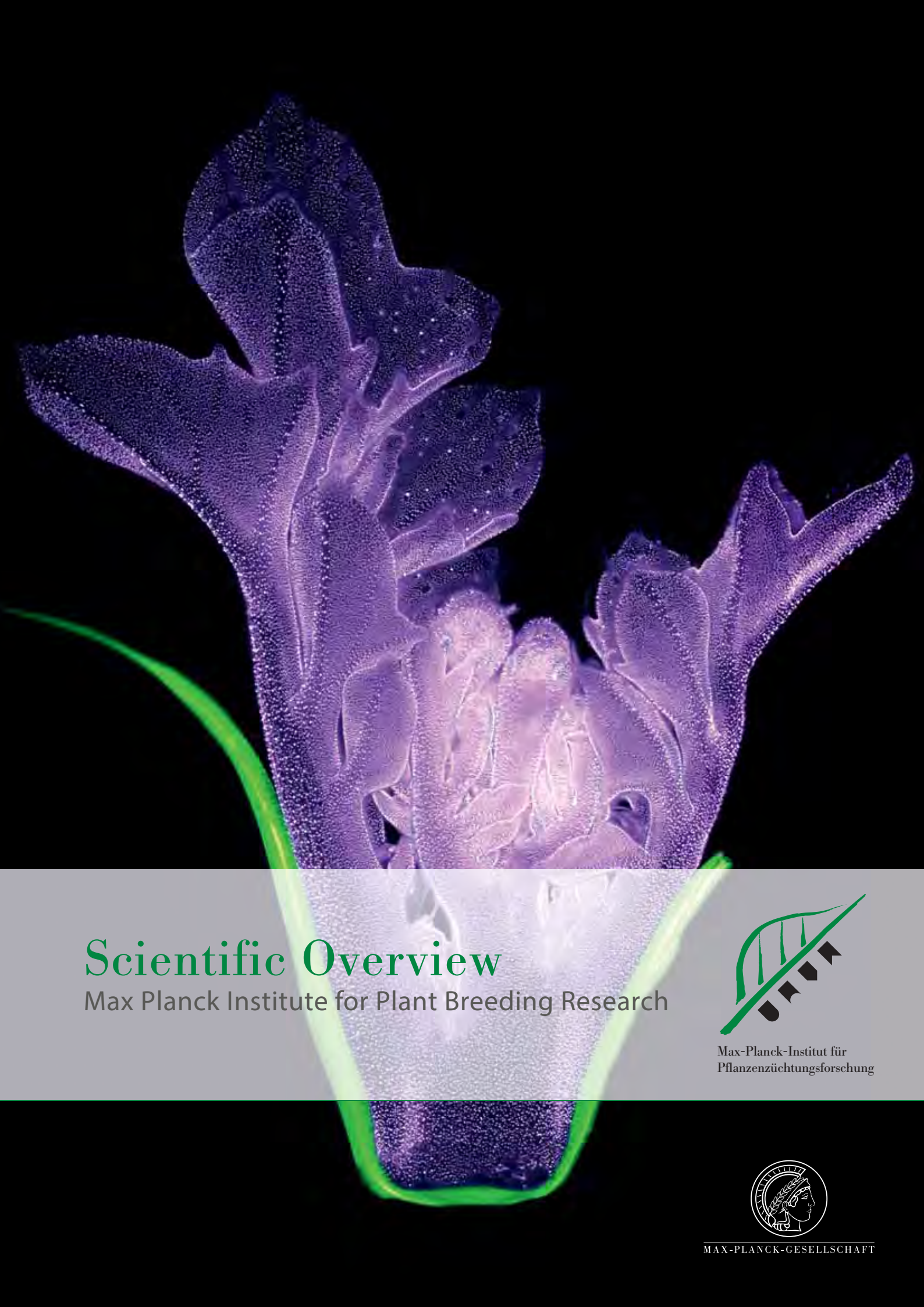
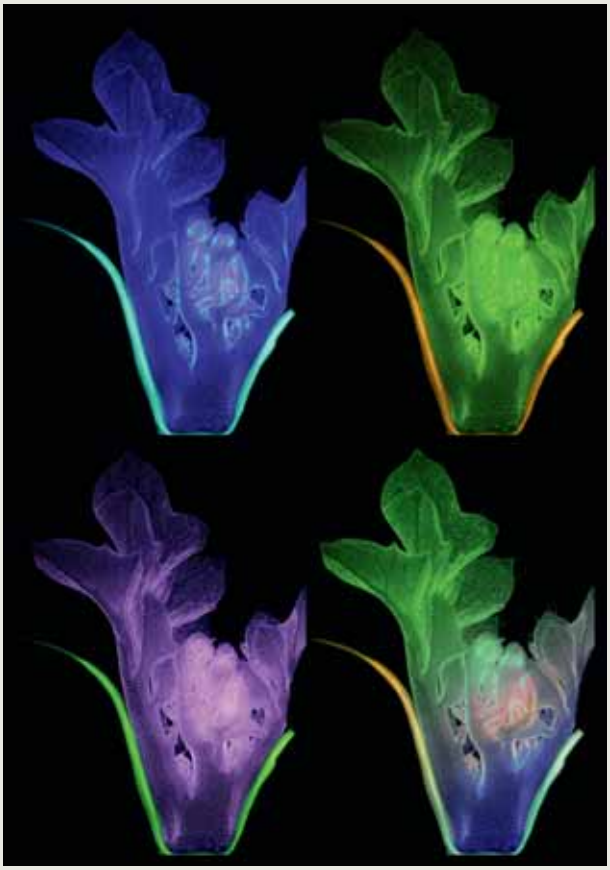


