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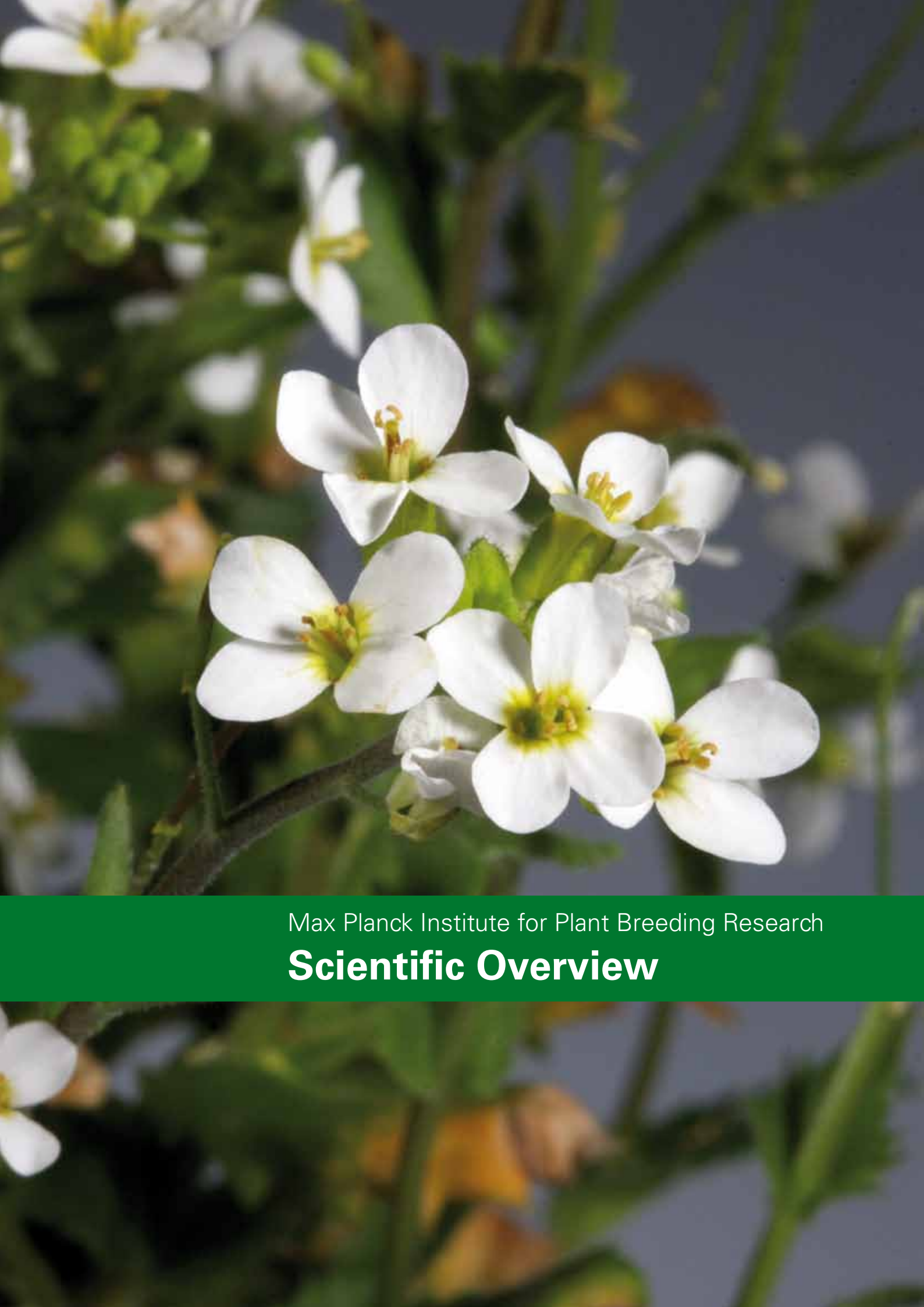
Max-Planck-Institut für
Züchtungsforschung



Max Planck Institute for Plant Breeding Research

Scientific Overview

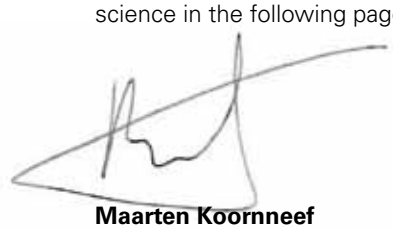
Welcome to our Institute

We have compiled this overview to provide an introduction to the scientific work of our Institute. In it we summarise the organisation and scientific aims of the Institute, and outline the projects being pursued by 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 report describes the work of 25 research groups. Half of the Directors, Group Leaders and PhD students, and two-thirds of the postdocs, come from abroad, underlining the international character of the Institute. In addition to the departmental groups, the Institute houses three independent research groups working on topics that are not covered by any of the four departments, and we hope to increase the number of such groups in the future. We place great emphasis on the training and mentoring of PhD students, and we have greatly extended our activities in these areas in recent years. The over 60 PhD students at the Institute represent a sizeable fraction of the 350 staff working here. Many of them participate in coordinated programmes such as the International Max Planck Research School (IMPRS). The progress of all students is followed closely by our Student Coordinator, who offers courses and advice, and organises retreats and annual student meetings.

Members of our Institute play important roles in plant science at both national and international levels, and we are indebted to the organisations that make this possible. We have particularly close links with the University of Cologne, which participates in our IMPRS and provides the academic framework for our PhD students. In addition, we collaborate with the University in the context of four Special Research Areas (Sonderforschungsbereiche) funded by the German Research Society (DFG). We are especially 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.



Maarten Koornneef
Managing Director

Historical background of the Institute

The Institute was originally founded in 1928 as part of the Kaiser-Wilhelm-Gesellschaft, and was then located in Müncheberg (Brandenburg). The founding Director, Erwin Baur, initiated breeding programmes with fruits and berries, as well as basic research on *Antirrhinum majus* and the domestication of lupins. After the Second World War, the Institute moved West to Voldagsen (Niedersachsen), and was relocated to new buildings on the present site in Cologne in 1955. 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 broadened 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 were constructed for the departments led by Schell, Hahlbrock and Salamini, in addition to a new lecture hall and the Max Delbrück Laboratory building that housed independent research groups over a period of 10 years.

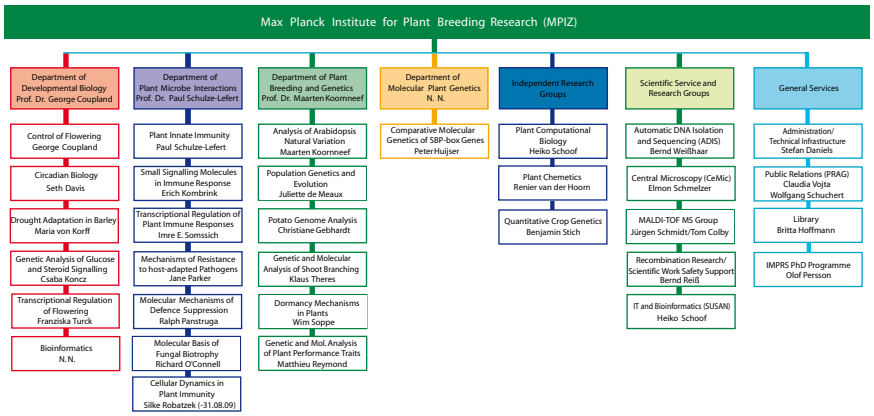
A new generation of Directors was appointed starting with the year 2000 with the approaching retirements of Klaus Hahlbrock and Jeff Schell. Paul Schulze-Lefert and George Coupland were appointed in 2000 and 2001, respectively, and Maarten Koornneef arrived three years later upon the retirement of Francesco Salamini. The new scientific departments brought a strong focus on utilising model species to understand the regulatory principles and 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 arrival of a new generation of Directors also required modernisation of the infrastructure. So far, this has involved complete refurbishment of the building that houses the Plant Developmental Biology laboratory (2004), construction of a new guest house and library (2005), as well as new buildings for the administration and technical workshops (2009), and the planning of a new laboratory building for the Koornneef Department (to be completed in 2012). The new laboratory building will include a section that will link all four scientific departments and house meeting rooms, offices and the Bioinformatics Research Group.

Directors at the Institute in Cologne (since 1955)

1936 - 1961	Wilhelm Rudorf
1961 - 1979	Josef Straub
1967 - 1978	Wilhelm Menke
1978 - 2000	Jeff Schell
1980 - 2009	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, three independent research groups, three scientific service groups, the greenhouse service group, an outreach department that presents plant science to the public and press, and the administrative department, which includes the technical workshops and library.



The Board of Directors is responsible for the management of the Institute. The Board is made up of the Directors of the four scientific departments and the Head of Administration. It meets once a month under the chairmanship of the Managing Director. The Board of Directors takes 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 provide a broader basis for decision making.

Each Director is also head of a scientific department and is responsible for the scientific programme, budget and personnel of that department. Each department comprises several research groups that are led by research scientists, who in turn 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 Coordinator, who manages the Graduate Schools and is responsible for student welfare, is formally attached to the administrative 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 meets monthly to discuss issues raised by its members. Major issues discussed have been future scientific strategy, particularly how to incorporate crop-plant research within the Institute, purchase of large pieces of equipment, and new recruitments. This committee has proven to be an important conduit for channelling the views of research scientists to the Board of Directors, and has helped create 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 links with local and national organisations within the sphere of activity of the Institute.

Research objectives and major emphases

Background and present status

"How can plant breeding be transformed into a rational, predictive science?" This question motivates all of the research programmes at the Institute. We wish to determine whether and how a detailed understanding of molecular mechanisms defined in model plant species can be used to rationally manipulate selected traits in crop plants.

The last 15 years have seen a tremendous increase in our knowledge of the molecular mechanisms underlying plant biology. This progress is largely based on studies on model species, principally *Arabidopsis*. However, the deeper knowledge of the regulatory components and mechanisms controlling plant traits that has resulted from these studies has not had 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. This also requires the study of biological processes in crop plants, with particular emphasis on understanding the variation present within each species. Genetic variation is the raw material with which plant breeders work. Greater knowledge of the processes and genes that control trait variation will allow much more efficient breeding, using either marker-assisted selection or the transfer of genes to 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 proteins they encode are often poorly understood. Therefore, focused programmes have been established within the Institute to elucidate the molecular mechanisms controlling traits of agronomic importance. These programmes investigate 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 an understanding of the natural plasticity

of complex traits under the influence of quantitative genetic variation. To study this variation in more detail, molecular population genetics has become an important tool in addition to quantitative genetics. Comparative studies of the function of similar genes in different species help us 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*, on the basis of their relatedness (*Arabis alpina*) or because they display specific genetic complexities or properties, as in the case of the tetraploid potato. It is hoped that a better understanding of this Darwinian variation will teach us how known regulatory networks can 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 that bridge the boundaries between traditional research disciplines like genetics, molecular biology, biochemistry, cell biology and computational biology are crucial for 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 help answer such questions. In recent years, we have greatly improved our technology platforms in protein mass spectrometry and confocal and electron microscopy. In addition, by setting up a dedicated independent research group and heavily investing in new computer infrastructure, the Institute has significantly enhanced its resources in the area of bioinformatics, annotation of genome sequences and interspecies sequence comparisons. Furthermore, the establishment of an independent research group in crop genetics has increased the expertise available in quantitative genetics. In addition, the infrastructure for DNA sequencing, Affymetrix microarrays, and polymorphism detection has been continually modernised. Continuous development of this complex technological infrastructure is critical for a rational understanding of plant traits. It is heavily utilised by all four departments and provides attractive training opportunities for students, most of whom will have encountered only the traditional research disciplines at university.

Future orientation

The Institute's mission requires a more concentrated and co-ordinated effort to balance research in model and crop plants to complement the ongoing work in potato and tomato. In particular, a major focus on barley that would be studied across all departments is expected to provide momentum in this area.

The development of "next-generation" sequencing technology provides novel opportunities for genome-based research that also deals with the issues of natural variation and biodiversity. Currently these technologies are being used for genome sequencing of several fungal species, and genome sequencing of *Arabis alpina* has been initiated. They also find application in the area of gene expression. The establishment of a Genome Centre where next-generation sequencing will be used is in preparation. As well as the existing departments, the new research group on fungal biodiversity recently approved by the Max Planck Society in the department of Plant Microbe Interactions will benefit from these facilities. Furthermore, a new department in the field of crop genetics and evolution, to be led by a newly recruited Director, will be founded to succeed the department of Plant Molecular Genetics. The latter development requires an update of our plant growth facilities, and in the near future our phenotyping facilities will also be extended to complement the laboratory phenotyping facilities such as luciferase imaging and the Opera system for large-scale screening of plants based on microscopic observations.

Co-operation and communication within the Institute and with Cologne University

Interactions within the Institute

The Departments of Plant Pathogen Interactions and Plant Developmental Biology have co-operated to establish the field of chemical genetics in 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 to screen a small library provided by the partner, in order to probe plant biology.

All departments have collaborated closely with the two graduate schools present in the Institute.

Co-operation with the Cologne University

Groups from the Institute take part in four Special Research Area (SFB) programmes with the University that are funded by the DFG (Deutsche Forschungsgemeinschaft). One of these programmes focuses on Cell Specification (SFB 572) and another on Post-translational Control of Protein Function (SFB 635). SFB 680 deals with the Molecular Basis of Evolutionary Innovation and SFB 670 with Cell-autonomous Immunity. In total, these programmes fund twelve research projects in the Institute.
The Institute's Graduate School is run as close collaboration with the University. The International Max Planck Research School (IMPRS) includes faculty from both the Institute and the University. It provides a forum for scientific communication, as it improves contacts and collaborations between plant science groups in both organisations on a daily basis, giving a thorough overview of all plant research activities through the yearly IMPRS retreats involving both faculty and students.

Ph.D. programme and education

Providing high-quality education for young researchers is of particular concern to the MPIZ. We support the future of plant science by producing well-educated young scientists with new ideas, novel concepts, unconventional approaches, creativity, and scientific curiosity, who can promote multilateral collaborations in an increasingly complex scientific network. Young scientists from all over the world with diverse scientific backgrounds find here a research environment that supports their development as researchers. The Institute's research would be unthinkable without the contribution of our students. Moreover, the international character of the Institute offers valuable insights into intercultural differences that allow the students to reconsider their position in a globalised world and to prepare for the international challenges to come.

The doctoral programmes, especially the graduate programme IMPRS, also promote scientific collaboration among European institutions. Training in modern plant sciences thus contributes to the future of the whole continent.

Our Research School helps to curb the loss of scientific talent and counteract the diminishing interest in plant science as a career path. This interdisciplinary approach ensures that the students not only obtain a Ph.D. degree, but also have the opportunity to learn complementary skills that will be recognised as a valuable career qualification. During the entire graduate programme each student receives scientific support from a Ph.D. Advisory Board, but also general support from fellow students and scientists from neighbouring disciplines. The demands of the modern scientific community are met through complementary training, including training in the communication of science and preparation for an increasingly dynamic and flexible global job market.

The statistical data for the past several years reveal a continuously high level of interest in joining our research programmes among applicants from all parts of the world. This clearly demonstrates that the Ph.D. education provided at the Max-Planck Institute for Plant Breeding Research is internationally recognised, and underlines the MPIZ's reputation as an attractive institution for a qualified Ph.D. education.

Overview: Department of Plant Developmental Biology

Director: George Coupland

Plants can thrive in locations in which 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 redirect plant development in response to environmental signals, and focus on the effect of environment upon flowering. Many plants flower in response to environmental signals, and these responses permit plants to adapt to growth conditions at particular locations and help maximise yields 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. Our 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, the importance of chromatin structure in controlling the transcription of flowering-time genes and how abiotic stresses influence flowering. We also study how these processes have evolved in other plant species. Here, we focus particularly on the modifications of flowering pathways that took place during domestication of barley and on the mechanisms by which distinct life histories, such as perennialism, have emerged during evolution.

Control of flowering: Molecular mechanisms and phenotypic diversity

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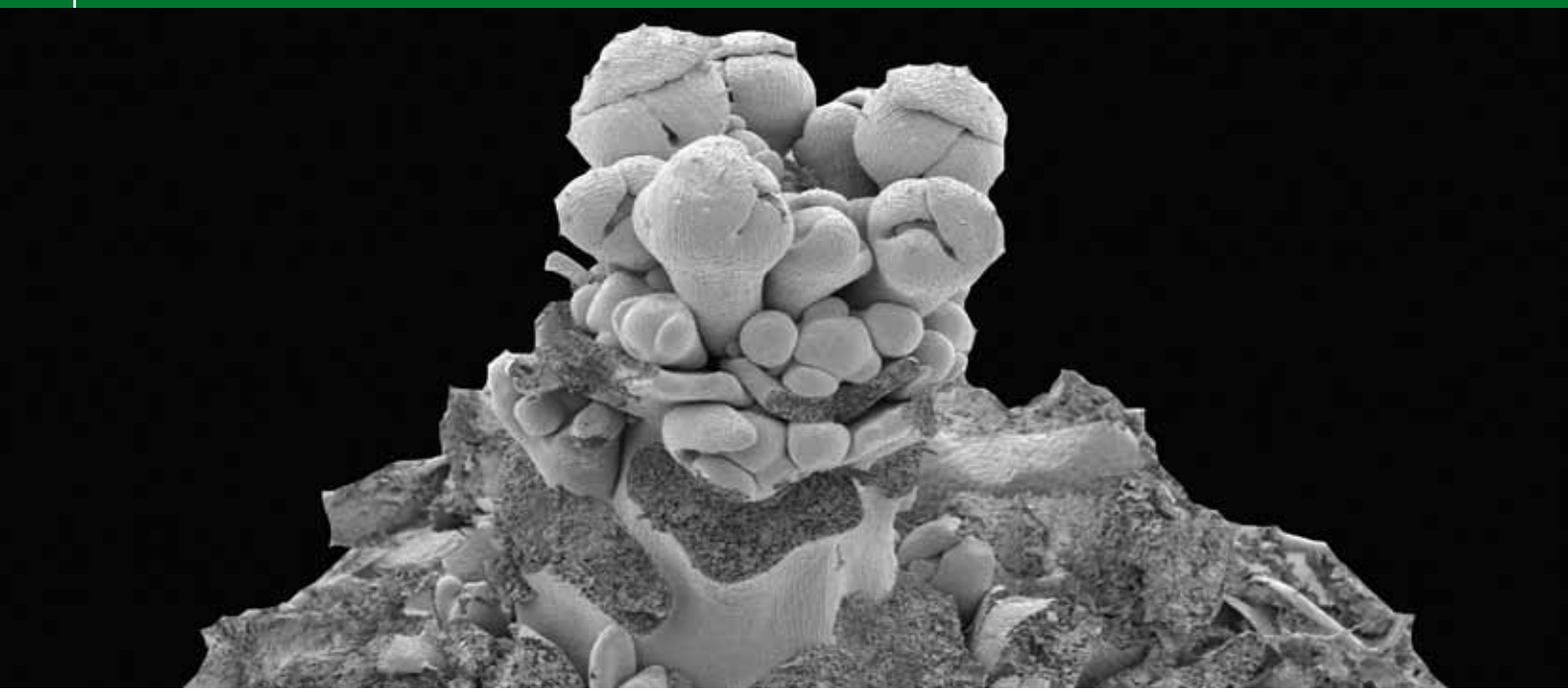
George Coupland

Department of Plant Developmental Biology



Plants flower at characteristic times of the year in response to seasonal changes in day length or temperature. These responses are exploited in agriculture so that crops flower synchronously at the optimal time to ensure maximal seed production. In contrast, in natural populations, there is tremendous variation in flowering time even within a single species, where genetic differences between individuals can radically change flowering time.

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. Such responses to environmental stimuli are responsible for the familiar seasonal patterns of flowering seen in nature and exploited in agriculture. We have defined a regulatory pathway that allows Arabidopsis to discriminate between short and long days, and trigger flowering in response to the latter. The difference in day length is perceived in the leaves through regulation of the *CONSTANS* gene: exposure to light late in the day causes the level of *CONSTANS* mRNA to rise and stabilises its protein product. These two layers of regulation ensure that CONSTANS protein accumulates in nuclei specifically under long summer days, and activates transcription of two closely related genes, *FT* and *TSF*. The FT protein is exported from the leaves to the shoot apical meristem via the vascular tissue of the plant. At the shoot apical meristem, FT triggers changes in gene expression so that sets of transcription factors specifically associated with flowering become active. Within a few hours of exposure to long days, transcription of genes that induce flower development, such as *SOC1* (which encodes a transcription factor), is initiated in the meristem.

We have also studied elements of this system in *Pharbitis nil* (Japanese morning glory) and shown that the same basic mechanisms defined in Arabidopsis are utilised in this species as well. However, they are modified to allow Pharbitis to flower in the autumn.

We have developed Arabis alpina, a close relative of Arabidopsis, as a model system to study perennialism.

The Pharbitis *FT* gene is expressed under short days of autumn, and the mechanism that measures day length is altered so that the length of the night rather than of the day is measured.

Arabidopsis provides a powerful system in which to study environmental control of flowering and 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. We have developed *Arabis alpina*, a close relative of Arabidopsis, as a model system for the study of perennialism. *A. alpina* plants show classical features of perennialism. For example, they flower for only a few weeks before reverting to vegetative growth, and only a small number of branches of the plant undergo the floral transition in a single flowering season. We identified a mutant of *A. alpina* called *perpetual flowering 1* that does not alternate between vegetative and reproductive development, but flowers continuously through the summer and autumn, with many more shoots undergoing the floral transition than in wild-type plants. The PERPETUAL FLOWERING 1 protein is a transcription factor that represses flowering. In wild-type plants levels of the protein are transiently reduced to allow flowering, but rise again soon after flowering has occurred, thus ensuring that plants revert to vegetative growth. We are also studying other aspects of perennial flowering in *A. alpina*, including control of the juvenile phase during which plants do not flower even if they are exposed to appropriate environmental conditions.

Selected publications

Wang, R., Farrona, S., Vincent, C., Joecker, A., Schoof, H., Turck, F., Alonso-Blanco, C., Coupland, G., Albani, M. (2009) *PEP1* regulates perennial flowering in *Arabis alpina. Nature* 459, 423-427.

Jang, S., Marchal, V., Panigrahi, K.C.S., Wenkel, S., Soppe, W., Deng, X-W., Valverde, F. and Coupland, G. (2008) Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. *EMBO J.* 27, 1277-1288.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C. and Coupland, G. (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* 316, 1030-1033.

Hayama, R., Agashe, B., Luley, E., King, R. and Coupland, G. (2007). A circadian rhythm set by dusk controls the expression of FT homologues and the short day photoperiodic flowering response in Pharbitis. *The Plant Cell* 19, 1 - 13.

Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Kröber, S., Amasino, R.A. and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes & Development* 20, 898-912.

The plant circadian clock

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Seth J. Davis

Department of Plant Developmental Biology

Most organisms experience a dramatic change in light during the 24 hours of a day. Our group is interested in the circadian clock that enables plants to anticipate the predictable oscillations of light and temperature, and to modulate their metabolic and developmental processes as a response to these environmental cycles. A complex network of interconnected-feedback loops constitutes the heart of the *Arabidopsis thaliana* clock. The core loop generates the 24-hour rhythmicity of the oscillating mechanism, and morning and evening loops fine tune rhythmicity in a temporally specific manner (Figure 1).

Our current efforts focus on understanding the clockwork with regards to input-signaling pathways to the oscillator, the oscillation-mechanism, and those output processes that originate from the oscillator. These outputs include the capacity for photon capture in the day and carbon fixation at night, rhythmic growth and development, and circadian control of seasonal timing. The first clock model was constructed on the basis of expression circadian mutants. In this model, *TIMING OF CAB2 EXPRESSION 1 (TOC1)* serves as a positive factor that regulates expression of *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)*, related genes that function as redundant negative factors in *TOC1* regulation. Mathematical approaches led to a four-loop model that explains most data (Figure 1). We tested this clock model genetically, and provided direct experimental support for the cog-wheels of the circadian oscillator. We also supported the notion of interconnections within the molecular loops. In separate work, another gene was placed into an intersection with both the morning and the evening arms of the oscillator, with the demonstration that *EARLY FLOWERING 4 (ELF4)* acts as a dose-dependent clock component (Figure 2).

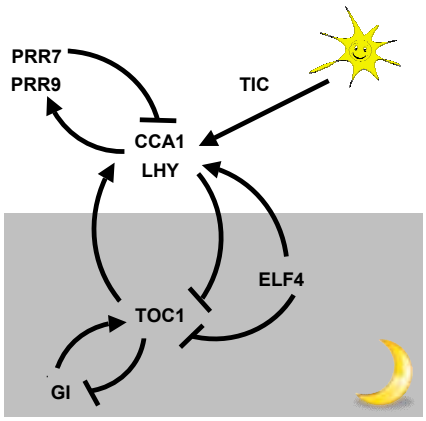


Figure 1. A molecular-genetic model for the *A. thaliana* circadian clock

The core of the oscillating mechanism is the morning genes CCA1 and LHY and the evening gene TOC1. Secondary loops that operate at dawn require PRR7 / PRR9, and a tertiary loop at dusk requires GI. We have now proposed that ELF4 interconnects the morning and evening arms and have defined how TIC integrates light to the clock at dawn.

