are met through complementary training that comprises training in scientific communication and the preparation for an increasingly dynamic and flexible global job market.
The statistical data over the last years reveal a continuous increase in the number of applications and an extending diversity of the countries of origin of the applicants. This clearly demonstrates that the Ph.D. education at the Max Planck Institute for Plant Breeding Research is meanwhile internationally recognized and promotes the positioning of the MPIZ as an attractive institution for a qualified Ph.D. education.

Overview Department Plant Developmental Biology

Director: George Coupland

Plants thrive in locations where they are exposed to a wide variety of environmental conditions. This versatility is possible because they continuously monitor and respond to environmental stimuli such as light, temperature and nutrient availability. Such responses alter the growth habit and form of plants. We study the molecular mechanisms that alter plant development in response to environmental signals, and focus on the effect of environment on flowering. Many plants flower in response to environmental signals, and these responses adapt plants to growth at particular locations and help maximize the yield of crop plants. Our studies employ molecular-genetic, biochemical and cell biology based approaches in the model species *Arabidopsis thaliana* to investigate the roles of key regulatory proteins in flowering. Particular interests are the mechanisms by which seasonal changes in day length control flowering, the role of the endogenous circadian clock in measuring day length, how the functions of regulatory proteins are modified by phosphorylation or attachment of small proteins and the transcriptional regulation of flowering-time genes. In addition, we study how these processes are modified in other plant species to create flowering behaviours, such as perennialism, that are not shown by *Arabidopsis*.

Protein Modifiers

Andreas Bachmair

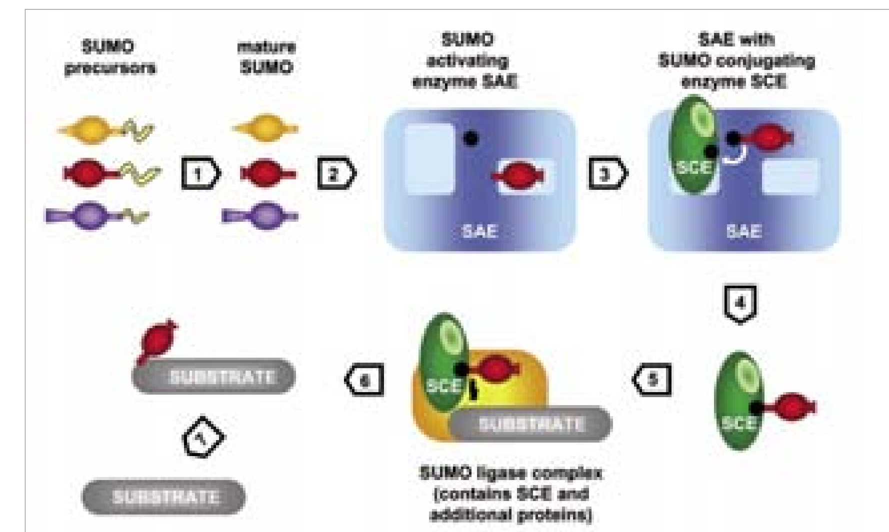
Modification of proteins after their synthesis is a recurrent theme in biology. Apart from small groups, such as phosphate or acetate, so-called modifier proteins can also be covalently attached to other proteins in a reversible manner. The reaction scheme, called conjugation, is shown for the small ubiquitin-related modifier (SUMO): SUMO proteins are synthesised as precursors. SUMO-specific proteases cleave off a small piece from the carboxyl terminus (step 1, see figure). Mature SUMO binds to SUMO activating enzyme SAE (step 2). The carboxyl terminus of SUMO forms first a thioester bond with SAE and then with SUMO conjugating enzyme, SCE (step 3). SCE carries SUMO to substrates (step 4). SUMO linkage to substrates usually requires additional proteins called ligases (steps 5 and 6). SUMO-specific proteases can restore the previous state of the substrate (step 7). While protein modifiers have been known for some time, it was realised only recently that plant cells make extensive use of conjugation reactions in their regulatory circuitry. We investigate conjugation reactions, focusing on the protein modifiers ubiquitin and SUMO.

Ubiquitin is best known for its role in degradation of cytoplasmic and nuclear proteins. Efforts to characterise novel ubiquitin conjugation reactions are concentrated in two areas. One project explores contributions of ubiquitin to plant cell death processes, using mutants and assays developed over the last five years. A second project investigates the function of so-called ubiquitin Lys 63 chains, in which several ubiquitin moieties are linked via Lys 63 of ubiquitin. To that end, enzymes involved in formation of such chains are being characterised.

SUMO conjugation (SUMOylation) is often important for association or dissociation of protein assemblages. Compared to ubiquitin transfer reactions, knowledge of SUMOylation is at an early stage. Our major efforts in this area concentrate on the development of reliable methods to identify SUMOylation targets. A procedure to enrich SUMO conjugates allowed the identification of potential substrates by mass spectrometric methods. The same procedure is also used to study the *in vivo* SUMOylation state of proteins with relevance to flower induction. Antibodies directed against candidate proteins are used to probe fractions enriched in SUMOylated proteins. A complementary route to understand protein SUMOylation uses mutants in pathway components.

Another focus is the investigation of retrotransposition. The plant retrotransposon Tto1 is analysed to understand regulatory steps of its life cycle. One goal of these investigations is the use of Tto1 for insertional mutagenesis in plants with large genomes. We have modified Tto1 so that it can be transcribed from a chemically inducible promoter. The inducible construct shall serve to further optimise transposition.

"Protein modifiers have been known for some time, but it was realised only recently that plant cells use them extensively in their regulatory circuitry."



Selected Publications

Böhmdorfer, G., I. Hofacker, K. Garber, S. Jelenic, V. Nizhynska, H. Hirochika, P. Stadler and A. Bachmair: Unorthodox mRNA start site to extend the highly structured leader of retrotransposon Tto1 mRNA increases transposition rate. RNA 11, 1181-1191 (2005).

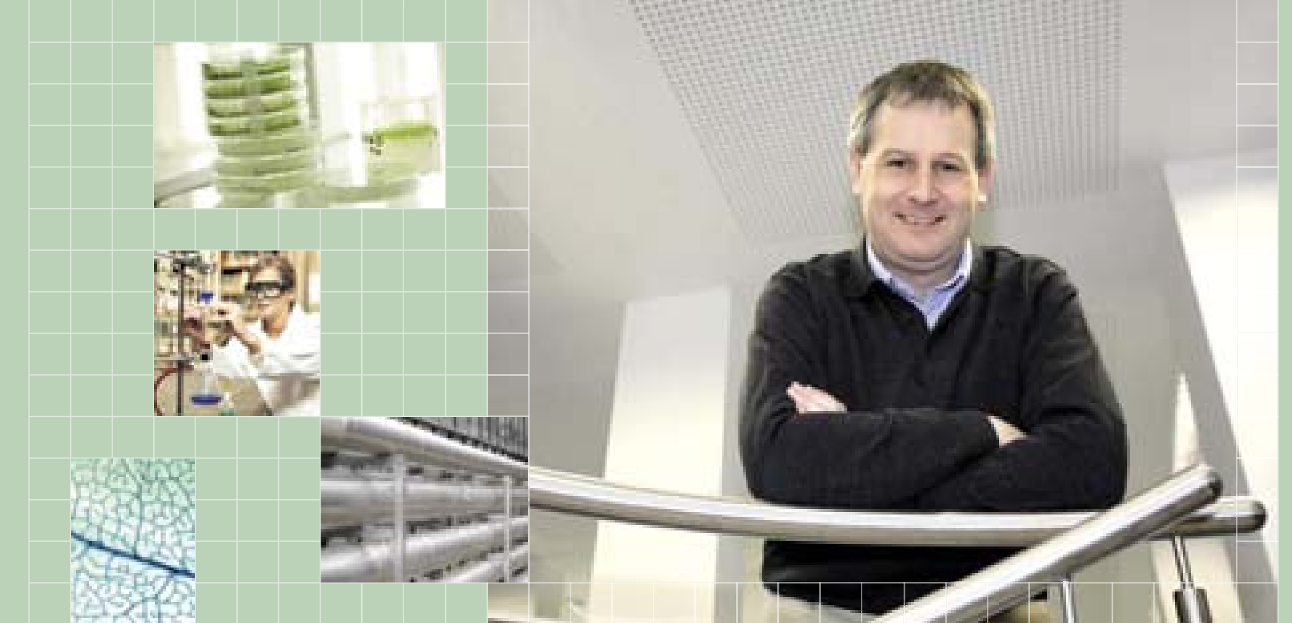
Novatchkova, M., R. Budhiraja, G. Coupland, F. Eisenhaber and A. Bachmair: SUMO conjugation in plants. Planta 220, 1-8 (2004).

Schlögelhofer, P., M. Garzón, C. Kerzendorfer, V. Nizhynska and A. Bachmair: Expression of the ubiquitin variant ubR48 decreases proteolytic activity in *Arabidopsis thaliana* and induces cell death. Planta 223, in press (2006).

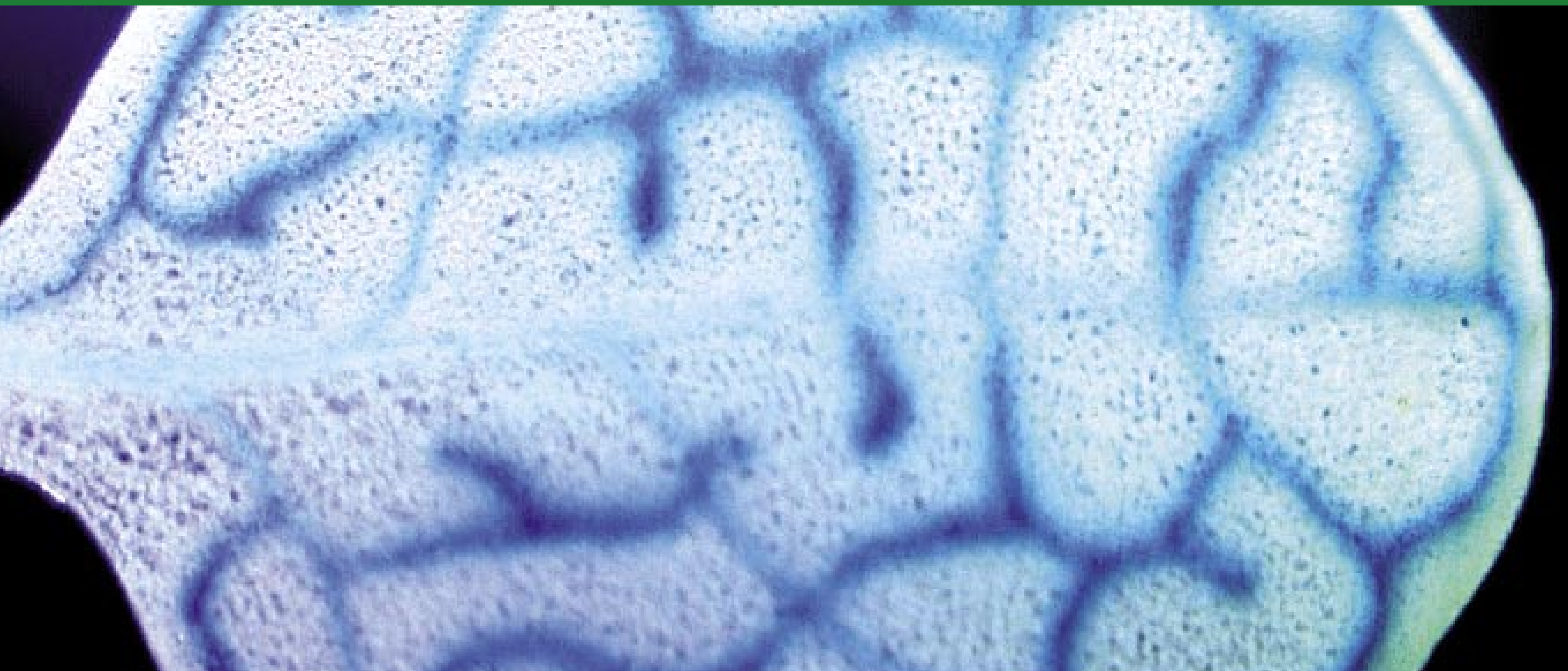
Stary, S., X.-J. Yin, T. Potuschak, P. Schlögelhofer, V. Nizhynska and A. Bachmair: PRT1 of *Arabidopsis* is a ubiquitin protein ligase of the plant N-end rule pathway with specificity for aromatic amino-terminal residues. Plant Physiol. 133, 1360-1366 (2003).

Control of Flowering:

Molecular Mechanisms and Phenotypic Diversity



George Coupland



The flowering behaviour of most plants is highly predictable. Seasonal changes in day length or temperature induce the transition to flowering at characteristic times of the year. These responses are used in agriculture so that crops flower synchronously and at the optimal time to ensure seed maturation. In contrast, in nature, there is tremendous variation in flowering time even within a single species where genetic differences between individuals can radically change flowering time. Furthermore, different species can show distinct flowering strategies, such as between annual plants that flower only once and perennial plants that flower repeatedly over many years.

We have used the model plant *Arabidopsis* to identify molecular mechanisms underlying flowering control. *Arabidopsis* plants grown under optimal summer day lengths can flower a few days after germination, but if the same plants are exposed to short winter days they take 1-2 months to flower. Similarly, winter-annual varieties of *Arabidopsis* found in the far North or at high altitude require vernalisation (extended exposure to winter temperatures) before they will flower even under summer day lengths, and these varieties are genetically distinct from summer annual varieties that do not require vernalisation. Such responses to environmental stimuli are responsible for the familiar seasonal patterns of flowering seen in nature and used in agriculture. We have defined a regulatory pathway that allows *Arabidopsis* to discriminate between long and short days, and in response to the long day signal, to trigger flowering. The difference in day length is perceived in the leaves through the regulation of the *CONSTANS* gene, and in response to the appropriate light signal, the CONSTANS protein accumulates in nuclei and activates transcription of the *FT* gene. The FT protein then activates a long-distance signal that is transported from the leaves to the apex. Within a few hours of exposure to long days, this signal has reached the apex and activates the transcription of genes that induce flower development, such as *SOC1* that encodes a regulatory protein.

We have also studied the conservation of this system in *Pharbitis nil* (Japanese morning glory) and barley and have shown that the same basic mechanisms defined in *Arabidopsis* occur in these species as well; although in *Pharbitis*, these are modified to allow the plant to flower in short autumn days.
Arabidopsis has proven to be a powerful system to study environmental control of flowering and to identify central components that regulate flowering time in annual plants. However, *Arabidopsis* lacks regulatory mechanisms that are of great significance in the plant kingdom. Of particular importance are those associated with perennialism. Whereas *Arabidopsis* and other annual plants complete their life cycle within one year and typically die soon after seed formation, perennial plants survive for many years and will often alternate periods of vegetative and reproductive development, suppressing senescence after flowering. We have developed *Arabis alpina*, a close relative of *Arabidopsis*, as a model system to study perennialism. *Arabis alpina* shows classical features of perennial plants including suppression of senescence after flowering that extends life span, a juvenile phase during which flowering will not occur even if the plant is exposed to favourable environmental conditions and conservation of meristems after floral induction, so that meristems that do not undergo flowering are available for further rounds of vegetative growth. The close phylogenetic relationship with *Arabidopsis* allows rapid isolation of flowering-time genes from *Arabis alpina* and comparison of their regulation with the defined patterns in *Arabidopsis*. Comparison of flowering behaviour in accessions of *Arabis alpina* and isolation of mutants allow testing of the importance of individual flowering genes in the perennial habit.

"We have defined a regulatory pathway that controls flowering in *Arabidopsis* and this pathway is widely conserved in flowering plants."

Selected Publications

Mizoguchi, T., L. Wright, S. Fujiwara, F. Cremer, K. Lee, H. Onouchi, A. Mouradov, S. Fowler, H. Kamada, J. Putterill and G. Coupland: Distinct roles of GIGANTEA in promoting flowering and regulating circadian rhythms in *Arabidopsis*. The Plant Cell 17, 2255-2270 (2005).

An, H., C. Roussot, P. Suárez-López, L. Corbesier, C. Vincent, M. Piñeiro, S. Hepworth, A. Mouradov, S. Justin, C. Turnbull and G. Coupland: CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131, 3615-3626 (2004).

Valverde, F., A. Mouradov, W. Soppe, D. Ravenscroft, A. Samach and G. Coupland: Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303, 1003-1006 (2004).

Hepworth, S.R., F. Valverde, D. Ravenscroft, A. Mouradov and G. Coupland: Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs. EMBO J. 21, 4327-4337 (2002).

Mizoguchi, T., K. Wheatley, Y. Hanzawa, L. Wright, M. Mizoguchi, H.-R. Song, I.A. Carré and G. Coupland: LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in Arabidopsis. Developmental Cell 2, 629-641 (2002).

The Circadian System of Plants

Seth Davis

Introduction

Plants anticipate altering daily conditions by integrating the perception of the environment to a circadian clock. Endogenous factors are likely coupled to these responses. My group uses the molecular-genetic tools available in *Arabidopsis thaliana* to probe the plant-circadian system. What good is it to molecularly understand an integrated clock? Plants relate the environment and subjective time of day to dramatically control growth and development. Thus, our circadian work provides key information into a central molecular-integration pathway that is of relevance to agriculture.

Projects

Hormonal regulation of the clock

Phytohormones and the circadian clock coordinate endogenous physiology with the external environment by what has been perceived as distinct mechanisms. This distinction was directly assesed. Phytohormones were pharmacologically tested to see whether any influences clock function. Very excitingly, many do, and do so in discrete ways. We found that cytokinins delay circadian phase, auxins regulate circadian amplitude and clock accuracy, and brassinosteroid and abscisic acid shorten circadian periodicity. We confirmed the pharmacology in hormone synthesis and perception mutants. We went on to dissect one mechanism that integrates hormone signals to the clock by showing that ARABIDOPSIS RESPONSE REGULATOR 4 (ARR4) and PHYTOCHROME B (phyB) are elements in the integration of the cytokinin signal to clock phase. The transcriptional targets of this signal were identified.

A Rosetta structural model of ELF4. Note the alpha-helical bundles "zipped" together down the central axis.

Light regulation of the clock

We previously described the *ELF4* locus as mediator of proper clock rhythmicity (Doyle *et. al*, 2002). The timing of action and the molecular-signaling network was not established from these studies. For this, we characterized the expression patterns of clock genes in various *ELF4* genotypes. It was found that *ELF4* promotes *CCA1* expression, and represses *TOC1*. *ELF4* functions at dusk in this clock modulation, and integrates the dark-to-light perception by acting on the expression of core-clock genes. ELF3 and ELF4 function as non-homologous proteins that both act at dusk to regulate *CCA1*. However, their modes of action are not known (Hanano and Davis 2005). We have assembled resources to elucidate the signaling modules of these genes. For this, a collection of *elf4* and *elf3* alleles was isolated (Figure 1). They are being characterized under a battery of assays. Knowing the amino-acid residues required for function will provide a start towards understanding activity. The approach is showing great promise as we found an *elf3* allele specifically defective in red-light signaling to the clock. To fully understand ELF3 and ELF4, we have employed computational approaches to predict their molecular structure. Combining this with phylogenetics, our group works towards a comprehensive structure-function map of the ELF3 and ELF4 family of proteins.

A screen for circadian mutants identified *TIC*. A detailed analysis revealed that its normal function is in dawn entrainment. To molecularly expand its function, we cloned the gene. The nature of the protein directs us to test whether TIC functions to de-repress proteolysis. This is plausible as TIC interacts with, and is phosphorylated by, a kinase reported to be proteosome associated. TIC may function at dawn as a light-activated entrainment factor that stabilizes morning-acting proteins within the plant oscillator. Consistent with this idea, TIC is expressed at dawn.

Future goals

Our unifying goal is to establish the molecular basis of how hormone and light factors are integrated to the clock. In the next years, we should have a firm grasp on the loops that connect hormone signaling to the clock. As well, our *ELF3* and *ELF4* genetic work on dusk perception should lead us towards establishing the biochemical activity of these gene products. For dawn detection, we imagine that TIC biochemistry should firmly establish mechanistically how this daily boundary is sensed.

"Our unifying goal is to establish the molecular basis of how hormone and light factors are integrated to the clock."

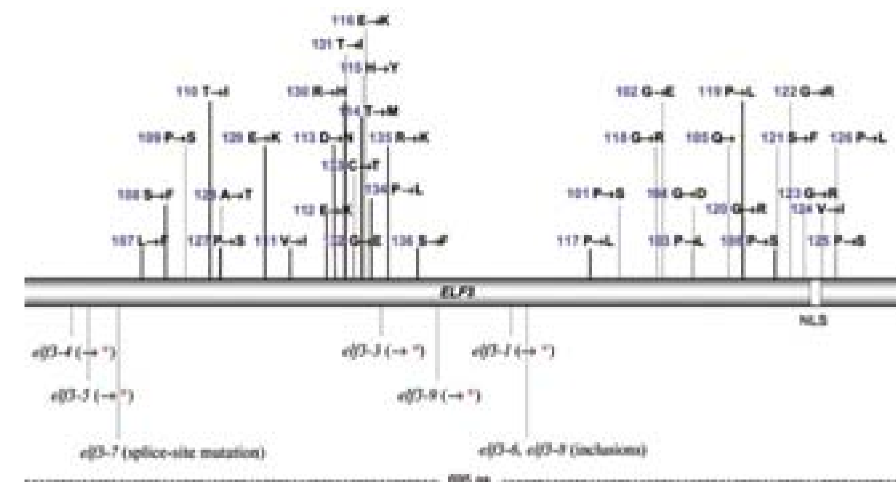


Figure 1. ELF3 TILLING Alleles.
*The ELF3 TILLING alleles are numbered 101 to 136. The relative positions of the point mutations are shown along the ELF3 protein. The locations of 9 previously published elf3 mutant alleles are also shown and, except for elf3-7, these previously characterised alleles all have a strongly reduced function of ELF3. *: Stop codon. NLS: Nuclear localisation signal (putative).*

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Hanano, S., Davis, S. J. (2005) Pseudo-response regulator genes "tell" the time of day: multiple feedbacks in the circadian system of higher plants. In: Endogenous Plant Rhythms (A. Hall and H. McWatters, eds.) Blackwell Publishing, Sheffield, UK.

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Doyle, M. R.*, Davis, S. J.*, Bastow, R. M., McWatters, H. G., Kozma-Bognár, L., Nagy, F., Millar, A. J., Amasino, R. M. (2002) The *ELF4* gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. Nature 419: 74-77. *co-first authors.