Adapted from: Plant Signaling and Behavior (2007) 5: 370-372.

As the plant circadian clock has at its core a number of proteins whose biochemical activities are not obvious from their sequences, we

The circadian clock of plants allows these autotrophic organisms to properly coordinate the reactions of photosynthesis to the natural light - dark cycle of one day.

have used structural approaches to predict functions. In this way, several biochemical functions were proposed for TOC1 and ELF4. Phylogenetic interpretations facilitated structural calculations. Based on our structural models, we suggested that TOC1 participates both in metal binding and in transcriptional regulation, and separately, that ELF4 is a molecular „key" that activates a „lock" that represses morning and evening arms of the clock. This created a platform for how analyses on circadian factors can be progressed from genetics to biochemical investigations.

The clock is an interconnected-feedback loop that impinges on parallel-signaling systems to ensure proper synchronization to the ambient-diurnal environment in a process referred to as entrainment. Here, various clock-input factors are required for normal perception of dawn and dusk. As an example, the morning-acting factor *TIME FOR COFFEE (TIC)* regulates dawn inputs. Our cloning of *TIC* provides new insight into dawn perception. Furthermore, a new line of experiments has revealed that *ELF4* is critical for dusk inputs to the clock. Collectively these input factors regulate clock resetting in response to changing light environments. We reported the discovery of transcription factors that mediate such responses.

Previous work in our group included an examination of the effects of phytohormones on rhythms. We found that many phytohormones control specific features of the circadian system. From there, our recent studies revealed that steroid signaling establishes an unexpected and previously unidentified genetic pathway in the developmental-timing transition. These findings led us to propose that plant steroids repress a timing repressor through chromatin remodeling, particularly in genetic situations where the timing repressor is activated.

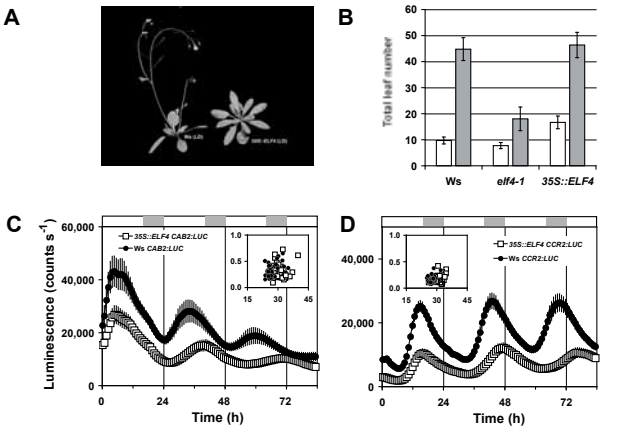


Figure 2. Dose-dependent effect of *ELF4*

(A, B) 35S::ELF4 plants flower late under long days (LD, white bars), but not short days (SD, grey bars). In 35S::ELF4 plants kept under continuous light, a long-period phenotype was observed for (C) CAB2:LUC and (D) CCR2:LUC. (C, D) Grey bars indicate subjective night. (Insets) Error plots of luminescence rhythms (Relative Amplitude of Error vs. period length; each period estimate corresponds to one seedling relative to its circadian precision). Error bars represent S.E.M. All seedlings were entrained under 16:8 light-dark cycles. Time is zeitgeber time. Adapted from: Plant Phys. (2007) 144: 391-401.

Selected publications

Hanano, S., Stracke, R., Jakoby, M., Merkle, T., Domagalska, M. A., Weisshaar, B., and Davis, S. J. (2008) A systematic survey in *Arabidopsis thaliana* of transcription factors that modulate circadian parameters. *BMC Genomics* 9:182 (This paper is noted as *„highly accessed"*).

Ding, Z., Doyle, M. R., Amasino, R. M., and Davis, S. J. (2007) A complex genetic interaction between *Arabidopsis thaliana* TOC1 and CCA1/LHY in driving the circadian clock and in output regulation. *Genetics* 176: 1501-1510 (*Cover image* & an *Issue Highlight*).

Domagalska, M. A., Schomburg, F. M., Amasino, R. M., Vierstra, R. D., Nagy, F., and Davis,S. J. (2007) Attenuation of Brassinosteroid Signaling Enhances *FLC* Expression and Delays Flowering. *Development* 134:2841-2850 (Faculty of 1000 review: *Recommended*) (*Cover image* & *In This Issue* featured).

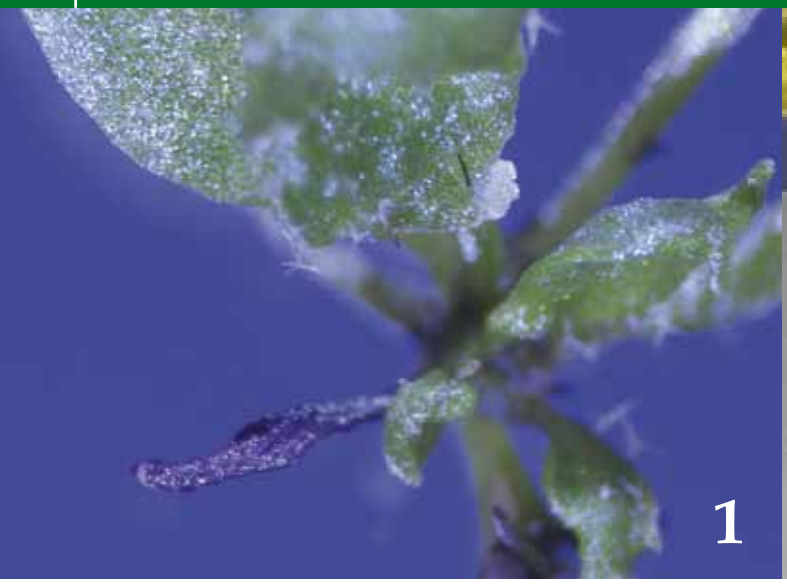
Ding, Z., Millar, A. J., Davis, A. M., Davis, S. J. (2007) *TIME FOR COFFEE* encodes a nuclear regulator in the *Arabidopsis thaliana* circadian clock. *Plant Cell* 19: 1522-1536.

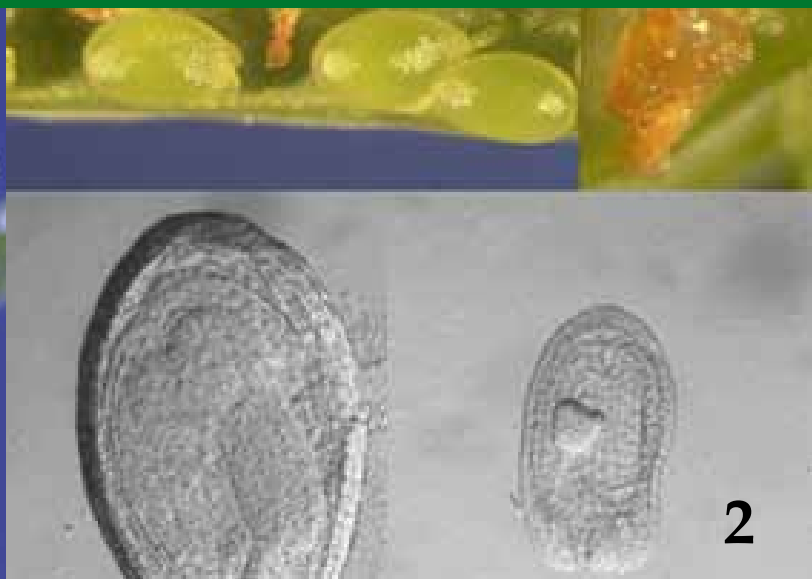
McWatters, H. G., Kolmos, E., Hall, A., Doyle, M. R., Amasino, R. A., Gyula, P., Nagy, F., Millar, A. J., and Davis, S. J. (2007) *ELF4* is required for oscillatory properties of the circadian clock *Plant Physiol.* 144: 391-401 (*Cover image*.)

RNA processing and regulation of stress responses

Csaba Koncz

Department of Plant Developmental Biology





Assembly of the genetic information carried by mRNAs and many regulatory non-coding RNAs is dependent on co-transcriptional processing of primary nuclear transcripts by the spliceosome. Recognition of exon-intron junctions and excision of intron lariats, yielding either regular or alternatively spliced transcripts, requires the ATP-dependent activation of spliceosome assembly, which is stimulated by the PRL1/CDC5/PRP19 complex. PRL1, a conserved WD-40 protein subunit of the spliceosome-activating complex, was first identified by T-DNA insertion mutations in the Arabidopsis gene *Pleiotopic Regulatory Locus 1* that caused dramatic changes in development (Figure 1) and enhanced signalling responses to glucose, sucrose, ABA, cold, cytokinins and auxins. In plants, PRL1 function overlaps with that of a paralogue, PRL2, as indicated by the finding that chimaeric proteins in which heterogeneous N-terminal and conserved C-terminal sequences of PRL1 and PRL2 have been swapped complement *prl1* mutations. PRL1 is expressed at higher levels than PRL2 in all organs, but PRL1 expression appears to be limited in embryos, as *prl2* mutations cause embryonic arrest at the heart stage (Figure 2). PRL orthologues (PLRGs) are encoded by unique genes in eukaryotes other than plants. Knockout or siRNA-mediated silencing of PLRG1s in mouse and zebrafish leads to induction of apoptosis, arrest of cell division and early lethality of embryos, due to hyperphosphorylation and consequent stabilisation of p53, accompanied by an increase in DNA damage, indicated by a rise in levels of phosphorylated histone H2A.X.

The WD40 repeats in PRL1/PRL2 interact with the Myb transcription factor CDC5, which can bind directly to DNA. Suppression of DNA damage and cell death by animal PRL orthologues is dependent on the function of the ubiquitin ligase Prp19/Pso4/

Figure 1. *In combination with the as1 (asymmetric leaf 1) mutation, the prl1 mutation results in dramatic alteration of leaf development, characterised by formation of finger-like filamentous leaves, induction of anthocyanin production, and cell proliferation at the leaf margins.*

Figure 2. *Inactivation of PRL2 by T-DNA insertion mutations results in arrest of embryo development at the heart stage (lower panel, compared to wild-type embryo of the same age), leading to seed abortion in the silique (upper panel).*

SNEV, which binds the PRLG1/CDC5 complex and interacts with the Werner syndrome (WRN) RecQ helicase, a key regulator of DNA repair pathways. In plants, no functional p53 or WRN homologue exists and, remarkably, the *prl1* mutation does not stimulate, but suppresses, the cell death pathways, while enhancing sensitivity to oxidative stress and pathogenic infections. Leaky mutations of the single-copy *CDC5* gene mimic the *prl1* phenotype, whereas plants bearing mutations in either of the duplicated *PRP19*

PRL1 can recruit and inhibit the activity of the plant AMP-activated SnRK1α protein kinases AKIN10 and AKIN11.

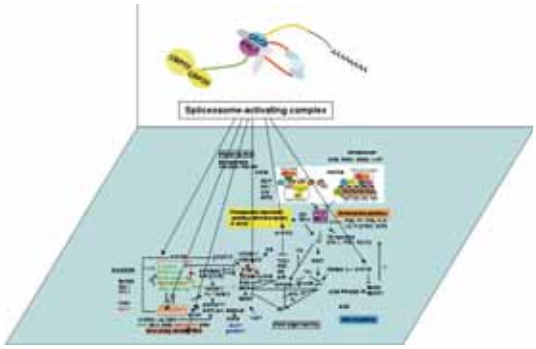


Figure 3. *Mutations affecting the function of the spliceosome-activating complex have simultaneous regulatory effects on specific functions controlling several developmental pathways, such as differentiation of apical meristem and flowering time.*

genes show wild-type phenotype. Unfortunately, *cdc5* null and double *prl1, prl2* and *prl19a, prp19b* mutants are not available, because no viable male gametes are formed in such mutants.

Interaction of the spliceosome-activating PRL1/CDC5 complex with DNA repair pathways in plants is indicated by the fact that PRL1 functions as a substrate-binding subunit for the E3 ubiquitin ligase CULLIN4 (CUL4)-DDB1 (Damaged DNA Binding factor 1), which serves as a co-regulator of the TFIIH complex of RNA polymerase II in DNA repair. PRL1 can also recruit and inhibit the activity of the plant AMP-activated SnRK1αprotein kinases AKIN10 and AKIN11 that play important roles in transcriptional control of cellular energy homeostasis, glucose repression, and biotic and abiotic stress responses. The increase in stability of AKIN10 observed in the *prl1* mutant suggests that this kinase is a potential target of the ubiquitin ligase CUL4-DDB1-PRL1. By binding to SKP1, AKIN10 can target SCF (CULLIN1-SKP1-RBX1-F-box protein) ubiquitin ligases to the α7/PAD1 subunit of the 20S proteasome cylinder. This suggests that stress/DNA damage-dependent regulation of AKIN10 stability by CUL4-DDB1-PRL1 may modulate SCF-mediated proteolysis of chromatin-associated transcription and splicing factors. Whether AKIN10 is also recruited to the CDC5-PRP19a/b ubiquitin ligase by PRL1 is still unknown. Nevertheless, phosphorylation of CDC5 indicates that stress-activated AKIN10 and CDKF-activated TFIIH-associated CDKD kinases, which phosphorylate the C-terminal repeated domain (CTD) of the largest subunit RNA polymerase II, may contribute to regulation of the spliceosome-activating complex. Down-regulation of the activity of PRL1/CDC5/PRP19 by the *prl1* and leaky *cdc5* mutations results in altered stress-regulation of transcription, inefficient removal of introns from specific pre-mRNAs, changes in alternative splicing, and changes in the abundance and processing of small silencing RNAs and microRNAs (Fig. 3). Whether, and how, stress stimuli regulate the processing of specific sets of co-regulated transcripts, and how these processes are coordinated with the control of cell death and DNA damage responses, represent major questions that are being addressed in ongoing studies. Some other projects directed towards the characterisation of novel regulators of plant stress responses are described in the references listed below.

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Drought adaptation and flowering time control in barley

20

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Figure 1. *Barley is characterised by a high degree of genetic and phenotypic variability. The spikes of wild barley from the Fertile Crescent carry long awns and are characterized by anthocyanin coloration. Anthocyanins may act as antioxidants and thus protect the plant from the effects of strong radiation. Image: Maria von Korff*

Introduction

Drought stress has always played an important selective role in the evolution of plant growth, development, and physiology, and has been a major limiting factor in crop production. Global warming and an expected increase in the incidence of extreme weather conditions are forcing agricultural research to focus even more on making plants resilient to environmental stresses, such as drought. Our group uses natural genetic diversity in barley to dissect the quantitative genetics of drought adaptation in this crop plant. The primary objective is to map and identify genomic regions and characterize genes and gene networks that function in drought adaptation in barley. In drought prone environments, the timing of flowering is crucial for reproductive success, and has thus a major impact on grain yield in crop species. Consequently, we wish to elucidate the regulation of flowering time in barley and its modulation by environmental cues.

Drought adaptation in barley

Among the major crops, barley shows superior drought adaptation and is an excellent model in which to study physiological, genetic, and breeding aspects of drought tolerance. We have identified candidate genes involved in adaptation to water limitations in drought susceptible and tolerant barley lines based on a microarray experiment. In addition, we demonstrated that the expression of drought candidate genes is regulated in *cis*, indicating that regulatory variation plays a major role in stress tolerance. In cooperation with the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, we are currently analysing a barley RIL (recombinant inbred line) population derived from a cross between a drought adapted landrace and a high-yielding cultivar.
The objective is to identify quantitative trait loci (QTLs) that contribute to a difference in water use efficiency in this cross. Preliminary results from a QTL analysis based on field data in Syria indicated that loci influencing plant development have a major impact on grain yield in very dry environments. The parental lines are being tested for differences in physiological traits and gene and protein expression with the aim of extending this analysis to the derived population.

The regulation of flowering plays a crucial role for the reproductive success of barley.

Flowering time control in barley

The genetic dissection of flowering time control has been facilitated by the elucidation of floral pathways in Arabidopsis, and orthologs of the Arabidopsis flowering genes have been cloned from cereal crops. However, the majority of these homologues show altered positions in the flowering pathways in Triticeae and their response to external stimuli differ from those described for Arabidopsis. In addition, a thorough analysis of genetic variation and detection of functional polymorphisms is still lacking for most candidate genes.

We are analysing flowering time behaviour and differential gene expression in diverse barley lines, landraces and wild barley, in order to establish a causative link between variation at candidate loci and flowering time. In addition, we use a transgenic approach to explore the effect of candidate genes in barley. Furthermore, we have identified flowering time QTLs which do not coincide with known genes. These will be subjected to fine-mapping with the aim of detecting novel flowering time genes in barley.

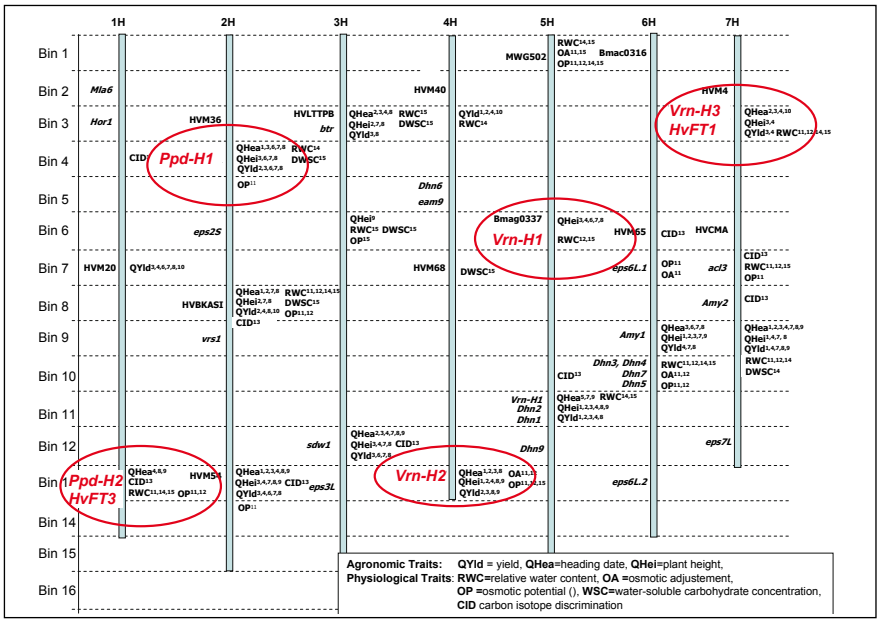

Figure 2. *Consensus map - QTLs for agronomic performance under drought coincide with flowering time genes. Genetic map of barley with locations of QTLs for agronomic and physiological traits detected in Mediterranean environments or in populations derived from drought adapted germplasm. Genomic locations of major vernalisation (VRN) and photoperiod genes (PPD) are indicated in red and circled. Marker names are given to the left, and QTL for agronomic traits and physiological traits are listed to the right of the bar. References: 1 Pillen et al., (2003), 2 Pillen et al., (2004), 3 Baum et al., (2003), 4 Talame et al., (2004), 5 Hori et al., (2005), 6 Li et al., (2005), 7 Li et al., (2006), 8 von Korff et al., (2006), 9 Teulat et al., (2001b), 10 Long et al., (2003), 11 Teulat et al., (1998), 12 Teulat et al., (2001a), 13 Teulat et al., (2002), 14 Teulat et al., (2003), 15 Diab et al., (2004)*

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Chromatin structure and transcriptional control in plant development

Franziska Turck

Department of Plant Developmental Biology

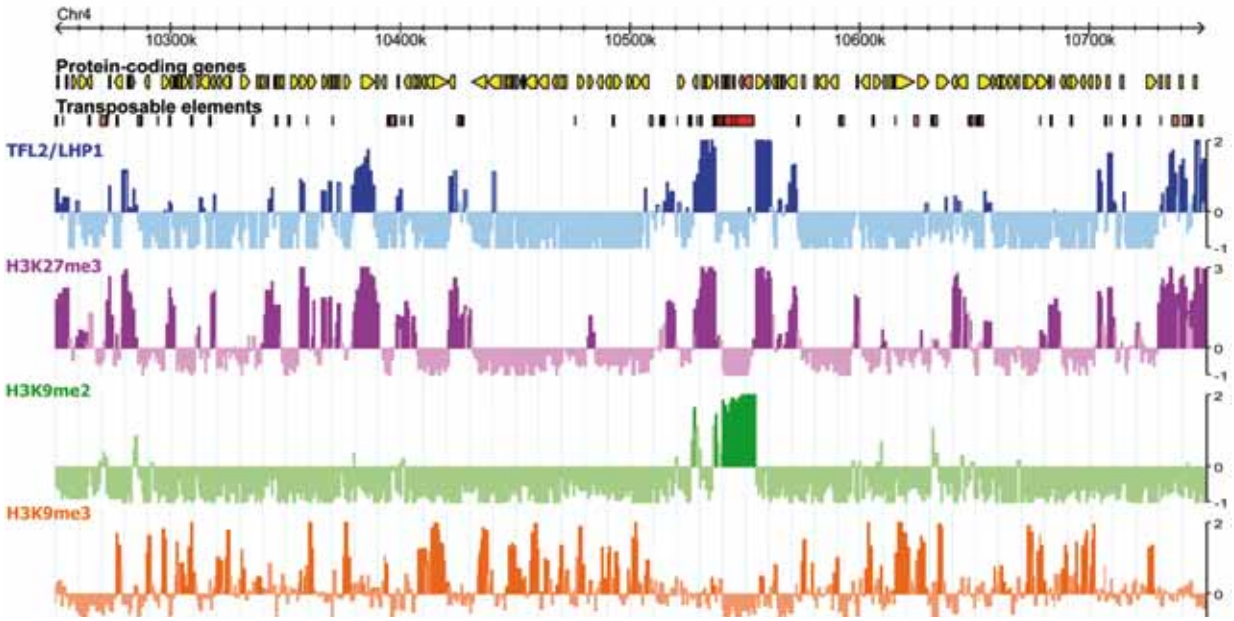

Figure 1. *Chromatin landscapes in Arabidopsis thaliana*

Introduction

The fundamental importance of nuclear DNA organisation is illustrated by the observation that histones, the building blocks of chromatin are among the most conserved eukaryotic proteins. However, the order conferred by these proteins comes at a price. While the naked DNA of bacteria is exposed directly to DNA-interacting proteins, the packaged DNA of eukaryotes is in large part inaccessible, although access to DNA is a prerequisite for transcription and replication. Eukaryotes have dealt with this problem by inventing a multilayered molecular machinery that regulates chromatin organisation and thereby participates in gene regulation.

Our goal is to learn how the enzymes responsible for chromatin organisation work together with transcription factors to control plant gene expression. We want to know why genes become targets of repressive chromatin complexes and how this repression can be reversed.

Histone marks and chromatin landscapes

Differential methylation of Lysine residues 9 and 27 of Histone 3 (H3) acts as a signal to recruit chromatin-associated proteins that repress target loci. Polycomb Repressive Complex 2 (PRC2) catalyses tri-methylation of H3K27 (H3K27me3) in animals and plants. Recently, we have shown that LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) is recruited to genes marked with H3K27me3 (Figure 1). LHP1 is also called TERMINAL FLOWER 2 (TFL2), and mutation of the corresponding Arabidopsis gene causes early flowering and formation of a terminal flower.

LHP1 is likely to interact with other proteins that participate in H3K27me3-mediated gene repression. A genetic screen for enhancers of the *lhp1* phenotype is being carried out to identify interaction partners for LHP1 and, as a complementary strategy, biochemical approaches to the purification of LHP1 complexes are being pursued.

H3K27me3-marked genes and plant development

H3K27me3-marked genes fall into two main groups: (i) genes that are never expressed and (ii) genes that are expressed only in a limited set of tissues or at a specific developmental stage. The second group of H3K27me3 targets includes a particularly large fraction of genes that are known to be important for Arabidopsis development.

Eukaryotes have invented multilayered molecular machinery that regulates chromatin organisation and thereby serves to control gene expression and cell division.

We argue that H3K27me3-marked genes that are expressed in a specific plant organ but are of unknown function are candidates for a role in development that has not yet been identified. To test this hypothesis, we make use of the public collections of T-DNA insertion mutants for Arabidopsis to systematically inactivate H3K27me3-marked genes and assess their function.

Attracting the PRC2 to target loci

Results from work carried out in fruitflies suggest that transcription factors interact with proteins of the PRC2 and thereby recruit the complex to targets in chromatin. The PRC2 is composed of four different proteins, each of which is encoded by a small gene family in Arabidopsis. Thus, many different versions of PRC2 may co-exist and interact with different transcription factors.

We have begun to identify target genes for specific PRC2 complexes in the hope that DNA-encoded targeting signals can thus be identified. In addition, we study natural variation at H3K27me3 target regions, in order to identify DNA sequence changes that underlie this variation.

Chromatin-mediated regulation of *FLOWERING LOCUS T*

Arabidopsis flowers earlier in long-day than in short-day conditions. Perception of long days in the leaves leads to the expression of the gene *FLOWERING LOCUS T (FT)* in the phloem, which promotes flowering (Figure 2). The putative transcription factor CONSTANS (CO) is required for long-day-mediated *FT* induction, whereas LHP1 represses *FT* expression irrespective of day length.