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Genetic and Biochemical Study of AMP-activated Protein Kinases

Csaba Koncz

Research

Our past research contributed to the development of several genetic approaches and technologies in plant molecular biology. We studied the function and regulation genes that are transferred by the T-DNA of *Agrobacterium* Ti and Ri plasmids into plants; analysed the mechanism by which the T-DNA is integrated into plant chromosomes; developed versatile plant transformation vectors and technologies; exploited light-emitting bacterial luciferase enzymes for visualization of gene activities in intact organisms; converted the T-DNA to a useful mutagenic agent for dissection of plant gene functions; constructed large insertion mutant collections, and characterised gene mutations influencing the activity of metabolic enzymes and regulatory proteins in the model plant *Arabidopsis* and other species. These activities led us to study hormonal and metabolic control of plant development. Our work on the *Arabidopsis cpd* mutant contributed to the recognition of essential functions of plant steroid hormones brassinosteroids (BRs); whereas, identification of the *PLEIOTROPIC REGULATOR LOCUS 1 (PRL1)* provided an insight into functions of a regulatory protein acting in the control of metabolic, stress and hormonal responses. The *CPD* gene codes for a microsomal P450-type C-23 steroid hydroxylase (CYP90A1) which fails to function in combination with known P450 electron donors. Similarity of dwarf phenotypes of *cpd* (Fig. 1) and *bri1* brassinosteroid receptor mutants suggests that CYP90A1 may also play a role in BR signalling. CYP90A1 is an extremely unstable membrane protein that can only be studied using highly sensitive molecular indicators. Therefore, to analyse the CYP90A1 membrane complex, we take advantage of a conditional *cpd* mutant complementation system using a CYP90A1 fusion with the green fluorescent protein GFP (Fig. 2). This system allows the analysis of cell autonomy and developmental regulation of the *CPD* function. The *PRL1* gene encodes a nuclear interacting partner of AMP-activated protein kinases (AMPKs) that represent key regulators of energy homeostasis and cellular responses to stress in all eukaryotes. Our main research interest is directed towards systematic analysis of AMPK-regulated nuclear factors involved in the co-ordination of metabolic, stress and hormonal regulatory pathways. Our data show that plant AMPKs are integral components of proteasomal SCF (Skp-Cullin-F-box protein) ubiquitin ligase complexes, and thus play a role in stress-mediated regulation of proteolysis. We also found that monomeric and trimeric AMPKs interact with regulatory proteins involved in chromatin remodelling and transcription. The AMPK-binding nuclear PRL1 protein is also a multi-protein interacting factor, inactivation of which leads to complex developmental (Fig. 3) and signalling defects causing hypersensitivity to, e.g. glucose and sucrose as well as plant hormones abscisic acid (ABA) and ethylene (Fig. 4). We aim to define the position and function of PRL1 in the co-regulated sugar, ethylene and ABA signal transduction pathways using a combinatorial analysis of mutations that affect subunits, substrates and interacting partners of AMP-activated kinases and signalling partners of PRL1. The major goal of our genetic studies is to functionally dissect components of the putative AMPK-PRL1 regulatory network that was identified by our previous protein interaction, biochemical and immunological studies. Purification of native and cross-linked AMPK and PRL1 complexes will assist the analysis of protein interactions by mass spectrometry. In addition, we use a conditional PRL1 genetic complementation system and inducible expression of dominantly active forms of AMPKs to classify nuclear AMPK substrates and AMPK/PRL1 regulated target genes by the analysis of nuclear phosphoproteins and changes in transcription profiles.

Figure 1. Inactivation of CYP90A1 C23-steroid hydroxylase causes a defect in brassinosteroid hormone biosynthesis leading to dwarfism in the cpd mutant.

Figure 2. Membrane localisation of unstable CYP90A1 protein using a fusion with the green fluorescent protein (GFP). Confocal microscopic fluorescent (left) and light images (right) of CYP90A1-GFP overexpressing cells.

Figure 3. Inactivation of the PRL1 nuclear regulatory protein results in severe root developmental defects. prl1 mutant root (left); wild-type root (right).

"Our main research interest is directed towards systematic analysis of AMPK-regulated nuclear factors involved in the co-ordination of metabolic, stress and hormonal regulatory pathways."

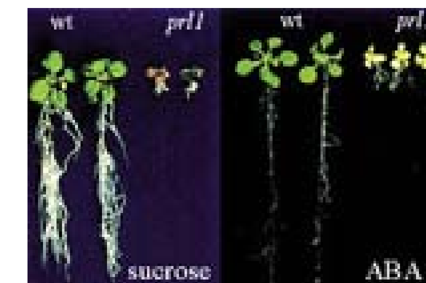

Figure 4. The prl1 mutant is hypersensitive to sucrose (accumulation of red anthocyanin pigments and growth reduction [left]) and the plant hormone abscisic acid (loss of green chlorophyll pigments in leaves [right]).

Selected Publications

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Bernd Reiss | DEPARTMENT PLANT DEVELOPMENTAL BIOLOGY

Comparative Approaches to DNA-damage Repair and Flowering Time Control in Plants

Bernd Reiss

Studies of the same process in different organisms can reveal important conserved features as well as adaptations to special functions. We focused on two model plants greatly differing in phylogenetic position, the flowering plant *Arabidopsis thaliana* and the moss *Physcomitrella patens*. While *A. thaliana* is a typical flowering plant with a complex body plan and elaborated developmental patterns, the moss *P. patens* is a lower plant with comparably simple patterns. The development of *P. patens* begins with a haploid, filamentous structure, the protonema which is characterised by one-dimensional growth. Three-dimensional structures develop later giving rise to the adult gametophyte, the moss plant with leafy shoots. These shoots produce male and female reproductive organs.

We are interested in two processes: DNA-damage repair and the control of flowering time. *A. thaliana* and *P. patens* differ fundamentally in the efficiency of gene targeting, a process generally considered to reflect the use of homologous recombination (HR) over the non-homologous end-joining pathway (NHEJ) in somatic cells, and thus a preference for error-free over error-prone DNA-damage repair. Further, although *A. thaliana* is a typical flowering plant and *P. patens* is a non-flowering plant, both undergo a transition from vegetative to generative growth. This observation suggests the presence of similar regulatory features that make this system attractive for comparative approaches of flowering-time control.

DNA-damage repair is involved in the maintenance of genome instability, and in humans, errors in this process are associated with cancer. NHEJ is considered to contribute to genome instability, whereas HR leads to precise DNA-damage repair and thus contributes to the prevention of cancer. *RAD51*, a homologue of the bacterial *RecA* gene, plays a key role in eukaryotic HR and is essential for DNA-damage repair and meiosis in yeast. In vertebrates like human, mouse and chicken, *RAD51* seems to have acquired an additional function at the interface of DNA-damage repair and cell cycle control. To analyse this process in plants with a special attention to the additional function, mutants were produced that lost *RAD51* function in both *A. thaliana* and *P. patens*. In contrast to animals, these mutants are fully viable indicating that the role of *RAD51* in plants is restricted to recombination functions. This feature is probably typical for all plants because it is conserved across a large phylogenetic distance and independent of the use of DNA-repair pathways. In addition, these data imply that plants and animals differ in important aspects in the link of DNA-damage repair to cell cycle control. However, *RAD51* has important functions in meiosis in *A. thaliana*. For the first time in a higher eukaryote, it was shown that the recognition of allelic chromosomes requires RAD51 function at the onset of meiosis, and thus, this recombinase has an essential function for the ordered inheritance of genetic material to the next generation.

The transition from vegetative growth to flowering is under environmental control in *A. thaliana*. The *CONSTANS (CO)* gene plays a central role in the pathway and promotes flowering specifically in response to long days. The *CO* pathway is highly conserved among the angiosperms and some of these genes complement the *A. thaliana co* mutation suggesting functional conservation. In *A. thaliana*, *CO* is part of a gene family with 17 members of *CO* and *CO-Like* genes. Although some of these CO-Like proteins are highly similar to CO, they have no apparent function in flowering time regulation. To address the question of the origin of *CO* and to identify features important for the regulation of flowering time, the role of *CO* in *P. patens* was analysed. Three genes with close homology to *CO* were found in the *P. patens* genome. However, although closely related to *CO*, they are more similar to a specific group of *CO-Like* genes suggesting that a genuine *CO* orthologue does not exist. Therefore, CO is likely to have acquired its specific role in flowering time after the divergence of mosses and flowering plants.

"We are interested in two processes: DNA-damage repair and the control of flowering time."

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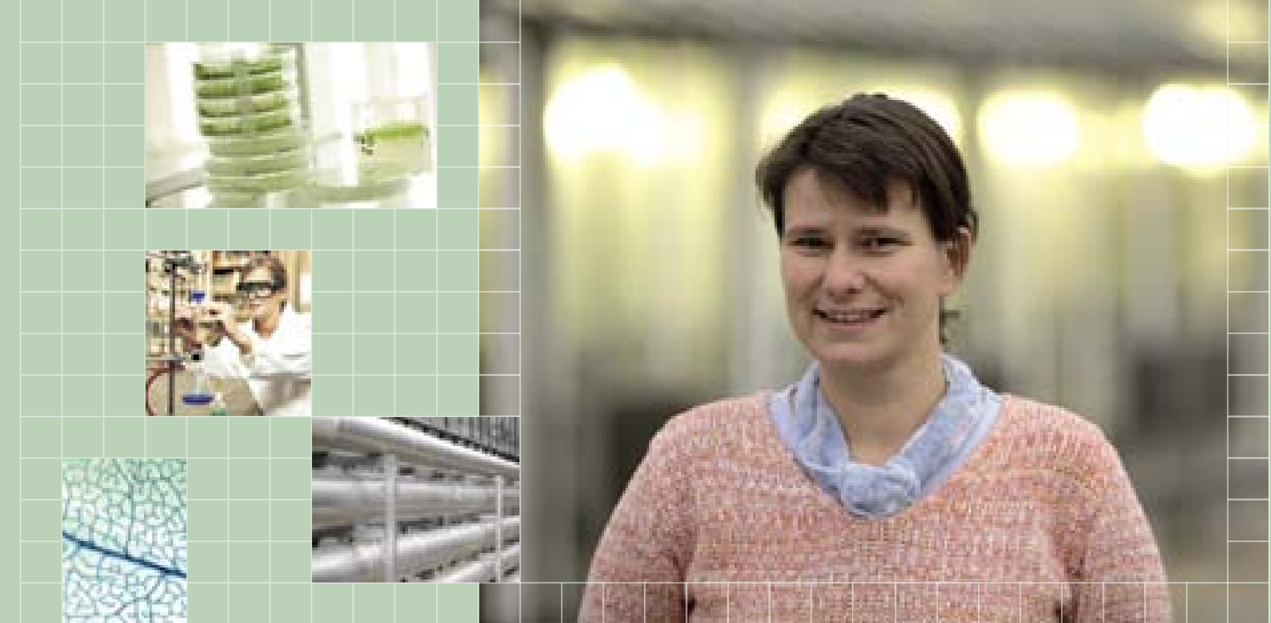
Transcriptional Networks and Mechanisms of Flowering Time Gene Regulation



Franziska Turck

Introduction

In recent years comparative genome analyses has demonstrated the importance of transcription factor evolution to the emergence of higher plants. Entire families of transcription factors with important functions in plants are absent from other branches of eukaryotic life, and most plant transcription factor families have greatly expanded during evolution of the higher plants. This complexity provides an extra challenge, in deciphering the interconnections of transcriptional networks. Clearly, the development of new tools is needed to complement genetic approaches in unravelling the components of partially redundant transcriptional networks. Our group is interested in generating new tools to enable these studies and then to applying them to further understand the transcriptional networks important in the transition from vegetative development to flowering. We are particularly interested in the transcriptional regulation of the floral integrator gene *FT* which is a convergence point of multiple signalling networks that control flowering time.

Phylogenetics – a tool to accelerate the identification of *cis*-regulatory elements

Cis-regulatory elements are short stretches of DNA of defined sequence that influence the expression level of genes because they are recognised by specific proteins, termed trans-acting factors. In the past, *cis*-regulatory elements were identified by dissecting promoter function by deletion and mutation analysis. We hope to accelerate the identification of novel *cis*-regulatory elements by phylogenetic analysis. Sequences that are not within the protein-coding sequence of genes evolve rapidly. However, regulatory sequences of orthologous genes isolated from closely related species are sufficiently conserved to be aligned and tend to form "shadows" of clustered, hyper-conserved residues. We are identifying such sequences by analyzing the promoters of flowering-time genes from members of the Brassicaceae and plan to validate the functional importance of "phylogenetic shadows" found in several promoters by applying targeted mutagenesis coupled with reporter gene and complementation approaches.

Systematic yeast one-hybrid assay - a tool to find novel *cis/trans* regulators

Knowledge of *cis*-regulatory element/*trans*-activating factor pairs is still in its infancy in plants. We have made a systematic, mating-compatible yeast collection expressing approximately 700 of the estimated 2000 *Arabidopsis* transcription factors as fusion proteins with a yeast transcriptional activator domain fusion. The library will be used to screen confirmed *cis*-regulatory elements for its cognate trans-acting factor. The systematic nature of our library will allow us to evaluate the possible redundancies within a transcription factor family. In a promoter context, transcription factors tend to bind as complexes or in a synergistic manner, possibly via recruitment to an adjacent *cis*-regulatory element within a promoter. Our systematic yeast library will also allow the testing of interactions between transcription factors in a systematic manner.

ChIP to chip – holistic validation of *in vivo* transcription factor binding sites

Only a fraction of putative transcription factor binding sites are occupied in the living cell. Often a potentially perfect binding site will not be accessible, either because the surrounding chromatin structure does not allow access to the DNA or the site is already occupied. In collaboration with two groups at the CNRS in Evry, France, we are currently developing a method that combines chromatin immunoprecipitation (ChIP) with array analysis. Two arrays are currently available allowing either comprehensive analysis of *Arabidopsis* chromosome 4 with overlapping DNA fragments or simultaneous validation of 20,000 *Arabidopsis* promoter sites. Using ChIP to chip analysis, we have started to identify *in vivo* target sites for the MADS-box transcriptional repressor FLC and the heterochromatin protein related factor TFL2, both of which have important regulatory roles in flowering time control.

"Clearly, the development of new tools is needed to complement genetic approaches in unravelling the components of partially redundant transcriptional networks."

Interplay between chromatin and transcription factors

In the past years, the relationship between the structure of chromatin and the transcriptional state of a gene has been correlated with patterns of covalent DNA and histone modifications. Transcription factors are both dependent on chromatin structure and potentially involved in the modification of chromatin via the recruitment of chromatin-modifying enzymes. We are elucidating the interplay between chromatin-modifying enzymes and transcription factors by (1) exploiting our yeast library in systematic screenings for protein-protein interactions between putative chromatin-modifying enzymes and transcription factors, and (2) characterising the mechanistic interplay between transcriptional activators and chromatin-associated repressors at the FT locus.

Selected Publications

Turck F, Zhou A, Somssich IE. Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to Its native promoter and the defense-related gene PcPR1-1 in Parsley. Plant Cell. 2004 Oct;16(10): 2573-85.

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Overview Department Plant Breeding and Genetics

Director: Maarten Koornneef

In addition to the large diversity between plant species also within species substantial genetic variation is present in nature or has been generated (selected for) by breeders and researchers. The use of molecular genetics led to improved understanding of the processes underlying plant growth and development and the interaction of plants with their abiotic and biotic environment. This knowledge provide new tools for plant breeders allowing a more efficient breeding by using genetic markers that “tag” traits of interest. For further development of these tools a more in-depth knowledge on the genes showing genetic variation for specific traits. Many of these traits for which variation is found in nature, are so-called quantitative traits due to their polygenic nature and large environmental effects on the expression of the traits. Their analysis therefore requires procedures of computational genetics such as quantitative trait locus (QTL) mapping and association mapping.

The objective of the department is to further develop knowledge on important processes determining plant growth and development including plant architecture, plant metabolism and mechanisms of developmental arrest by making use of genetic and genomic tools available and being developed, both for crop plants and models plants.

Population Genetics and Evolution of *cis*-regulatory DNA

Juliette de Meaux

The question that motivates research in my group is that of the evolution of DNA regions that regulate the first step towards protein synthesis. These regions are described as cis-regulatory regions and share the control of mRNA synthesis with *trans*-regulatory proteins. How much functional *cis*-regulatory variation is found within a species? What is its evolutionary fate? How does it participate in phenotypic evolution? My group aims to improve our understanding of DNA evolution by focusing on DNA regions which, by contrast with protein-coding regions, have not been the focus of as much interest.

The evolution of *cis*-regulatory DNA is particularly interesting to study because it is suspected to evolve faster than protein-coding DNA. Indeed, its functional architecture is based on nucleotide stretches (binding-sites) scattered throughout a background of apparently non-functional DNA. *Trans*-regulatory proteins bind specifically to binding sites and jointly regulate the expression of adjacent genes. Because these binding sites are short, numerous and often redundant, *cis*-regulatory DNA is suspected to play a predominant role in phenotypic changes.
Large-scale genomic comparisons have shown that *cis*-regulatory DNA is subject to constraints in its evolution that tend to maintain function. However, the extent to which function may be modulated in the course of evolution is not known. Because of this, the importance of cis-regulatory DNA for the generation of phenotypic novelties and for species adaptation to new environments is poorly understood. To address this issue, approaches are required that focus on functional change rather than conservation.
Previous work conducted at the MPI for Chemical Ecology in Jena has investigated both nucleotide and functional variation of a cis-regulatory region within and among three closely related species of the *Arabidopsis* genus (de Meaux *et al.* 2005, de Meaux et al. in preparation).
By combining population genetics

"*Cis*-regulatory DNA is suspected to evolve faster than protein-coding DNA."

methods with a functional assay, ecologically relevant variation in a *cis*-regulatory region was identified and its recent evolutionary fate reconstructed. The focus gene of the study was *Chalcone Synthase (CHS)*, a gene coding for the branch point enzyme of a pathway involved in the interaction between the plant and its environment.
Functional *cis*-regulatory variation for *CHS* expression in response to light and/or insect feeding was found to segregate within *Arabidopsis thaliana, Arabidopsis lyrata* and *Arabidopsis halleri*. Furthermore, large interspecific changes in *CHS cis*-regulation were detected in response to light or insect feeding. This study thus demonstrated that abundant *cis*-regulatory variation segregates *within* and *between* species and alters the expression of *CHS* in response to a variety of ecological cues (de Meaux *et al.* 2005, de Meaux *et al.* in preparation).
Yet, a population genetics analysis indicates that this variation has not been the target of selection in any of these species.
Population genetics methods examine the frequency distribution of nucleotide polymorphism and compare it to expected distributions in the absence of selection (neutral evolution). Patterns of diversity in the *CHS cis*-regulatory region were found to be consistent with neutral expectations, revealing that functional *cis*-regulatory variation does not correlate with fitness.
CHS cis-regulation thus appears to continuously drift, in an undirected manner, although the response to environmental cues is changing.
This neutral variation could, however, constitute a functional reservoir which would provide the raw material for selection to proceed and adaptive evolution to occur. This work forms the basis of the research the Population Genetics and Evolution Group is performing within the Department of Genetics and Plant Breeding. Is *CHS cis*-regulatory evolution typical of functional non-coding DNA evolution? How can we better understand the evolutionary constraints placed on *cis*-regulatory DNA? Can *cis*-regulatory changes permit the emergence of fitter, better adapted phenotypes? How do they participate in the evolution of complex phenotypic traits required for species adaptation such as seed dormancy? By addressing these and other questions related to the role of *cis*-regulatory DNA in species adaptation, we hope to unravel the evolutionary mechanisms underpinning the phenotypic diversity that is the research focus of our department.

Selected Publications

Chalcone Synthase *cis*-regulatory evolution in the *Arabidopsis genus*. J. de Meaux, U. Goebel, A. Pop and T. Mitchell-Olds (in preparation).

Allele-specific assay reveals functional variation in the *Arabidopsis thaliana* chalcone synthase promoter region that is compatible with neutral evolution. J. de Meaux, U. Goebel, A. Pop and T. Mitchell-Olds. (2005) Plant Cell, 17, 676-690.

Structure and Function of the Potato Genome

Christiane Gebhardt

Figure 1. Solanum tuberosum and Solanum nigrum, cultivated and wild Solanum species.

The group's research activities concentrate on the potato (*Solanum tuberosum*), the most important crop species of the Solanaceae family worldwide, which comprises more than 3000 species (Figure 1). The potato is most closely related to about 200 tuber-bearing *Solanum* species native to Mexico, Middle or South America, and represents a rich source of biodiversity. Potatoes have with humans in common that both are outcrossing species and that phenotypic and genotypic variation is natural. However, unlike humans, the cultivated potato is tetraploid and each heterozygous genotype is fixed by vegetative propagation.

Our long-term goals are (1) to elucidate the genetic basis of complex agronomic characters of potato at the molecular level, (2) to contribute to the better understanding of structure, evolution, function and natural diversity of crop plant genomes and (3) to contribute to the genetic improvement of the cultivated potato by developing molecular diagnostic tools to assist the breeding of new cultivars. To reach our goals, we exploit, as in human genetics, the natural diversity present in the potato species and its close relatives, and we adapt principles and approaches commonly used in human population genetics. Our outputs are (1) knowledge of genomic positions and identity of genes that control qualitative or quantitative agronomic characters, (2) DNA-based markers that can be used for marker-assisted selection of superior cultivars and (3) cloned genes and superior alleles of agronomic characters that may be transferred into cultivars by genetic engineering.