Transcriptional regulation of *FT* is a case study for the interplay of chromatin and transcription factors in gene regulation. The regulatory regions required for *FT* regulation by CO and LHP1 have been mapped. Currently, we are assessing whether changes in chromatin precede or follow the change in *FT* expression.

Figure 2. *Expression pattern of FT in Arabidopsis.*

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Overview: Department of Plant Breeding and Genetics

Director: Maarten Koornneef

In addition to the breadth of interspecies diversity found in plants, substantial genetic variation is present within species in nature or has been generated (selected for) by breeders. The application of molecular genetic methods has led to detailed insights into the molecular nature of the genetic differences and to a much better understanding of the processes underlying genetic differences in plant growth and development.

This knowledge base provides new tools for plant breeders that will make plant breeding more efficient by using genetic markers that 'tag' genes for traits of interest. For further development of these tools, more detailed knowledge of the function of the genes that display variation in nature and of the molecular basis of agronomically relevant traits is needed. Much of the natural variation in traits of interest is determined by multiple genes, and therefore shows complex genetic behaviour. Hence, methods of computational genetics, such as quantitative trait locus (QTL) analysis and association mapping, are indispensable.

The objective of the department is to extend our knowledge of processes that determine crucial aspects of plant growth and development, including plant architecture, plant metabolism and seed dormancy, using genetic and genomic tools. We expect that this knowledge will find practical application in plant breeding and will allow further improvement of crop plants.

Complex traits in potato: Molecular basis and diagnosis

Christiane Gebhardt

Department of Plant Breeding and Genetics

Introduction

The potato (*Solanum tuberosum*) is the most important crop species of the Solanaceae family worldwide. Of these, some 200 tuber-bearing *Solanum* species native to Mexico, Central and South America are closely related to *S. tuberosum*. The amount of DNA variation present in the potato gene pool is tremendous, and some cause - in combination with environmental factors - the natural phenotypic variation between potato genotypes.

The long-term goals of our research are (1) to elucidate the molecular basis of complex agronomic characters of potato, (2) to contribute to the understanding of structure, evolution, function and natural diversity of crop plant genomes, and (3) to develop molecular diagnostic tools to facilitate the selection of improved cultivars. To achieve these goals, we exploit the natural variation present in *S. tuberosum* and related species, using strategies developed for human population genetics. The outputs of our research are (1) knowledge of the genomic location and identity of genes that control qualitative or quantitative agronomic characters, and the allelic variation present at these loci, (2) diagnostic DNA-based markers that can be used for marker-assisted selection of superior cultivars ('precision breeding'), and (3) cloned genes and superior alleles for agronomic characters that may be transferred into cultivars by genetic engineering.

Disease resistance

Numerous genetic factors associated with qualitative and quantitative resistance to pathogens, as well as positions of candidate genes (genes already known to function in pathogen recognition, defence signalling and defence responses) have been identified in the potato genome by molecular mapping (see the 'Solanaceae function map for resistance' at http://www.gabipd.org/database/maps). The pathogen of greatest relevance to potato cultivation is *Phytophthora infestans*, which causes late blight. A 600-kbp section of chromosome V of potato that encompasses a hot-spot for resistance to late blight and other pathogens has been sequenced, revealing a high degree of structural divergence between homologous potato chromosome segments, and syntenic relationships with Arabidopsis (Ballvora et al. 2007). The sequence information was instrumental in identifying single-nucleotide polymorphisms (SNPs) that are highly diagnostic for resistance to the root-cyst nematode *Globodera pallida* (Achenbach et al. 2009). In collaboration with potato breeders, we used single-nucleotide polymorphisms (SNP) and microsatellite markers to perform an association mapping experiment for field resistance to late blight. A major association was found on chromosome XI, at the *StAOS2* locus encoding allene oxide synthase 2 (Fig. 1) (Pajerowska-Mukhtar et al. 2009). *StAOS2* is a key enzyme in the biosynthesis of jasmonates, plant hormones that function in defence signalling. Furthermore, dsRNAi-mediated silencing of *StAOS2* in potato diminished jasmonic acid production and compromised quantitative resistance to late blight. *Arabidopsis thaliana* was employed for functional analysis of natural variants of the potato *StAOS2* gene. Five *StAOS2* alleles were expressed in the null Arabidopsis *aos* mutant, and tested for complementation of mutant

Population genetic studies based on candidate genes can uncover the molecular basis of complex traits and make 'precision breeding' possible in potato.

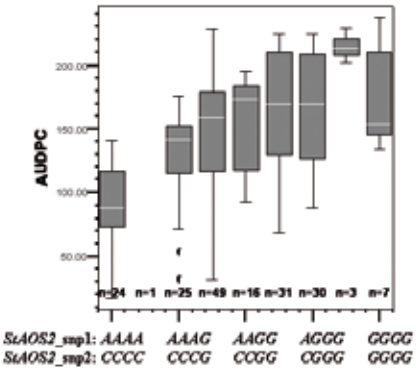

Figure 1. *SNPs in the StAOS2 coding sequence are associated with variation in resistance to late blight. The box plot shows the distribution of the 'area under disease progress curve' (AUDPC) in 8 genotypic classes (n = number of individuals per class) observed for the combination of SNP1 (alleles A and G) and SNP2 (alleles C and G). Tetraploid individuals homozygous for the AC combination (left box) are, on average, more resistant (lower AUDPC values) than individuals homozygous for the GG combination (right box). The effect depends on the dosage of the SNP alleles (boxes in the middle).*

phenotypes. *StAOS2* alleles linked to increased disease resistance in potato more effectively complemented *aos* mutant phenotypes than did *StAOS2* alleles associated with increased susceptibility (Pajerowska-Mukhtar et al. 2008). The results therefore support the conclusion that *StAOS2* is one of the factors that control natural variation of pathogen resistance in potato, and demonstrate how a candidate-gene approach, in combination with the use of Arabidopsis as a reporter of function, can help to dissect the molecular basis of complex traits in crop plants.

Tuber quality traits

Tuber yield, starch and sugar content are complex traits controlled by multiple genetic and environmental factors. The genes and biochemical pathways

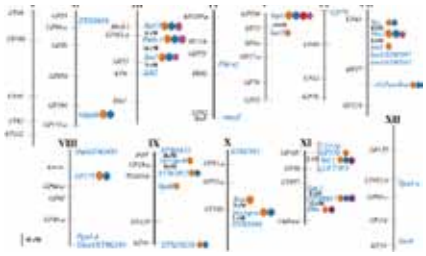

Figure 2. *Molecular linkage map of potato showing the positions of candidate loci associated with tuber chip quality (orange circles), starch content (blue circles), yield (red circle) and starch yield (purple circles). Stp23, StpL and StpH encode starch phosphorylases; Pain-1, Inv-ap-a and Inv-ap-b: invertases; G6pdh: glucose-6 phosphate dehydrogenase; SssI: soluble starch synthase I; Sut2: sucrose transporter 2: Sps: sucrose phosphate synthase; Sus3: sucrose synthase 3; Pha2: plasma membrane H+ ATPase 2; AGPaseB: ADP-glucose pyrophosphorylase B; Rca: Rubisco activase; Dbe: debranching enzyme. Associated loci GP171, STM3012, STM3023b STM0037 do not encode candidate genes.*

involved in synthesis, degradation and transport of carbohydrates are among the best studied in plants. In an association mapping experiment we tested whether natural DNA variants of genes encoding enzymes involved in carbohydrate metabolism are associated with differences in tuber yield, starch content and chip quality, which itself depends on the content of reducing sugars in tubers. Highly significant and robust associations were identified with DNA variants in genes that act in starch and sugar metabolism (Fig. 2). Most frequent were associations with chip quality and tuber starch content. Alleles that increased tuber starch content improved chip quality, and vice versa (Li et al. 2008). Invertases and starch phosphorylases are currently being studied to confirm the functional relevance of allelic variation.

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Natural variation in Arabidopsis and barley

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Maarten Koornneef

Department of Plant Breeding and Genetics

Figure 2. *Seeds from different barley recombinant inbred lines.*

Plants belonging to the same species can differ in their properties owing to genetic differences. This can be observed among wild plants, but also among cultivated forms. In nature this variation is assumed to be related to the ability of specific plants to adapt to specific growing conditions, although other mechanisms such as genetic drift also shape genetic differences between and within plant populations. The study of natural variation requires methods of quantitative genetics, and for this a range of so-called mapping populations, including collections of accessions, can be used. Our group is developing novel types of mapping populations based on natural variants both in Arabidopsis and barley, where combinations of genes from more than one parent can be combined. It is expected that novel combinations of alleles may result in novel phenotypes. Novel phenotypes for flowering time and plant architecture (Fig. 1) have been identified, and screens for stress tolerance and metabolic compositions of seeds are underway. To analyse the data, statistical mixed-model approaches have been developed in collaboration with the group led by Prof. F. van Eeuwijk at Wageningen University.

Cultivated barley is assumed to derive from a limited part of the large gene pool of the wild barley species *Hordeum spontaneum*, the ancestor of the cultivated *H. vulgare*. Thus, germplasm that has not been used in barley domestication or in modern breeding programs might provide agronomically useful alleles. Therefore we have combined different wild accessions with a barley cultivar with a view to identifying interesting allelic variants or variant combinations in a predominantly cultivated barley background.

The traits that we study in barley are seed dormancy (a trait exhibited in more extreme forms by wild barley) and seed longevity.

The combination of genetic variants from different origins can result in the expression of novel traits.

Figure 1. *An Arabidopsis plant with an altered architecture (reduced number of side shoots in the inflorescence).*

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The molecular basis of genetic adaptation in plants

Juliette de Meaux

Department of Plant Breeding and Genetics

Introduction

TThe aim of our research is to gain insight into the molecular basis of genetic adaptation. Our research combines various population-genetic approaches with molecular techniques, and focuses on genetic changes occurring either in specific genetic elements (cis-regulatory DNA, miRNAs) or specific phenotypes (seed dormancy, innate immunity).

Cis-regulatory evolution

The evolution of *cis*-regulatory DNA is particularly interesting to study because it is suspected to evolve faster than protein-coding DNA. However, little is known about the short-term evolutionary dynamics of these regions. Previous work in the genus *Arabidopsis* has shown that *cis*-regulation of genes for chalcone synthase (CHS) appears to vary continuously, in an undirected manner, although the response to environmental cues is changing (de

Meaux et al. 2005, 2006). Is the pattern displayed by *CHS* typical of *cis*-regulatory variation?

Theoretical work performed in our group predicts that continuous selection for phenotypic changes of small magnitude will fix many mutations of small effect (Collins et al. 2007). Many of these mutations will occur in *cis*-regulatory regions. We have developed a method that allows us to scan the genome for *cis*-regulatory variation affecting functionally connected genes. The distribution of *cis*-regulatory mutations across the gene regulatory network may in turn reveal adaptive phenotypes that have been subjected to constant divergent selection since speciation (He et al., in preparation). This work will illuminate the role of molecular changes of small phenotypic effect in adaptive processes, as well as the part played by *cis*-regulatory variation in phenotypic diversification.

MicroRNA evolution

In plants and animals, gene expression can be down-regulated at the post-transcriptional level by microRNAs (miRNAs), a class of small endogenous ribonucleic acids. We have investigated the micro-evolutionary dynamics of

We wish to identify the spectrum of mutations that underlie adaptive changes in phenotype and characterise the ecological conditions that favor such adaptive novelties.

these genetic elements in *A. thaliana*. We found that one miRNA (miR824) displays strong signs of balancing selection on two alleles that encode RNAs with significantly different secondary structures. This work was the first to highlight the relevance of RNA structure in miRNA sequence evolution, suggesting that the evolutionary dynamics of miRNA-encoding loci may be more complex than suggested by the constraints associated by the requirement for interaction between processed 22-bp fragments of miRNA and their target exon(s) (de Meaux et al. 2008).

Evolutionary genetics of two adaptive phenotypes

We are accumulating evidence that seed dormancy is an adaptive trait in *A. thaliana* (I. Kronholm, submitted and M. Debieu, PhD project). This provides us with an excellent model to investigate in detail the ecological causes and molecular consequences of the evolution of a complex trait, which is evolving primarily in response to climate. Concomitantly, we have analysed variation in a second trait whose molecular basis is extremely well understood: flagellin sensing. *A. thaliana* possesses a specific receptor that triggers multifaceted defence responses when it binds the bacterial protein flagellin. This is the primary hurdle encountered by bacterial pathogens when they attempt to invade a potential host. In collaboration with Silke Robatzek and her group, we show that, despite strong constraints exerted on flagellin sensing, it is a dynamic function (M. Vetter, PhD project). We are currently examining the extent to which life-cycle variation may influence quantitative variation in flagellin sensing.

Selected publications

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Dissecting genetic and molecular bases of plant performance using natural variation in *Arabidopsis thaliana*

32

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Introduction

Arabidopsis thaliana accessions have been collected from various environments in the Northern hemisphere (Figure 1). Genetic diversity present among these accessions is assumed to reflect adaptation to local environments. This genetic diversity leads to variation in many traits. When grown under laboratory conditions, overall rosette size in accessions from distant geographical locations is variable (Figure 1). In addition, responses of growth-related traits to environmental factors can also differ between accessions. Work in our group is devoted to revealing the genetic and molecular bases of growth and its responses to environmental factors by exploiting the natural variation present in *A. thaliana*. Understanding the effect of this genetic variation on plant performance under different environmental scenarios is also relevant for plant breeding, as it impinges on traits that determine yield and yield stability in crops.

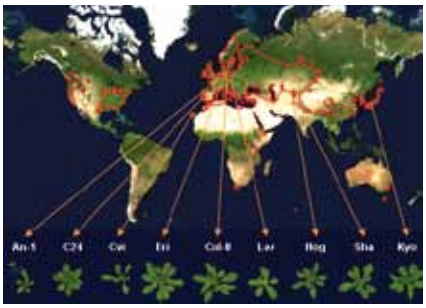

Figure 1. *Geographical distribution of Arabidopsis thaliana (regions surrounded in red) and variation of overall rosette morphology and size in selected accessions and laboratory strains originating from contrasting habitats (An-1: Antwerp – Belgium; C24: Portugal; Cvi: Cape Verde Islands; Eri: Eringsboda – Sweden; Col-0: Columbia – Poland; Ler: Landsberg erecta – Poland; Hog: Hodja-Obi-Garm – Tadjikstan; Sha: Shakdara – Tadjikistan; Kyo: Kyoto – Japan).*

Projects

Growth-related traits are quantitative traits and, using natural variation, the genetic determinants involved in the variation of such traits can be pinpointed by mapping Quantitative Trait Loci (QTLs) (Figure 2). A QTL is detected by establishing associations between defined genetic markers and

Understanding the genetic variation involved in plant performance is relevant for plant breeding.

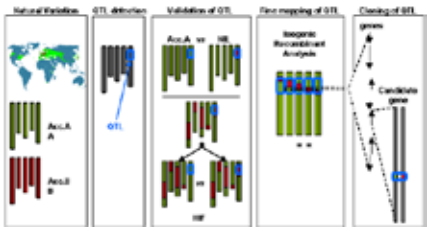

Figure 2. *Flow diagram depicting the different steps involved in using QTL (Quantitative Trait Loci) detection to identify the genetic and molecular bases of any trait that segregates between different accessions (Acc.A & Acc.B). NIL: Near Isogenic Line; HIF: Heterozygous Inbred Families. The asterisks indicate recombinant lines that have the same phenotype as the NIL or the HIF carrying the alleles from Acc.B at the QTL under consideration.*

the variation of the trait of interest. This usually permits mapping of the QTL to a chromosomal region that may encompass several hundred genes. The genetic and molecular bases of the phenotypic effect of the QTL can then be precisely localised and elucidated by detailed studies of that region (Figure 2 – from Reymond et al., 2007).

Growth is a dynamic process that can be quantified by estimating the accumulation of biomass or the increase of area with time. Nowadays, by using digital analysis software, it is possible to quantify plant area, and consequently plant growth, in a non-destructive manner and for large numbers of plants. Our aim is to discover genes involved in the variation of growth-related traits by quantifying the increase in rosette size with time. QTLs have already been identified, using various mapping populations (biparental and multiparental recombinant inbred lines). Growth responses to a given environmental factor are also under strong genetic control (genotype by environment interactions – GxE). Our goal is to dissect the genetic modules that determine how growth rates change in response to environmental factors. By growing *A. thaliana* in controlled and contrasting environments (various temperature regimes in growth chambers, nutrient regimes in hydroponic systems, salt concentrations in agar-based medium, etc), we have been able to detect QTLs involved in the growth responses to these various environments.

The genetic basis of the major QTLs detected is being investigated by selecting suitable lines (Near Isogenic lines – NILs, or Heterozygous Inbred Families – HIF; Figure 2). Using this approach, we showed that the genetic mechanism involved in cases of hybrid necrosis in response to temperature found in two mapping populations involves a cluster of resistance genes (Alcázar et al, 2009). We are currently identifying interactors for these genes and will characterise this system in more detail at the molecular level.

All these responses are relevant in nature, and allow plants to cope with environmental changes. Knowing the genetic networks underlying these responses will allow us to ask the question whether the corresponding genes have been subject to selection during evolution and contribute to enhancing plant performance in specific environments.

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Tisné, S., Reymond, M., Vile, D., Fabre, J., Dauzat, M., Koornneef, M. and Granier, C.: Combined genetic and modelling approaches reveal that epidermal cell area and number in leaves are controlled by leaf and plant developmental processes in A. thaliana. Plant Physiology 148, 1117–1127 (2008).

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Dormancy mechanisms in plants

34

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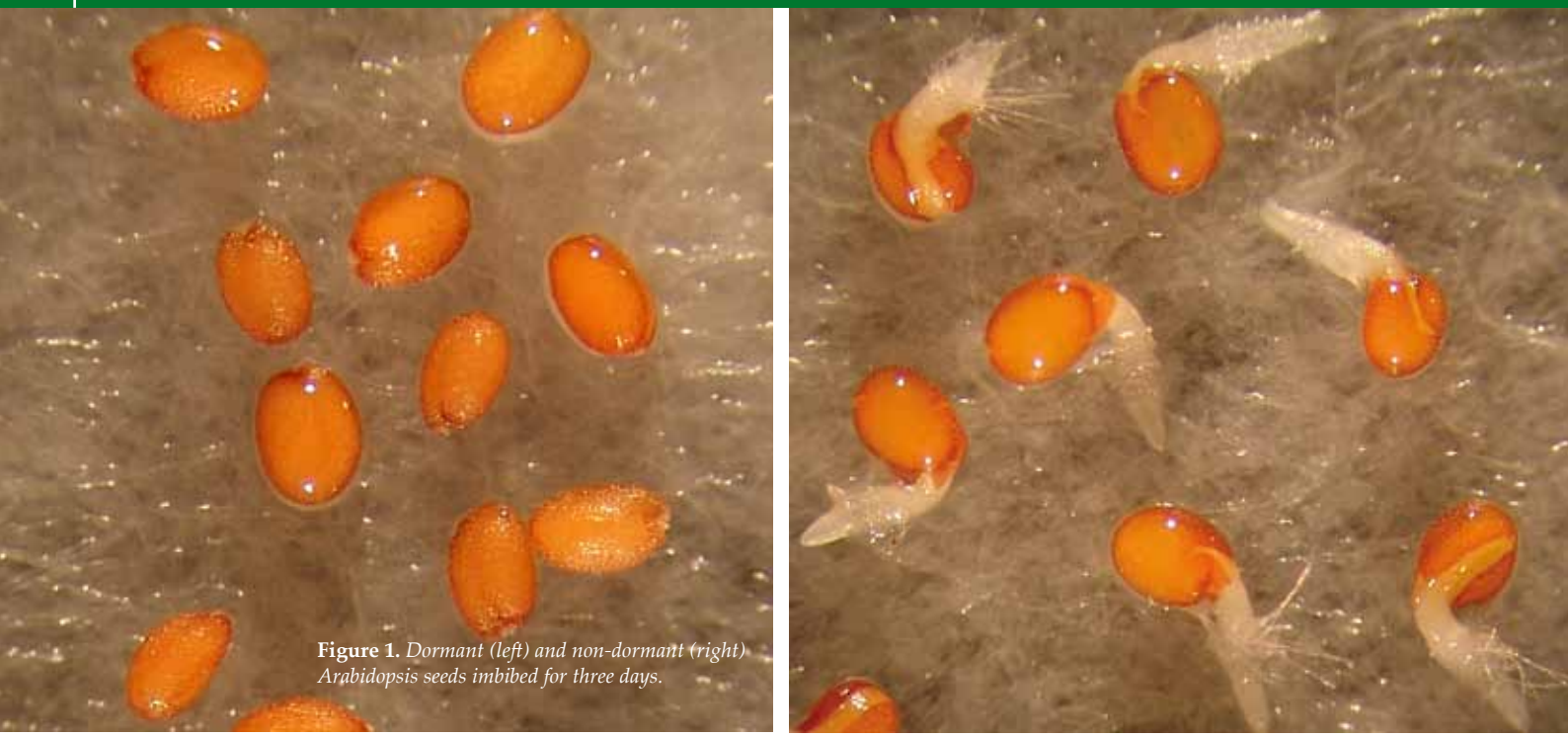

Figure 1. *Dormant (left) and non-dormant (right) Arabidopsis seeds imbibed for three days.*

Dormancy is defined as the absence of development under (temporarily) favourable environmental conditions and can prevent the germination of seeds or the outgrowth of buds at the wrong time of the year. Our research focuses on seed dormancy in Arabidopsis.

Non-dormant seeds germinate in the presence of water, light and favourable temperatures, but dormant seeds are unable to do so (Figure 1). The transition between dormancy and germination represents a critical stage in the life cycle of higher plants, and is an ecologically and commercially important trait.

Dormancy induction occurs during the maturation of seeds in the silique (Figure 2) and is strongly determined by abscisic acid levels. Seed dormancy

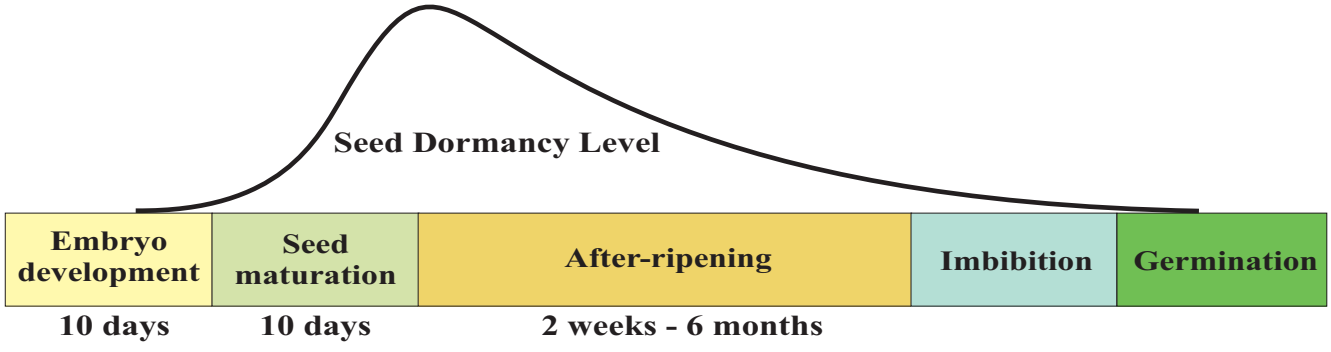

Figure 2. *A model depicting levels of dormancy during the life cycle of a seed.*

We wish to determine the interrelationships among the different dormancy proteins and their interplay with environmental factors, and thus construct a model of the pathways and mechanisms involved in seed dormancy.

in Arabidopsis can be released by low temperatures (stratification) or dry storage (after-ripening). Physiological and genetic research on seed dormancy has identified additional roles for gibberellins, ethylene, sugars, phytochrome, brassinosteroids and nitrate. However, our knowledge of the molecular regulation of seed dormancy is limited, and lags behind that of other developmental processes.

In order to reveal the molecular mechanisms of seed dormancy, we are taking a molecular genetic approach. As a first step, we performed mutagenesis screens on dormant Arabidopsis seeds in order to obtain mutants affected in seed dormancy. This led to the isolation of several novel mutants that lack dormancy or that fail to respond to stratification. The first of these mutants are being cloned at present.

During the past four years, we have cloned the genes responsible for several previously identified mutants with reduced dormancy (*rdo2*, *rdo3* and *rdo4*), as well as a mutation (*sua*) that suppresses the *abscisic acid insensitive3* mutation (which affects a major regulator of seed maturation). The cloned genes encode proteins with general functions in transcription regulation, chromatin remodelling, splicing and hormone regulation, and do not have a seed-specific expression pattern. The *RDO4* gene, for instance, encodes a C3HC4 RING finger protein that is required for monoubiquitination of histone H2B (Liu et al., 2007). This gene was renamed *HISTONE MONOUBIQUITINATION 1* (*HUB1*). H2B monoubiquitination is absent in the *hub1* mutant. In yeast and human, this histone modification is a prerequisite for methylation of Lys4 and Lys79 of histone H3, which is associated with actively transcribed genes. We found that expression levels of several dormancy genes were altered in the *hub1* mutant. Further investigation of the role of HUB1 in seed dormancy is in progress.