Current research areas

Late blight of potato caused by the oomycete *Phytophthora infestans* is the most important disease in potato cultivation. The combination of durable resistance to late blight with good agronomic quality is still a major target of variety development, particularly for production systems that cannot or must not use chemical control. Resistance to late blight can be classified at the phenotypic level as qualitative resistance controlled by single *R* genes or as quantitative or field resistance controlled by an unknown number of genes. Identification of the genes that control both types of resistance to *P. infestans* can reveal new strategies and diagnostic markers for molecular breeding.
We have cloned *R1*, the first gene for resistance to late blight and completed several QTL (quantitative trait locus) mapping or tagging experiments for quantitative late blight resistance (Bormann *et al.* 2004). Candidate genes for quantitative resistance loci (QRL) have been identified and a model for the molecular basis of QRL has been proposed (Gebhardt and Valkonen 2001). Natural variation at candidate loci is currently being tested for association with quantitative resistance evaluated under field conditions. For genetic material and phenotypic analysis of field resistance to late blight, we are collaborating with potato breeding companies. First associations between candidate loci and field resistance to late blight were found (Gebhardt *et al.* 2004). *R1* homologous sequences have been isolated from other *S. tuberosum* genotypes as well as other Solanum species and these are being characterised for their expression and resistance function.
Damage caused by two root cyst nematode species, *Globodera rostochiensis* and *Globodera pallida*, is relevant to middle European potato cultivation. Genetic dissection has previously shown that resistance to these nematodes is controlled by *R* genes and/or by major QTL. Recently, we cloned the *Gro1-4* gene for resistance to *G. rostochiensis* based on a candidate gene approach (Paal *et al.* 2004). The *Gro1-4* gene is one member of a gene family, which we will further characterise for gene-specific expression and function. Diagnostic PCR markers for resistance to *G. pallida* were developed that are being evaluated in collaboration with potato breeders for general applicability in marker-assisted selection. Tuber yield and tuber starch and tuber sugar content are complex quality traits, which are all functionally connected with carbohydrate metabolism. The genes and biochemical pathways for synthesis, degradation and transport of carbohydrates are among the best studied in plants. Therefore, QTL (quantitative trait loci) for tuber starch and sugar content are a model system for testing the candidate gene approach to identify genes that control complex agronomic traits. QTL for tuber starch and sugar content (cold-sweetening) have been mapped previously using molecular markers, as well as genes functional in carbohydrate metabolism and transport. A number of positional candidate genes were identified.

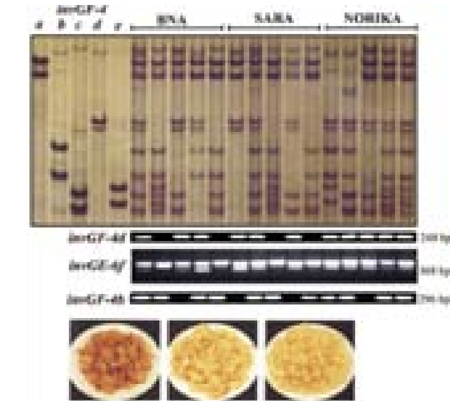

Figure 2. Alleles 4d, 6f and 4b at the invertase locus InvGE/GF on potato chromosome IX are associated with chip quality or tuber starch content of 179 tetraploid breeding clones of the potato breeding companies Böhm-Nordkartoffel Agrarproduktion (BNA, Ebstorf), Saka-Ragis (SARA, Windeby) and NORIKA (Groß-Lüsewitz). Presence of alleles 4d and 6f is associated with, on average, better chip colour (golden yellow), whereas presence of allele 4b is associated with, on average, lower tuber starch content.

These candidate genes are now being studied for natural variation associated with tuber quality traits in tetraploid potato breeding populations (Li *et al.* 2005) (Figure 2).

Selected Publications

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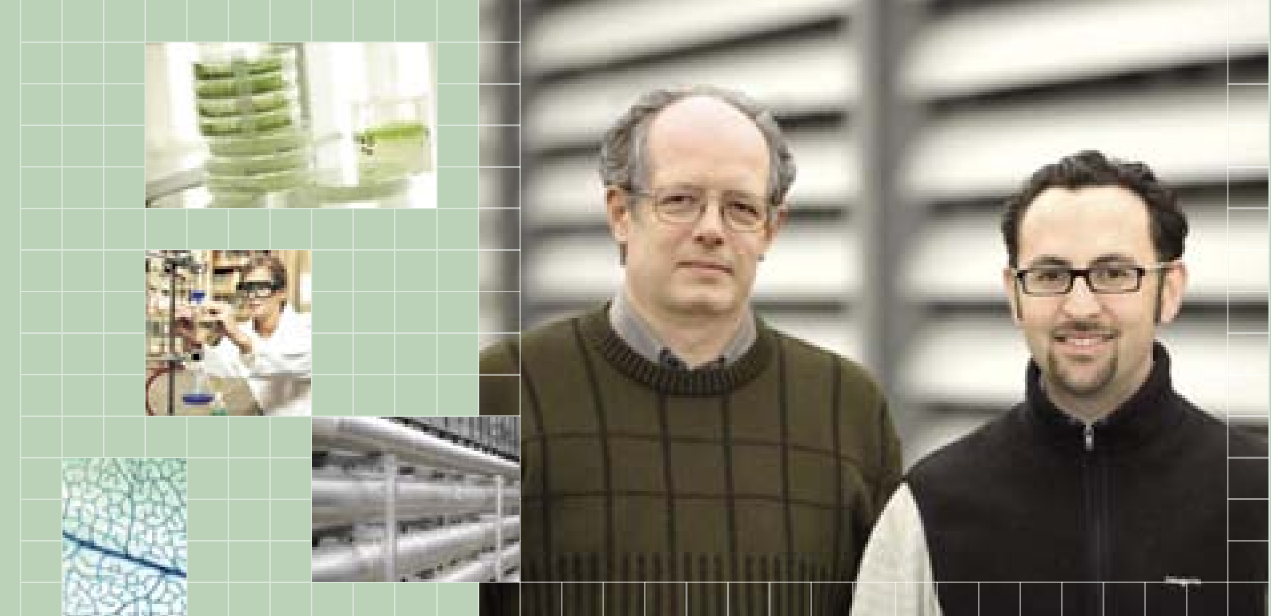
Genetics of Plant Performance



Maarten Koornneef & Matthieu Reymond

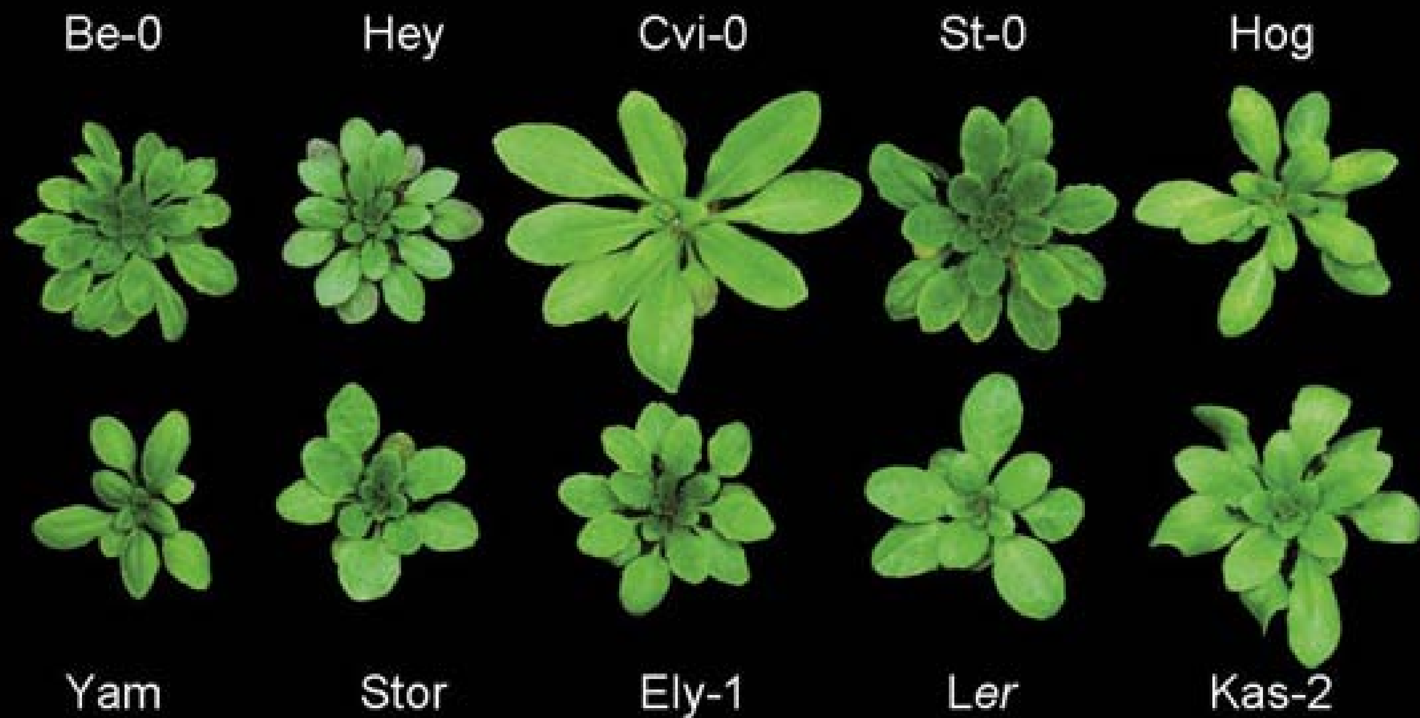


Figure 1. Overall rosette shape of several accessions grown on soil (18°C – 12 h day length)

Introduction

That *Arabidopsis* plants collected in nature can differ considerably was already recognised and described by Laibach in 1943. It is assumed that the genetic variation found in nature (Fig 1) is under natural selection for adaptation to the local environment. Therefore, better understanding of the molecular basis of this variation is an interesting research topic.

A complication in the analysis of natural variation is mainly caused by so-called quantitative traits that require QTL analysis. This approach allows an estimate of the number of genes segregating together with their map positions. For this QTL analysis, immortal mapping populations, such as recombinant inbred lines and introgression lines, have been developed for several crosses. Novel and more complex mapping populations derived from intercrosses of eight *Arabidopsis* accessions (plant collected in nature, in geographical contrasted regions) are being generated at present (Dr. Xueqing Huang). Working with these populations requires the development of additional QTL analysis tools (Dr. Joao Paulo). QTL analysis and subsequent QTL cloning of genetic variation for growth and plant performance traits in *Arabidopsis* is the main topic of the research group of Dr. Matthieu Reymond.

Previous work in Wageningen (El-Lithy *et al.* 2004) had shown that genetic variation for plant performance traits, which include growth (under various conditions) and allocation of resources (sink-source relations), can be found. The identified QTL in the Ler x Sha population will be further analysed (Bjorn Pieper, PhD student). Starting with near isogenic lines, recombinants in the QTL region have been isolated and undergo detailed physiological analysis in a range of environmental conditions.

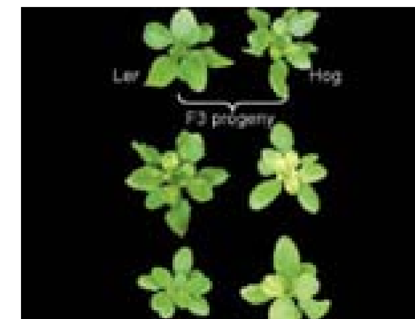


Fig 2. Segregation of the "pale green leaves" phenotype in Ler and Hog accessions and in a F3 progeny of a cross between these accessions.

An evaluation of a large number of accessions at two temperatures (below and above what is commonly used for *Arabidopsis*) showed considerable differences in responsiveness of the tested accessions. This genetic variation for growth and photosynthetic performance at low temperature is studied by Dr. Anna Ihnatowicz. Chlorophyll content and fluorescence parameters are tested on a new population showing contrasting responses to low temperature (Fig 2). QTL analysis of a new RIL population (Ler x Kas-2) in hydroponic cultures (Fig 3) under controlled temperature and light conditions has been initiated. Other environmental variations that will be tested deal with light quantity and supply of minerals (N, P, K and Mg) in the new Ler x Kas-2 populations (Dr. Hugues Barbier). The content of various compounds such as C, N, P, amino acids, etc. is being analysed using high throughput spectrophotometric analysis.

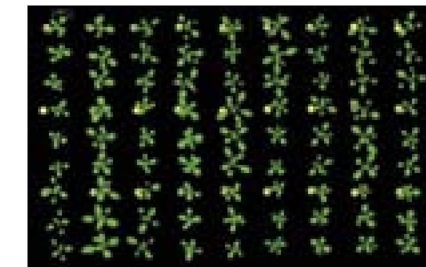


Fig 3. A set of 20 recombinants inbred lines (RIL) from Ler x Kas-2 grown in a hydropomic system

The challenge of this research programme is to understand this genetic variation, which is also very relevant for plant breeding, since it involves the traits determining yield and yield stability. It is difficult to predict which processes underlie this genetic variation, but candidate processes are primary and secondary metabolism, nutrient uptake, transport processes and aspects of development. This implies that a thorough and broad (whole plant) approach needs to be applied to identify the nature of the observed variation. Apart from being relevant for plant breeding, it is assumed that the effect of genetic variation on plant performance contributes to the adaptation of specific genotypes to a specific ecological system, and therefore has ecological and evolutionary relevance.

"The challenge is to understand this genetic variation, which is also very relevant for plant breeding, since it involves the traits determining yield and yield stability."

Selected Publications

T El-Assal, S.E., C. Alonso-Blanco, A.J.M. Peeters, V. Raz and M. Koornneef: The cloning of a flowering time QTL reveals a novel allele of CRY2. Nature Genetics 29, 435-440 (2001).

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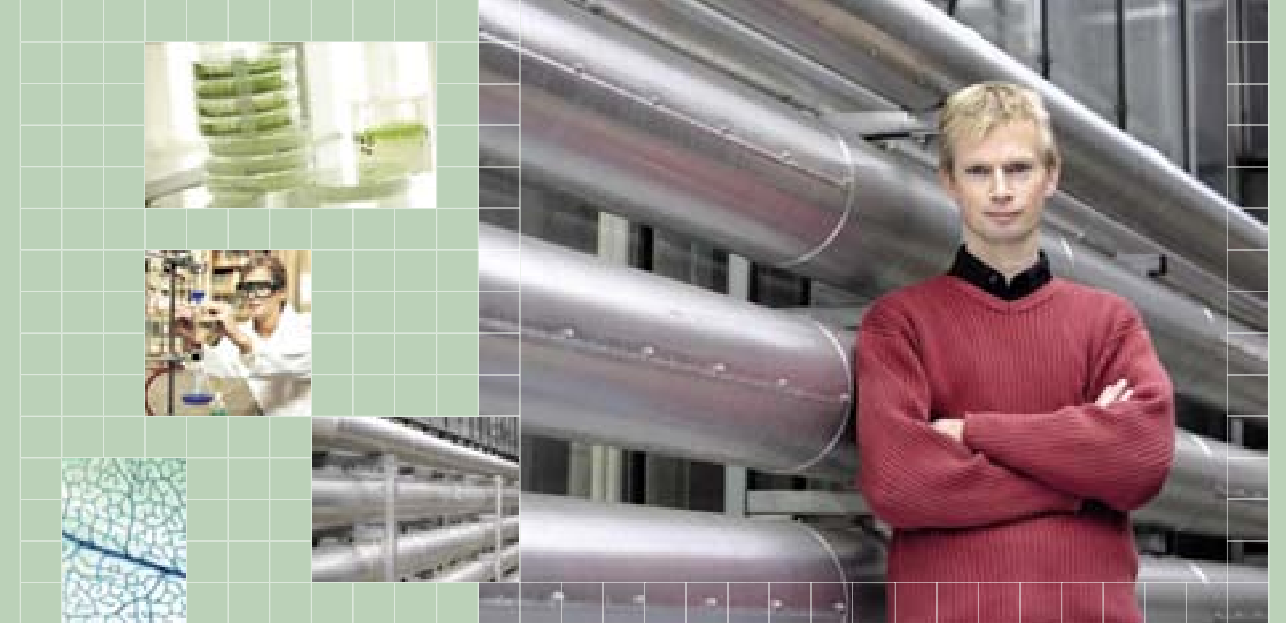
Wim Soppe | DEPARTMENT PLANT BREEDING AND GENETICS



Dormancy Mechanisms in Plants

Wim Soppe

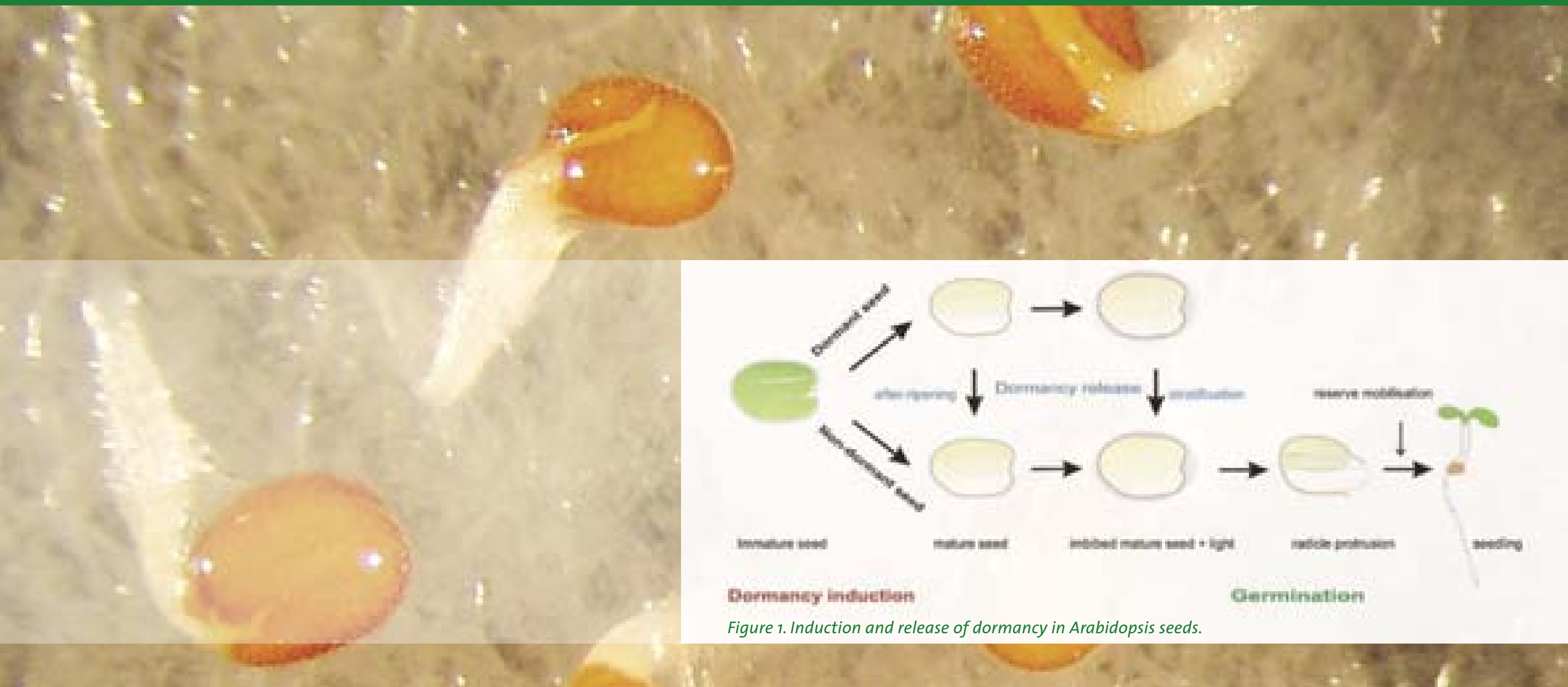


Figure 1. Induction and release of dormancy in Arabidopsis seeds.

Dormancy is the absence of development under (temporary) favourable environmental conditions and can prevent the germination of seeds or the outgrowth of buds at the wrong time of the year. Our research is focussed on seed dormancy in *Arabidopsis thaliana*. Non-dormant *Arabidopsis* seeds germinate in the presence of water, light and favourable temperatures, but dormant seeds are unable to do so (Figure 1). The dormancy of *Arabidopsis* seeds can be released by low temperatures (stratification) or dry storage (after-ripening). Both environmental and endogenous factors play a role in the induction and release of seed dormancy.

Extensive physiological research has been performed on seed dormancy; however, the molecular knowledge of this process is still limited. Indeed, only a few genes that influence seed dormancy have been isolated to date and the molecular mechanisms of the induction of dormancy and the release by low temperatures and dry storage are still unknown. Therefore, our aim is to clone and characterise novel genes that are involved in dormancy. The ultimate goal is to unravel the molecular pathways that induce and release dormancy, as has for instance been done for flowering initiation in *Arabidopsis*. In Maarten Koornneef's laboratory in Wageningen, a wealth of genetic resources has been generated which we will use to study seed dormancy. This material includes previously identified dormancy mutants, quantitative trait loci (QTLs) and near isogenic lines (NILs).

In the past year, we have cloned two genes of which the mutants show reduced dormancy (*rdo*). *RDO2* encodes for a TFIIS transcription elongation factor and *RDO4* for a RING finger-containing protein. Both genes will be further characterised. In addition, fine mapping and cloning of a third *rdo* mutant and of a dormancy QTL (*DELAY OF GERMINATION 3; DOG3*) has been started. Another dormancy QTL (*DOG1*) has been cloned in Maarten Koornneef's lab in Wageningen. Although a mutation in this gene demonstrated that *DOG1* is essential for dormancy, the DNA sequence did not give any clues about its function (L. Bentsink, Utrecht University, pers. comm.). A molecular and biochemical characterisation of *DOG1* is in progress in our lab.
In addition to the study of previously isolated mutants, we would also like to isolate novel dormancy mutants. Two different mutagenesis screens are currently being carried out to isolate mutants that specifically affect the stratification or after-ripening process. Furthermore, we will also start gene expression and DNA chip experiments to identify additional genes involved in dormancy and germination.

Dormancy release determines the beginning of the period in which seeds are able to germinate, whereas loss of viability (often after long-term storage under less favourable conditions) determines the end of this period. In Maarten Koornneef's lab in Wageningen, mutants with increased seed storability were identified in a screen for longer surviving seeds in an *abscisic acid insensitive3* (*abi3*) or *leafy cotyledon1* (*lec1*) background (mutants without dormancy and with low storability). Mapping and characterisation of several of these mutants is currently underway.

"Extensive physiological research has been performed on seed dormancy; however, the molecular knowledge of this process is still limited."

Developmental phase transitions require a highly co-ordinated expression of many genes, which is linked to chromatin dynamics and organisation. Preliminary observations showed differences in chromatin organisation between immature, dormant and non-dormant *Arabidopsis* embryos. Moreover, between 10 and 15 days after fertilisation, nuclei of the developing embryo were seen to decrease in volume (Figure 2). We will further study chromatin organisation using FISH and immunolabelling techniques.

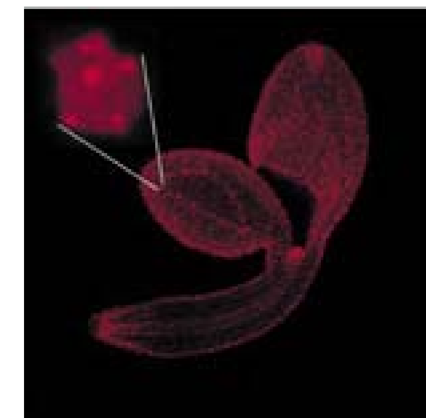


Figure 2. Mature Arabidopsis embryo of which the DNA is stained with propidium iodine. The inset shows a single nucleus.

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Genetic and Molecular Analysis of the Shoot Branching in Seed Plants

Klaus Theres

In higher plants, the primary axis of growth with the shoot apical meristem (SAM) at one pole and the root apical meristem at the opposite pole is laid down during embryogenesis. After germination, secondary axes of growth originate from lateral meristems that are established in the axils of leaves. Axillary meristems function like the SAM of the primary shoot initiating the development of lateral organs, a process resulting in the formation of an axillary bud. In many plant species, further development of axillary buds into shoots is blocked to different extents by the influence of the primary shoot apex, a phenomenon that was termed apical dominance. Therefore, the pattern of shoot branching depends not only on the formation of axillary meristems but also on the regulation of bud outgrowth. However, despite the importance of shoot branching for plant development, very little is known about the molecular mechanisms controlling this process. The aim of our work is to understand at the molecular level the mechanisms regulating the formation of axillary meristems. As model systems, we are using *Arabidopsis thaliana* and tomato.

The *Arabidopsis LATERAL SUPPRESSOR (LAS)* gene is required for the initiation of axillary meristems during the vegetative phase of development. To identify novel genes involved in formation and specification of axillary meristems, we have performed second-site EMS mutagenesis and screened for modifiers of the *las-4* branching phenotype. Nine enhancer and five suppressor candidates have been identified and are currently being characterised. RNA *in situ* hybridisation experiments have demonstrated that the *LAS* gene is expressed in the axils of all primordia originating from the SAM. To characterise upstream regulators that delimit the LAS expression domain, we have analysed the *LAS* promoter and screened for trans-acting factors. These experiments revealed that 820 bp upstream of the ATG and a 3,5 kb downstream DNA fragment are sufficient for complementation of *las-4*. Candidate regulators of *LAS* are currently being characterised.

Figure 1. Comparison of axillary bud formation in Col wild-type (A) and myb37 myb38 myb84 (B) mutants. Both pictures show close ups of rosette leaf axils.

The cellular distribution of *LAS* mRNA and LAS protein is being compared by expressing different marker gene constructs. *LAS::GFP-ER* transgenic plants show that the *LAS* promoter directs *GFP-ER* expression to leaf axils and to root tips. However, no specific signals could be detected in plants containing *LAS::GFP* or *LAS::LAS-GFP* constructs.

The tomato *Blind* gene encodes a *MYB* transcription factor controlling the formation of axillary meristems. A group of three homologous genes from *Arabidopsis* has been characterised. A T-DNA insertion in *MYB37* led to a reduction in side shoot formation early in vegetative development. *myb37 myb38 myb84* triple mutants exhibited an almost complete inhibition of axillary bud formation during vegetative development (Fig. 1 and 2). *In situ* hybridisation experiments demonstrated that *MYB37* and *MYB84* transcripts accumulate in the axils of leaf primordia, whereas *MYB38* mRNA was found in all cells of the shoot tip.

Figure 2. Schematic representations of shoot branching patterns in Col wild-type and myb37 myb38 myb84 (B) mutants.

The tomato *lateral suppressor (ls)* mutant is not useful for breeding purposes because of its negative effects on fertility and yield.
To achieve a reduction in shoot branching without the undesired side effects, we have characterised transgenic lines showing a reduced Ls activity due to the expression of an *Engrailed-Ls* construct or an RNAi construct. Several of these lines showed a strong reduction in shoot branching in conjunction with normal flower development. This trait was stably inherited over five generations.

The tomato *super-determinate (sde)* mutant does not develop axillary meristems in most leaf axils, whereas inflorescence development is not compromised. Genetic analysis revealed that the *sde* mutant phenotype is caused by mutations in two genes. One of the mutated loci could be mapped to the middle of chromosome 4. FISH analysis revealed that this locus is positioned in a heterochromatin-rich region of about 90 Megabases. Therefore, the isolation of this gene is currently not feasible.

"*Blind*-homologous *R2R3 MYB* genes control the pattern of lateral meristem initiation in *Arabidopsis*."

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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

Overview Department Molecular Plant Genetics

Director: Heinz Saedler

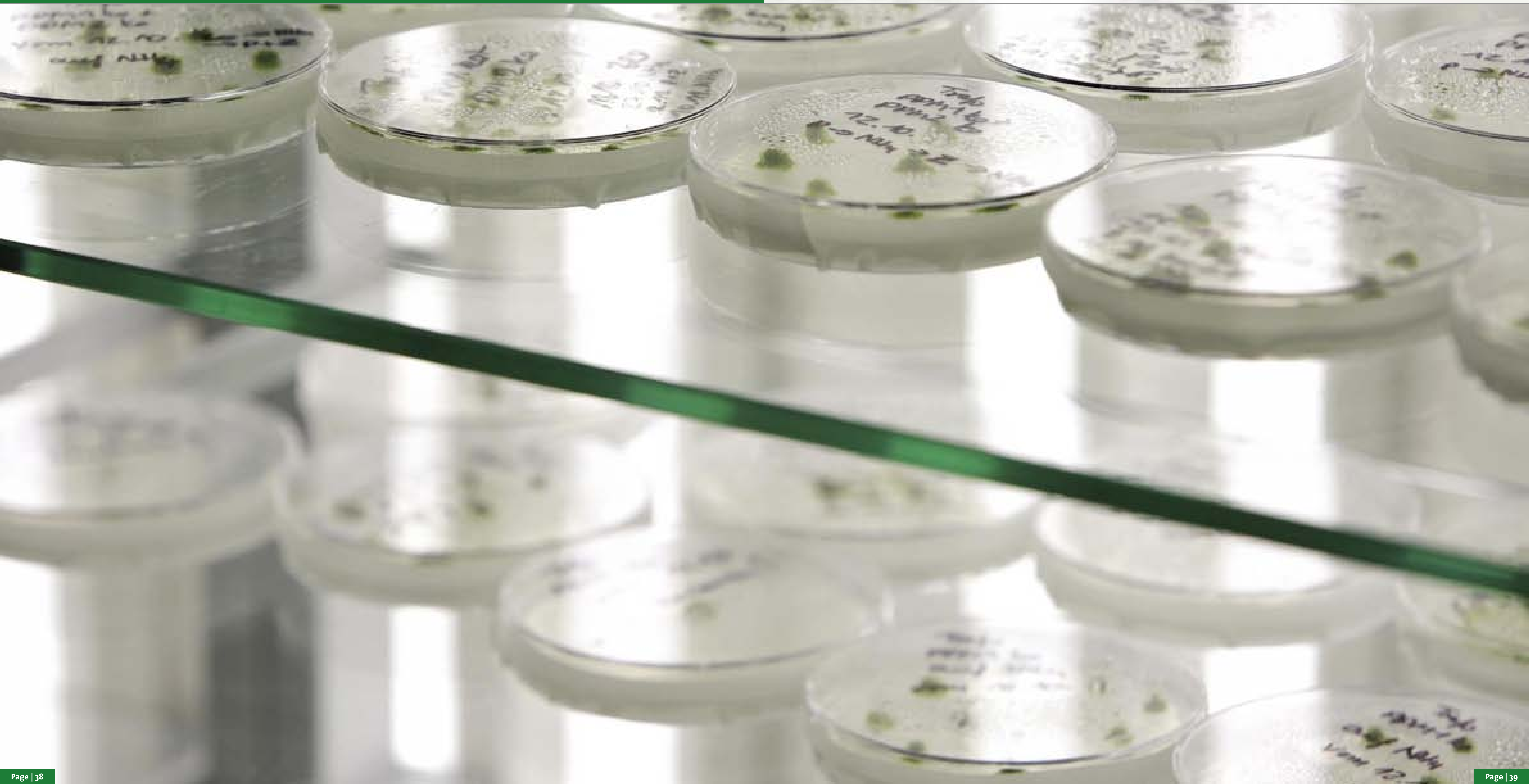
The primary scientific goal of the department is to unravel molecular details of flower development, the evolution of some transcription factors involved in this process and to study the mechanisms underlying the evolution of novel floral traits. In this context the following items are central to a few lines of research:

Control of gene expression: We plan to better understand how the expression of particular MADS-box genes, which control flower organ identity, is regulated. The integration of such regulators into the network of gene functions controlling flower development is also studied.

Signaling and cell fusion: Using combined genetics, molecular and biochemical approaches, we study the signaling pathways leading to cell fusion especially in floral organs.

Organogenesis: We plan to extend the current lines of research asking for more components like SPB-box and TCP transcription factors, that can be assembled into genetic circuits guiding petal and stamen development. To achieve this combined genetic and molecular approaches are used.

Molecular Evolution: We began a new line of research designed to isolate functional components required for the evolution of novelties, including sexual processes and morphological features of the flower.



Peter Huijser | DEPARTMENT MOLECULAR PLANT GENETICS

Comparative Genetics of SBP-box Genes

Peter Huijser

Figure 4

Comparative genetics of SBP-box genes: A family of plant-specific transcription factors

Proper development of all organisms requires a temporally and spatially co-ordinated expression of their genes. As transcription factors are known to play key roles in gene regulation, their study is thus assumed to contribute to a better understanding of the molecular genetic mechanisms underlying development. For this reason, the SBP-box gene family of plant-specific transcription factors has become the focus of our studies. We originally identified SBP-box genes by means of their proteins which are capable of recognising and binding a defined sequence motif within the promoter region of the *Antirrhinum* floral meristem identity gene *SQUAMOSA*, hence their name SQUAMOSA-PROMOTER BINDING PROTEINS. Since then, we have learnt that SBP-box genes are ubiquitously present in the plant kingdom, from unicellular algae to seed plants. However, despite their wide distribution, only a few mutant phenotypes are currently known that may shed light on the role of SBP-box genes in plant development. The main objectives of our current research are (1) to assign functions to SBP-box genes in plant development, (2) to gain insights into the regulatory networks they are involved in and (3) to elucidate their origin and evolution in the plant kingdom.

Figure 4. The autotrophic gametophyte produces leafy gametophores and represents the dominant life form during the life cycle of the moss Physcomitrella patens. The yellowish brown and globular sporophytes formed after zygosis remain nutritionally dependent on these gametophores. This interdependence of the alternating generations has become reversed in the evolution of seed plants.

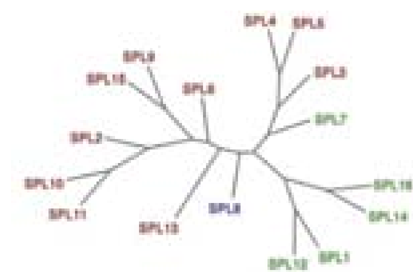


Figure 1. The Arabidopsis SBP-box genes are known as SPL genes. A phylogenetic reconstruction based on a comparison of their DNA-binding domains shows the evolutionary relationships within the family. SPL genes carrying a putative miRNA response element are depicted in red.

To this end, we exploit the unique opportunities for reverse genetics offered by the model species *Arabidopsis thaliana* and, more recently, also by the moss *Physcomitrella patens*. The choice to study simultaneously a seed and a non-seed plant is based on the assumption that in the course of evolution, the present SBP-box genes arose through duplication, recombination and mutation events. Evolutionary conservation of functional domains, in addition to the DNA-binding SBP-domain, thus may serve as a basis for grouping SBP-box genes in sub-families. Such sub-family members are expected to share, to some degree, common functions that may become evident as soon as for one of the members a mutant phenotype can be uncovered.

Figure 2. Transgenic Arabidopsis plants constitutiv expressing the SBP-box gene SPL3 (pots with red tags) flower early in comparison to wild-type plants (blue tag).

SBP-box genes in *Arabidopsis* are known as *SPL* genes and comprise a family of 16 members (Fig. 1). An original question concerning the developmental functions of SBP-box genes is „do SBP-box genes play a role in flowering?". The way in which the first SBP-box gene family members were identified together with their temporal expression patterns already suggested such a link. In support of this is the early flowering phenotype conferred to *Arabidopsis* by constitutive expression of an *SPL3* transgene (Fig. 2). Furthermore, screening for *SPL* mutants has provided phenotypes affected in the floral transition, inflorescence architecture and fertility. *Spl8* mutants, for instance, display severe anther defects (Fig. 3). Future research may uncover further roles of particular *SPL* genes in the process of flowering.

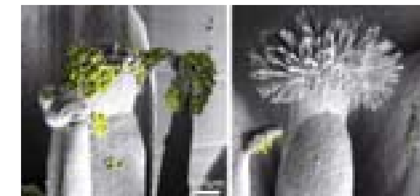


Figure 3. Mutations in the Arabidopsis SBP-box gene SPL8 result in strongly reduced fertility. Whereas anthers of wild-type flowers produce ample pollen (artificially coloured yellow in this scanning electron microscope image), those of the spl8 mutant produce only a few.

For comparative studies that may help to understand the origin and evolution of SBP-box genes and their functions in the plant kingdom, we started to isolate and characterise SBP-box genes from the non-flowering plant *Physcomitrella* (Fig. 4). A first feature revealed to be of ancient origin concerns a microRNA-dependent regulation of certain SBP-box gene family members. Mosses do not flower but like flowering plants they alternate between gametophytic and sporohytic generations. Further comparative studies may reveal whether control over the transitional processes involved, i.e. meiosis and zygosis, could be a common theme in SBP-box gene function in the plant kingdom.

"SBP-box genes represent plant-specific transcription factors and we aim to elucidate their role in development as well as their evolution in the plant kingdom. "

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Dollo's Law and Phenotypic Reversibility in *Misopates*

Wolf-Ekkehard Lönnig

Introduction

According to Dollo's law, evolution is irreversible, i.e. "[A]n organism cannot return, even partially, to a former state already realised in the series of its ancestors" –(Louis Dollo, 1893). Yet, applying this law to the eight derived features essentially distinguishing *Misopates orontium* from the closely related *Antirrhinum majus*, reveals that five differences have phenotypically been clearly diminished or fully overcome by mutant genes. These features are aspects of the life cycle, leaf form, flower size, flower colour and mode of fertilisation. Therefore, *Misopates orontium* outwardly approaches, meets or even overlaps the features of *Antirrhinum majus* or vice versa. Nevertheless, to date, the morphological key distinguishing feature between the two genera, the strongly elongated sepals in *Misopates*, intriguingly itself a feature being at odds with Dollo's law, could not be reduced to that of the length of *Antirrhinum* nor could the development of the short *Antirrhinum* sepals be extended to that of the length of *Misopates* despite extensive mutagenesis programmes with both species. Thus, these observations are in agreement with Dollo's law, i.e. as to the stasis of this dif-ference. The relevance of Dollo's law has been investigated for our mutants according to different evolutionary viewpoints as well as to solve the following basic questions: (1) to what extent can the hypothesis be substantiated that the long and short sepals could really constitute genuinely persistent ("immutable") characters; and (2) to what magnitude can the unexpected constancy of a feature distinguishing genera like the sepal difference be generalised for systematics and paleontology.

Projects

Project 1: Mutagenesis in *Misopates orontium* and *Antirrhinum majus.*
Altogether 335,000 plants of *Misopates* have been investigated for the project, including 10,800 M2-families. Moreover, during the last two decades, some 1.5 million *Antirrhinum* plants including 30,000 M2-families have been evaluated.

Project 2: Identifying the homologous genes by DNA sequencing
Since locus identity test crosses between *Misopates* and *Antirrhinum* are not possible, the genes so far identified were recognised by sequence analyses of the homologous *Antirrhinum/Misopates* wild-type genes and their mutant deviations. For measuring the genetical distance between the two taxa, a series of additional genes have been sequenced (also) by Dr. Jeong Hee Kim).

The experimental part of these two projects is nearly at an end, and the important *Misopates* mutants derived from this work have been accepted at the seed bank at Gatersleben.

Project 3: Mutagenesis in *Physalis pubescens*
The sepal-derived lanterns of *Physalis pubescens* of the Solanaceae offer a promising biological system (see He and Saedler, PNAS 102, 5779, 2005) to further investigate sepal development in plants. However, so far, no spontaneous or induced mutants have been described in *Physalis* that could help elucidate further intricacies of the system. Thus, to detect a range of mutant lanterns (among others) and then to identify the corresponding genes and their contributions to lantern development, a large mutation programme is now underway for the vegetation periods of 2006 and 2007.

"The relevance of Dollo's law has been investigated for our mutants according to different evolutionary viewpoints as well as to solve the basic questions."

Standing of the research projects in national and international comparison

Well-received, as stated by several scientific editors and reviewers (e.g. Species Concepts: "... a better reference book on the species problem is not to be found anywhere ... recommendable as an original, instructive and reliable contribution". – Dynamic Genomes: "...excellent and suggestive paper. We trust that your contribution will bring prestige to our book." – Carnivorous Plants: "The scientific editor felt that this was a well-written article." – Transposons, eukaryotic: "Article of the week" of Nature ELS, 20 May 2004). The genetic work has been the basis of much of the molecular investigations in *Misopates* and *Antirrhinum*.

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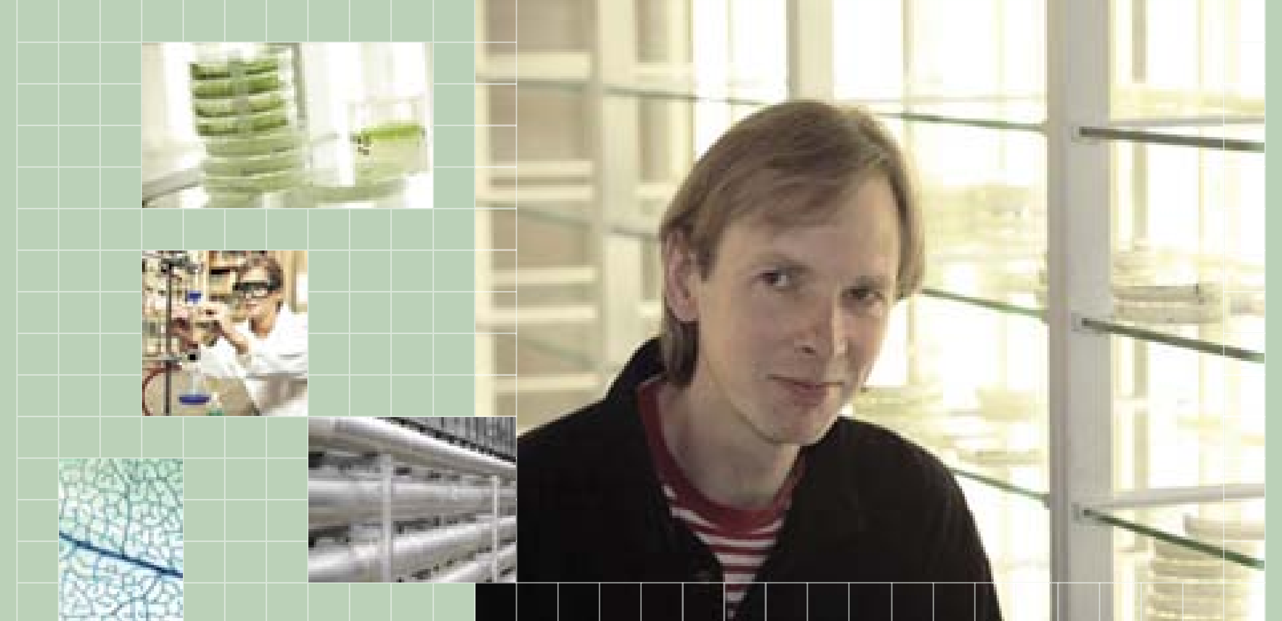
Developmental Biology of *Physcomitrella*



Thomas Münster

It is a corollary of evolutionary developmental genetics that major evolutionary changes in the morphology of organisms can be caused by changes of the function and/or expression of developmental control genes. The origin of new genes via gene duplication and the recruitment of already existing genes for new functions might have significantly contributed to the formation of evolutionary novelties. For the reconstruction of such "macroevolutionary" changes, it is therefore an important prerequisite to understand the action and function of the genes involved. One of the most prominent gene families that contribute to the understanding of the relationship between development and evolution in plants comprise the MADS-box gene transcription factors (see also Heinz Saedler Group). MADS-box genes are well known for the roles they exert as floral organ identity genes in flowering plants, which define the differentiation of the whorls of floral organs.

Our group is interested in multiple aspects of the functional evolution of MADS-box genes. In the past, MADS-box genes have been isolated from all groups of eukaryotic organisms. However, whereas in the genomes of animals and fungi only few MADS-box genes are present, in seed plants, MADS-box genes belong to large gene families. Recently, more than 100 MADS-box gene loci were identified in the model flowering plant *Arabidopsis thaliana* after sequencing of the complete genome. Representatives of the so far most intensely studied subclass of MADS-box genes in plants possess a common so-called "MIKC-type" structure, named after the four typical protein domains. For a better understanding of the evolution of MADS-box gene functions and networks in plants we initially characterised these genes in primitive and derived ferns. Nowadays, we are using the moss *Physcomitrella patens* as our favourite experimental system, because *Physcomitrella* has the advantage of efficient homologous recombination in the genome. This provides us with unique and powerful technical tools for our analysis.

In *Physcomitrella patens*, only 17 MADS-box genes coming in two different versions of the MIKC-type are present. Because of this limited number, we are currently reconstructing the complete network of the genes and their encoded proteins. Moreover, we are elucidating the different functions of these important transcriptional regulators in the moss model system. Thus far, the obtained results already provide a basis for a detailed comparative analysis of the evolution of MADS-box gene networks within land plants.

To date, we have shown that the *Physcomitrella patens* MADS-box genes possess broad expression domains; this is in strong contrast to the mostly temporally and spatially restricted transcriptional activities of the homologous seed plant MADS-box genes. Interestingly, some genes are predominantly or even exclusively expressed in the protonema. Protonema represents one of two gametophytic stages of the moss life cycle. It exhibits simple tip growth resulting in a filamentous tissue that in nature grows in the soil. The second gametophytic stage starts with the formation of buds from which the tiny "moss plants" develop. The complex moss gametophyte is of special interest to our analysis because it enables us to study the evolution of the haploid phase of plant life which is highly reduced to three or eight cells in flowering plants.

Currently, we are analysing transgenic mosses where selected MADS-box genes have been either functionally destroyed or tagged with a "molecular flag". These lines have been produced based on the targeted integration of foreign DNA at the original MADS-box gene loci in the genome. Using these moss lines, we are studying phenotypic changes caused by the loss of the specific gene function. Additionally, by detecting the tagged proteins, we are now able to determine the temporal and spatial expression patterns of the *Physcomitrella* MADS-domain proteins.