RDO2, *RDO3*, *HUB1* and *SUA* are expressed in all plant tissues and the corresponding mutants show pleiotropic phenotypes. In contrast, *DELAY OF GERMINATION1* (*DOG1*) is expressed specifically in the seed; *dog1* mutants completely lack dormancy and do not show pleiotropic phenotypes. The *DOG1* gene encodes a protein of unknown function (Bentsink et al., 2006). We believe that *DOG1* is a key player in seed dormancy and have therefore devoted a major effort to the analysis of the regulation and molecular function of the *DOG1* protein. *DOG1* RNA is alternatively spliced, and five different splicing variants have been found that code for three different proteins. We have obtained several genetic suppressors of the *dog1* phenotype, which are currently being analysed, together with a protein that interacts with DOG1 in the yeast two-hybrid system.

In the future, we will determine the functional relationships between the different dormancy genes and their products, and how these are modulated by environmental factors. This should lead to a model of the pathways and mechanisms that are involved in this process. When such a model is established, we would like to extend it to other plant species and to other types of dormancy. Such a dormancy model should enable predictions and manipulations of seed dormancy levels in crop plants.

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Bentsink, L., Jowett, J., Hanhart, C. J. and Koornneef, M.: Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 103, 17042-17047 (2006).

Shoot branching in seed plants

36

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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. Shoot branching is initiated by the formation of new meristems in the axils of leaves, which develop into secondary axes of growth. Axillary meristems recapitulate the function of the shoot apical meristem (SAM) by initiating several leaf primordia, resulting in the formation of axillary buds, which either grow out or remain dormant, depending on their position along the shoot axis, the developmental phase of the plant and environmental factors. The aim of our work is to understand at the molecular level the mechanisms that regulate the formation of axillary meristems. As model systems, we use *Arabidopsis thaliana* and tomato.

Figure 1. *Growth habit of a Wassilewskija wild type (left) and a cuc3-2 mutant*

New regulators of axillary meristem formation in *Arabidopsis*

Because of the similarity in expression pattern between the *CUP SHAPED COTYLEDON (CUC)* genes and the known branching regulator *LATERAL SUPPRESSOR (LAS)*, *cuc* mutants were tested for defects in axillary meristem development. These experiments demonstrated that *cuc3-2* mutants are impaired in axillary meristem initiation (Fig. 1). In addition, the redundant functions of *CUC1* and *CUC2*, as well as miR164, which regulates *CUC1* and *CUC2*, are required for a wild-type branching pattern. Expression studies suggested that *CUC1* and *CUC2* control axillary meristem development through regulation of *LAS*, whereas *CUC3* may function in an *LAS*-independent manner.

Axillary meristem formation and leaf architecture are controlled by similar mechanisms.

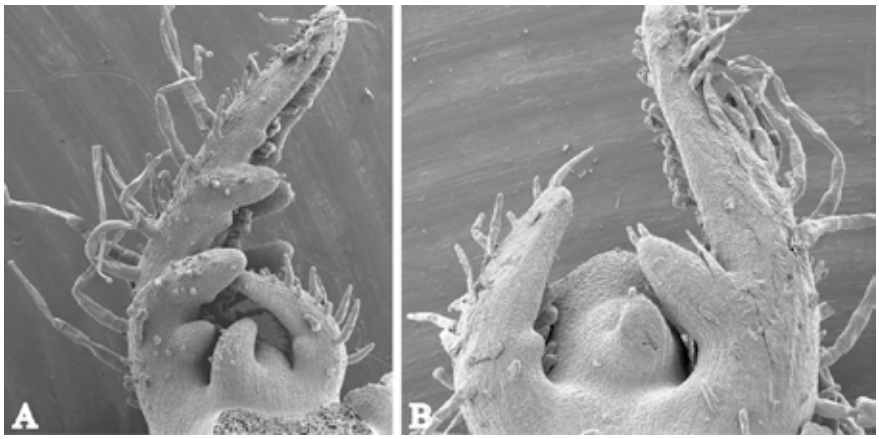

Figure 2. *SEM micrographs of wild-type (A) and trifoliate (B) shoot apices.*

Additional regulators of axillary meristem formation were identified in a screen for modifiers of the *las-4* branching phenotype. One of the enhancers, *eol5*, has been mapped to a short interval on chromosome 2. Currently a candidate gene is being tested for complementation of the mutant phenotype. Furthermore, the *las-4* mutant has been crossed to different *Arabidopsis* accessions to exploit natural variation. Several F2 populations segregated for modifiers of the *las-4* phenotype. These modifiers will be mapped and characterised further.

Three *GRAS* genes, *LOM1*, *LOM2*, and *LOM3*, were shown to be involved in the regulation of shoot meristem maintenance and axillary bud formation. When grown in short photoperiods, *lom1 lom2* double mutants and *lom1 lom2 lom3* triple mutants arrested shoot development after formation of about 22 leaves. In addition, these double and triple mutants do not form axillary buds. Using different markers for meristem development, we have shown that undifferentiated cells accumulate in the peripheral zone of the arresting meristems, which indicates that *LOM1* and *LOM2* play an important role in promoting the incorporation of cells into leaf primordia.

Mechanisms regulating axillary meristem formation and leaf architecture in tomato

Several tomato mutants exhibit defects in axillary bud formation and alterations in leaf architecture. This observation indicates that similar mechanisms act to regulate the formation of axillary meristems and the formation of leaflets in the compound leaf of tomato. We are studying such mutants with the aim of identifying genes involved in the underlying mechanisms. One of these mutants, *trifoliate*, has been characterised in detail (Fig. 2) and the gene has been mapped to a small region on chromosome 5. Currently a candidate gene from this region is being tested for complementation of the mutant phenotype.

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Jasinski, S., Tattersall, A., Piazza, P., Hay, A., Martinez-Garcia, J.F., Schmitz, G., Theres, K., McCormick, S. and Tsiantis, M.: *PROCERA* encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant J. 56, 603-612 (2008).

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Müller, D., Schmitz, G. and Theres, K.: *Blind* homologous *R2R3 Myb* genes control the pattern of lateral meristem initiation in Arabidopsis. Plant Cell 18, 586-597 (2006).

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Overview: Department of Plant Microbe Interactions

Director: Paul Schulze-Lefert

Research in the Department of Plant Microbe Interactions concentrates on 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 programme. We are pursuing an integrated approach that connects traditionally separate research territories like genetics, molecular biology, biochemistry, and cell biology. Much of our work is focused on interactions between plants and filamentous pathogens such as fungi and oomycetes, two widespread classes of pathogenic microbes.

Although the plant immune system ensures effective protection against most microbial pathogens, some intruders do succeed in colonising host plants. In such cases, plant immune receptors fail to recognise the pathogen, or the invader has evolved ways of suppressing immune responses. Our goal is to define the regulatory network of the plant immune system in such detail that we can predict how it will respond to specific changes in defined components. This should provide insights into how the plant immune system can be modified, using molecular breeding techniques, so as to improve plant protection.

Small signalling molecules in the plant immune response

40

Erich Kombrink

Department of Plant Microbe Interactions

Figure 1. *Screening for Arabidopsis thaliana mutants*

Introduction

Plants have evolved a wide 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 combatting pathogen attack. Key features of these active defence mechanisms are the induction of rapid cell death at the initial site of infection - the so-called hypersensitive response (HR), the production of reactive oxygen species (ROS) and nitric oxide (NO), followed by the activation of host gene expression and protein synthesis in neighbouring cells. We are interested in identifying signalling components that control early plant defence responses, with particular emphasis on mechanisms involved in the generation and perception of small signalling molecules.

Chemical genetics of plant immune responses

Chemical genetics is a scientific strategy that utilises small bioactive molecules as experimental tools to unravel biological processes. Bioactive chemicals occurring in nature represent an enormous diversity of structures that can be used as inhibitors or activators of biochemical pathways, transport processes, regulatory networks or developmental programmes. Work in my laboratory focuses on the discovery of novel bioactive molecules, which is achieved by high-throughput screening of diverse chemical libraries for the ability to influence a variety of phenotypes that are related to plant immunity, e.g. by modulating specific reporter genes or defence-related reactions (callose deposition, development of HR cell death, production of ROS or NO). Once the activity of a chemical compound has been critically evaluated, it can be applied in genetic screens to search for mutants that escape the chemically enforced phenotype, because they are affected either in the direct target of the small molecule or in intermediate steps in the signalling pathway leading to the phenotype. Clearly, chemical genetic approaches have the power to circumvent the inherent limitations of traditional forward genetic screens such as lethality, pleiotropy or redundancy of gene functions, because chemical intervention can be performed in a conditional, dose-dependent and reversible manner. Thus, chemical genetics allows the identification of new regulatory components of biological processes or signalling networks that are inaccessible by mutant analysis.

The yeast 3-hybrid system: A screening platform for targets of small molecules

Ultimately, the identification of the protein targets of small bioactive molecules is of fundamental importance for understanding the

We systematically search for bioactive chemicals to generate new tools for biological discovery.

molecular mechanisms of signal perception and transduction. Among the various strategies available for target identification, we have chosen to establish the yeast three-hybrid technology in our laboratory. This technique allows direct functional cloning of genes encoding proteins that interact with synthetic hybrid ligands *in vivo*. The adaptation of this new technology to plant systems has two important consequences: (1) the system provides us with a general experimental platform for identification of the targets of compounds that originate from chemical genetic screens, and (2) allows us to identify primary targets of established signal molecules in plant defence, such as salicylic acid (SA), jasmonic acid (JA) and its derivatives, or abscisic acid (ABA), whose immediate targets and modes of action are still largely unknown. We are currently using cDNA libraries, derived from various organs, tissues or plants that have been subjected to a variety of treatments, to systematically scan the whole expressed genome (proteome) of *Arabidopsis thaliana* for SA, JA and ABA targets. This approach should ultimately provide a system-wide overview of the corresponding binding proteins. We expect that elucidation of the biochemical functions of these proteins will afford exciting insights into the mechanistic details of how signalling networks operate.

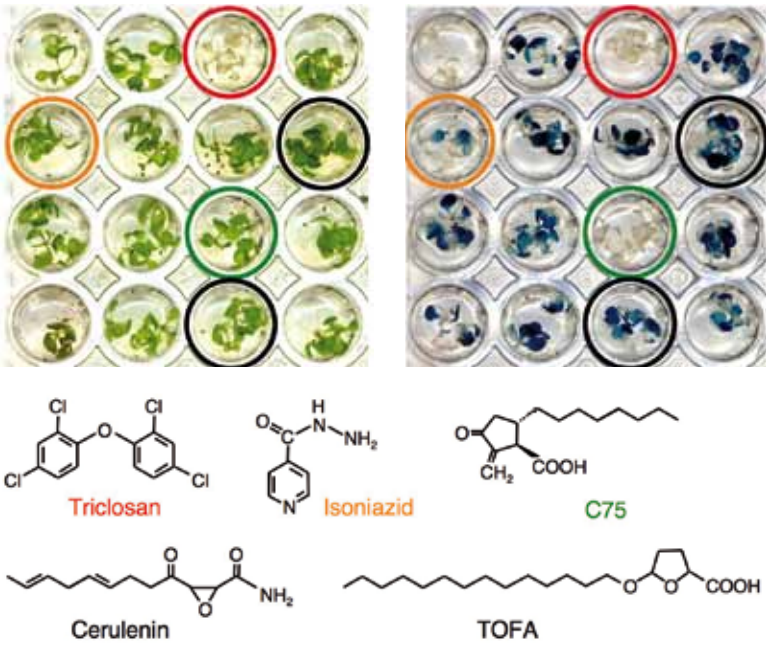

Figure 2. *Chemical genetic screening for compounds affecting jasmonate signalling in Arabidopsis thaliana. The impact of individual chemicals on plant viability is evaluated in the left panel, interference with jasmonate-mediated activation of reporter gene expression is assessed in the right panel. Compounds that provoke a strong response without impairing plant viability (green label) are subjected to detailed analysis before they are eventually employed in a genetic screen.*

Selected publications

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Molecular basis of fungal biotrophy

42

Richard O'Connell

Department of Plant Microbe Interactions

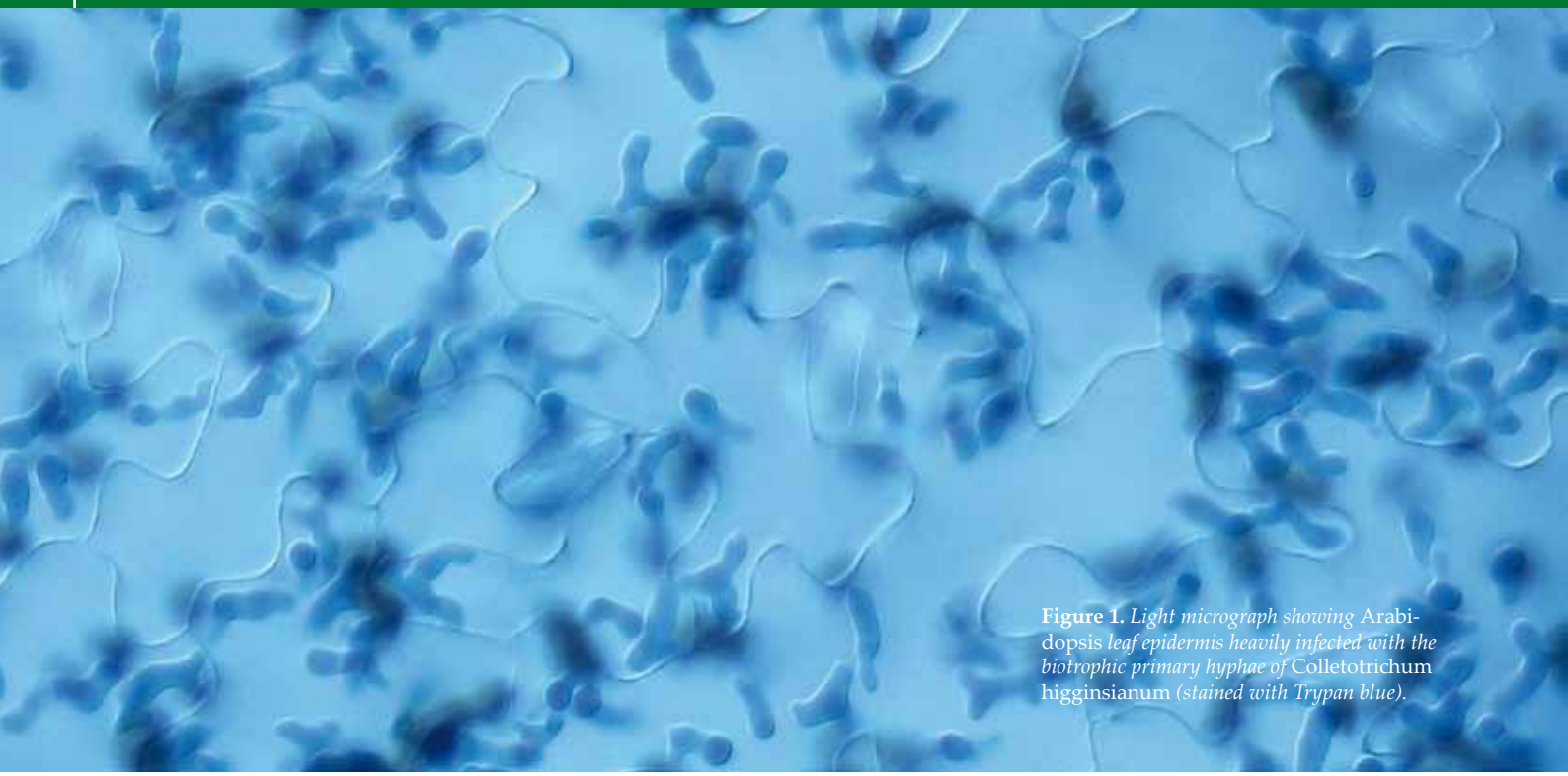

Figure 1. *Light micrograph showing* Arabidopsis *leaf epidermis heavily infected with the biotrophic primary hyphae of* Colletotrichum higginsianum *(stained with Trypan blue).*

Introduction

Biotrophic pathogens feed on living plant cells. They cause some of the most damaging plant diseases worldwide, but they are also sophisticated parasites, having evolved the remarkable ability to insert specialised feeding structures into plant cells, without killing them or triggering defence responses. However, the molecular analysis of biotrophs is difficult because they only grow on living host plants. The mechanisms underlying biotrophy are therefore largely unknown.

We use the interaction between *Arabidopsis thaliana* and *Colletotrichum higginsianum* as a model because the fungal partner can be cultured *in vitro* and genetically transformed, permitting analysis of gene function, e.g. by random mutagenesis, targeted gene disruption and overexpression. Moreover, we recently obtained the complete genome sequence of this fungus using next-generation sequencing technology. *Colletotrichum* uses a two-stage infection strategy: melanised appressoria first penetrate the plant epidermis to form bulbous biotrophic hyphae, similar to haustoria, inside living host cells (Figure 1). The fungus later switches to destructive necrotrophy, feeding on dead tissues. Our goal is to make use of this model pathosystem to dissect the molecular basis of biotrophy.

Discovering novel pathogenicity genes

One approach we are using to identify genes required for pathogenicity is random insertional mutagenesis, where fungal genes are tagged with T-DNA via *Agrobacterium*-mediated transformation (ATMT). After screening 8,850 transformants on *Arabidopsis*, we found 40 pathogenicity-deficient mutants with a range of abnormal phenotypes, e.g. impaired appressorium formation or host penetration, induction of host defense responses and inability to switch from biotrophy to necrotrophy. Analysis of T-DNA flanking sequences led to the identification of 14 putative pathogenicity genes, including

Our goal is to exploit the Colletotrichum-Arabidopsis pathosystem to dissect the molecular basis of biotrophy.

homologues of importin-beta2, ornithine decarboxylase, a phosphate uptake transporter and genes required for arginine biosynthesis. Further characterisation of these genes should give new insights into the molecular basis of fungal pathogenicity. Overall, ATMT will be a powerful tool for functional genomics in this model pathogen.

Hunting for secreted effectors

Many plant pathogens establish disease by secreting effector proteins that manipulate host cell structure and metabolism and disable plant defences, creating an environment favourable to the pathogen. However, little is known about the effectors used by biotrophic fungi. To search for *C. higginsianum* effectors, we generate stage-specific cDNA libraries from the fungal cell types that set up biotrophy, namely appressoria and biotrophic hyphae. After sequencing, we use computational prediction tools to mine the data for genes encoding small, soluble secreted proteins that may function as effectors.

Sampling the transcriptome of biotrophic hyphae is problematic because they only develop inside host epidermal cells (Figure 1). In collaboration with Dr Elmar Endl (University of Bonn), we have developed a novel method to purify hyphae from infected leaves by fluorescence-activated cell sorting (Figure 2). This eliminated plant and fungal contaminants, allowing construction of a stage-specific cDNA library. The library was enriched for plant-induced and pathogenicity-related genes, including some encoding candidate effectors. Genes related to nutrition (e.g. amino acid and vitamin biosynthesis, amino acid and sugar uptake) were also highly represented.

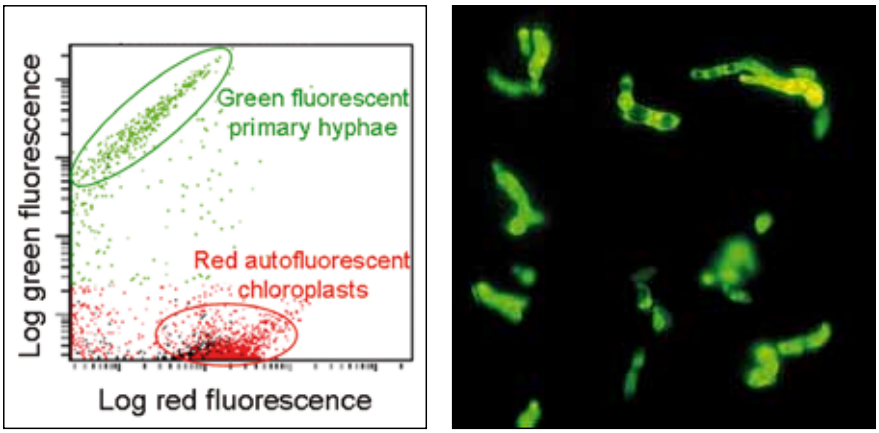

Figure 2. *Fluorescence-activated cell sorting (FACS) of* Colletotrichum higginsianum *biotrophic hyphae. Hyphae were enriched from homogenates of infected leaves by centrifugation, labelled with the vital dye fluorescein diacetate (FDA) and separated from other fungal cells and plant contaminants by FACS. (A) Cytogram showing FACS separation of green FDA-stained hyphae from red autofluorescent chloroplasts. (B) Purified hyphae are alive, as shown by FDA staining.*

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Host cell manipulation and defence suppression in plant/powdery mildew interactions

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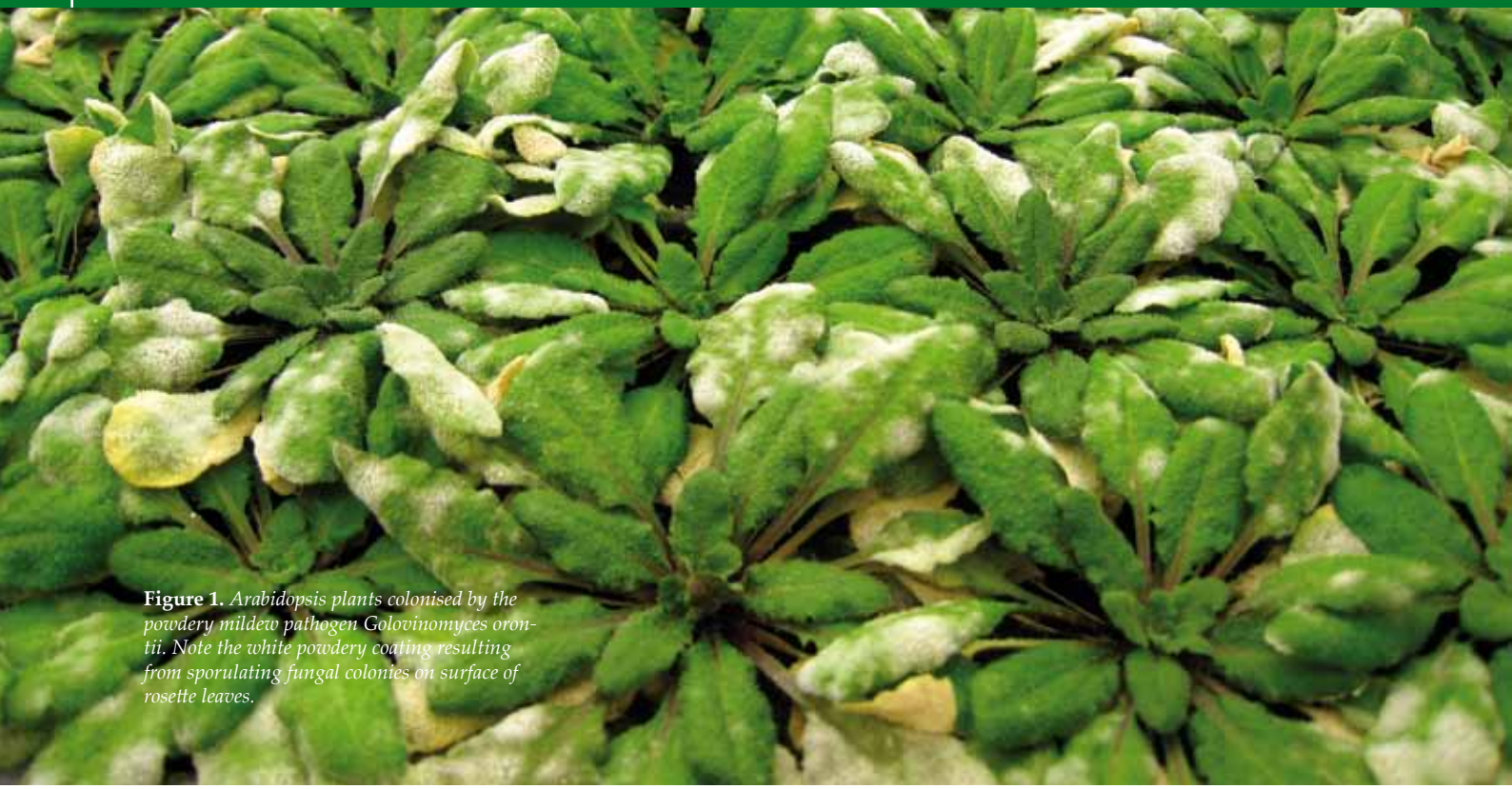

Figure 1. *Arabidopsis plants colonised by the powdery mildew pathogen Golovinomyces orontii. Note the white powdery coating resulting from sporulating fungal colonies on surface of rosette leaves.*

Plants are very well equipped to defend themselves against all types of microbes. They possess a surveillance system comprising dedicated immune sensors, some of which are localised at the cell periphery. These constantly monitor the exterior for the presence of pathogen-derived ("non-self") molecules. Upon recognition of signatures that are typical for a potential intruder, the immune sensors initiate intracellular signalling cascades to activate a plethora of defence responses. These comprise the secretion of antimicrobial proteins and toxic metabolites, which serve to arrest growth of the invader. Successful plant pathogens, however, have found ways to circumvent these elaborate defence mechanisms. They either elude recognition or suppress the activation and/or execution of defence responses, e.g. by interfering with intracellular signalling or detoxifying secreted antimicrobials. The molecular mechanisms of this "defence suppression" are the focus of my research programme.