"We are currently reconstructing the complete network of MADS-box genes and their encoded proteins."

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Evolution of Morphological Novelties in Solanaceae

Heinz Saedler

The origin of morphological novelties is a long-standing problem in evolutionary biology. Their understanding demands elucidation of developmental and genetic mechanisms that produce such new structures. Two likely processes that may be involved are heterochrony, which causes phylogenetic changes in the timing and rates of ontogenetic processes, or heterotopic expression of existing functions. In these scenario, changes in *cis*-regulatory elements of genes and/or changes in *trans*-acting transcriptional regulators could be involved.

The molecular mechanisms underlying the evolution of the inflated calyx syndrome (ICS) found in several genera of the Solanaceae is central in our studies. In addition, since the trait occurs many times within the family, the mechanism concerning polyphyly is also of key interest to our group.

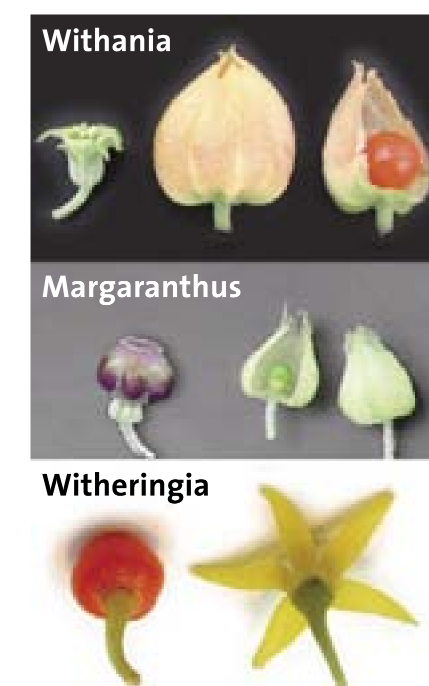
Combinations of certain MADS-box transcription factors determine floral organ identity. Changes in these combinations lead to alterations in floral organ identity, i.e. sepals are replaced by petals or vice versa. While most genera of the Solanaceae feature small sepals, some display more spectacular changes, like a balloon-like calyx, which encapsulates the entire mature fruit.

In *Physalis*, this structure is often termed “Chinese lantern”, but it also has been called “inflated calyx syndrome” (ICS). Recently, we have reported that the recruitment of a transcription factor from vegetative development into a floral context via heterotopic expression generated this novel morphological trait.

The MADS-box transcription factor STMADS16 is solely observed in vegetative tissues of *Solanum tuberosum*. Conversely, its orthologue *MPF2* from *Physalis pubescens* is also expressed in floral tissues. Transgenic *Physalis* plants in which *MPF2* expression was knocked-down via RNAi technology did not feature ICS suggesting that MPF2 function is essential for ICS formation. Sequence deviations in the promoter region of the MPF2 and STMADS16 genes seem to be responsible for the heterotopic expression of *MPF2* in *Physalis*. This perhaps reflects the exposure to different selection pressures in the two species during evolution. Although heterotopic expression of *MPF2* is crucial for the realisation of the ICS formation, fertilisation is also required to trigger the process.

"Molecular phylogenies, promoter sequence comparison of *MPF2*-like genes and functional tests are next."

At least two factors are involved in the evolution of ICS: (1) promoter changes to provide heterotopic expression of an MPF2-like function in the floral context and (2) a signal provided by fertilisation or developing fruit. Due to this complexity, one would expect to find ICS only very rarely in the Solanaceae. However, this is not the case. Among the 96 genera within the Solanaceae, at least 5 have evolved ICS or ICS-like calyces. These include *Exodeconus*, *Margaranthus*, *Physalis*, *Withania* and some of the *Hyoscyamae*. Clearly, the origin of ICS is polyphyletic. Studies concerning the mechanism of polyphyly have been initiated by phylogenetic reconstructions of the ca. 100 species of Solanaceae to determine the closest relatives, like *Witheringia* and *Capsicum*, of the above-mentioned genera. The next step will involve DNA sequence comparisons of the promoter regions of *MPF2*-like genes from these species. This approach might reveal a mechanism for polyphyly of ICS.



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Antirrhinum Flower Development

Genetic Control of Floral Gene Expression Boundaries

Zsuzsanna Schwarz-Sommer

Introduction

Floral homeotic genes and the spatial control of their expression

Floral organ primordia become determined to a particular organ type shortly after their appearance on the floral meristem. This process is regulated by homeotic organ identity genes, whose combinatorial relation is summarised in the textbook 'ABC model'. In this model, the C-function genes (which encode MADS-box transcription factors) govern male and female reproductive development in the inner whorls of the flower. Mutations in C-function genes result in homeotic conversions of the reproductive organs to perianth-like structures, normally present in the two outer whorls. Conversely, misexpression of C-function genes in the outer whorls converts the sepals and petals into reproductive organs (Fig. 1). We would like to understand the molecular details of this control by identifying the genes involved.

Projects

Project 1: *STYLOSA* - More than a factor in controlling flower development

We have cloned *STYLOSA* and showed that it is structurally related to the TUP1/GRO proteins, which belong to a well-known group of transcriptional co-repressors and regulate by interacting with various DNA-binding proteins a broad range of processes in yeasts and animals. In fact, we found that repressing the C-function during flower development is just one aspect of the *STY* function; the *sty* mutant also displays fascination, alteration in leaf venation patterns and hypersensitivity towards auxin and polar auxin transport inhibitors. Thus, *STY* has a general role in plant development controlling both vegetative and reproductive processes. We identified several proteins which can interact with STY and in an endeavour to better characterise the *in vivo* relevance of these protein interactions, we are currently analysing corresponding mutants for these STY interaction partners. Interaction between the GRAMINIFOLIA (GRAM) protein and STY *in vivo* is supported by the partially overlapping mRNA and protein expression patterns of STY and GRAM. The functional association of these two proteins is corroborated by enhancement of vegetative and reproductive features of the *sty* or *gram* single mutant phenotypes in the *sty gram* double mutant. Ongoing projects aim at the isolation of mutants in other genes whose protein products interact with STY.

Project 2: *FISTULATA*: Establishing cloning by a map-based approach in *Antirrhinum*

FISTULATA is a gene that interacts with STY and several other known factors involved in the spatial control of the C-domain. Attempts to clone *FIS* by classical strategies failed and therefore we initiated a map-based approach. For this purpose, we generated an *Antirrhinum* BAC library and established a high-resolution physical map of the *FIS* locus by obtaining recombinant individuals within a range of ~3 cM from 1600 segregating *fis* mutants. Currently, after finding a region of zero recombination, we are attempting to identify the *FIS* gene within a region of about 400 kb. 'Side-products' of this project are our BAC library that is now available for the *Antirrhinum* community as well as a CAPS-based physical map of *Antirrhinum* for the efficient mapping of mutants. Additional genomics tools, such as our previously established *Antirrhinum* EST collection, also facilitate chromosome walking in *Antirrhinum* as well as determining the similarity of overall gene distribution (synteny) between *Antirrhinum* and other species.

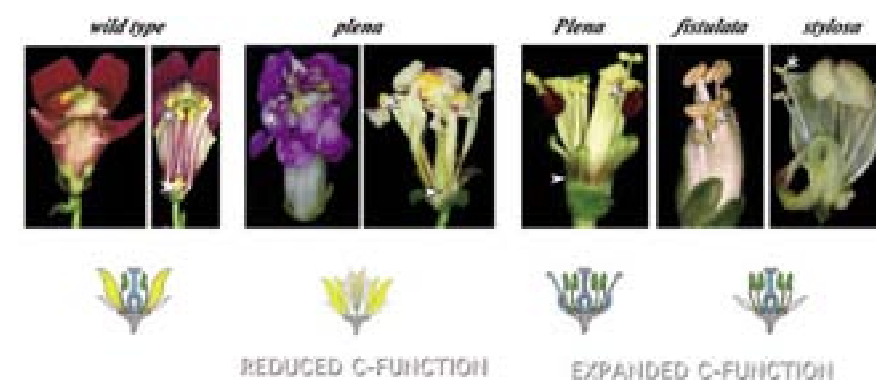

Figure 1. Mutants of Antirrhinum with aberrant phenotypes due to changes in the C-function. The name of the mutants is indicated above the photographs. Asterisks mark stamens and arrows highlight carpels in the wild-type flower and indicate the homeotic conversion of these organs in the mutants when the C-function is abolished or expands. The coloured flower diagrams illustrate schematically the changes in organ identity displayed by the flowers in the photographs.

Standing of the research project in national and international comparison

Our work in establishing and providing genomics tools is much appreciated in the *Antirrhinum* community. Acknowledgement by the national/international research community is reflected by the broad forum for written and oral presentations and by collaborations with scientists in Europe, Canada and Australia.

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Cell Signalling

Alexander Yephremov

Cuticle is the outer protective layer of the plant epidermis, containing wax and insoluble cuticular biopolymers, mainly polyesters. As the major protective barrier of aerial organs against environmental stresses, including drought, cold, UV radiation and chemical pollutants, as well as pathogens, cuticle is very important for the plant; however, its biological significance probably extends well beyond this.
Mutational analysis has suggested that defects in the formation of the cuticle affect not only features that may be easily associated with improper cuticle formation, such as growth, sensitivity to low humidity and chemicals, as well as cell-cell interactions, but also morphology of organs and single cells and cell differentiation in the epidermis.
A paradoxical increase in disease resistance levels has been found in *Arabidopsis* cuticular mutants and plants expressing a secreted fungal cutinase that is capable of breaking down cutin. To date, it remains to be determined how defective cuticle induces developmental and defence responses.
The Cell Signalling Group focuses on the molecular characterisation of *Arabidopsis* cuticular mutants showing various defects in development. Our aim is to understand how cuticle is synthesised, formed and how it controls morphogenesis and cell differentiation and also signals the plant to activate its defences.
The use of the model species *Arabidopsis* has enabled us to clone by transposon tagging and molecularly characterise four epidermis-specific genes involved in cuticle formation: *FIDDLEHEAD (FDH)*, *LACERATA (LCR)*, *BODYGUARD (BDG)* and APB24, the latter of which appeared to be allelic to *HOTHEAD (HTH)*. Examination of the corresponding mutants with transmission electron microscopy (in collaboration with Dr. Christiane Nawrath, University of Lausanne) revealed drastic changes in cuticle structure for all four mutant types.
In the leaf of wild-type *Arabidopsis*, the cuticle, like in other plants, has a continuous and regular layered structure; however, unlike in other plants, it is ten times thinner than those studied to date. Therefore, biochemical characterisation of cuticular polyesters in *Arabidopsis* was not a trivial task. Our major efforts (in collaboration with Prof. Dr. Lukas Schreiber, University of Bonn) were directed at developing a method that would allow us to rapidly compare the composition of lipid cuticular polyesters in mutants and wild type using gas chromatography/mass spectrometry analysis. Results obtained with this method are very similar to those when pure cutin is analysed, and cuticular polyesters in several mutants have been studied in our lab. Unexpectedly, however, we found that α-,ω-dicarboxylic fatty acids and ω-hydroxy fatty acids are the major depolymerisation products in leaf cuticular polyesters and cutin in *Arabidopsis* (Franke *et al.* 2005). Thus, aliphatic composition of *Arabidopsis* leaf cutin is more similar to that of suberin which is a root-specific polyester in all plants.
We showed that the APB24 mutant, designated *hth-12*, accumulates less α-,ω-dicarboxylic fatty acids and, respectively, more ω-hydroxy fatty acids in cuticular lipids due to a defect in the ability to oxidize long-chain ω-hydroxy fatty acids to ω-oxo fatty acids (Kurdyukov *et al.* 2006a). Based on the amino acid sequence similarity of *HTH*, we hypothesise that it may catalyse the next step after cytochrome P450 fatty acid ω-hydroxylases, such as LCR, in the ω-oxidation pathway (Fig. 1). We showed that *HTH* is the epidermis-specific gene (Fig. 2). These results are particularly interesting when one considers the attention that the *hothead* mutation received after a recent report in Nature about cases of non-Mendelian inheritance in this mutant (Lolle *et al.* 2005).
Mutation in *BDG* also causes organ fusions and other dramatic changes in the morphology of the plant. Characterisation of the epidermis-specific *BDG* gene upon cloning showed that it encodes a polarly localised extracellular protein, which is required for cuticle formation rather than for biosynthesis of cutin monomers (Kurdyukov *et al.* 2006b).

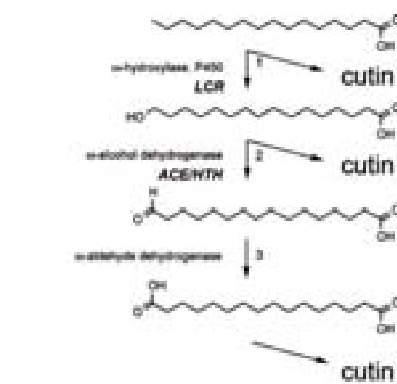

Figure 1. GFP reporter gene expression driven by the ACE/HTH promoter in the epidermis of transgenic plants. | Scale bars are 200 µm for (A), (C), (F); 100 µm for (D), (E); 400 µm for (B). | (A and B) Longitudinal sections through floral buds showing ACE/HTH is relatively strongly expressed in petals in mature buds (B, arrow). | (C and D) Cross-section through the central part of the pistil shows the obvious expression in the septum (arrow) and ovules (D). | Cross-sections through anther (E) and flower stalk (F).

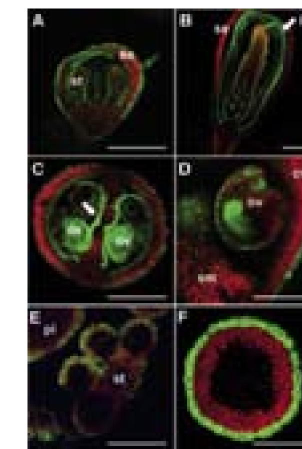

Figure 2. Proposed fatty acid ω-oxidation pathway for biosynthesis of cuticular polyesters in Arabidopsis. ACE/HTH may also act on other substrates but, for simplicity's sake, other routes except through palmitic (C16:0) acid are not shown. Biosynthetic step 1 is probably common to all plant species, while the roles of steps 2 and 3 are species specific. A parallel pathway through a cytochrome oxidase capable of performing reactions 1 to 3 may contribute to the amount of α-,ω-dicarboxylic fatty acids in cuticular polyesters.

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Regulation of Petal and Stamen Organogenesis

Sabine Zachgo

Figure 1

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. Floral class B MADS-box genes control the organogenesis of petals and stamens. In the *Antirrhinum* class B *DEF* null-mutant, second and third whorl organs are homeotically transformed. Sepals replace petals in the second whorl and instead of stamens a fused carpeloid structure is formed in the centre of the flower. Although class B genes have been intensively studied during the last decade, little is known about the target genes that realise their regulatory potential. Therefore, we have established a macroarray expression profiling technique that enables the investigation of the expression of over 11000 unigenes from *Antirrhinum* in parallel. Given the large size of *Antirrhinum* flowers, floral organs can be dissected and stage-specific probes can be prepared allowing highly specific profiling experiments to be conducted. We performed a detailed analysis of petal development and unravelled the complex transcriptome changes that take place during late petal morphogenesis. Furthermore, we exploited a

Figure 1. Brassicaceae flowers with different petal symmetries. Flowers from left to right: Arabidopsis thaliana, actinomorphic; Iberis amara, zygomorphic; mutant flower from Iberis amara, actinomorphic.

well-characterised conditional class B mutant *def-101* to isolate a large number of petal target genes of the class B gene *DEFICIENS*.
A key feature of the *Antirrhinum* corolla is its bilateral symmetry. The zygomorphy of the flowers reflects morphological distinctions between the upper (dorsal) and lower (lateral and ventral) petals. The TCP transcription factors *CYCLOIDEA* and *DICHOTOMA* control the formation of their different shapes. Lack of function of these genes gives rise to a ventralised flower displaying a radial (actinomorphic) symmetry; whereas, in gain of function mutants, all petals become dorsalised. The *Antirrhinum* macroarray system can be used to study transcriptome changes between these different mutants and to identify target genes acting downstream of the floral symmetry regulatory genes, and thus contribute to generate the complex corolla shape.
It is assumed that monosymmetry evolved several times independently from actinomorphic plants. Key regulatory genes controlling floral monosymmetry are known to be TCP transcription factors. Interestingly, within the Brassicaceae, only species in the genus *Iberis* show a monosymmetry in the second whorl. Therefore, isolation and analysis of *Iberis* TCP genes will reveal whether the same genes and molecular networks have been independently recruited to establish monosymmetry in different plant families.

Although MADS-box and TCP transcription factors control petal organogenesis and shape, little is known about the genes involved in the correct initiation of their organ primordia. Therefore, we isolated an *Arabidopsis* T-DNA mutant from the GABI-KAT T-DNA collection, named *roxy1*, that forms a reduced number of petal primordia. This mutant also reveals abnormalities during late petal development such as bending of the petal blade. Surprisingly, the effected gene, named *ROXY1*, encodes a glutaredoxin (GRX) and belongs to a large *Arabidopsis* gene family for which information is scarce. GRXs are oxidoreductases that oxidise or reduce conserved cysteine-containing motives. Thus far, GRXs have been associated with the response to oxidative stress. Our data indicate for the first time that a GRX plays a crucial role during flower development and that this is most likely via conserved cysteine-mediating posttranslational modifications.

Future goals

By analysing posttranslational modification processes and performing comparative studies of key regulatory genes controlling petal organogenesis and shape formation, we aim to better understand the regulatory mechanisms that govern the whole organ formation process from the early stages when organ primordia are initiated, to the differentiation stages and finally to late development when the characteristic petal shape is established.

"Our data indicate for the first time that a GRX plays a crucial role during flower development and that this is most likely via conserved cysteine-mediating posttranslational modifications."

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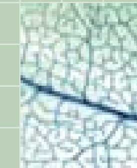
Overview Department Plant Microbe Interactions

Director: Paul Schulze-Lefert

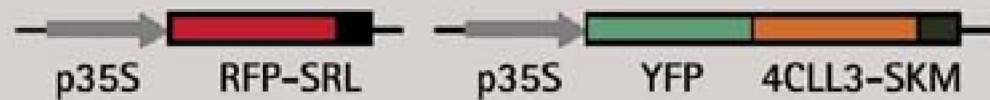
Research in the department of Plant Microbe Interactions engages in fundamental molecular processes underlying interactions between plants and pathogens. The innate immune system of plants and mechanisms of microbial pathogenesis have a central role in our discovery program. We are pursuing an integrated approach bridging traditional research territories like genetics, molecular biology, biochemistry, and cell biology. Much of our work is focused on interactions between plants and fungi, a common class of pathogenic microbes.

Although the structure of the plant immune system ensures effective protection against most microbial pathogens, few intruders succeed to colonize plants. In such cases plant immune receptors fail to recognize a pathogen or the invader evolved mechanisms to suppress immune responses. Our goal is to define a regulatory network of the plant immune system that can make predictions which components and how component activities can be changed to modulate immune responses. This should provide insights as to how the plant immune system can be modified to improve plant protection using molecular breeding techniques.

Small Signalling Molecules in the Plant Immune Response



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. However, inducible mechanisms, which are initiated after successful recognition of the invading pathogen, are considered to be more important for combating pathogen attack. We are interested in the identification of signalling components controlling early plant defence responses, with particular emphasis on mechanisms involved in the generation and perception of small signalling molecules.

Projects

Structure-function relationship and expression of 4-coumarate:CoA ligase

We have extensively studied the structure-function relationship of 4-coumarate:CoA ligase (4CL), a key enzyme of the general phenylpropanoid pathway, which is involved in the synthesis of secondary plant products with important biological function, such as lignin, wall-bound phenolics and flavonoids. The structural principles determining substrate specificity of the four 4CL isoforms encoded in the *Arabidopsis* genome (At4CL1-4) have partially been unravelled by homology modelling and mutant analysis. Current work focuses on the expression analysis of the *Arabidopsis 4CL* gene family, individual members of which show unique expression patterns. For example, *At4CL2* is constitutively expressed in vascular tissues, whereas *At4CL1* is strongly up-regulated by the bacterial elicitor flg22 but only moderately responds to treatment with salicylic acid (SA) or methyl jasmonate. *At4CL3* is the only family member that is light-activated and restricted to epidermal cells.

Functional characterisation of acyl-activating enzymes

Arabidopsis thaliana contains a large family of carboxylic acid-activating enzymes, which in addition to nine long-chain fatty acyl-CoA synthetases and the four characterised 4CLs, comprises twenty-five 4CL-like proteins of unknown function. Following heterologous expression, purified 4CL-like proteins were subjected to a large-scale substrate screen which uncovered significant activity of four proteins with jasmonic acid (JA) precursors. Using fluorescent-tagged variants, we demonstrated that these 4CL-like proteins are targeted to leaf peroxisomes. These results show that specific 4CL-like proteins have the capacity to contribute to JA biosynthesis by initiating the β-oxidative chain shortening of its precursors. Current work aims at elucidating the *in vivo* function of these putative JA biosynthetic enzymes using reverse genetic tools. In addition, miss-expression experiments of selected genes using strong constitutive or inducible promoters are being carried out which are complemented by expression analyses using reporter genes. Collectively, this work is expected to contribute to our understanding of the JA biosynthesis and signalling network.

Future goals

Organic small molecules have been widely used as cell permeable ligands to elucidate mechanisms of signal transduction. Having touched upon JA biosynthesis, we now plan to dissect the signalling function of different oxylipins, i.e. JA precursors and metabolites, using chemical genetic approaches. We are currently establishing suitable screening methods by generating appropriate transgenic reporter lines consisting of different jasmonate-responsive promoters that drive expression of glucuronidase or luciferase. Screening of available chemical libraries will be designed such that both agonists and antagonists of jasmonate function can be identified. Ultimately, the identification of the protein targets of small molecule probes is of fundamental importance for understanding the molecular mechanism of signal perception and transduction. This can be achieved by the yeast three-hybrid technology which we are currently establishing in our laboratory as a prelude (1) to identify primary targets of different signal molecules derived from the JA pathway which still remain elusive despite JA biosynthesis and function having been extensively studied and (2) to establish a general experimental platform for target identification which will be a useful tool for numerous ongoing projects in the Department that utilise chemical genetic screens.

"Ultimately, the identification of the protein targets of small molecule probes is of fundamental importance for understanding the molecular mechanism of signal perception and transduction."