The molecular basis of broad-spectrum resistance to powdery mildew

We address this topic by detailed analysis of the interaction of plants, in particular the monocotyledonous grass barley (*Hordeum vulgare*) and the dicotyledonous model plant thale cress (*Arabidopsis thaliana*), with a class of pathogenic fungi called powdery mildews. These fungi are agronomically and economically important, since they cause serious damage to a range of cereals, crops and ornamentals. We found that a polytopic plant membrane protein termed MLO is a key component that is required to enable entry of powdery mildew fungi into plant host cells. Mutants that lack, or synthesise defective, MLO proteins are highly resistant to powdery mildew disease. MLO proteins are a pre-requisite for successful powdery mildew pathogenesis in both mono- (barley) and dicotyledonous (Arabidopsis, tomato and pea) plant species. Although the biochemical

Knowing the pathogen's effector arsenal is key to understanding defence suppression.

function of MLO proteins is still elusive, we hypothesise that powdery mildew fungi may exploit plant MLO proteins for defence suppression. The recent discovery of an Arabidopsis *mlo* mutant, which is resistant to the powdery fungus that is normally pathogenic on Arabidopsis, will enable us to dissect the molecular basis of the highly effective and agronomically durable *mlo* resistance by using the comprehensive tool-box available for Arabidopsis. Initial findings based on genetic epistasis analysis indicate that *mlo* resistance is largely based on the arsenal that is also employed during defence against other adapted and non-adapted pathogens. It will thus be intriguing to learn why the antifungal defence response of *mlo* mutants is so much more effective than that of wild-type plants. To understand how powdery mildew is perceived by the plant, we plan to identify the relevant pathogen-derived molecular patterns and their cognate plant receptors that initiate early defence signalling. We are also interested in unravelling the core biochemical function of MLO proteins by studying other physiological processes in which members of this plant-specific membrane protein family play a decisive role (e.g. root thigmomorphogenesis).

Powdery mildew effectors

A comprehensive understanding of the suppression of plant defence responses also requires detailed knowledge of the specific pathogen at the molecular level. We have recently initiated genome sequencing of two powdery mildew species that are pathogenic on Arabidopsis and pea, respectively. This approach is being complemented by deep transcriptome analyses of the two fungi. The sequence information obtained in this project will enable us to identify and study the set of effector proteins secreted by powdery mildew fungi at various stages of pathogenesis. These effectors comprise comparatively small polypeptides of unknown function. We hypothesise that several effectors might function in defence suppression, e.g. by interfering with defence signalling or inhibiting the execution of defence measures. We hope to identify the host targets of these proteins and so obtain further insights into how powdery mildews manipulate their host cells.

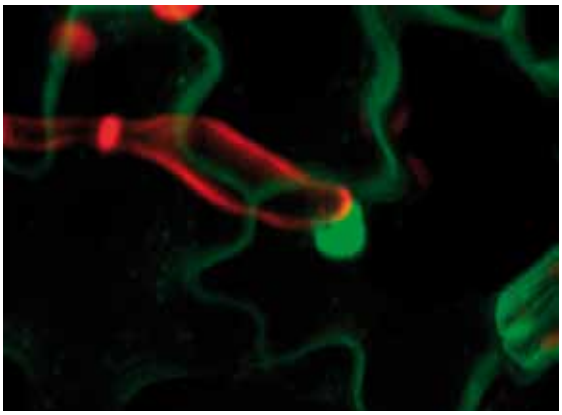

Figure 2. *Focal accumulation of the GFP-tagged syntaxin PEN1 at a site where a fungus is trying to pierce the plant cell wall. The confocal micrograph depicts a leaf epidermal cell of a transgenic Arabidopsis plant expressing the fluorophore-tagged PEN1 syntaxin (green), a defence component required for mlo-mediated resistance. The protein is found all along the cell periphery, but focally accumulates at the site where the fungal germ tube (highlighted in red) attempts to penetrate the plant cell wall.*

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Regulation of plant resistance to biotrophic pathogens

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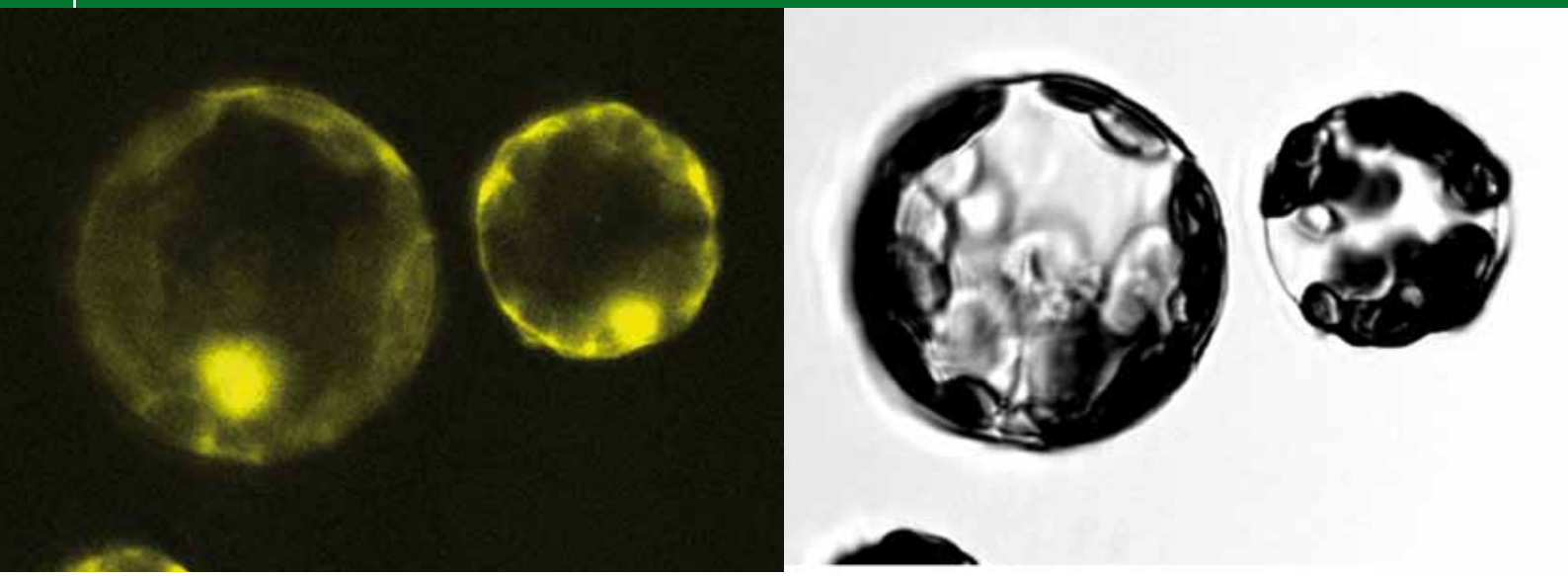

Introduction

We seek to understand how plants control innate immune responses to invasive biotrophic pathogens. The balance between disease resistance and susceptibility is finely poised, and pathogens manipulate it by altering thresholds for activation of host anti-microbial and cell death pathways. Plants have evolved mechanisms to counteract infection, including the recognition of specific pathogen effectors by intracellular immune receptors. Complementary approaches from genetics, genomics, protein biochemistry and cell biology in *Arabidopsis* are enabling us to characterise regulatory steps in host immunity, build a better picture of how plants coordinate stress signalling networks and identify cellular processes that are vulnerable to interference by pathogen effectors.

Figure 2. *Hpa is highly adapted to its Arabidopsis host. Sporulation on leaves.*

Resistance pathways converging on EDS1

EDS1 complexes that shuttle between the cytoplasm and the nucleus form a central regulatory node in resistance to biotrophic pathogens. They control production of the phenolic defence signal salicylic acid (SA) and other stress metabolites, and are necessary for triggering resistance and cell death upon activation of intracellular TIR-NB-LRR-type immune receptors. Early pathogen-induced accumulation of EDS1 in the nucleus correlates with transcriptional defence reprogramming, and we are investigating whether EDS1 behaves as a transcriptional switch, since this would explain its ability to coordinate responses to multiple stimuli. Expression and metabolite profiling of wild-type and mutant leaf responses is revealing new components of EDS1-dependent local and systemic immunity. In collaboration with Dr. Karsten Niefend (Dept. of Biochemistry, University of Cologne) we are measuring physicochemical properties of purified recombinant EDS1 complexes and performing crystallisation experiments.

Plant immune receptor activities inside nuclei

Receptor activation triggers a strong local resistance response and induces systemic immunity. We are studying the sequence of events between activation of an *Arabidopsis* TIR-NB-LRR receptor (RPS4) and initiation of EDS1-dependent defence and cell death. A small nuclear pool of RPS4 is needed to

The need for plants to respond in a flexible manner to different pathogens requires exquisite tuning of their stress signalling networks.

trigger resistance. Using a combination of chromatin immunoprecipitation (Ch-IP) and sequencing we are examining whether RPS4 and associated chaperones contact the host transcriptional machinery and at what level nuclear RPS4 activity is regulated by EDS1 complexes.

Resistance regulation as an adaptive trait

A collaboration with the group of Matthieu Reymond (Dept. of Plant Breeding and Genetics) was established to discover which molecular processes underlie hybrid incompatibility associated with temperature-dependent epistatic interactions between *Arabidopsis* accessions. We find that the extent of incompatibility in several epistatic combinations can be explained by EDS1/SA pathway activation and, in some instances, by the balance between SA signalling and other stress-hormone pathways. We are exploring further the relationship between immunity and environmentally conditioned hybrid incompatibility as major determinants of plant growth and survival and, potentially, speciation.

Activities of oomycete effectors

Together with the group led by Dr. Guido van den Ackerveken (Utrecht University), we have identified secreted proteins that are expressed during infection by the biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*), which is pathogenic on *Arabidopsis*. We initially focus on effector-like protein families that are polymorphic between different *Arabidopsis*-infecting *Hpa* isolates, and are now testing their effects on disease severity and, in some host genotypes, their roles in triggering resistance. The aim of the study is to define the range of host processes manipulated by invasive biotrophic pathogens and elucidate how the plant counteracts such interference.

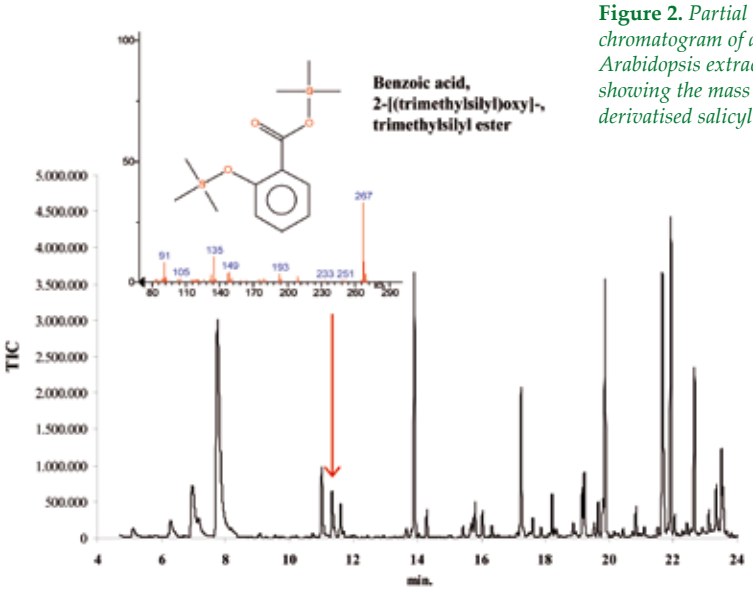

Figure 2. *Partial GC-MS chromatogram of a semi-polar Arabidopsis extract with inset showing the mass spectrum of derivatised salicylic acid.*

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Alcázar, R., Garcia, A.V., Parker, J.E. and Reymond, M. Incremental steps towards incompatibility revealed by Arabidopsis epistatic interactions modulating salicylic pathway activation. Proc. Natl. Acad. Sci. USA 106: 334-339 (2009).

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Cellular dynamics in plant-microbe interactions

48

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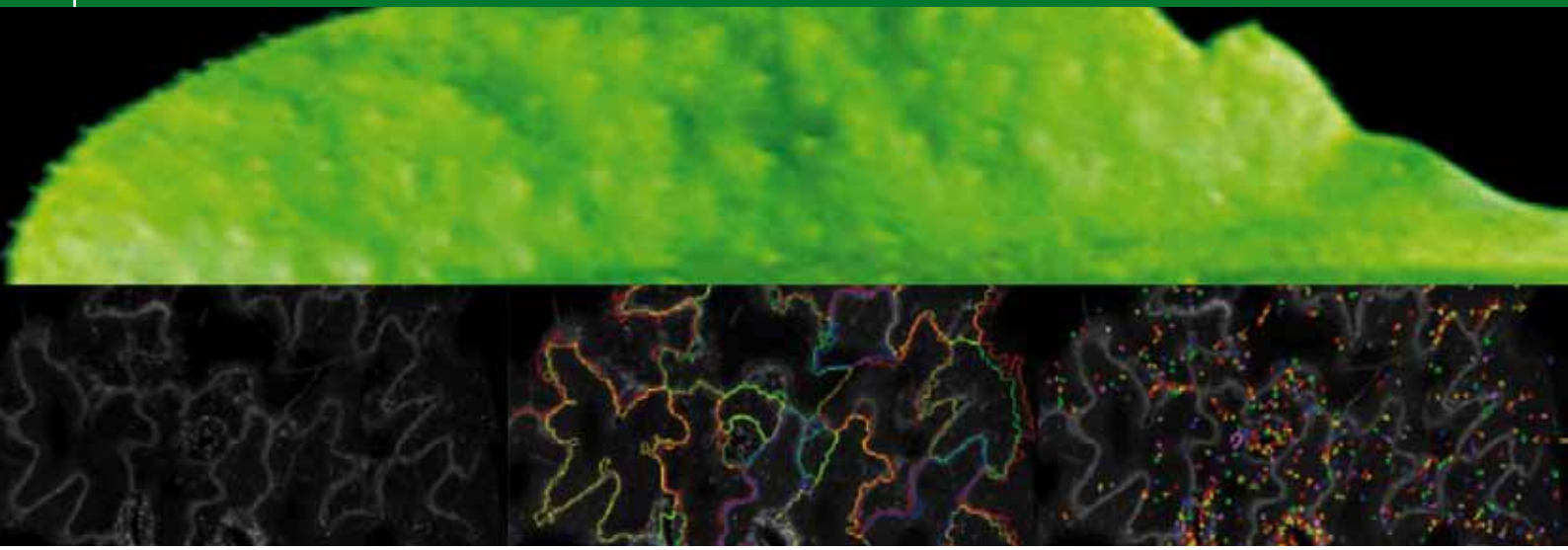

Introduction

Intracellular membrane compartmentalisation and trafficking are pivotal for eukaryotic life. Plant defence responses to pathogen infection are tightly associated with a global reprogramming of membrane trafficking pathways (Fig. 1). Several plasma membrane-resident proteins focally accumulate at pathogen penetration sites, where most organelles aggregate and to which secretory pathways are redirected. This hallmark of microbial attack has been known for decades, and is thought to strengthen the plant cell wall and deliver toxic compounds to disable the pathogen. However, only now are we beginning to identify the molecular components and secreted cargo molecules.

One of the first lines of active defence is made up of cell surface pattern recognition receptors that detect conserved microbe-associated molecular patterns (MAMPs). Upon perception of MAMPs such as bacterial flagellin (flg22), a plethora of defence responses are stimulated that lead to plant immunity. The plasma membrane receptor responsible for flg22 recognition in Arabidopsis is the leucine-rich-repeat (LRR) transmembrane kinase FLS2, which inducibly interacts with the receptor kinase BAK1 to signal detection of flg22 and activate the appropriate response. Furthermore, activated FLS2 is internalised and enters the endocytic pathway, which appears to be connected with flg22 signalling and targets FLS2 for degradation (Fig. 1). This suggests an important role for endocytic trafficking in plant immunity.

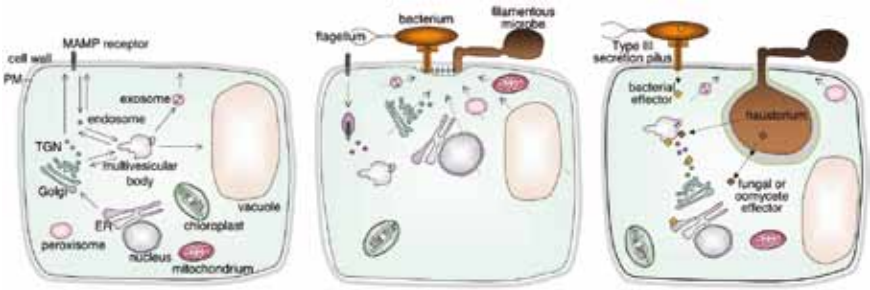

Figure 1. *Schematic representation of the changes in cellular dynamics induced by microbial attack. Right panel: Plant organelles and vesicle trafficking pathways are shown. Middle panel: Focal accumulation of membrane compartments at the site of attempted microbial penetration. Cell wall reinforcement and secretion of harmful compounds arrest microbial ingress. Left panel: Successful pathogens deliver effector molecules into the host cell and subvert immune responses. Effector-triggered reprogramming of host processes allows proliferation of the pathogen.*

Projects

MAMP-triggered responses are known for their rapid induction and transient behaviour, which suggests that a tight regulatory process is required to efficiently defend against pathogens

If we are to understand plant immunity and pathogen-induced disease, we need to investigate the associated subcellular rearrangements at the molecular level.

and maintain host-cell viability. My laboratory seeks to understand the dynamic nature of this MAMP response during signalling and at the subcellular level. We have employed a variety of genetic screens and isolated collections of mutants that are impaired in MAMP responses at different levels, e.g. mutants (*rio* = reduced in oxidative burst) defective in the oxidative burst, one of the earliest MAMP responses. Interestingly, some of these mutants appear to interfere with *FLS2* expression via crosstalk with plant stress-hormone pathways. Similarly, collaborative work identified mutants impaired in MAMP receptor abundance. Another mutant set (*fli* = flagellin insensitive) was isolated by screening for alterations in late MAMP responses and, importantly, these show enhanced susceptiblity to bacterial infection. Both mutant collections should yield insights into the regulation of MAMP responses.

With respect to subcellular changes during plant-microbe interactions, my laboratory mainly focuses on the endocytic pathway. Multivesicular bodies, which are late endosomal compartments, are of particular interest, because they constitute an intersection of several different vesicle trafficking routes (Fig. 1). If we are to understand plant immunity and pathogen-induced disease, we need to investigate the associated subcellular rearrangements at the molecular level. We have therefore performed a genetic screen based on quantitative high-throughput confocal laser microscopy, and identified a unique collection of altered endocytosis mutants (Fig. 2). These *fel* mutants (= FYVE endosome levels) are currently being investigated, and appear to provide a valuable tool for studying vesicle traffic during biotic stress. Furthermore, to specifically address FLS2 endocytosis, we identified a number of FLS2 interacting proteins (FIPs), which we are testing for activity during bacterial infections and responses provoked by flg22.

Successful pathogens depend on their ability to overcome MAMP-triggered immune responses. Bacterial pathogens such as Pseudomonas syringae pv tomato DC3000 (PtoDC3000) inject a battery of so-called effector proteins into the plant cell via their type III secretion system (Fig. 1). One of these effectors is AvrPtoB, initially identified as being recognized by the soluble kinase Pto in tomato, which triggers resistance and carries a eukaryotic E3 ubiquitin ligase domain at its C-terminus. We discovered that AvrPtoB reduces the level of FLS2 and thereby promotes PtoDC3000 virulence in Arabidopsis. AvrPtoB interacts with FLS2 and BAK1 through its N-terminus. Moreover, interaction with FLS2, but not its co-receptor BAK1, is strongly enhanced upon activation by flg22. Furthermore, we detected that AvrPtoB polyubiquitinylates FLS2 in vitro and in vivo, which targets FLS2 for degradation. We are now in the course of identifying the amino acid residues ubiquitinated by AvrPtoB, and are also searching for plant E3 ligases, which ubiquitinate FLS2.

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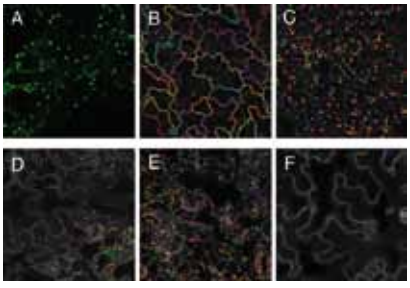

Figure 2. *Application of advanced confocal laser microscopy in a genetic screen. A transgenic Arabidopsis line expressing the endosomal marker FYVE-GFP was used as a reference. Conventional confocal laser imaging of FYVE-GFP endosomes (A). High-throughput confocal laser microscopy is programmed to automatically merge pictures from several Z-sections, recognise cell boundaries (B) and spots within recognised cells (C) to quantify endosomes. Spot quantification was used to identify mutants with increased (E) or reduced numbers of endosomes (F) compared to the reference line (D).*

The innate immune system of plants

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Paul Schulze-Lefert

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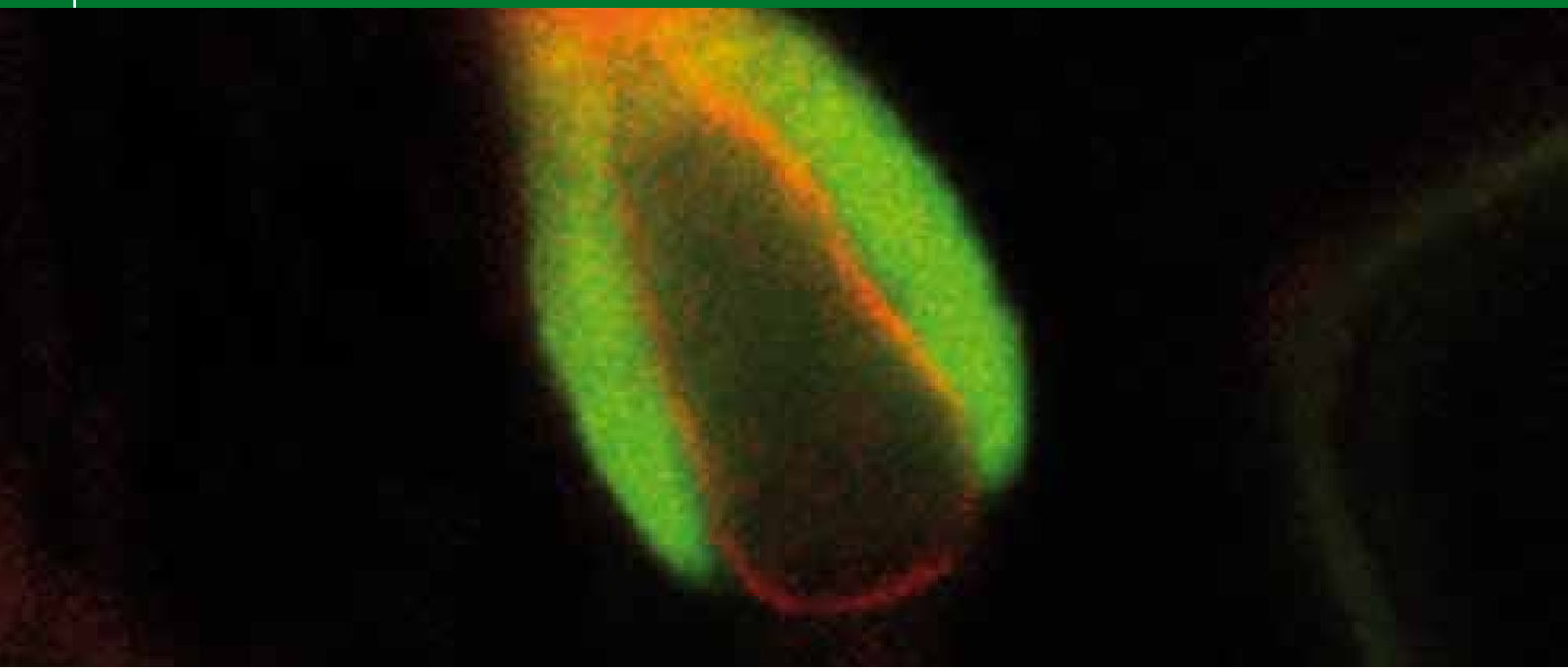

Introduction

Molecular research during the past decade has revealed the existence of an elaborate innate immune system in plants that recognises the presence of potential pathogens and mounts powerful immune responses to microbial intruders. Two classes of immune receptors detect pathogen-derived molecular structures outside and inside of plant cells. One class comprises membrane-resident pattern recognition receptors (PRRs) that recognise widely conserved microbe-associated molecular patterns (MAMPs) such as bacterial flagellin or fungus-derived chitin on the external surface of plant cells. A second class of mainly intracellular immune sensors, designated disease resistance (R) proteins, sense either the structure or function of isolate-specific pathogen effectors that are delivered into host cells.

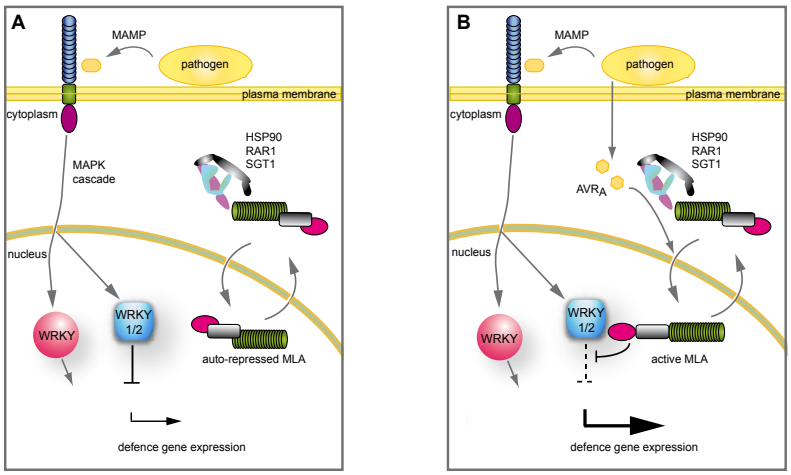

Figure 1. *Nuclear action of MLA links effector-specific and MAMP-triggered immune responses. One or several MAMP receptors initiate MAMP signaling via intracellular MAPK cascades, which in turn stimulate the induction of unknown WRKY transcriptional activators (pink) and WRKY1/2 repressors (blue). Auto-repressed MLA receptors are folded by RAR1, SGT1, and cytosolic HSP90, and might continuously cycle between nucleus and cytoplasm. (B) Integrated MAMP- and MLA-triggered immune response upon co-activation of MAMP receptors and MLA by cognate powdery mildew effectors (designated AVRA). Activated MLA associates with WRKY1/2 in the nucleus, thereby de-repressing MAMP-triggered immunity. Whether AVRA is directly or indirectly recognised by the cytoplasmic and/or nuclear MLA pool remains unknown.*

Fundamental insights into the plant immune system provide a conceptual framework for rationally directed efforts to breed or engineer crops for durable and broad-spectrum disease resistance.

Regulatory logic of integrated immune responses triggered by cell-surface and intracellular immune sensors

Intracellular and cell-surface immune receptors are known to trigger similar immune responses upon perception of non-self patterns. This suggests a convergence of their signalling pathways. We have shown that, in barley, intracellular MLA R proteins function in the nucleus to confer immunity against the powdery mildew fungus. Recognition of the fungal AVRA effector by MLA induces associations between receptor and WRKY transcription factors in the nucleus. These WRKY proteins act as repressors of MAMP-stimulated defence (Figure 1). MLA interferes with this repressor function, thereby de-repressing MAMP-triggered immune responses. This derepression is thought to amplify expression of defence-related genes (Fig. 1). Our findings point to the existence of a double-negative regulatory circuit that connects immune responses triggered by R proteins and PRR receptors. Ongoing research activities aim to solve the crystal structure of the MLA immune sensor and determine its exact mode of action in the nucleus. We are also investigating whether MLA binds to specific chromatin sites to reprogram plant cells for immune responses.

Figure 2. *Focal secretion at contact sites between plant and pathogen (target) cells. Genetic studies have shown that the PEN1-dependent and vesicle-mediated secretory pathway functions independently from the PEN2/PEN3 pathway. PM, plasma membrane; NE, nuclear envelope.*

Targeted secretion in plant immune responses

Our studies revealed the existence of a secretory machinery that participates in the execution of extracellular immune responses. A vesicle-associated and SNARE protein-mediated exocytosis pathway drives the focal secretion of vesicle cargo comprising proteins and cell-wall building blocks into the extracellular space (Figure 2). This pathway has additional functions in plant development and might have been co-opted for immune responses. Components of bacteria and fungi that are delivered into host cells can interfere with the secretion machinery by blocking vesicle formation from intracellular membranes. A parallel secretory pathway in the model plant *Arabidopsis* delivers intracellular enzymes that release antimicrobials from protoxins (indole glucosinolates) for translocation into the extracellular space by plant plasma membrane-resident efflux pumps (Figure 2). Future research will seek to study the regulation and assembly of the multicomponent secretory machineries that mediate targeted release of vesicle cargos and antimicrobials at pathogen contact sites.

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Transcriptional regulatory networks governing the plant immune response

52

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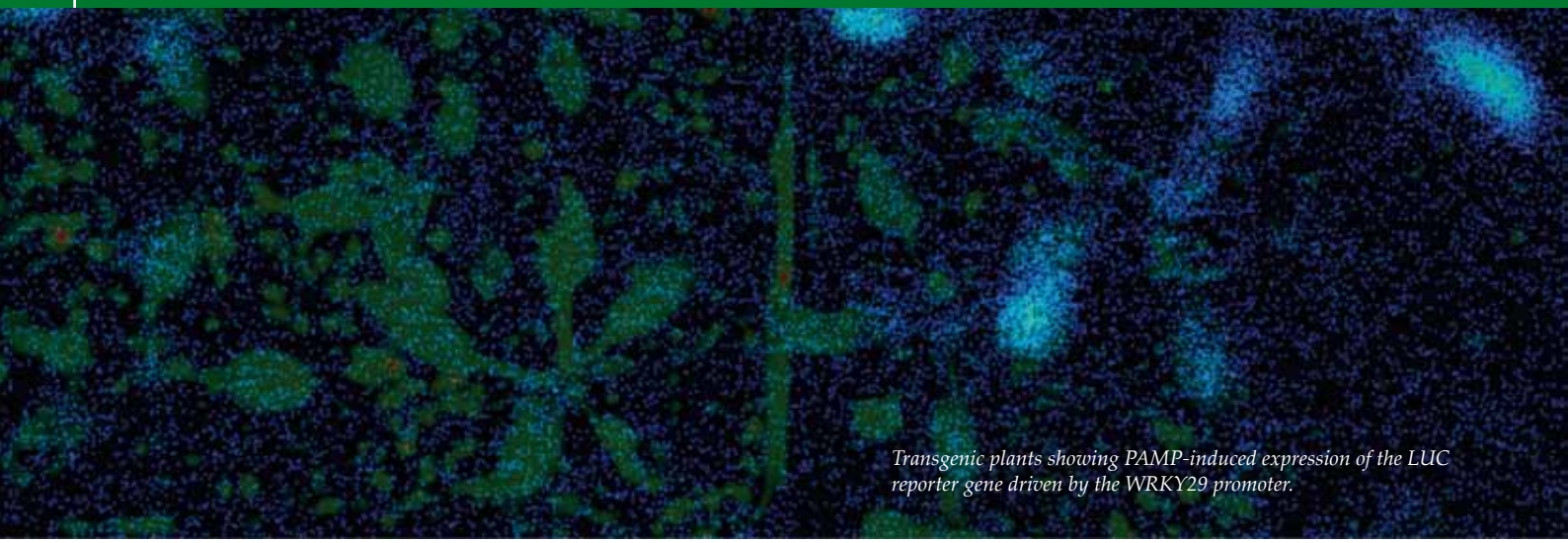

Transgenic plants showing PAMP-induced expression of the LUC reporter gene driven by the WRKY29 promoter.

Introduction

The plant innate immune system consists of two interconnected branches termed PTI (PAMP-Triggered Immunity) and ETI (Effector-Triggered Immunity) that initiate massive transcriptional reprogramming. PTI is provoked by pathogen-associated molecular patterns (PAMPs), molecular signatures that are ubiquitous features of the surfaces of many microbes. Several microorganisms secrete effector proteins into host cells that interrupt PAMP-triggered defence-signal relays and thereby attenuate PTI. Co-evolution of virulent pathogens with their hosts has resulted in the establishment of ETI, a manifestation of so-called gene-for-gene resistance. ETI is triggered by plant resistance (R) proteins that provoke highly efficient defence responses upon specific detection of pathogen effectors. The major differences between PTI and ETI appear to be quantitative and/or temporal rather than qualitative, suggesting that most pathogens trigger a common/interconnected plant signalling network. The graded transcriptional responses associated with immunity clearly indicate the existence of a complex regulatory circuitry comprised of transcriptional activators and repressors that act to fine-tune the expression of defence genes. The nuclear end of the signaling cascades is less well studied, but members of several transcription factor (TF) families are known to modulate the defence transcriptome. In particular, zinc-finger-type WRKY factors appear to play a broad and pivotal role in regulating host defences.

WRKY transcription factors as positive and negative regulators of plant immunity

Our current research is focused on defining how *Arabidopsis* members of the WRKY TF family re-program the defence transcriptome. Two kinds of interactions are being studied. 1) Negative regulation of defences: Loss of *AtWRKY18/40* functions renders plants highly resistant to the powdery mildew fungus *Golovinomyces orontii* (Figure 1; M. Schön, A. Töller). 2) Positive regulation of defences: Loss of *AtWRKY33* function leads to susceptibility to the necrotrophic fungus *Botrytis cinerea* (Dr. R. Birkenbihl).

In both cases, detailed comparative analyses of global expression profiles have been performed, using microarrays, to define gene networks controlled by these WRKY factors. Transgenic lines expressing functional epitope-tagged versions of the respective WRKY TFs have been generated. These are currently being used to define the set of target genes directly recognised by these TFs in vivo, by means of chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-Seq). Moreover, attempts to identify additional nuclear components that interact with these WRKY proteins are underway.

Finally, our earlier hypothesis (Shen et al. Science 315, 1098, 2007) that

Understanding how the various defense signaling cascades are linked to the transcriptional regulatory network governing cellular responses remains a major challenge.

specific associations with still unknown *Arabidopsis* CC-NB-LRR-type resistance (R) proteins modulate the activities of WRKY18 and WRKY40 is currently being tested using a targeted approach based on the yeast 2-hybrid system.

Novel pathogen-responsive cis-regulatory DNA elements

This project aims to identify novel *cis*-acting regulatory DNA elements that respond to abiotic and biotic stimuli using an unbiased approach (Dr. M. Roccaro). We have developed a novel screening method that exploits the fact that the transition from transcriptional initiation to elongation correlates with phosphorylation of Ser5 of the heptad repeats in the CTD of the largest subunit of RNA polymerase II (RNApol-II). Randomised oligonucleotide libraries were constructed that carry synthetic elements upstream of a minimal promoter driving the expression of a reporter gene, and transformed into plant cell protoplasts. Following PAMP stimulation, chromosomal proteins are cross-linked to the DNA, and fragmented chromatin is immunoprecipitated using a monoclonal anti-Ser5 antibody. The DNA in this first enriched fraction is cloned and subjected to two additional rounds of transformation and enrichment. Solexa paired-end sequencing of a series of enriched pools and the initial library has been performed. We are currently applying bioinformatic tools to identify candidate elements, which will subsequently be rigorously tested with respect to functionality.

Future goals

WRKY factors appear to form a complex regulatory network involving both positive and negative feedback loops. Dissecting this network will require a combination of genetics, biochemistry, bioinformatics and modern technological approaches including ChIP-Seq and mass spectrometry. Moreover, it is now evident that the nucleus is a major battleground in the co-evolutionary struggle for survival between the host and pathogen. Thus, identification of nuclear components/sites targeted by pathogens and definition of pathogen-dependent, temporally specific changes in the defence transcriptome will be of utmost importance.

Figure 1. *The double mutant wrky18/40, lacking the negative transcriptional regulators of basal defence WRKY18 and WRKY40, is strongly resistant to the otherwise virulent powdery mildew pathogen Golovinomyces orontii (wildtype Col-0). Re-introduction of WRKY40 into this mutant restores susceptibility (wrky18/40 + 35S:WRKY40).*

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Overview: Independent Research Groups and Research of Service Groups

Giving talented young scientists from diverse backgrounds the opportunity to prove themselves as leaders of independent research groups complements and expands the focus of the departments. The groups directed by these younger scientists operate outside the departmental structure and can pursue their own research topics for a period of up to five years.

Currently three independent research groups supply extra know-how in bioinformatics, chemical and quantitative crop genetics. The Plant Computational Biology group is headed by Heiko Schoof, who is also the leader of the Institute's IT infrastructure group. Renier van der Hoorn leads the Plant Chemetics group. Benjamin Stich is head of the Crop Genetics group, which is based on a strong background in quantitative genetics.

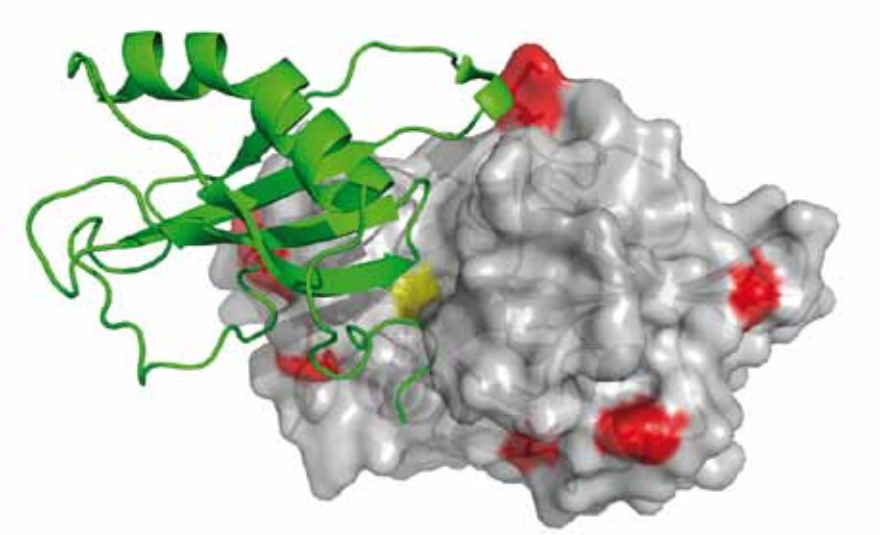
A new department devoted to the field of crop genetics and evolution, to be led by a newly recruited Director, will be founded to replace the Plant Molecular Genetics department. Peter Huijser from the Plant Molecular Genetics department will continue his research in the new department.

Service groups are also independent of the departments and are headed by tenured scientists who also perform research tasks. Elmon Schmelzer's group deals with microscopy, Jürgen Schmidt's group with mass spectrometry, while Bernd Reiss' group studies recombination and also takes care of issues relating to laboratory biosafety.

Plant Chemetics

Renier van der Hoorn

Independent Research Group



The Plant Chemetics Lab is part of the Chemical Genomics Centre of the Max Planck Society, and is staffed by organic chemists (located in Dortmund) and plant biologists (located in Cologne), who collaborate closely to generate new approaches to study plant biology.

Introduction

High-capacity profiling approaches have generated a tremendous wealth of information on genomes, transcriptomes and proteomes, providing insights into many biological processes. Activities of enzymes, however, are post-translationally regulated and thus cannot be predicted solely on the basis of the presence of proteins or transcripts. The Plant Chemetics Lab develops and applies Activity-based Protein Profiling (ABPP) to make this important layer of functional proteomic information accessible.

ABPP is based on the use of labelled, mechanism-based inhibitors that react with active-site residues of whole enzyme classes in an activity-dependent manner. Labelling results in the formation of a stable covalent bond, which facilitates detection on protein gels, and purification and identification by mass spectrometry. Over 150 probes are available for diverse enzyme classes, including cysteine proteases, lipases, carboxyesterases, phosphatases and glycosidases (Fig. 1).

Projects

The first challenge faced by the Plant Chemetics Lab is to *establish* ABPP in plants by identifying at least 1000 probe targets. This goal can be reached in close collaboration with

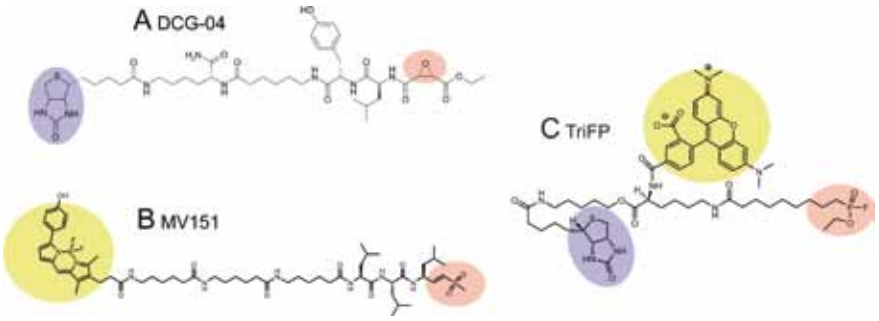


Figure 1. *Examples of activity-based probes for papain-like cysteine proteases (A), catalytic subunits of the proteasome (B), and serine hydrolases (C). The probes contain a covalent inhibitor (red) flanking a binding group and linker; and a reporter group for purification (biotin, blue) and/or fluorescence detection (yellow).*

Mining the active proteome reveals a new layer of functional genomic information.

organic chemists, chemical biologists and proteomics experts, by varying probes, proteomes and conditions, and by applying *in vivo* profiling using click chemistry (Kaschani et al., 2008). Using probes for cysteine proteases, the proteasome and serine hydrolases, we can now monitor the activity of over 100 enzymes (e.g. Kaschani et al., 2009). Some probes, however, target unexpected sites. ß-lactone probes, for example, were found to label the N-termini of proteins. This N-terminal labelling is mediated by an Arabidopsis protease that can ligate peptides to the N-termini of other proteins (Wang et al., 2008).

The second goal is to *apply* ABPP to investigate of plant-pathogen interactions by 1) discovering differential enzyme activities that are induced during defence responses following infection of Arabidopsis and tomato by various pathogens; and 2) identifying pathogen proteins that inhibit plant enzymes. These approaches have revealed dynamic changes in multiple Arabidopsis serine hydrolase activities upon infection with *Botrytis cinerea* (Kaschani et al., 2009; Fig. 2). It was also found that papain-like cysteine proteases from tomato are inhibited by effectors produced by oomycete, fungal and bacterial pathogens. AVR2, for example, is a fungal effector protein that selectively targets diversifying, defence-related cysteine proteases of tomato (Shabab et al., 2008).

Future challenges

We are further expanding ABPP using new probes and other proteomes. ABPP will be used to screen for pathogen-derived inhibitors of plant enzymes, and the role of pathogen-derived inhibitors and their host targets in pathogenicity will be investigated by reverse genetics and targeted chemical reverse genetics ('chemetics').

Selected publications

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Wang, Z., Gu, C., Colby, T., Shindo, T., Balamurugan, R., Waldmann, H., Kaiser, M., and Van der Hoorn, R. A. L. (2008) Beta-lactone probes identify a papain-like peptide ligase in *Arabidopsis thaliana*. Nat. Chem. Biol. 4, 557-563.

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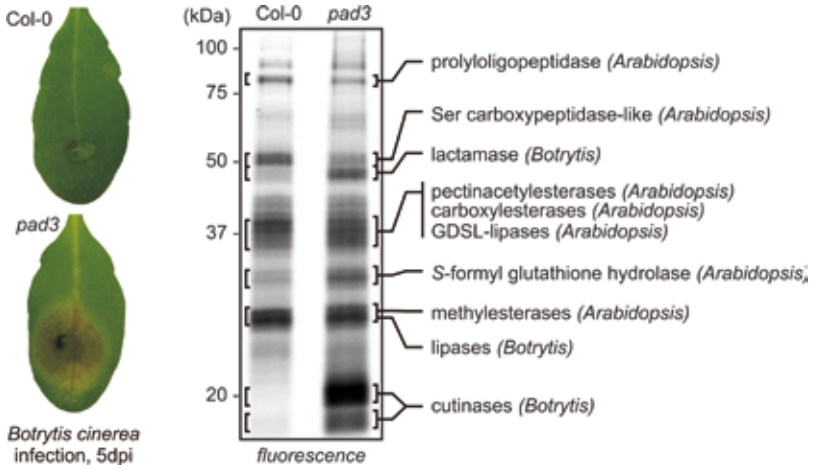


Figure 2. *Serine hydrolase activities expressed upon infection of Arabidopsis with Botrytis cinerea. Leaves of wild-type and phytoalexin-deficient (pad3) Arabidopsis plants were inoculated with a droplet containing Botrytis spores. Leaf extracts isolated five days after infection (left) were labelled with TriFP and biotinylated proteins were purified, visualised by fluorescence scanning and identified by mass spectrometry (right).*

Plant Computational Biology

Heiko Schoof

Independent Research Group

Introduction

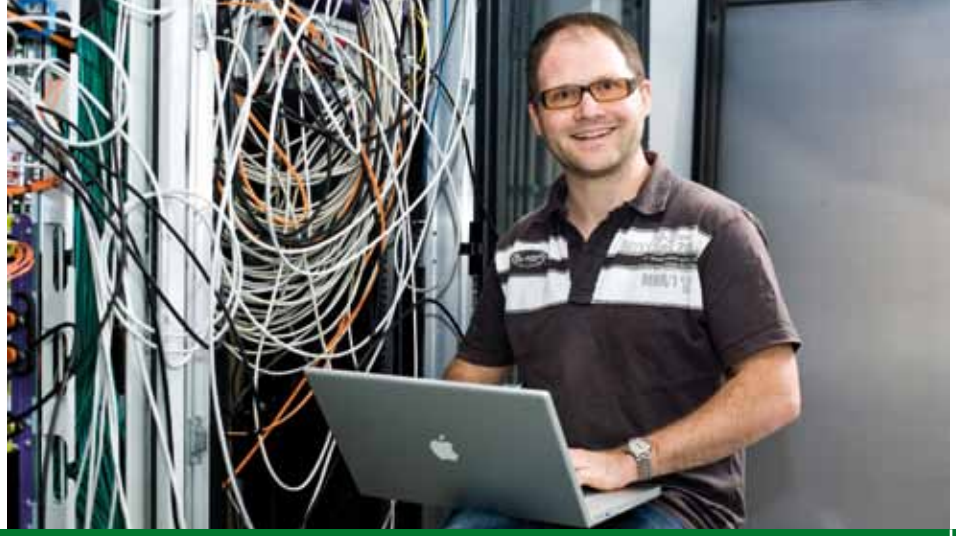
My aim is to contribute to a vision of data-driven, systematic, quantitative and predictive biology by computationally linking genomic data to specific life processes. Genomic approaches in recent years have generated large data sets for many aspects of plant biology, and the complete genome sequences now available for more and more plant species have become both a rich source for information and a framework upon which to organise data on plant biology. Many levels of information need to be integrated. Sophisticated methods for genome sequence analysis, extraction of patterns from gene regulatory networks, and computer-tractable representation of phenotypic data are developing rapidly. Data are being generated and computational analysis is progressing on many levels, but bringing individual bodies of information together so as to obtain a comprehensive overview remains a major challenge.

Current Projects

My group addresses three key problems commonly encountered in the comprehensive integration of genomic data: (1) how to meet the technological challenge of facilitating integration of high-throughput data, (2) how to distill biologically meaningful interpretations from large integrated datasets, and (3) how to exploit comparative genomics for evolutionary analysis.

(1) To facilitate integration of high-throughput data, we are pursuing a distributed, service-oriented approach to data availability and compatibility, on the basis of internet technologies such as web services or Semantic Web,

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



e.g. within the BioMoby framework. We provide aggregators that allow users to query multiple distributed databases and automatically include new BioMoby data sources. Many of our tools are implemented as reusable workflows.

(2) As an example of comprehensive correlative analysis, we have implemented a system for integrative prediction of gene function in a phylogenomic framework (see Fig. 1). We provide genome-wide functional annotation within the tomato and barrel medic genome projects. We have analysed sequence motifs, RNA structure and protein function within *Arabidopsis thaliana* 3'UTRs with the goal of identifying elements that control post-transcriptional regulation. We are working on tools for analysing next-generation sequencing data, e.g. for expression analysis (RNA-Seq), chromatin immunoprecipitation (ChIP-Seq) or genome annotation.

(3) We utilise evolutionary relationships both as an essential tool and as a source of important new knowledge. In the context of the tomato genome project, and as a partner in an international consortium (EU-SOL, funded by the EU Sixth Framework Programme), the group is involved in the annotation of new sequence information and its integration with data from functional genomics and phenotypic analyses. We have performed an analysis of genetic redundancy based on the relationship of knockout phenotypes and transcriptional coexpression of duplicated genes in *Arabidopsis thaliana* and *C. elegans*.

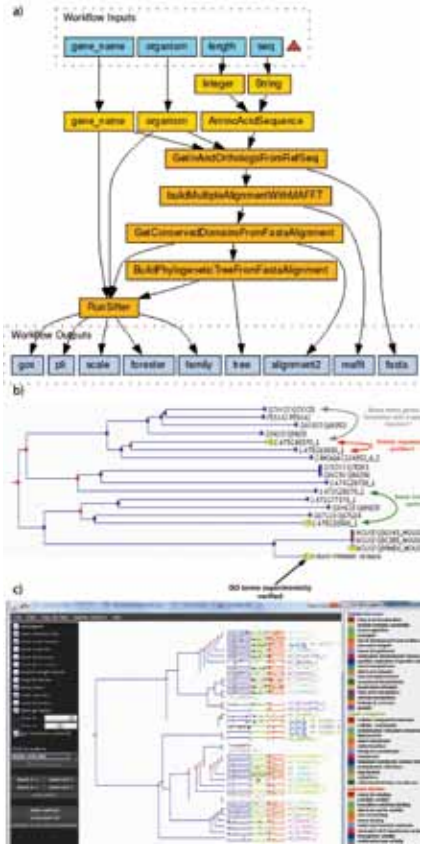


Figure 1. *Automatic function prediction in a phylogenomic context*
a) We have implemented a workflow which, for a given query sequence, selects putative orthologues and a complete set of paralogues from fully sequenced genomes, computes an alignment, selects conserved regions and computes a phylogenetic tree. b) Functional annotation is transferred within this tree from protein nodes with verified functions, with the probability of function transfer being weighted by branch length, duplication vs. speciation nodes, and a functional mutation rate computed from shared functional attributes, protein interaction and protein domain data. c) Our tree viewer displays functional attributes such as Gene Ontology terms for proteins. The data is loaded dynamically using BioMoby web services and visualised for rapid comparison.