Selected Publications

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Molecular Basis of Fungal Biotrophy

Richard O'Connell

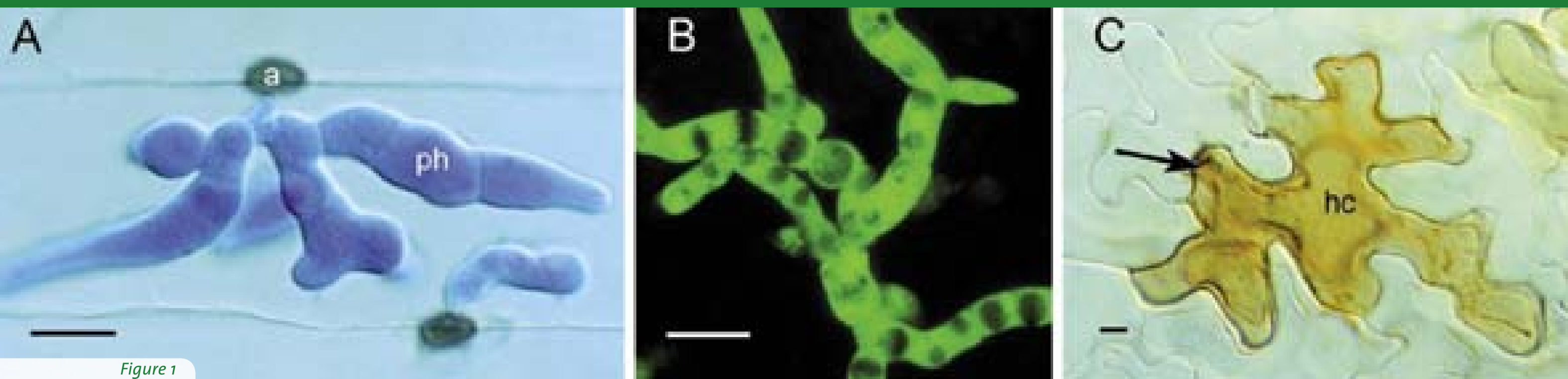

Figure 1.
(A) The infection of susceptible Arabidopsis plants by Colletotrichum higginsianum begins when spores germinate to form melanized appressoria (a), which adhere firmly to the plant surface. The appressorium penetrates through the plant cuticle and cell wall to form large primary hyphae (ph) inside the leaf epidermis. These specialized hyphae feed biotrophically inside living host cells and the host plasma membrane expands and invaginates around them.

(B, C) higginsianum can be easily transformed genetically using Agrobacterium tumefaciens. Here, fungal hyphae are expressing the green fluorescent protein gene in their cytoplasm, allowing their development in planta to be followed by confocal microscopy.

(C) During infection of the resistant Arabidopsis ecotype Wassilewskija, host epidermal cells die soon after penetration and the development of C. higginsianum hyphae (arrow) is entirely restricted to the dead hypersensitive cell (hc). Bars = 10 µm.

Introduction

Biotrophic fungal pathogens establish intimate relationships with their host plants, having evolved the remarkable ability to insert specialised feeding structures called haustoria into plant cells, without killing them or triggering defence responses. Obligate biotrophs, which require a living host plant for their growth and reproduction, have enormous economic impact, causing disease in most major crop plants throughout the world. However, techniques for the molecular analysis of these organisms are severely limited because they are not amenable to genetic transformation. As a consequence, the mechanisms underlying biotrophy remain one of the largest gaps in our understanding of plant-fungal interactions.

The brassica pathogen, *Colletotrichum higginsianum*, was recently shown to infect the model plant *Arabidopsis thaliana*. This ascomycete fungus uses a two-stage, 'hemibiotrophic' infection process, initially growing biotrophically inside living host cells, before switching to destructive necrotrophic growth, when it feeds on dead host tissues. During the biotrophic phase, *Colletotrichum* produces specialised intracellular hyphae (Fig. 1A), similar to the haustoria of rusts, powdery mildews and downy mildews. However, unlike these obligate biotrophs, it is a haploid organism that can be easily cultured *in vitro* and genetically transformed (Fig. 1B), facilitating insertional mutagenesis and the assessment of gene function by targeted disruption. We are exploiting the many advantages of this new model pathosystem to dissect the molecular basis of biotrophy using a range of complementary approaches.

Discovery of fungal biotrophy genes

Random insertional mutagenesis is a powerful tool for analysing fungal pathogenicity and we are using this approach to identify *C. higginsianum* genes required for biotrophy. A library of insertional mutants has been generated by *Agrobacterium tumefaciens*-mediated transformation and these are being screened for defects in their pathogenicity that occur after penetration into host cells, e.g. failure to differentiate specialised intracellular hyphae, maintain host cell viability or complete the transition from biotrophy to necrotrophy.

In a parallel approach, we are using suppression subtractive hybridisation to search for fungal genes that are differentially expressed *in planta* during the initial biotrophic phase of infection. For this, cDNA libraries prepared from biotrophic hyphae isolated from infected leaves will be subtracted with cDNAs derived from pre-penetration infection structures and the later-formed necrotrophic mycelium to enrich for fungal genes upregulated during biotrophy.

> **"Obligate biotrophs, which require a living host plant for their growth and reproduction, have enormous economic impact, causing disease in most major crop plants throughout the world."**

Assessing the plant's contribution to biotrophy

In contrast to the wealth of information available on plant resistance genes and defence signalling pathways, little is known about the contribution made by plants to susceptible interactions with biotrophs. In collaboration with Jane Parker's group, we are using a forward genetic screen to identify plant genes required for susceptibility. Mutagenised plants of a susceptible *Arabidopsis* accession are being screened for a gain of resistance to *Colletotrichum*, without constitutive expression of plant defence genes. Putative susceptibility genes will be isolated by positional cloning. Naturally-occurring recessive resistance genes may also encode plant susceptibility factors and these are being sought in wild-type *Arabidopsis* accessions that vary in susceptibility to *Colletotrichum* (Fig. 1C).

Internalisation of *Colletotrichum* hyphae involves invagination and expansion of the plant plasma membrane, creating a large surface area for molecular exchange between the two organisms. Host cells undergo an inverted form of polarised growth, with targeted secretion of new plasma membrane, membrane recycling by endocytosis and re-arrangement of the actin cytoskeleton. Confocal microscopy of GFP-tagged plants is being used to study how components of the plant endomembrane system and cytoskeleton become remodelled during development of the plant-fungal interface.

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Host Cell Manipulation and Defence Suppression in Plant/Powdery Mildew Interactions

Ralph Panstruga

Plants resist attempted attacks of most microbes due to the presence of a highly evolved innate immune system that constantly monitors the environment for the presence of so-called pathogen-associated molecular patterns (PAMPs), highly conserved pathogen-derived molecules. PAMP recognition triggers (a) signal cascade(s) that ultimately lead(s) to an activation of a set of defence responses that suffice to keep the majority of "wanna-be" pathogens at bay. Suppression of preformed or induced host defences appears to be an integral part of the pathogenicity mechanisms of a range of truly pathogenic microbes, including phytopathogenic fungi and bacteria. However, little is currently known about how exactly this proposed "defence suppression" functions at the molecular level. Despite the identification of a broad range of effector proteins from phytopathogenic bacteria, such as *Pseudomonas syringae*, the precise role of most of these effectors is largely unknown. Moreover, in the case of biotrophic fungal pathogens, only a few effector proteins are known to date. This is at least partly because biotrophic pathogens like powdery mildew fungi are at present hardly amenable to *in-vitro* cultivation and/or genetic manipulation.

Host cell polarisation including polar secretion appears to represent a fundamental principle in plant defence against microbial intruders including pathogenic fungi. The heptahelical barley MLO protein constitutes a potential host target for defence suppression that presumably modulates defence-related and focal secretory processes in the host plant. Presence of the plasma membrane-resident polypeptide is an absolute requirement for compatible barley-powdery mildew interactions. Consequently, lack of functional MLO (as in *mlo* mutant genotypes) results in full resistance against the fungus. Genetic screens identified a SNARE domain protein as a key component of *mlo*-based disease resistance. SNARE proteins are essential mediators of membrane fusion events, e.g. during vesicle transport in both yeast and animal cells. It is thus currently thought that the fungal pathogens might corrupt MLO function(s) to suppress a SNARE protein-dependent and vesicle-associated defence response at the cell periphery. Consistent with this hypothesis, a further SNARE protein, the SNAP25 homologue HvSNAP34, is also required for *mlo*-mediated disease resistance in barley.

Work in my laboratory is primarily devoted to the processes of "compatibility" and "defence suppression" in the context of plant-powdery mildew interactions. We are particularly interested to unravel the exact molecular role of MLO during the early phases of fungal pathogenesis. In addition, we plan to identify the fungal effector proteins that potentially mediate such pivotal functions as defence and cell death suppression. The molecular basis of natural genetic variation of powdery mildew resistance within the reference dicot plant species *Arabidopsis thaliana* is another research topic. Finally, we are interested to learn more about the role of MLO proteins in other biological processes. To reach these goals, my laboratory uses a combination of genetics, molecular biology, biochemistry and advanced non-invasive quantitative imaging technologies to determine *in planta* dynamics of protein complexes.

"Host cell polarisation including polar secretion appears to represent a fundamental principle in plant defence against microbial intruders including pathogenic fungi. "

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Bhat, R. A., Miklis, M., Schmelzer, E., Schulze-Lefert, P., and Panstruga R. Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane micro-domain. Proc. Natl. Acad. Sci. 102, 3135-3140 (2005).

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Regulatory Protein Complexes in Plant Defence against Pathogens

Jane Parker

In plants, effective resistance against pathogens is expressed at the cell surface as well as inside attacked cells. One of the most obvious forms of defence is controlled by intracellular immune receptors (known as Resistance or R proteins) that recognise specific pathogen effectors. The recognition event triggers dramatic cellular reprogramming that normally culminates in localised plant cell death and the production of important signalling molecules.

Our interest is in the regulation of plant defence after cells have been invaded. Genetic analyses in the model plant *Arabidopsis* show that a large number of plant components contribute to post-invasion ("basal") resistance that limits the spread of disease. These basal defence systems are strengthened by immune receptor-mediated pathogen recognition. How the recognition events connect molecularly to basal defence pathways is not clear. However, we know that certain regulators of basal defence are recruited very early by immune receptors in the resistance pathway. We have focussed on a particular type (so-called TIR-NB-LRR) of intracellular R protein that depends on the basal defence component EDS1 and its interacting partners, PAD4 and SAG101 (Fig. 1). These components are soluble proteins controlling plant cell death and the production of defence signalling intermediates that act locally and systemically. Recent analysis of the EDS1 pathway has led to several major findings. First, EDS1 acts as a kind of scaffold that stabilises PAD4 and SAG101 inside the cell. Evidence from pathology assays of wild-type and mutant plants shows that PAD4 and SAG101 are very important for defence signal relay. Before pathogen attack, EDS1 forms a number of complexes with PAD4 and SAG101 (Fig. 2). The presence of distinct but co-regulated EDS1 complexes in the cell's nucleus and cytoplasm suggests that a certain dynamic of EDS1 with its partners is important for their signalling activities. Current experiments are aimed towards establishing where their primary site of action is, whether movement between the nucleus and cytoplasm is necessary for signal transduction and importantly, whether changes in EDS1 or its partners occur once pathogens attack the cell.

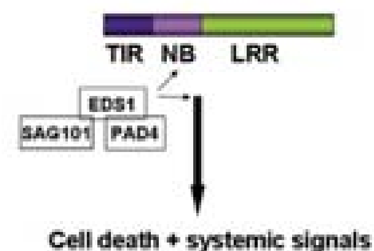

Figure 1. Certain plant immune receptors trigger cellular defences through EDS1

A number of pieces of evidence indicate that EDS1 and its partners, PAD4 and SAG101, act very early in TIR-NB-LRR receptor triggered disease resistance, controlling programmed plant cell death at pathogen infection sites and the production of signals that induce systemic defences.

Another approach that we adopted to unravel mechanisms of plant immunity was to use *Arabidopsis* whole genome expression microarrays that show the mode of expression of nearly all the plant genes in a given tissue after a particular treatment. We designed a microarray experiment in such a way that we could extract a few genes whose expression is tightly regulated by EDS1 and PAD4 after pathogen invasion. This analysis was very informative and led to the identification of both positive- and negative-acting components of the plant defence system.

"We designed a microarray experiment in such a way that we could extract a few genes whose expression is tightly regulated by EDS1 and PAD4 after pathogen invasion."

In other studies, we aim to understand more fully the roles of two plant assembly factors, RAR1 and SGT1, in disease resistance. Evidence from our experiments and from other groups points to interactions between these proteins and chaperones (proteins that assist protein folding and protein complex maturation) in stabilising R proteins and other cellular complexes. We found that one chaperone, HSP70, interacts with SGT1 in plant cells and this may be important in generating correctly primed "pathogen sensing" platforms. Many unanswered questions prompt us to examine more closely the nature of two *Arabidopsis* TIR-NB-LRR protein complexes and use a combination of genetics and protein biochemistry to understand how these are formed, what cellular processes they protect and how they become activated upon pathogen recognition.

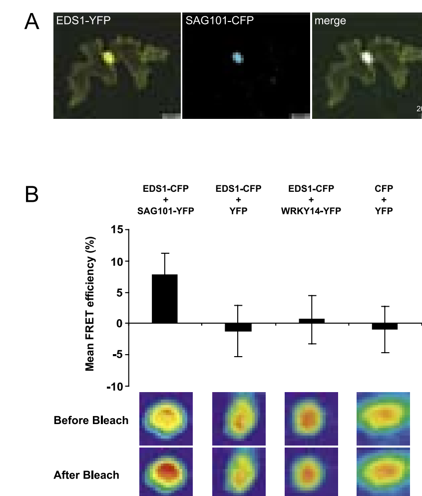

Figure 2. Visualising defence regulatory complexes inside a living plant cell.
(A) Arabidopsis epidermal cells were bombarded with DNA of fluorescent protein-tagged EDS1 and SAG101 to transiently express these proteins. The cells were then analysed by confocal laser scanning microscopy. EDS1 is present in both the cytosol and the nucleus whereas SAG101 accumulates only inside the nucleus. Images shown are three-dimensional reconstructions from individual image stacks.
(B) Fluorescence Resonance Energy Transfer-Acceptor Photobleaching (FRET-APB) analysis of the interaction in nuclei between EDS1-Cyan Fluorescent Protein (CFP) and SAG101-Yellow Fluorescent Protein (YFP). This method can be used to measure the extent of physical interaction between individual proteins by monitoring how bleaching of the "acceptor" protein (in this case SAG101-YFP) affects the fluoresence characteristics of the "donor" protein (EDS1-CFP). The average FRET efficiencies ± standard deviations from multiple sample sites are shown. Pictures of pseudo-coloured nuclei show CFP donor fluorescence before and after bleaching of individual cells. An increase in donor fluorescence (red) is seen only if protein-protein interaction occurs. The FRET technique coupled with various molecular ana-lyses has allowed us to visualise EDS1-SAG101 complexes inside the nucleus that are molecularly and spatially distinct from EDS1-PAD4 interactions in the cytosol and the nucleus. Our data suggest that a certain dynamic between cellular compartments is important for proper plant defence signal relay.

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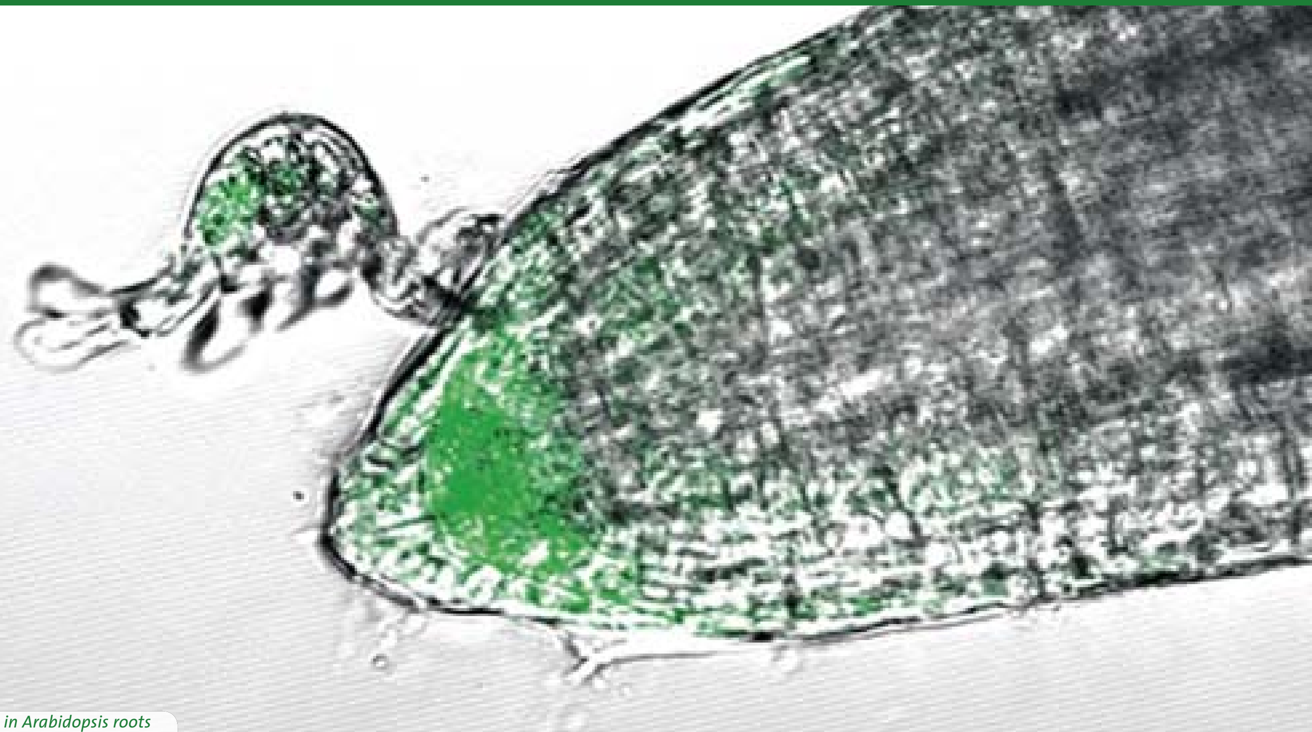
Silke Robatzek | DEPARTMENT PLANT MICROBE INTERACTIONS

Ligand-induced Endocytosis of the Pattern Recognition Receptor FLS2 in *Arabidopsis*

Silke Robatzek



FLS2-GFP expression in Arabidopsis roots

Plants are constantly exposed to microbial growth on all their root, leaf and flower surfaces. However, disease appears to be the exception rather than the rule. The principle behind this phenomenon is the ability of plant cells to sense the presence of non-self, a hallmark of general host defence mechanisms. So-called pathogen-associated molecular patterns (PAMPs), which are universally conserved molecules in whole classes of microbes, are perceived by cognate pattern-recognition receptors (PRRs) in host cells. This recognition triggers a general innate immune response that increases the plant's resistance to microbial infection (Fig.1). So far, only two plant PRRs have been reported, one of which is encoded by the *Arabidopsis* transmembrane receptor kinase Flagellin Sensing 2 (FLS2), a paradigm of investigated plant receptors.

FLS2 recognises a highly conserved epitope of bacterial flagellin (flg22), which is the main building Block of the bacterial motility organ. Flg22 induces numerous changes such as generation of reactive oxygene species, kinase activation, gene expression, and callose deposition. Remarkably, expression of about 1000 genes was up-regulated after only 30 min of flg22 treatment (Zipfel *et al.* 2004). Moreover, flg22 perception has been shown to be essential for efficient plant defence. *Arabidopsis* plants carrying a mutation in *FLS2* appear to be more susceptible to bacterial infection than wild-type plants (Fig. 2). Although FLS2 signalling plays a key role in plant defence, it is not yet clear what is the exact molecular mechanism of ligand-induced receptor signalling and receptor downregulation. In the case of innate immune resistance, host cell responses have to be effective in defeating microbial infection, but also have to ensure host cell survival. Therefore, the signals emitted by FLS2 have to be tailored to mount dose-dependent cellular responses within a certain time frame.

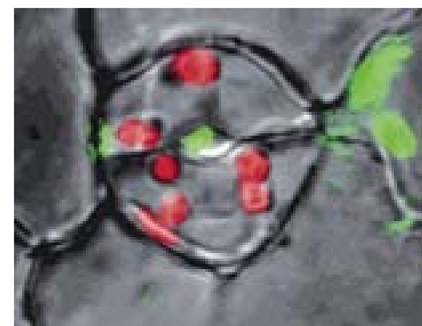


Figure 1. Pseudomonas GFP-bacteria penetrates Arabidopsis leaf stomata.

To address possible subcellular dynamics of FLS2, we have applied *in vivo* cell imaging techniques. Therefore, we established transgenic *Arabidopsis* lines (ecotype Ws-0, an *fls2* mutant [Zipfel *et al.* 2004]) expressing a functional FLS2 fusion protein joined to the green-fluorescent protein (GFP) under the control of the FLS2 promoter. Confocal microscopy revealed that FLS2-GFP was expressed in all plant organs and was always localised to plasma membranes (Fig. 3).

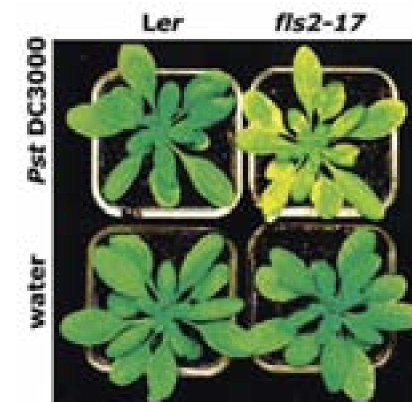


Figure 2. Susceptibility of fls2-mutants

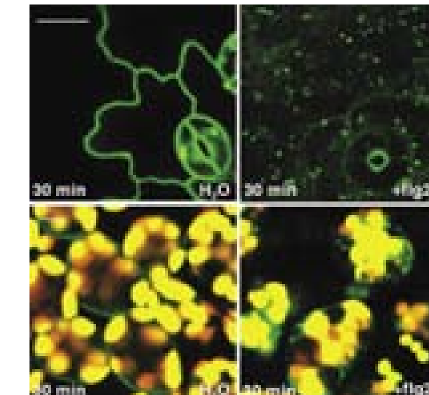


Figure 3. Ligand-induced endocytosis of FLS2

We discovered that flg22 induces FLS2-GFP disappearance from the plasma membrane and accumulation into numerous distinct intracellular vesicles (Fig. 3). Prolonged incubation with flg22 led to a complete loss of the GFP signal indicating FLS2 protein degradation. In contrast, prolonged incubation after flg22 pulse-treatment revealed a recovery of the plasma membrane FLS2-GFP signal due to protein synthesis. Experiments using pharmacological inhibitor molecules suggest that flg22-induced FLS2 internalisation follows an endocytic pathway and involves proteasome and kinase activities.