Selected publications

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Quantitative Crop Genetics

Benjamin Stich

Independent Research Group

Figure 1. *Phenotypic variation in Brassica napus napobrassica*

Introduction

In natural environments, individuals belonging to the same species tend to display variation in most traits. This variation is partly attributable to genetic differences. Natural variation between different genotypes can be classified as qualitative or quantitative. Qualitative traits are characterised by distinct phenotypic classes and are often a result of single-gene differences. In contrast, quantitative traits display continuous variation in phenotypes, because multiple genes are involved and environmental factors have a relatively large effect on the expression of the trait. Most traits that are important for fitness and agricultural value in plants are quantitative in character. It is therefore important to improve our understanding of the genetics underlying such traits.

Statistical aspects of quantitative trait dissection

We are developing new biometrical methods for dissecting quantitative traits using linkage as well as association mapping approaches. Furthermore, a major objective is the elaboration of strategies to optimise the design of QTL (quantitative trait locus) mapping projects.

Associative expression and systems analysis of quantitative traits in rapeseed

Rapeseed (*Brassica napus*) was chosen as the focus for this case study for two

With the mining of *omics information to map molecular variation onto trait variation, quantitative genetics will move even more to the forefront.

Figure 2. *Maize plant showing symptoms of iron deficiency.*

reasons. 1) Rapeseed is the leading European oilseed crop for nutritional and renewable energy purposes. 2) The plant possesses a large and complex, highly duplicated, polyploid genome, which presents a particular challenge to the use of traditional genomics-based breeding techniques. On the other hand, *B. napus* is very closely related to *Arabidopsis thaliana* and, using comparative genomics approaches, it is possible to exploit the vast array of genome resources that are available for the model crucifer.

In rapeseed, seedling establishment plays an extremely important role in the optimisation of nutrient uptake and plant development prior to flowering (Figure 1). In this project, we therefore focus on seedling development traits as a case study for a highly complex, interactive system that is genetically very poorly understood, but is agronomically very important. The objectives of this project are to identify markers for seedling development by integrating transcriptome data with quantitative metabolite and phenotype data, using a systems-genetics approach.

Iron homeostasis in graminaceous monocots

Iron (Fe) is involved in many essential metabolic processes in plants, including hormone synthesis, DNA synthesis, chlorophyll biosynthesis, and various other fundamental redox reactions. Insufficient Fe uptake by plants leads to chlorosis (Figure 2) and significantly reduces yields. Worldwide, this problem occurs on about one-third of the arable land, illustrating both its importance and the potential benefits to be gained by improving the Fe supply of plants.

Fe is not only important for plant nutrition, it is also an essential micronutrient for humans. Worldwide, as many as 3 billion people are affected by Fe deficiency. This has manifold negative impacts on human health and well-being, decreasing work capacity and slowing the cognitive development of iron-deficient children. Africa, Latin America, and the developing countries in Asia are the regions in which the proportion of the population affected by Fe deficiency is particularly high. In these regions, cereals provide between 35 and 60 % of the daily calories. Therefore, an increase in Fe levels in the kernels of cereals has the potential to dramatically improve the supply of dietary Fe to the human population. Currently, we are applying a map-based cloning approach in maize to identify a gene involved in the mobilisation and uptake of Fe.

Selected publications

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

Peter Huijser

Figure 1. *Release of pollen grains (yellow) from floral anthers is a major consequence of the vegetative to reproductive phase change, and is highly dependent on several SBP-box genes acting at different stages of plant development.*

Introduction

SBP-box genes, which are the central interest of the group, code for transcription factors that are ubiquitous in green plants ranging from algae to trees. Plants generally display remarkable developmental plasticity. However, the degree of phenotypic variation in different environments is limited by developmental homeostasis. Recent studies by others and ourselves indicate that SBP-box genes play central roles in controlling certain homeostatic processes. SBP-box genes seem not only to be involved in maintaining, but also in overcoming, the limits imposed by homeostasis as required to allow developmental phase changes.

In the short term, the unique opportunities for reverse genetics offered by the model species *Arabidopsis thaliana* are being exploited to assign functions to SBP-box genes (known in Arabidopsis as *SPL* genes), and to integrate them in regulatory networks. In the long term, comparative genetics of SBP-box genes should contribute to a better understanding of the molecular, genetic and evolutionary mechanisms that drive plant diversification and distribution in relation to ecological factors.

SBP-box genes and the vegetative to reproductive phase change

In recent years, numerous small RNAs, known as microRNAs, have emerged as important regulatory components in different aspects of plant development. In Arabidopsis, eleven out of the seventeen *SPL* genes are targeted by the microRNA miR156. The interaction is also found in the moss Physcomitrella and thus reflects an evolutionarily ancient feature. In Arabidopsis, this interplay has turned out to be highly relevant for shoot maturation and flowering. In these developmental processes, several miR156-targeted *SPL* genes act redundantly in controlling the juvenile-to-adult phase transition, which renders plants sensitive to photoperiodic induction of flowering. Disruption of particular *SPL* genes prolongs the juvenile phase, whereas precocious expression of others causes early flowering. Ongoing research seeks to link these SBP-box genes to known flowering pathways.

SBP-box genes and fertility

Not all SBP-box genes in Arabidopsis are under the control of miR156. One of the exceptions, the SBP-box gene *SPL8*, has been found to function in the reproductive organs of the flower. In particular, *spl8* mutant flowers produce less pollen and set less seed. *SPL8* expression is associated with the differentiation of the sporogenic tissues in the anthers, possibly in response to the plant hormone gibberellin. How *SPL8* affects sporogenesis and why *spl8* mutants

Acting at the interface between homeostasis and phase change, SBP-box genes may lead to better understanding plant diversification.

are not completely sterile is currently being investigated as part of a project supported by the DFG.

SBP-box genes and copper homeostasis

Unlike *SPL8* and the miR156-controlled *SPL* genes, the remaining *SPL* genes are more deeply conserved within green plants, and are expressed constitutively and in a wider range of tissues. In the single-celled alga Chlamydomonas, a key factor in nutritional copper signalling has been identified as a member of this subfamily of SBP-box genes. More recently, a homologous Arabidopsis gene, *SPL7*, was also found to play a central role in copper homeostasis. In collaboration with other groups specialised in the study of metal homeostasis, *spl7* mutants are currently being exploited to study plant growth under limiting copper availability.

Future goals

Comparative studies will reveal whether the control of homeostatic responses and the regulation of growth transitions are common themes in SBP-box gene function in the plant kingdom, and whether they represent two sides of the same coin.

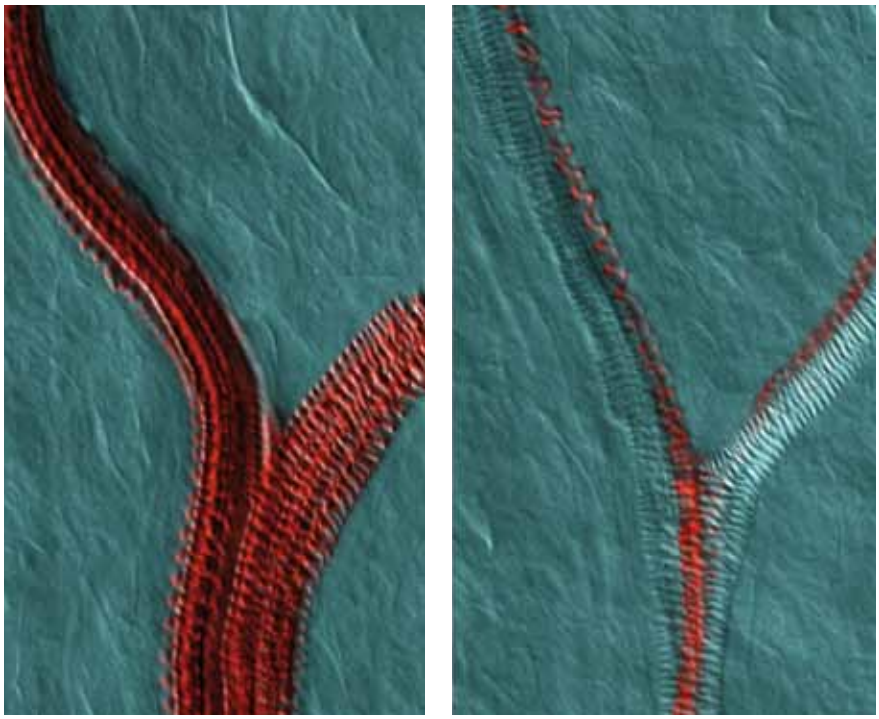

Figure 2. *Xylem vessels (ribbon-like structures) stain red due to the presence of lignin. Perturbation of copper homeostasis, as a result of an SBP-box gene mutation, results in aberrant lignification (right panel).*

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DNA recombination and its application in plants

Bernd Reiss

Service and Research Group

Introduction

The repair of damaged DNA is crucial for cell survival. An essential part of DNA damage repair is double-strand break (DSB) repair by genetic recombination. DSBs can be repaired either by non-homologous end-joining (NHEJ), an imprecise repair process that contributes to genome instability, or by homologous recombination (HR), a precise repair process that preserves genome stability. We are interested in how these pathways are used in two plants that differ greatly in their phylogenetic position and in their efficiency of gene targeting, a process that depends on HR. One is the flowering plant *Arabidopsis thaliana*, a rapidly evolving species, and the other is the moss *Physcomitrella patens*, a slowly evolving species in which high-efficiency gene targeting is possible. In addition, we are interested in the control of HR by chromatin structure.

The role of RAD51 in development and DNA damage repair in P. patens and A. thaliana

The RAD51 recombinase is at the centre of the HR pathway, but it also has another, vital function in vertebrates that was believed not to exist in lower organisms. In our analysis of *RAD51* function in *A. thaliana* we found that this gene is completely dispensable for vegetative development, a finding that was compatible with the above hypothesis. However, the same mutation in *P. patens* caused a significant vegetative phenotype, demonstrating that RAD51 has an important, though not essential, function *in P. patens*. Therefore, the importance of RAD51 for development is not a simple function of the complexity of an organism.

Repair of DSBs is essential for cell survival. Repair can occur by HR or NHEJ. To analyse which repair pathway operates in *A. thaliana* and *P. patens*, rad51 mutants were exposed to a DSB-inducing agent. While the mutation barely affected survival in *A. thaliana*, it caused marked hypersensitivity in *P. patens*. These findings imply that NHEJ is the predominant pathway for somatic DSB repair in higher plants,

We are interested in plant-specific aspects of DNA recombination.

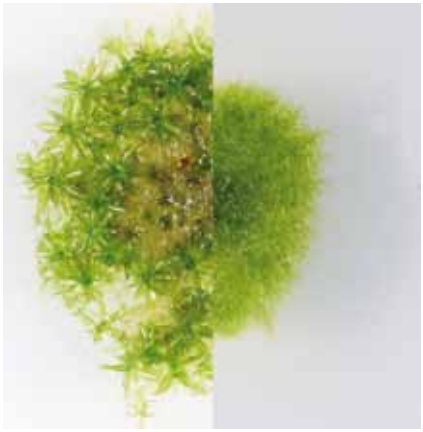

Figure 1. *Loss of RAD51 function causes a marked developmental phenotype in P. patens. Left: Wild type. Right: Loss-of-function rad51 mutant*

while HR acts in the same capacity in *P. patens*. This outcome correlates with the different gene targeting efficiencies observed in the two species. Nevertheless, these results are surprising, since the efficiency of gene targeting in animals is not correlated with the choice of HR as major repair pathway. This finding suggests that plants may differ fundamentally in their use of recombination pathways, while this does not seem to be the case in animals. The striking correlation between evolutionary rate and the relative importance of recombination pathways suggests that imprecise repair of DNA damage is a driving force for evolution in *A. thaliana*, while precise repair by HR in *P. patens* acts to inhibit the generation of biological diversity.

Control of homologous recombination by chromatin structure

The low gene targeting frequencies encountered in plants stand in sharp contrast to high efficiencies of extrachromosomal HR. This observation suggests the involvement of chromatin structure in the control of HR. To address this question we have analysed an *A. thaliana* mutant that is defective in the re-establishment of chromatin after replication, *fasciata1-4* (*fas1-4*). The mutation had a persistent effect on chromatin that was apparent in hetero- as well as euchromatin. Most importantly, we were able to show that all the chromatin remained in the open conformation that is permissive for gene activity. As shown by whole-plant intrachromosomal recombination assays, HR was stimulated almost 100-fold in *fas1-4*. Therefore chromatin is directly involved in the control of HR in *A. thaliana*, especially since other changes that might be relevant to HR were not detectable in the mutant. These findings differ from data obtained in analogous studies in yeast, suggesting that plants have evolved specific, chromatin-related HR control mechanisms.

Figure 2. *Stimulation of HR in the mutant fas1-4, which is defieicient in a chromatin assembly factor. The efficiency of intrachromosomal recombination in whole plants is represented by the number of blue spots in wild type (left) and fas1-4 (right).*

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Kirik, A., Pecinka, A., Wendeler, E. and Reiss, B.: The chromatin assembly factor subunit FASCIATA1 is involved in homologous recombination in plants. Plant Cell 18, 2431-2442 (2006).

Zobell, O., Coupland, G. and Reiss, B.: The family of CONSTANS-like genes in Physcomitrella patens. Plant Biol. 7, 266-275 (2005).

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Central Microscopy (CeMic) in plants

Elmon Schmelzer

Service and Research Group

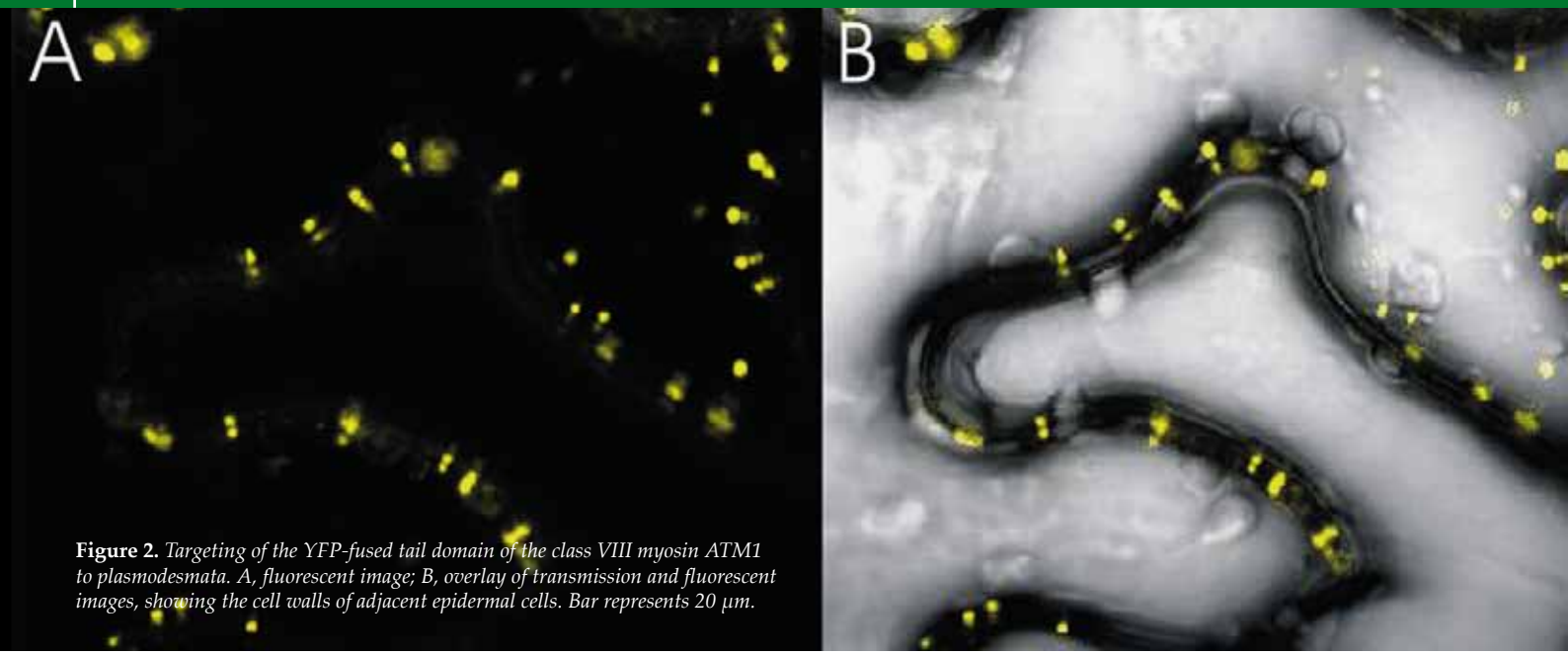

Figure 2. *Targeting of the YFP-fused tail domain of the class VIII myosin ATM1 to plasmodesmata. A, fluorescent image; B, overlay of transmission and fluorescent images, showing the cell walls of adjacent epidermal cells. Bar represents 20 µm.*

Introduction

New imaging and computer technologies, as well as new biochemical, biophysical and molecular-genetic methods, have led to striking advances in our ability to observe the dynamics and interactions of biological macromolecules in the context of the multiple processes and pathways that occur in cells, tissues and organs. Cell biology has begun to serve an integrating function for classical disciplines in biological sciences, such as anatomy, physiology, biochemistry and genetics. To meet the increasing demands for microscopical imaging, the Institute has made substantial investments in recent years in order to provide the appropriate instrumentation. Ongoing projects in our Institute utilise the entire spectrum of imaging technologies. These include up-to-date light microscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The service and research group Central Microscopy (CeMic) manages imaging technology and microscopical equipment at the MPIZ. CeMic supervises equipment use, and assists and trains researchers in instrument operation. CeMic also consults and instructs researchers concerning cytological issues and methodology, and offers practical courses in applications of microscopy. Furthermore, CeMic carries out collaborative research on cytological aspects of ongoing research projects and conducts its own investigations.

Service and research
Live-cell imaging

Fluorescent protein tagging in combination with *in vivo* imaging by confocal laser scanning microscopy has become a standard technology for many users in our Institute. Applications arise in a multitude of projects in all four departments, including elucidation of the control of flowering time and analysis of the control of flower development, the dissection of plant-microbe interactions and the investigation of dormancy mechanisms. A key aspect of confocal laser scanning microscopy is the *in vivo* monitoring of protein interactions by measurement of fluorescence resonance energy transfer (FRET) using acceptor photobleaching (APB) and fluorescence lifetime imaging (FLIM). Fluorescence correlation spectroscopy (FCS) is also employed for the determination of diffusion coefficients of membrane-associated proteins.

The Institute's facilities for live cell imaging are considerably augmented by an automated confocal microplate imaging reader. This robot combines high-speed and high-resolution confocal

Up-to-date microscopic imaging: watching cells at work and analysing the mysteries of molecular interplay.

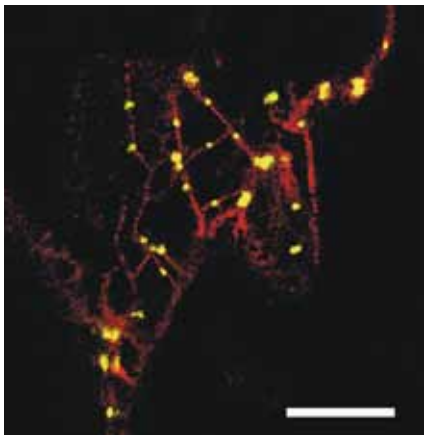

Figure 1. *Golgi stacks labelled with the YFP-fused tail region of a class XI myosin (AT1g17580) localised along actin filaments visualised with RFP-talin. Bar represents 20µm.*

imaging with high-throughput, high-content image processing and analysis. By permitting the comprehensive and reproducible characterisation and profiling of tissues or cells on the basis of highly quantitative data, this instrument provides a novel methodology for assessing physiological states.

Electron microscopy

An up-to-date Scanning Electron Microscope (SEM) equipped with advanced electron optics (field emission gun) enables high-resolution/magnification imaging at low voltage, which is especially suitable for beam-sensitive biological probes. Rapid handling of probes is made possible by a linked cryopreparation and transfer system. Even the immediate examination of uncoated specimens in a gaseous environment is possible in the so-called variable pressure mode. The instrument is widely used for detailed 3-dimensional analysis of all kinds of morphological phenotypes.

Transmission electron microcopy (TEM) remains the only means of obtaining high-resolution information on scales ranging from whole cells down to individual molecules. The resolving power of TEM represents the ultimate tool for analysing the fine structure of cellular compartments and macromolecular complexes. Of particular interest in our Institute are investigations in relation to plant-microbe interactions and organ development. The application of high-pressure freezing and freeze-substitution technology ensures the preservation of biological samples in as close to their native states as possible.

Collaborative and own research projects

The group is involved in numerous cooperations both inside and outside the Institute. Together with groups in all departments, CeMic performs live-cell imaging and electron microscopic investigations on a wide variety of topics. CeMic currently has external collaborations with groups from the Universities of Cologne and Aachen.

With respect to our own research, we are exploring the intracellular localisation, cargo-binding specificity and *in vivo* function of the two classes of higher-plant myosins, class VIII and class XI. Our data suggest that these two classes perform different functions. The members of class XI may be essential for the transportation of organelles such as Golgi stacks, peroxisomes and mitochondria, whereas those of class VIII are involved in endocytosis and plasmodesmatal targeting.

Selected publications

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Mass Spectrometry (MS)

Jürgen Schmidt and Thomas Colby

Service and Research Group

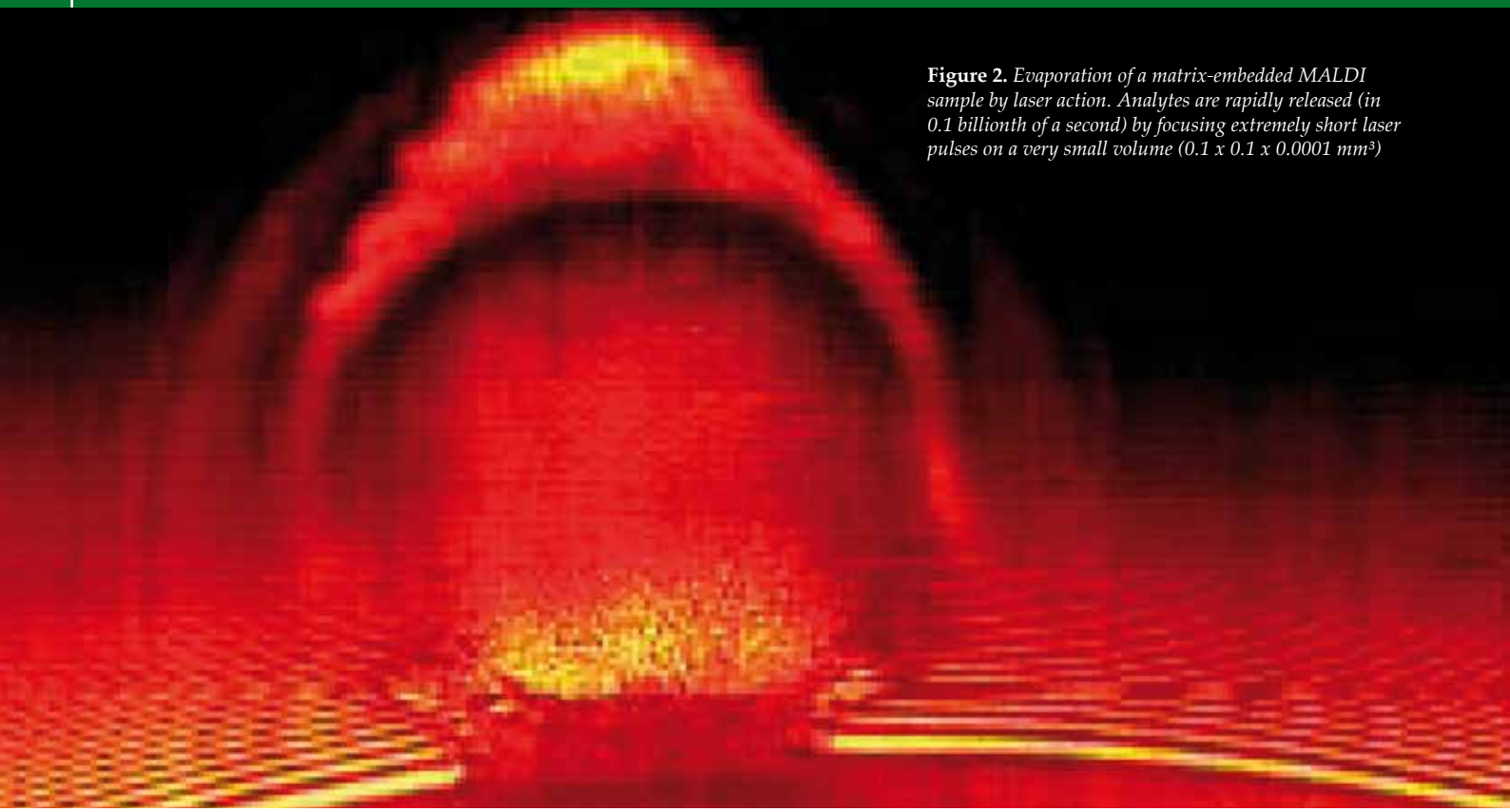


Figure 2. *Evaporation of a matrix-embedded MALDI sample by laser action. Analytes are rapidly released (in 0.1 billionth of a second) by focusing extremely short laser pulses on a very small volume (0.1 x 0.1 x 0.0001 mm³)*

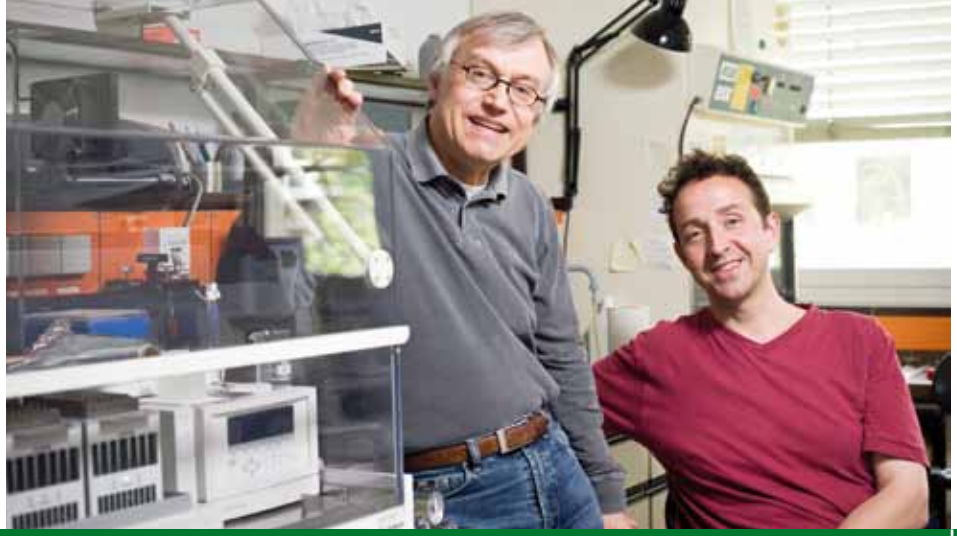
Staff and facilities

The four departments at the MPIZ carry out projects designed to obtain a more detailed understanding of regulatory pathways and their functions in plant cells. These projects have been facilitated by the availability of complete genomic sequences, and utilise a range of technologies. One of the most important post-genomic methodologies is proteomics, which can be defined as the large-scale analysis of proteins, protein-protein interactions and post-translational modifications. For this purpose a MALDI-TOF, a MALDI-TOF/TOF MS as well as two LC-MS/MS systems (ion trap and Q-TOF MS/MS) are run by our group, which consists of two scientists and two technical assistants.