Substitutions of three threonine residues have been identified that strongly affected FLS2 response, but still exhibited clear flg22 binding. One of those has been further investigated and shown that it links flg22 response and FLS2 endocytosis.

Our findings provide the first demonstration of ligand-induced internalisation of a PRR within the newly emerging field of plant endocytic processes. In future, we aim to define PAMP-mediated PRR internalisation and its physiological significance in the innate immune response by genetic, molecular, biochemical and microscopical *in vivo* imaging approaches. Unravelling how endosome dynamics are linked to signalling networks and to the nature of PAMP-mediated responses promises to provide many exciting discoveries in the future.

"Unravelling how endosome dynamics are linked to signalling networks and to the nature of PAMP-mediated responses promises to provide many exciting discoveries in the future."

Selected Publications

Robatzek S, Chinchilla D and Boller T (2005) Ligand-induced endocytosis of the pattern recognition receptor FLS2 in *Arabidopsis*. (Genes & Development, in press).

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Recognition and Signalling in Plant Innate Immunity

Paul Schulze-Lefert

Figure 1. Focal accumulation of PEN2-containing peroxisomes at a fungal entry site.
Confocal microscopic image of an Arabidopsis leaf epidermal cell expressing PEN2-GFP. The fungal structure (appressorium at the tip of a fungal germ tube) was visualized by propidium iodide (red colour). Note the cluster of peroxisomes at the tip of the appresorial germ tube.

The genetic and biochemical basis of immunity to non-adapted parasites

Nonhost resistance describes the immunity of an entire plant species against non-adapted pathogens. Although this form of resistance is widespread in nature, it is still poorly understood. We have begun to genetically dissect this form of immunity by searching for *Arabidopsis* mutants that support growth of fungal parasites which in nature colonise grass or pea species. These fungi typically fail to enter attacked epidermal cells on wild-type *Arabidopsis* leaves. The underlying resistance mechanisms involve specific isoforms of a plasma membrane resident syntaxin (PEN1) and an ABC transporter (PEN3) as well as a peroxisome-associated glycosyl hydrolase (PEN2). PEN proteins become recruited to pathogen entry sites and are thought to direct secretory processes that terminate fungal pathogenesis. Interestingly, host (virulent) powdery mildews of *Arabidopsis* appear to have evolved mechanisms to subvert PEN-dependent immunity for pathogen entry. The immune response suppression includes *Arabidopsis* MLO proteins and is investigated by Ralph Panstruga's research group. PEN1 appears to be required for SNARE protein-dependent vesicle tethering and discharge of vesicle cargo at pathogen entry sites. Identification of endosomal vesicles captured by the plasma membrane resident PEN1 syntaxin and molecular characterisation of their cargo represent one focus of ongoing research. PEN2 and PEN3 appear to be involved in a distinct secretory process. One hypothesis is that the peroxisome associated glycosyl hydrolase PEN2 releases small molecules that are transported to the extracellular space *via* the PEN3 ABC transporter (Fig. 1). Characterisation of these secreted small molecules and elucidation of their mode of action is one subject of ongoing research.

Recognition and signalling in plant innate immunity triggered by allelic NB-LRR immune receptors

A plethora of race-specific (*R*) genes conferring immunity to parasitic powdery mildew fungi have been genetically characterised in the crop barley (Fig. 2). Each of these *R* genes confers immunity to a powdery mildew isolate carrying a cognate avirulence gene (*AvrMla*). The complex *Mla* locus is unusually polymorphic and encodes the majority of known *R* gene specificities to the fungal pathogen. Molecular isolation of several *Mla R* gene specificities (e.g. *Mla1, Mla6, Mla10, Mla12, Mla13*) has shown that the encoded gene products belong to the CC-NB-LRR subclass of intracellular immune receptors. The availability of several allelic MLA immune receptors, the powdery mildew effector AVRMLA10 (recognised by the MLA10 receptor), as well as components required for MLA function (RAR1, SGT1, cytosolic HSP90), provides the basis for detailed mechanistic studies that should unravel effector recognition by MLA immune receptors and subsequent initiation of immune responses.

Figure 2. Sequence diversity of allelic MLA immune receptors is confined to the C-terminal region.

Top: Graphic representation of the modular structure of MLA immune receptors. CC=coiled coil, NB=nucleotide binding domain, LRR= Leucin rich repeats, CT= C-terminus.

The graph below illustrates distinct regions of sequence conservation (CC and NB domains) as well as sequence divergence (LRR and CT regions) that were identified by comparison of known MLA1, MLA3, MLA6, MLA7, MLA10, MLA12, MLA13, and MLA22 sequences. The sequence divergent LRR-CT region determines recognition specificity.

"Deciphering the regulatory logic of the plant immune system remains a major future challenge."

Chemical genetics of immune responses

Although mutational studies are powerful to identify genes required for plant innate immunity, such approaches have inherent limitations in detecting all components of the plant immune system due to functional redundancy and lethality of many genes. We have begun to utilise small molecule libraries as a novel tool to dissect plant immune responses. We have developed various reporter systems that enable *in planta* monitoring of pathogen-triggered defence reactions and these can be used for high-throughput analysis in microtiter plates. By this means, we identified candidate agonists and antagonists that complement our genetic tools. Such small molecules offer the advantage to conditionally block or auto-activate subsets of immune responses. Our long-term objective is to identify cellular targets of the agonists/antagonists using biochemical and genetic techniques. This project is currently underway as part of the Max Planck Chemical Genomics Initiative http://www.cgc.mpg.de/.

Selected Publications

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Huckelhoven R, Stein M, Freialdenhoven A, Somerville SC, et al.: SNARE-protein-mediated disease resistance at the plant cell wall. Nature 2003, 425:973-977.

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Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P: The RAR1 Interactor SGT1, an Essential Component of *R* Gene-Triggered Disease Resistance. Science 2002, 295:2073-2076.

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Transcriptional Regulation of the Plant Immune Response

Imre E. Somssich

Tissue specific expression of various WRKYpromoter:GUS reporter lines

Introduction

Plants have evolved a complex signalling network enabling them to perceive and to respond optimally to environmental changes and when challenged by phytopathogens. A crucial part of these multifaceted responses centres on the ability of the plant to transcriptionally modulate gene expression patterns both temporally and spatially. The importance of transcriptional re-programming in the activation of plant defence responses was illustrated by global expression studies in *Arabidopsis thaliana* revealing that up to 20% of all genes show altered transcript levels upon pathogen infection. Such drastic re-programming requires a sophisticated regulatory system involving the interplay of both positive and negative transcriptional modulators acting within regulatory circuits to finetune the expression of the defence transcriptome. Currently, the contribution and interplay of specific transcription factors to defence gene regulation remains ill-defined. We have identified a family of transcription factors named WRKY, which bind to a functionally important DNA element (W box; 5'C/TTGACC/T-3') that is present within the promoters of numerous defence genes. WRKY proteins are zinc finger-type transcription factors that have enormously expanded and diversified within the plant lineage. Expression analyses suggest that environmental factors, in particular exposure to abiotic and biotic stresses, have significantly contributed to this expansion.

Projects

WRKY transcription factors modulating plant defence responses

A. thaliana contains 74 *WRKY* genes, 54 of which respond to biotic/abiotic stresses. Using reverse genetics, we identified four *WRKY* genes whose functions influence the outcome of *Arabidopsis*-microbe interactions. Loss-of-function of *AtWRKY40* and *AtWRKY53* renders *Arabidopsis* (Col-0) plants highly susceptible to the pathogen *Pseudomonas syringae* DC3000. In contrast, mutations within *AtWRKY18*, the closest related member to *AtWRKY40*, showed no effect towards this pathogen. However, *AtWRKY18/40* double mutants exhibit a more drastic susceptibility phenotype than *AtWRKY40* mutants, suggesting a contribution of *AtWRKY18* in modulating defence responses (Dr. B. Ülker). Disruption of *AtWRKY27* rendered plants more resistant to *Ralstonia solanacearum*. Complementation of such mutants with an *AtWRKY27* cDNA controlled by the native *AtWRKY27* promoter restored the wild-type phenotype, whereas ectopic expression driven by the strong *35S CaMV* promoter resulted in plants with increased susceptibility (M. S. Mukhtar). A second major focus of our work is to define *in vivo* target genes for *AtWRKY* factors. For this purpose, the chromatin immunoprecipitation (X-ChIP) method is being employed. The use of defined *Atwrky* knock-out lines expressing epitope-tagged versions of the respective *AtWRKY* transgene under the control of inducible promoters, in combination with global expression arrays and X-ChIP, should help to identify primary targets (G. Rivory, Dr. R. Birkenbihl).

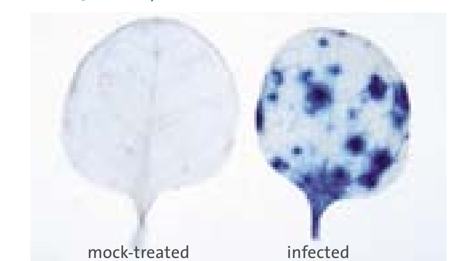
Novel pathogen-responsive promoter elements in wheat

This new project aims at identifying novel pathogen responsive *cis*-acting regulatory DNA elements that are functional in wheat (Dr. Roccaro). For this, synthetic libraries consisting of random oligomers inserted upstream of the minimal *35S CaMV* TATA box-containing promoter linked to a selectable marker gene are being constructed. The libraries will be transformed into wheat cells that respond strongly to a fungal-derived elicitor.

Future goals

Future work will focus on the search for in vivo targets of *AtWRKY* genes involved in plant defence. Such studies will include genome-wide location analysis to define the transcriptional regulatory networks governed by these factors. To gain access to upstream components within WRKY-dependent signalling pathways, genetic screens and chemical genetic approaches will be used.

WRKY19::GUS reporter lines treated with SA



WRKY33p::GUS reporter line treated with P. parasitica

"Currently, the contribution and interplay of specific transcription factors to defence gene regulation remains ill-defined."

Selected Publications

Andreasson, E., Jenkins, T., Brodersen, P., Thorgrimsen, S., Petersen, N.H.T., Zhu, S., Qiu, J.-L., Micheelsen, P., Rocher, A., Petersen, M., Newman, M.-A., Nielsen, H.B., Hirt, H., Somssich, I., Mattsson, O. and Mundy, J.: The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J., 24,2579-2589 (2005).

Turck, F., Zhou, A., and Somssich, I.E.: Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to its native promoter and the defense-related gene *PcPR1-1* in parsley. Plant Cell 16, 2573-2585 (2004).

Ülker, B. and Somssich, I.E.: WRKY transcription factors: from DNA binding towards biological function. Curr. Opin. Plant Biol. 7, 491-498 (2004).

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

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., 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).

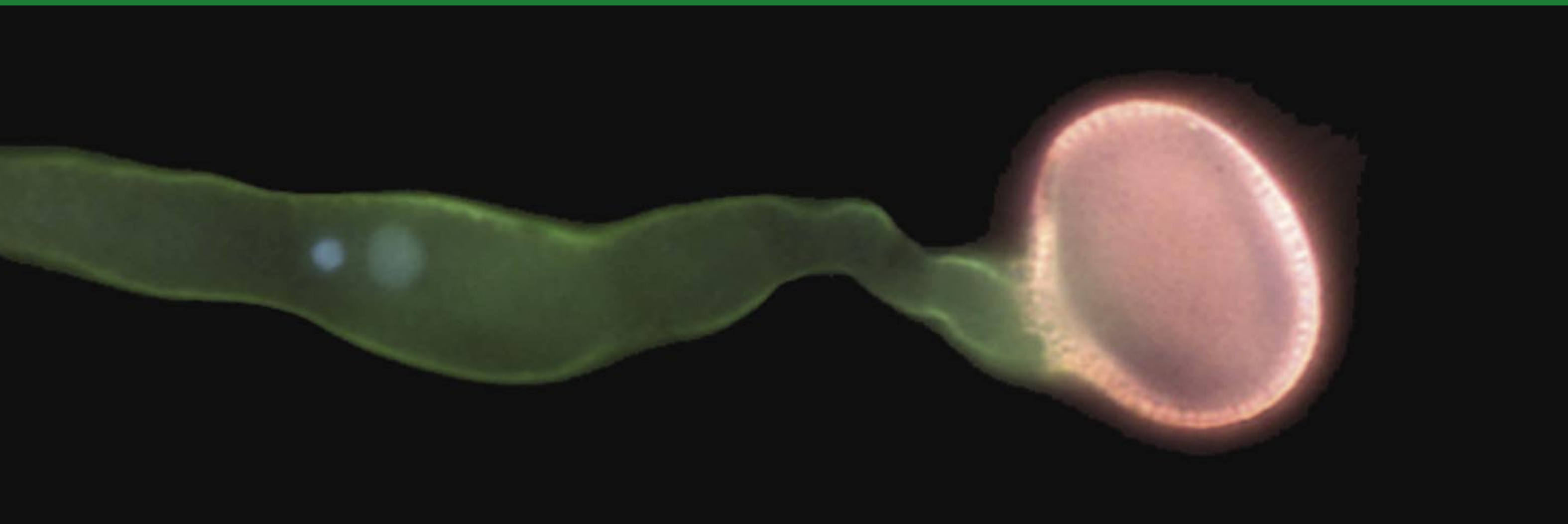
Overview Independent Research Groups

Attracting talented young scientists from diverse backgrounds that complement and expand the focus of the four departments, the institute supports independent junior research groups. These operate outside of the departmental structure, have their own research focus and are led by young scientists for a limited period of five years.

It is essential for the institute to broaden the approaches and facilities available in the institute in order to bridge the gap to other disciplines and meet the vision of integrative biology. Currently, three independent research groups bring cell biology, bioinformatics and chemical genetics know-how and generate stimulating interactions with plant molecular biology, breeding and genomics. Arp Schnittger's group is a collaboration with the Department of Botany of Cologne University, strengthening links and interactions. As bioinformatics was perceived to have a growing impact on plant molecular biology, Heiko Schoof's group Plant Computational Biology has been empowered to install the computational infrastructure required for large scale analyses. As a participant in the Max Planck Society Chemical Genomics Centre, the Plant Chemetics group of Renier van der Hoorn will strengthen the institute's efforts in chemical genetics.

Plant Cell and Developmental Biology

Arp Schnittger



Introduction

Cell cycle control is a fundamental process of all cells. The cell cycle is connected to many physiologial functions, for instance stress responses, and developmental decisions, such as growth control. Especially in plants, cell cycle control appears to be of major importance since plants are immobile and rely on an iterative production of new organs.

We use *Arabidopsis* as a model plant to investigate the plant cell cycle. This exploration takes place at different levels. On a molecular level, we are interested in the plant-specific regulators of cell cycle control, e.g. large cyclin families with still unknown functions. On a cellular level, we make use of well-described model cells, such as trichomes, stomata and root hairs, to explore the connection between differentiation and cell cycle control. Finally, on an organ and organism level, we study the integration of cell cycle regulation in organ growth and function.

Projects

Transition from proliferation to differentiation

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 transition from a mitotic mode to a non-dividing mode is an integration point where developmental cues are read out and combined with signals from the environment. The result must be a rapid and stable decision. To gain a deeper insight into what causes the transition from proliferation to differentiation, we are examining the underlying molecular dynamics of the switch from a dividing to a differentiated cell.

Endoreplication

With the exit from a proliferative cell cycle mode, many animal and plant cells enter an endoreplication programme as they differentiate. In endoreplicating cells, DNA replication is continued in the absence of cell divisions leading to cellular polyploidy. A typical example for an endoreplicating cell are *Arabidopsis* leaf hairs (trichomes). It has been speculated that the function of endoreduplication is to supply enough DNA templates for an increased demand of transcripts for a higher or specialised metabolic activity. Importantly, the amount of DNA seems to be one major determinant of cell growth and form. We are interested to learn how the entry into, progression through and exit from an endoreplication cycle is regulated.

Cell cycle regulators

In general, the cell cycle control machinery is conserved throughout the kingdoms. However, plants have developed a few idiosyncrasies, one striking example are the large families of cell cycle regulators. We use loss-of-function as well as gain-of-function approaches, including the generation of mutant protein variants, to understand the function and regulation of important cell cycle regulators in the context of plant growth and development. Our current interests include CDKs, B-type cyclins and a class of CDK inhibitors called KRPs.

Future goals

One major goal is to understand the molecular dynamics and regulatory circuits of cell cycle regulation in the context of plant growth and development. For this, we are trying to better understand cell cycle processes at the biophysical level.

Standing of the research project in national and international comparison

Our group was the first to dissect cell cycle regulation and development in a cell type- and tissue type-specific way. For this, we have used trichome, stomata, and root hairs cells as model systems for studying cell cycle regulations. Recently, it has also become clear in animals that many cell cycle regulators have tissue or even cell type-specific functions underscoring that cell cycle control is intimately coupled to developmental and environmental cues.

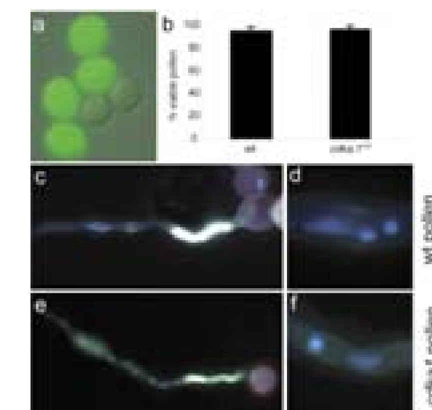


Figure: Viability and in vitro germination ability of cdka;1 mutant pollen.

"Especially in plants, cell cycle control appears to be of major importance since plants are immobile and rely on an iterative production of new organs."

Selected Publications

Nowack, M.K., Grini, P.E., Jakoby, M.J., Lafos, M., Koncz, C., and A. Schnittger: A positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis. Nat. Genet. 38 (1), 63 - 67 (2006).

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Weinl, C., Marquardt, S., Kuijt, S.J.H., Nowack, M.K, Jakoby, M.J., Hülskamp, M. and A. Schnittger: Novel functions of plant cyclin-dependent kinase inhibitors - ICK1/KRP1 can act non-cell-autonomously and inhibit entry into mitosis. Plant Cell 17 (2005).

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Plant Computational Biology

Heiko Schoof

Introduction

My aim is to contribute to a vision of data-driven, systematic, quantitative and predictive biology through computational linking of genomic data to life processes. Genomic approaches in recent years have generated large-scale data sets on many aspects of plant biology, and the complete genome sequence of *Arabidopsis thaliana* has been both a rich source for information as well as a backbone against which to organise plant biology data. The complete list of genes available through the genome represents the basic building blocks, but only through analysing how they interact and are regulated will we understand how the genetic information leads to a complex organism. Many levels of information need to be integrated: sophisticated genome sequence analysis, extraction of patterns from gene regulatory networks, and computer-tractable representations of phenotypic data. On many levels, data are being generated and computational analysis is progressing, but bringing individual efforts together for a more comprehensive overview remains a major challenge.

Current Projects

My group will address three key problems encountered in the comprehensive integration of genomic data: (1) the technological challenge of enabling high-throughput data integration, (2) biologically meaningful interpretation of large integrated datasets and (3) leveraging comparative genomics for evolutionary analysis. (1) To enable high-throughput data integration, we will aim at a distributed, service-oriented approach to data availability on the basis of internet technologies like web services or semantic web. (2) To exemplify comprehensive correlative analysis, we will aim to extract regulatory elements that affect posttranscriptional regulation from large-scale integrated genomic data. Posttranscriptional regulation has an important role in plants, yet little is known about regulatory elements in mRNA sequences. We will predict and analyse regulatory elements in the untranslated regions of plant mRNAs using statistical, machine learning and phylogenetic tools to correlate sequence conservation with gene expression, RNA structure and protein function.
(3) To utilize evolutionary relationships both as an essential tool as well as a source of important new knowledge, we apply (comparative) genome analysis. In the scope of the tomato genome project, the group is involved in annotation of new genomic sequences as well as integration of genome with functional genomics and phenotypic data in an international consortium (funded by the EU Sixth Framework Programme). However, the availability of multiple plant genomes will also contribute to the analysis of conserved regulatory elements.

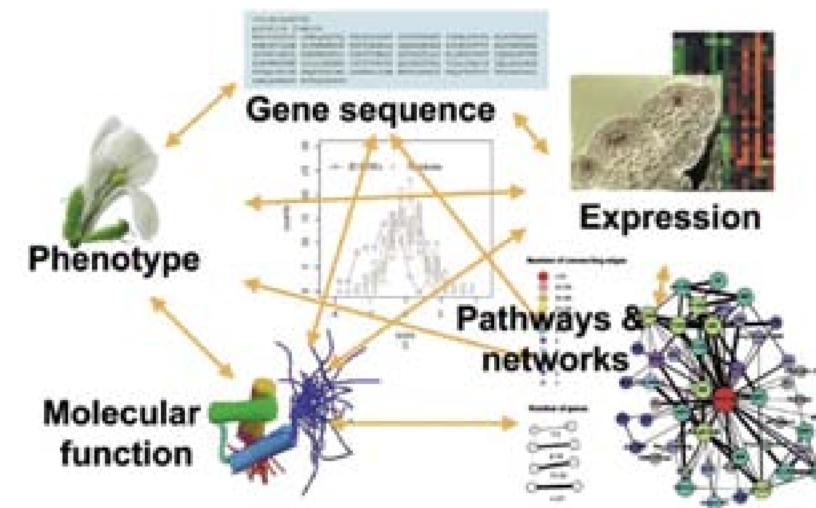

Figure 1.
Many levels of information and heterogeneous data types need to be integrated for a comprehensive understanding of how genetic information leads to complex organisms. While the initial focus in genome projects was on protein-coding genes and their function, we next need to understand their interactions and regulation. To some extent, genome sequences contain regulatory signals. By correlating sequence with function, metabolic pathways, expression data, phenotypes, interaction networks, we can try to select sequences with putative regulatory function. The graph in the centre shows, for example, correlation of a set of conserved sequence motifs (purple) in comparison to random motifs (sampled 10 times, black).

"Predictive biology through computational linking of genomic data to life processes. "

Selected Publications

Schoof H, Spannagl M, Yang L, Ernst R, Gundlach H, Haase D, Haberer G, Mayer KF.: Munich information center for protein sequences plant genome resources. A framework for integrative and comparative analyses. Plant Physiol. 38(3): 1301-1309 (2005).

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Plant Physiol. 38(1):5-17 (2005).

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Plant Chemetics

Renier van der Hoorn

Profiling approaches have generated a tremendous wealth of information on genomes, transcriptomes and proteomes for many intriguing biological processes. Activities of enzymes, however, are post-translationally regulated and cannot solely be predicted by the presence of proteins. The Plant Chemetics Group will apply activity-based proteomics to reveal this new and most useful level of functional information.

Activity profiling is a key technology in activity-based proteomics and is based on the use of biotinylated, mechanism-based inhibitors (probes, e.g. DCG-04; Fig. 1) that react with whole classes of enzymes in an activity-dependent manner. Probes for cysteine and serine proteases, glycosidases and phosphatases are already available, and more probes are being developed. As a proof-of-concept, activities of many papain-like proteases in plants have been displayed using activity profiling (Van der Hoorn *et al.* 2004).

Chemical tools to investigate plant-pathogen interactions

Within the Plant Chemetics Group, activity profiling is applied to investigate the role of enzymes (e.g. proteases) in the process of bacterial pathogenesis in plants. Diverse roles for protease activities can be expected during plant-pathogen interactions (Fig. 2A). However, despite huge efforts to understand plant immunity, mechanisms underlying the disease process, in particular reprogramming of host cells for colonisation, are intriguing but unresolved. Presumably, plant proteases act in pathogen perception, signalling or defence, whereas pathogen-derived proteases and inhibitors may interfere with these processes or release nutrients (see also Van der Hoorn & Jones, 2004). By applying activity profiling with DCG-04 on *Pseudomonas*-infected cell cultures, it was demonstrated that the activity of one of the papain-like cysteine proteases, called RD21, is increased during the plant defence response by avirulent *Pseudomonas*, but is post-translationally suppressed in the presence of virulent *Pseudomonas* (Fig. 2B). Importantly, preliminary data indicate that *Arabidopsis* plants lacking RD21 are more susceptible to various pathogens (Fig. 2C), suggesting that this enzyme plays a role in plant defence.

Future developments

The Plant Chemetics Group aims to reveal activities of additional enzyme classes during the *Arabidopsis-Pseudomonas* interaction,

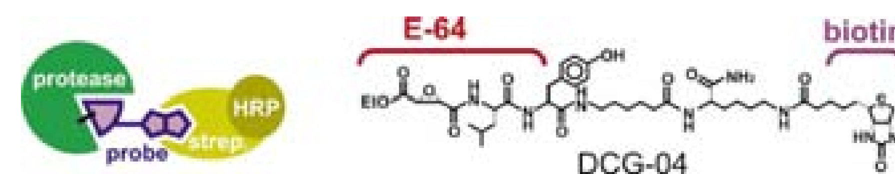


Figure 1. Principle of protease activity profiling. Left: A biotinylated protease inhibitor (probe) reacts covalently with active proteases and is detected with streptavidin-HRP conjugates. Right: DCG-04, an example of a mechanism-based probe for protease activity profiling.

including serine proteases, caspases, lipases, desumoylating enzymes, phosphatases and glycosidases. This requires (1) to further develop activity-based technology in order to design better probes (in collaboration with small-molecule chemists), (2) to establish labelling *in vivo* and (3) to launch high-throughput analysis. Differentially-activated enzymes of both plant and pathogen are further studied through multidisciplinary approaches, focussed on defining roles for these enzymes during pathogenesis. Probe-derived chemical compound libraries are used to screen for specific inhibitors to knockout enzymes chemically and to study their function in plant disease.

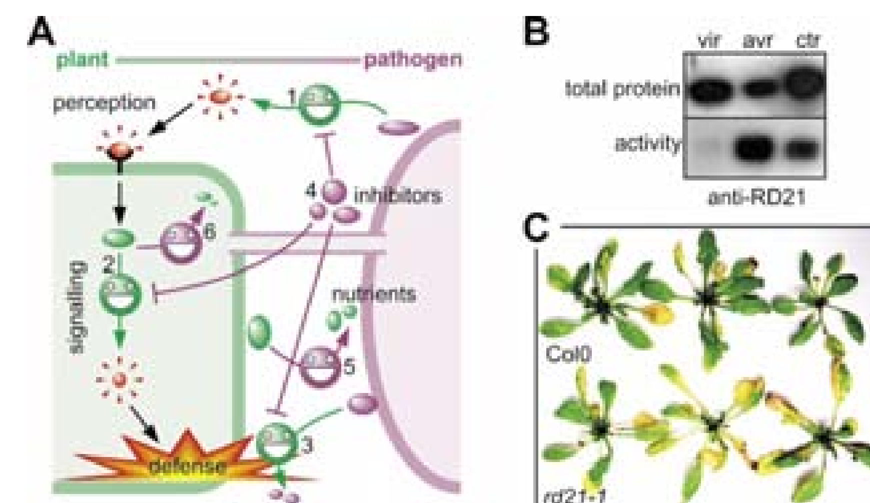


Figure 2. (A) Possible roles of proteases in plant-pathogen interactions. Plant proteases (green) can play a role in perceiving pathogens (1), defence signalling (2) or basal defence (3). Vice versa, pathogens may produce inhibitors (4) and proteases (purple) that release nutrients from the host (5) or interfere in host defence signalling (6). (B) Differential activation of RD21 protease during infection of Arabidopsis cell culture with Pseudomonas (Pst) bacteria. RD21 antibodies were used to display RD21 protein accumulation (top), and its activity (bottom), 24 h after infection. For activity display, total cell extracts were labelled with DCG-04, biotinylated protease were purified and detected with anti-RD21 antibody. vir, virulent Pst; avr, avirulent Pst; ctr, non-infected control. (C) Reverse genetic studies on RD21. The rd21-1 knock-out line appears more susceptible for virulent Pseudomonas bacteria. These experiments are being confirmed with an independent knockout line.

"We use activity based profiling and other chemistry tools to unravel functions of proteins in plants."

Selected Publications

Rooney, H., Van 't Klooster, J., Van der Hoorn, R. A. L., Joosten, M. H. A. J., Jones, J. D. G., and De Wit, P. J. G. M. (2005) *Cladosporium* Avr2 inhibits tomato Rcr3 protease required for Cf-2-dependent disease resistance. Science 308, 1783-1789.

Van der Hoorn, R. A. L., Wulff, B., Rivas, S., Durrant, M. C., Van der Ploeg, A., De Wit, P. J. G. M., and Jones, J. D. G. (2005) Structure-function analysis of Cf-9, a receptor-like protein with extracytoplasmic leucine-rich repeats. Plant Cell 17, 1000-1015.

Van der Hoorn, R. A. L., Leeuwenburgh, M. A, Bogyo, M., Joosten, M. H. A. J., and Peck, S. C. (2004) Activity profiling of papain-like cysteine proteases in plants. Plant Physiol. 135, 1170-1178.

Van der Hoorn, R. A. L., and Jones, J. D. G. (2004) The plant proteolytic machinery and its role in defence. Curr. Opin. Plant Biol. 7, 400-407.

Van der Hoorn, R. A. L., De Wit, P. J. G. M., and Joosten, M. H. A. J. (2002) Balancing selection favors guarding resistance proteins. Trends Plant Sci. 6, 67-71.

Service Facilities

Central Microscopy (CeMic)

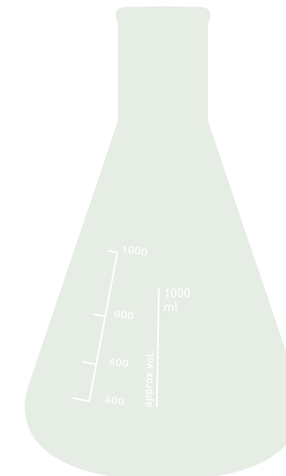
(Elmon Schmelzer)

The service group Central Microscopy (CeMic) provides comprehensive knowledge concerning cytology along with practical experience and technical skills to allow optimal cytological/microscopical work to take place at the MPIZ. CeMic is involved in studies of a wide range of living or fixed specimens for which conventional light or fluorescence microscopy, confocal laser scanning microscopy, and scanning as well as transmission electron microscopy are used. Besides performing collaborative research on cytological aspects, CeMic gives advice on cytological questions and instructs researchers in cytological methods, operation and usage of microscopes and accessories. In addition, CeMic is responsible for introducing new techniques and equipment.

Greenhouse Management

(Wolfgang Schuchert)

The greenhouse area comprises 5000 m^2 under glass with 2000 m^2 of workbenches, 7 saran houses and halls for growth chambers. Due to the increasing demand for growth chambers, we had applied for two further halls which were approved in accordance with the German Gene Technology Act (Gentechnikgesetz) in September 2003. The greenhouse staff includes 9 gardeners having to handle 80 to 100 culture orders per week. Around 3000 planting trays (size 0.25 m^2 each tray) are circulating in the greenhouse area. Each month 30 m^3 of soil substratum are used. By implementing integrated pest and disease management strategies, we try to reduce the application of chemical treatments. A thermal inactivating procedure for transgenic *Arabidopsis* plants (secondary transformants) complete with seeds and cultivating soil substratum as in accordance with §13 of the German Gene Technology Safety Decree (Gentechnik-Sicherheitsverordnung - GenTSV). This system replaces the excessive use of autoclaving, and thereby saves considerable energy costs.



Mass Spectrometry (MS)

(Jürgen Schmidt, Thomas Colby)

Interest in mass spectrometric analyses is steadily increasing in all Departments at the MPIZ, as is the demand for improved sample acquisition and proteomic data administration technologies. In addition to the MALDI-TOF and Q-TOF MS systems run since January 2002 in the Institute, we have installed two robots to prevent bottlenecks in large-scale sample preparation of high-throughput proteomics and metabolomics. Since February 2005, we have used a spot-picking robot to image gels, detect spots and then to cut them out and distribute them into 96 micro-well plates. These plates are moved to the digest and sample robot (installed 2003), which is used for automated in-gel digestion and target plate preparation prior to MALDI- and/or Q-TOF analysis. The MALDI-TOF MS and the sample robot are not only used for the identification of proteins and peptides, but also for the analyses of various biomolecules in metabolomics screening programmes. In the present configuration, spot detection can be carried out from Coomassie or silver stained gels using a daylight scanner, and also from gels labelled with fluorescent dyes by using a laser scanner for gel imaging. Since February 2005, the administration of proteomic data has been significantly improved. The data of all scanned gels are stored in a central repository and essential information about all spots analysed is accessible, e.g. general spot information, position of samples in digest plates and on MALDI targets, MS spectra, protein database search results from two search algorithms and information on the identified proteins.

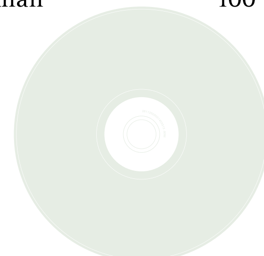
Service Facilities

Plant Bioinformatics
(Kurt Stüber)

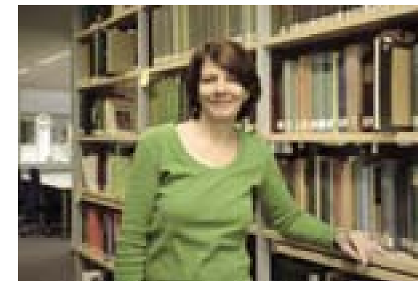
Bioinformatics services are provided for all scientists in the institute. This includes collaborations with other groups, development of software for specialised needs and an educational programme to introduce old and new colleagues to programs available in the central computing facilities. Current research concentrates on the evaluation of genomic and EST sequences from *Antirrhinum majus, Arabidopsis thaliana* and other plants. New algorithms for the prediction of signalling structures and for phylogenetic comparison of sequences are also being developed. As a service to the MPIZ, a data base to store and evaluate the results of chemical genetics experiments is currently under construction. Finally, an online library of historical biological manuscripts is maintained which receives much attention as a valuable resource for biological teaching and research (www.BioLib.de).

Computer Group (SUSAN)
(Guido Goebel)

The service unit SUSAN (scientific user support – system administration – networking) is responsible for the maintenance of all computing facilities at the MPIZ.
The network infrastructure consists of structured cabling, i.e. Gbit Backbone (fibre optic) and 100Mbit (twisted pair), to the labs and offices, along with a wireless local area network in the greenhouses and foyer of the lecture hall. We are also a part of the G-WIN of the DFN (German Research Network). The bandwidth of the gateway is 34Mbit. We support more than 40 servers with different functionalities (NIS, LDAP, Mail, Web, DHCP, Proxy, Firewall, File- and Printserver, Biosoft Applications) and about 20 Extreme Network Switches. Recently acquired equipment includes 6 transtec 4200L Servers all equipped with 4 Intel Xeon MP 2.7 GHz Processors and 8 GB ECC DDR-RAM and 3 transtec 6100 SATA Premium RAID Systems all equipped with 16 x 400 GB SATA HDs. Overall, we support about 500 devices running diverse OSs which are linked to the Ethernet and are involved in the ordering, installation and integration processes. Moreover, we also offer advice on computational issues and we generally deal with between 100 and 200 inquiries per month. To further support the researchers, SUSAN offers special courses for bioinformatic basics as well as for MicroSoft's office package.



Library
(Britta Hoffmann)



The library at the MPIZ contains around 20,000 journal volumes and 5,000 monographs. In 2003, we stocked 140 printed journals; however, we now subscribe to mostly electronic journals and have just 40 printed journal available. Currently, about 4,000 titles are available electronically, and between 2003 and 2005, 2,000 different electronic journals and over 43,000 articles were accessed from the Institute. In addition, the library obtains requested literature that is not available on site. For the researchers' convenience, users simply fill out a form available on the intranet which is then forwarded to the library's database. In the last two years, 1,760 articles have been ordered with this tool.

After extensive renovation in 2003, five PC workstations and an additional ten regular working desks have been made available for the users of the library. The number of PC workstations will be tripled to meet the growing demand for electronic information supply. The library perceives its task to be the continuing improvement of the information supply for the scientists. This task should be performed in close co-operation with other Max Planck Institutes and the Max Planck Society.

Automated DNA Isolation and Sequencing (ADIS)
(Diana Lehmann)

ADIS provides a DNA-related technological service at the MPIZ. The increasing automation of modern molecular biology techniques requires complex and expensive equipment. This is especially true for DNA isolation, DNA sequencing, quantitative PCR, clone and library handling, and the creation of DNA and clone arrays in combination with the respective bioinformatics tools. In 1995 and in accordance with the programme "Zukunftsperspektiven des MPIZ", a core facility was started to provide a service for highly requested and automatic modern techniques for all departments at the MPIZ. The unit was designated ADIS for "automated DNA isolation and sequencing".
In 2003 and 2004, about 150,000 sequences per year were operated by ADIS, half of them belonging to the GABI-Kat project. The large amounts of data resulting from these activities are handled by a "laboratory information management system" (LIMS) designated SeqOrderDB. ADIS provides a html/cgi-based oligonucleotide ordering software to purchase oligos. At present, the MPIZ orders about 260 oligonucleotides on average per week, mainly for PCR-related experiments.

Press and Public Relations

Press and Public Relations

(Claudia Vojta, Wolfgang Schuchert)

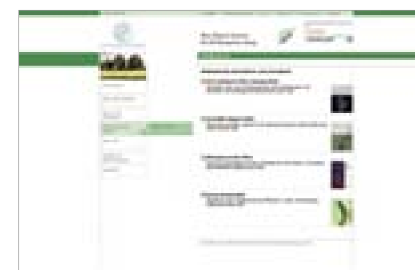
An open dialogue is the basis for a stable and long lasting partnership between our Institute and the public. It is of great importance to us that not only the scientific community but in fact anyone interested in modern science should have the opportunity to feel informed about the research activities at the MPIZ.
The Public Relations Office at the MPI for Plant Breeding Research was established in 1990 to ensure active and open dialogue with the public. It was initially set up as a response to criticism triggered by the first German field trial with genetically modified petunia plants at the MPIZ. The Public Relations Office is responsible for the central co-ordination and documentation of all PR activities and works closely with the PR Department of the Max Planck Society (MPG).

The main focus of the PR work is informing the public about the research at the Institute, creating a communication and contact network in NRW (the state of location), developing a concept for our new web site, improving internal communication through the internal web pages and the staff magazine MPIZinside. The MPIZ has adopted an active programme to increase public awareness and understanding of science, educate politicians with regard to the benefits of promoting and supporting science and basic research, and attracting the interest of companies and the business community in activities ongoing in the Institute. The reason for pursuing these programmes is the need for science to prove its value to the public, politicians and the private sector. This is essential if science is to receive the support it needs to continue to feed the market places with innovative opportunities for commercial development as well as to satisfy the core mission of research in the life sciences. These programmes are also entirely compatible with the core mission of the MPG, i.e. to generate knowledge for the public's benefit.

Internet presentation

The Institute serves an own homepage www.mpiz-koeln.mpg.de that is regularly updated and provides information about the Institute's structure, the various research groups and their main research interests, service groups, as well as PR activities such as news, seminar announcements, the online show garden with information about 80 different crop plants, our visitor's programme and also a collection of press releases and newspaper clippings. Our latest achievement is the collection and revision of the "Frequently Asked Questions" (FAQs) about the Max Planck Society, the MPIZ and about the sensitive issue of green gene technology. Moreover, essential information on the PhD programme of the IMPRS can be found on these pages. The MPIZ's homepage also has information on job opportunities and allows access to the Institute's library.

In May 2005, we took up the challenge to revamp the entire MPIZ's homepage. We were one of the first Max Planck Institutes to implement the new Content Management System (CMS) of the MPG. Thereby, guaranteeing browsing comfort for the user due to the clear and intuitive structure and allowing easy support and maintenance by the provider.



Visitors

There is a continuous flow of visitors to the MPI for Plant Breeding Research. We welcome members of the public or professionals from all sectors within the community to visit the Institute and learn about the profile of research conducted within the Institute, get an extensive look at the greenhouses and see the major visitor's attraction, the Institute's show garden.
As very few people know about agriculture, despite everyone using crop plant products every day, we try to demonstrate different aspects of biology and agriculture in our show garden. At present, about 100 agricultural and horticultural plants are growing in small plots in our exhibition of crop plants. The show garden also provides information on a number of topics about plant breeding research in both an agricultural and environmental context. The main topics covered are evolution, the Mendelian laws, breeding methods (including hybrid vigour), genetically modified plants, renewable resources and plant diseases. Visitors can compare wild forms, old land races and modern varieties. The Mendelian laws are demonstrated by flower colour of *Cosmea* and *Antirrhinum* to show inheritance of intermediate and dominant traits as the basis for classical breeding. Modern maize varieties in comparison to inbred-lines demonstrate the effect of hybrid breeding. The range of renewable resources for industrial purposes comprises typical oil, fibre and starch plants. The show garden also includes two examples of transgenic plants: (1) maize tolerant to the European corn borer and (2) maize tolerant to a broad spectrum herbicide. To demonstrate plant diseases and stress on the different resistant levels of the planted varieties, no chemical pesticides are used. In the past two years, approximately 1800 people have visited and toured the MPIZ. Apart from pupils and students, there have been representatives from business, politics, the church and the media, as well as from numerous other associations. The tours for politicians, professional organisations and special interest groups are conducted with the long-term goal of not only increasing awareness of science among political leadership but also to encourage politicians to adopt policies supporting basic research and to lobby for further funding from within the political system.
Many of our non-scientific guests – especially teachers – ask for laboratory visits and courses in biotechnology. Such lab-based training is given by our co-operational partner KölnPUB within the framework of teacher education.

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Consulting & information service for green gene technology

Apart from our regular PR work, we prepare application procedures and statements concerning the gene technology legislation (Gentechnikrecht). This includes applications for greenhouses to cultivate transgenic plants as well as statements with regard to amendment of the gene technology law. We also provide an information service concerning biotechnology and agriculture (regulation, field trials, commercialisation of transgenic plants, labelling, risks and benefits).

Internal PR work

Staff magazine - MPIZinside

MPIZinside is a magazine published quarterly that provides information on the activities of the Institute to all staff. The PR group has incorporated new ideas on the layout and on how to communicate news and views from within the Institute as well as highlighting recent research and publications. MPIZinside is one forum through which everyone should be informed of the developments at the MPIZ such as new research groups, internal information about works council as well as the ongoing construction. The content is also presented on the inter- and intranet.

Internal communication

For a good research climate, it is not only crucial to have the right equipment, but also to maintain a healthy working atmosphere. The TATA Bar was set up by the PhD students and is regularly used by all the Institute staff for small get-togethers. A major highlight of the social calendar at the MPIZ is the Summer Party held in September. This is always preceded by the Institute Run. Due to the success of this run, we now have another sporting event, namely the MPIZ Soccer Cup. Another way to improve internal communication is by running competitions. For example, in August 2005, we ran a coffee mug competition, in which staff members submitted possible designs for the new Institute mug. The Internal web pages are a useful source of information for our staff. These pages provide technical information, details of contact persons, service requests, information on new colleagues, picture galleries from the Institute run, the football cup and the summer party and also allow access to download areas, e.g. to the starter kit “visitor’s folder”.

How to get to the MPIZ

By car

Motorway A1:
Take the Lövenich exit (# 103), turn right at intersection, drive along “Aachener Straße”, direction to Köln-Zentrum.
After about 1 km turn right towards direction A1 (north), A57 (north), Ossendorf, (“Militärring”).
Turn left at next intersection (T crossing), take the third exit and follow the signs to the Max Planck Institute.

By train

Arrival at Cologne main station (Köln Hauptbahnhof):

- Underground #5 direction Ossendorf to station “Subbelrather Straße/Gürtel”, then transfer to
- Bus #141 direction Vogelsang (the bus stop is on the other side of the intersection on “Subbelrather Straße”) to station “Goldammerweg”.
- Walk (about 15 min.) straight on “Vogelsanger Straße” and “Carl-von-Linné-Weg” (cross railway and motorway, pass the farm on the right-hand side).

By plane

Airport Cologne/Bonn:
S-Bahn “S13” or train Regionalbahn „RE8“ to Cologne main station “Köln Hauptbahnhof”

Airport Düsseldorf:
S-Bahn „S7“ to Düsseldorf main station “Düsseldorf Hauptbahnhof”, then take train IC, ICE, EC to Cologne main station.

Then continue as described under arrival at Cologne main station.

Contact

Contact MPIZ

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Notes

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The MPIZ is continually growing and evolving.
For up-to-date information please see our website: www.mpiz-koeln.mpg.de

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