The MS technology established at the MPIZ has developed into a widely used technical platform in plant research, and is not only exploited for the identification of proteins and peptides, but also in metabolomic screening programs. Our facilities are centralised in a laboratory with adequate space, which enables us to welcome guest scientists and to offer training courses.

We constantly strive to improve our analytical capabilities and MS infrastructure. In addition to the MS systems mentioned above, we have installed two robots to avoid bottlenecks in large-scale sample preparation for high-throughput proteomics. We use a spot-picking robot to image gels, detect spots, isolate them and distribute them into microwell plates. These plates are then moved to the digest-and-sample robot, which is used for automated in-gel digestion and target-plate preparation prior to analysis by MALDI- and/or LC-MS/MS. With the current equipment the workflow from gel separation to mass spectrometric identification and characterisation is fully automated, enhancing reproducibility and minimising contamination. As an alternative to gel-based sample

The MS technology established at the MPIZ has developed into a widely used proteomics platform in plant research.



acquisition, we also apply MudPit as a gel-free technique in combination with LC-MS/MS for more sensitive quantitation.

All data are stored in a central data repository, and essential information about all spots or gel-free samples analysed by MALDI or LC-MS/MS is accessible, e.g. general spot information (coordinates, intensity, MW/pi), position of samples in digest plates and on MALDI targets, MS and MS/MS spectra, protein database search results obtained from the search algorithms and information on the identified proteins.

Projects

The group also carries out its own projects devoted to the identification of stress-related phosphoproteins, including the investigation of changes triggered by dehydration in the phosphoproteome of the resurrection plant *Craterostigma .plantagineum*. In this context, we employ gel-free approaches, including protocols for the enrichment of phosphoproteins and -peptides combined with subsequent nanoLC coupled to an ion trap for the detection of phosphorylation sites by neutral loss analysis. To meet the constantly increasing demand for proteomics analyses, the MS group is also engaged in various collaborative projects. Some of these are supported by joint grants. The corresponding projects are collaborations based on the MS facilities run by our group, and focus not only on the functional analysis of plant proteins that play a role in developmental processes, but also on various aspects of plant-pathogen interactions.
http://www.mpiz-koeln.mpg.de/english/services/Maldi/index.html

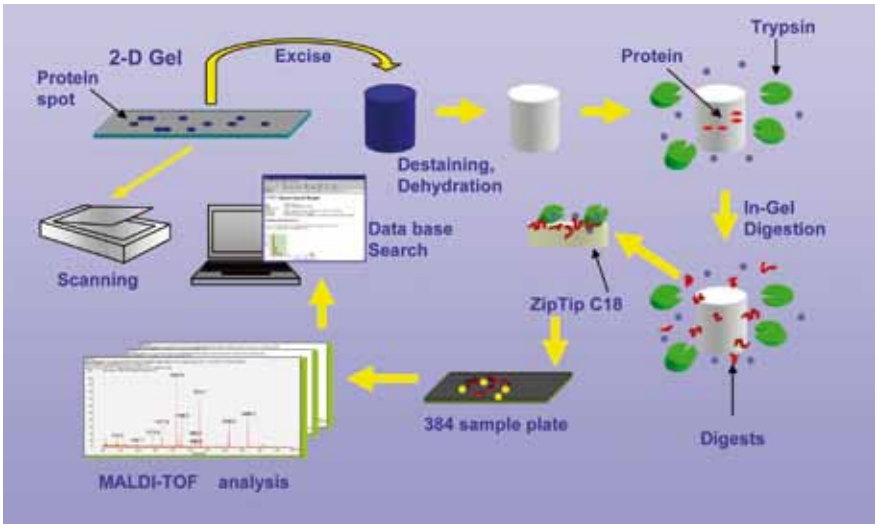


Figure 1. *Flow scheme for the identification of proteins by generating peptide mass fingerprints (PMFs) for sequence database searches.*

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Service and Facilities

SUSAN: Information technology and bioinformatics support

SUSAN

Modern plant biology is becoming more and more dependent on information technology, not only for communication via e-mail and internet, but also for storing and analysing large volumes of heterogeneous electronic data. Core computing and bioinformatics support have thus become more and more important. To address present and future needs, the Institute has restructured the bioinformatics support and computer groups, merging them and exploiting synergies with the Plant Computational Biology research group. The aim is to form a unit driven by scientist needs and dedicated to the efficient use of data and computational analysis.

The bioinformatics support unit is now staffed by two full-time scientists and two students. It can handle larger projects, e.g. the assembly of fungal genomes from 454 sequencing data or the analysis of microarray and ChIP-Chip/ChIP-Seq data. Tool development has become an important task, not only to provide an in-house BLAST server, but also user-friendly, web-based interfaces to new methods of analysis developed e.g. for ChIP data.

The information technology branch is headed by Tobias Brisach. It maintains core infrastructures and provides user support. One important task is the consolidation of essential services for greater reliability, security and reduced maintenance. This will free human resources required to support scientific data management and analysis tasks. On a new hardware platform based around enterprise class Sun Microsystems servers, mail, groupware, file and backup services are being restructured. An emphasis lies on easier data sharing and expandability to meet the demands of high-throughput data generation. A reorganisation of the network seeks to increase security and performance, as well as making the resources of the bioinformatics group, e.g. a 30 processor computing cluster, available to any scientist in the Institute. On this foundation, scientific services can be set up, including automated archiving and processing of primary data generated at the Institute, e.g. by high-throughput sequencing, microarray experiments or automated microscopy.

Library

The Library of the Max Planck Institute for Plant Breeding provides literature, electronic media and information services to its scientific staff and guests. The collection is focused on the fields of research covered by the departments and the research groups at the Institute.

At present the library's holdings comprise approximately 22,500 print-journal volumes and 5,000 monographs.

The MPIZ Library, together with the Max-Planck-Digital Library of the Max-Planck-Society, provides a wide selection of electronic journals and other scientific information resources. The Institute devotes 0.9 % of its budget to this service. A committee of scientists provides support to the library on questions related to research needs.

About 24,000 journals are now available electronically. In addition to this, the library can obtain any literature that is not available on site, either in electronic form or as hard copy. Users can fill out a form available via the intranet, which forwards the data to a database. During the last two years some 1,000 articles and books have been ordered with this tool.

Furthermore the library collects all institute publications for institutional self-archiving of research output on the eDoc server of the Max-Planck-Society, and provides all publications as PDFs on the web site (with a one-year delay for copyright reasons).

Britta Hoffmann

Technical equipment

The number of PC workstations has increased to ten and there are twelve additional workplaces for private laptops. There are also two separate rooms with PCs available for temporary staff.

Service and Facilities

ADIS

Bruno Hüttel

At the MPIZ, a core facility for DNA- and RNA-related technology has been serving all departments by providing highly requested and automated modern technologies since 1995. The unit was designated ADIS for "Automated DNA Isolation and Sequencing". The increasing automation of modern molecular biological techniques requires complex and expensive equipment. This is especially true for DNA and RNA isolation, DNA sequencing, expression profiling, clone and library handling, and the production of DNA and clone arrays in combination with the respective bioinformatics tools. The large amounts of data resulting from these „omics" activities (genomics, transcriptomics) are handled by a Laboratory Information Management System (LIMS). This database also takes care of the approximately 320 oligonucleotides ordered at MPIZ as a whole on average per week. In 2008, ADIS extended its service to include gene expression profiling and single-nucleotide polymorphism (SNP) analysis with different Affymetrix gene-chip platforms (expression and SNP chips, tiling arrays). Expression profiling and DNA sequence analyses are being extended further in 2009 to incorporate novel high-throughput sequencing platforms such as the GS FLX from 454/Roche.

Greenhouse management

In the greenhouses we can at present cultivate plants for scientific experiments on an area of 1,300 m^2 (net), including 400 m^2 with automatic cooling. There is a growing demand for growth chambers and additional cooling space (300 m^2), primarily for Arabidopsis and barley. Five Saran houses have been demolished and replaced by new transparent greenhouses (300 m^2) with drip irrigation and automatic climate conditioning. One older greenhouse was modernised for the cultivation of Arabidopsis (60 m^2) and tomatoes (250 m^2). For projects with Arabis, tomatoes and barley we have rented greenhouses (net space 500 m^2) at the Horticultural Experimental Station of the NRW Chamber of Agriculture in Köln-Auweiler.

The greenhouse staff comprises 9 gardeners, 2 horticultural apprentices and 2 helpers. The greenhouse staff handles 300-400 culture orders/ month, in close cooperation with the scientists. The main tasks are the preparation of soil, sowing, transplantation, nutrient supply, watering, plant protection measures, seed harvesting, plant propagation and control of growing conditions. Around 150 m^3 of standardised soil substratum is used per year. To minimise energy costs and reduce autoclave loads, transgenic Arabidopsis plants with seeds and soil material are inactivated at the end of the experiments by a thermal procedure using steam at 200 °C. The cleaning of planting trays and the sterilisation procedure take place in a separate greenhouse department.

In addition, a "green group" is responsible for the following: preparing and conducting field trials practically, cultivating the green area around the Institute (including the Demonstration Garden), inactivating plant and soil material from greenhouses, disposing of waste paper, maintenance of agricultural implements and winter maintenance. In 2007 we conducted field trials with transgenic potatoes for the University of Cologne, and provided support for scientists and students of Brown University (Providence, Rhode Island, USA) on Arabidopsis field trials.

Wolfgang Schuchert

IMPRS

The International Max Planck Research School

Olof Persson

The International Max Planck Research School on „The molecular basis of plant development and environmental interactions“ started in September 2002, and is organised by the Max Planck Institute for Plant Breeding Research together with the University of Cologne. The programme offers a three-year curriculum with courses on plant science and methodology, as well as on soft skills like scientific writing and presentation. Yearly retreats at which IMPRS students present their work, and close supervision by thesis committees seek to ensure a high standard of Ph.D. training within the programme.

The IMPRS is currently collaborating with science centres in Poznań (Poland), Wageningen (The Netherlands), Gif-sur-Yvette (France), Ghent (Belgium) and Norwich (UK) to improve training and opportunities for its Ph.D. students. By March 2009, 35 Ph.D. students had graduated from the IMPRS programme and 36 students are still pursuing their Ph.D. studies within the IMPRS. Another 12 students are expected to join the IMPRS in 2009.

From the Demonstration Garden to the WissenschaftsScheune

From the Demonstration Garden to the WissenschaftsScheune (Heinz Saedler)

To intensify our dialogue with the public we encourage interested groups to visit the Institute. We inform them about current research projects at the Institute and discuss questions of modern plant breeding research, including its applications to agriculture and the GMO debate. We also offer guided tours of the Institute, the greenhouses and the demonstration garden. Our Demonstration Garden is a very helpful tool for conveying information on various aspects of biology and agriculture, from basic research to practical use. It is an exhibition of about 100 agricultural and horticultural plants, which are grown in small plots. The main topics covered are domestication and evolution, biodiversity, plant development and environment, the Mendelian Laws as the basis for classical breeding, hybrid vigour in comparison to inbred lines, plant pests and diseases (susceptible and resistant varieties), renewable crops (fibre-, energy-, starch- and oil-plants) and crops that have fallen into disuse.

In the past few years we have extended the demonstration garden to include otherwise forgotten fruit varieties bred by MPIZ staff members in the 1950s and a collection of genetic resources of fruit trees grown in the "Kölner Bucht" region over the past several centuries.

Several botanical gardens have drawn on our experience to establish similar exhibitions of crop plants. The MPIMP in Golm has used it as a model to develop the successful project "Komm ins Beet". The Botany Institute of the University of Cologne has integrated excursions to our Demonstration Garden into their training courses for students. We also participate in annual events such as "Girls Day" and "Kölner Kinder Uni" (Children's' University), to bring young people into contact with modern plant science and become acquainted with education and training programmes.

In 2007 and 2008 approximately 1,500 people visited the MPIZ and took part in guided tours. Most of these were schoolchildren and students, but various interest groups and representatives from business and politics also paid visits. In May 2008 the Parliamentary Secretary of the Federal Ministry for Consumer Protection, Nutrition and Agriculture (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz) together with German Members of Parliament and local politicians were guests at the Institute to discuss general questions on future developments in plant sciences.

At present, we are developing the "WissenschaftsScheune" (Science Barn). This is an extensive project, which aims to give the public an opportunity to experience aspects of science from basic research to practical agriculture. The preparatory work started in 2007. An agricultural park, the Landwirtschaftspark Belvedere, will be established around the Institute. This is the result of a competition in the context of the Strukturförderprogramm Regionale 2010 initiated by Cologne City Council and the State of NRW, together with citizen organisations and the MPIZ.

The Institute and its adjoining farm are the centrepiece of this park, into which neighbouring fields and demonstration gardens will be integrated. A farm building of 500 m^2 is being converted into the "WissenschaftsScheune", with exhibitions and interactive stations documenting several fields of plant science. This is our approach to combining indoor and outdoor activities. Feedback from our visitors will be used to improve and enlarge hands-on stations. Well educated personnel will offer guided tours for different target groups. We intend not only to address upper school classes and adults but also to fascinate children and primary school classes.

A flyer and a special homepage (www.wissenschaftsscheune.de) with basic information for a visit already exist. In accordance with its statutes, a circle of friends of the MPIZ has been set up to promote and support these activities.

Press and Public Relations

Wolfgang Schuchert and Claudia Vojta

Public Relations Activities at the MPIZ

Public relations work has a long tradition at the MPIZ. The Public Relations Office (PRO) was established in 1990, when the first field trials with transgenic plants in Germany took place at the Institute. Its main focus is to inform not only the scientific community, but also the public at large, about the research activities and current developments in and related to plant sciences in a broad societal context. The other important task is to support internal communication among employees. Our approach to communication is based on an active and open dialogue, including several measures aimed at the different target groups.

Internal communication

Special events such as the weekly TATA-bar get-togethers, organised by PhD students, the summer party, the annual MPIZ concert in December or sporting events such as the MPIZ Institute Run or the Soccer Cup contribute to a pleasant working atmosphere. Foreign scientists and students starting their work at the institute are supported administratively and receive assistance in dealing with the relevant authorities.

Internet presentation and media activities

The MPIZ web pages (www.mpiz-koeln.mpg.de) are updated regularly. Over the past several years, the content management system (CMS) of the MPG has been implemented. Every user can find detailed reports from the scientific departments and research groups, the service units, the International Max Planck Research School (IMPRS) and the MPIZ Alumni Initiative. Press releases are listed chronologically to document scientific highlights, and journalists may use these for their reports.

The Federal Foreign Office (Auswärtiges Amt) and the German Academic Exchange Service (Deutscher Akademischer Austauschdienst, DAAD) for instance recently published a portrait of the MPIZ, and an interview with a postdoc from Kenya (who worked at the Institute on the structure and analysis of the potato genome), in which she discussed research conditions at the MPIZ and problems of food production in Africa.

Cooperation projects

The PRO works closely with the PR Department of the Max-Planck Society (MPG) and also cooperates with various committees and organisations. The Institute is represented in interest groups such as the "Informationskreis Grüne Gentechnik des Bundesverbandes Deutscher Pflanzenzüchter" (Advisory Group on Green Gene Technology in the Federal Association of German Plant Breeders), the "Wissenschaftlerkreis Grüne Gentechnik" (Science Forum on Green Gene Technology), the "Kölner Transferrunde der Industrie- und Handelskammer" (Cologne Exchange Forum of the Chambers of Industry and Commerce) and the "Kölner Wissenschaftsrunde" (Cologne Science Circle). It is also involved in the annual conference on teacher training organised by the "Fonds der Chemischen Industrie NRW". This enables the institute to establish contact with schoolteachers. As a consequence, many school classes visit the Institute. Special lab-based training and experimental courses in biotechnology for secondary-school classes are given by KölnPUB e.V., a joint initiative of the University of Cologne and the MPIZ.

Scientists from the MPIZ regularly give public lectures and take part in discussions with the general public at events like "Wissenschaft im Rathaus" (Science at the Town Hall) organised by the "Kölner Wissenschaftsrunde" or "Sustainable Development in Agriculture" initiated by the European Academy in Otzenhausen.

In cooperation with the MPG'S other "green" institutes in Golm and Jena, the Leibnitz Institut für Pflanzengenetik und Kulturpflanzenforschung and the Hochschule für Wirtschaft und Umwelt in Nürtingen, an educational package comprising six booklets and a CD-ROM on modern plant science and green biotechnology is in preparation. This project, which is being supervised

www.mpiz-koeln.mpg.de

by the MPIMP in Golm is primarily targeted to secondary school classes, and represents a further development and update of our booklet called "Grüne Gentechnik".

The "Cologne Science Center (CSC) Odysseum", an ambitious project of the Sparkasse KölnBonn (SK-Stiftung CSC) focusing on modern technologies and their long-term implications, is

expected to open in April 2009. The Scientific Advisory Board of the CSC includes members of the Institute, who will act as consultants, and will support science journalists and media agencies in producing articles and exhibitions dealing with green biotechnology.

Information service on green gene technology

We also prepare application procedures (cultivation of transgenic plants, field trials) and statements concerning the amendment of the German gene technology law.
This includes information on the commercialisation of transgenic plants, risk-benefit debate and labelling. We are also actively involved in training courses for project leaders and biosafety officers arranged by the University of Cologne.

Communication with the Public

We make every effort to encourage open dialogue between our Institute and the public. It is of great importance for the future that, not only the scientific community, but everyone interested in modern science should have the opportunity to obtain first-hand information on the research that is pursued in the Institute.

Since research in, and application of, plant breeding and plant molecular biology have been increasingly in the public eye, research institutes like the MPIZ have a responsibility to be a source of objective and well-founded information. Therefore, we need to make appropriate material available to our various target groups. Pupils and biology students, for example, often ask for more information on special research projects. This demand will be satisfied by additional easy-to-read publications, such as articles on our web site, brochures and leaflets.

Among the other channels used by the PRO to communicate science and/or improve internal communication are the Alumni Network and the magazine MPIZAlumniNews, newspaper articles and an interesting web site. In particular, our modern web site provides the public with information about the structure of the Institute and the main research interests of the various research groups and service units.

We have an extensive visitor's programme at the MPIZ. There is a continuous stream of visitors to the Institute. We welcome members of the public or professionals from all sectors of the community to visit the Institute, learn about the spectrum of research conducted there, tour the greenhouses and admire and enjoy the major attraction for visitors - the Institute's Demonstration Garden. School classes are especially frequent visitors.

Our most recent major innovation is the WissenschaftsScheune (WsS) - which can be described as a small science center. Its aim is to explicate the research carried out at the MPIZ and spark interest in plant science. The WissenschaftsScheune combines indoor and outdoor activities, enabling the public at large to experience all facets of plant science from basic research to practical agriculture. It is the centrepiece of the Landwirtschaftspark Belvedere, which is part of the regional development plan outlined in the "Strukturförderprogramm Regionale 2010".

Max Planck Institute for Plant Breeding Research

How to get to the MPIZ

By car

Motorway A1 (north): Take the Bocklemünd exit (# 102), turn left at crossroads, drive along Venloer Straße, direction Köln-Zentrum. After approx. 2 km turn right at crossroads, drive along „Militärring". After approx. 1 km turn right and follow the signs to the Max Planck Institute.

Motorway A1 (south): Take the Lövenich exit (# 103), turn right at crossroads, drive along Aachener Straße, direction 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 train station (Köln Hauptbahnhof)

- Underground #5 (direction Ossendorf) to stop Subbelrather Straße/Gürtel, then transfer to
- Bus #141 direction Vogelsang or bus # 143 direction Bocklemünd (the bus stop is on the other side of the intersection on Subbelrather Straße) to stop Goldammerweg.
- Walk (for approx. 15 min.) straight on Vogelsanger Straße and Carl-von-Linné-Weg (cross railway land motorway, pass farm on the right-hand side, cross Belvederestr.).

By plane

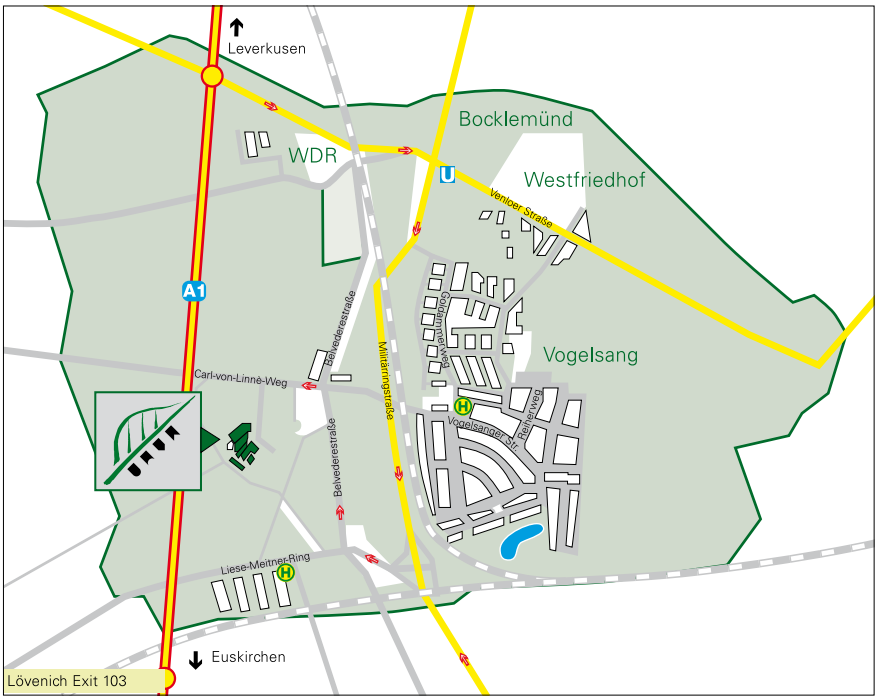
Cologne/Bonn Airport

- S-Bahn „S13" or train (Regionalbahn „R8") to Cologne main train station „Köln Hauptbahnhof"

Düsseldorf Airport

S-Bahn „S1" or „S7" (or a „Regionalbahn") to Düsseldorf main train station "Düsseldorf Hauptbahnhof", then take train IC, ICE, RE, RB to Cologne main train station.

Then continue as described under arrival at Cologne main train station.



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Notes