Scientific Overview

Max Planck Institute for Plant Breeding Research

Max-Planck-Institut für
Pflanzenzüchtungsforschung

MAX-PLANCK-GESELLSCHAFT



Cover picture (Peter Huijser):

False-colour fluorescence microscopy image of young compound leaves developing at the shoot apex of hairy bittercress *(Cardamine hirsuta)*. The nuclei of all epidermal cells light up as tiny dots due to the accumulation of an expressed transgenic fluorescent protein. This image illustrates the research in the new Department of Comparative Development and Genetics aimed at understanding how biological forms develop and diversify.

Max-Planck-Institut für
Pflanzenzüchtungsforschung

Scientific Overview 2014

Max Planck Institute for Plant Breeding Research

MAX-PLANCK-GESELLSCHAFT

The entire world we apprehend through our senses is no more than a tiny fragment in the vastness of Nature.

Max Planck (1858 - 1947)
from his book "The Universe in the Light of Modern Physics" (1931)
Page 8 (Translated by W.H. Johnston)

Table of Contents

Welcome to our Institute

We have compiled this overview to provide an introduction to the research work of our Max Planck Institute. It summarizes the Institute's scientific aims and organization, and outlines 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 you will find the text both absorbing and informative. We foster a curiosity-driven approach to plant sciences that stimulates collaborations and yet provides the freedom to shape individual careers. The report describes the work of 29 research groups. More than 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 independent research groups and service groups that focus on topics not covered by any of the four departments. We place great emphasis on the training and mentoring of PhD students. With over 60 members, this group represents a sizeable fraction of the 370 staff on the campus. Many of them participate in structured programmes such as the International Max Planck Research School (IMPRS) or the Centre of Excellence on Plant Sciences (CEPLAS), a regional research and training initiative undertaken jointly with the universities of Düsseldorf and Cologne. Our Student Coordinator follows the progress of all students closely, offering supplementary courses and advice, and organizing retreats and annual student meetings. In addition, for more than 110 post-doctoral scientists, the Institute's cutting-edge infrastructure and intellectual environment provide advanced training and serve as a springboard to a research career in academia or in the plant science industry.

Members of our Institute play important roles in plant science at both national and international levels, and make our campus a premier site for basic research on plants in Europe. We have particularly close links with the regional universities of Cologne and Düsseldorf, which participate in our IMPRS and provide the academic framework for our PhD students. In addition, we collaborate with them in the context of four Collaborative Research Centers (Sonderforschungsbereiche) funded by the German Research Society (DFG) and the CEPLAS. We are especially indebted to the Max Planck Society for the allocation of 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.

Paul Schulze-Lefert
Managing Director

Historical background to the Institute

The Institute was originally founded in 1928 as part of the Kaiser-Wilhelm-Gesellschaft, and was then located in Müncheberg (Brandenburg). Its 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 to Voldagsen (Niedersachsen), and was relocated to new buildings on the present site in Cologne in 1955. The modern era began in 1978 with the appointment of Jeff Schell as Director, 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 arrival of Klaus Hahlbrock in 1983 brought further expertise in the area of plant biochemistry, and the advent 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, which housed independent research groups over a period of 10 years.
In light of the approaching retirements of Klaus Hahlbrock and Jeff Schell, recruitment of a new generation of Directors began in 2000. 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. Miltos Tsiantis began to build up a Department of Comparative Development and Genetics in 2013, following the retirement of Heinz Saedler. The new science departments brought a strong focus on utilizing model species to understand the regulatory principles and molecular mechanisms underlying selected plant 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 leadership also necessitated modernization of the infrastructure. So far, this has involved complete refurbishment of the building that houses the Plant Developmental Biology Laboratory (2004) and the construction of a new guesthouse and library (2005), as well as new buildings for Stores, the Outreach Department and Workshops, along with a new entrance gate (completed in spring 2009). The new laboratory building for the Koornneef Department was completed in spring 2012, as was the communal building that links all four science departments and houses meeting rooms, offices and the bioinformatics groups. An extensive new space to accommodate the Genome-centre's servers was opened in spring 2012, while a complete overhaul of the former Saedler department building (now the Department of Comparative Development and Genetics) was begun in mid-2013. Modernization of the glasshouses has also been approved, started in spring 2013 and will be completed by the end of 2014.

Directors at the Institute in Cologne (since 1955)

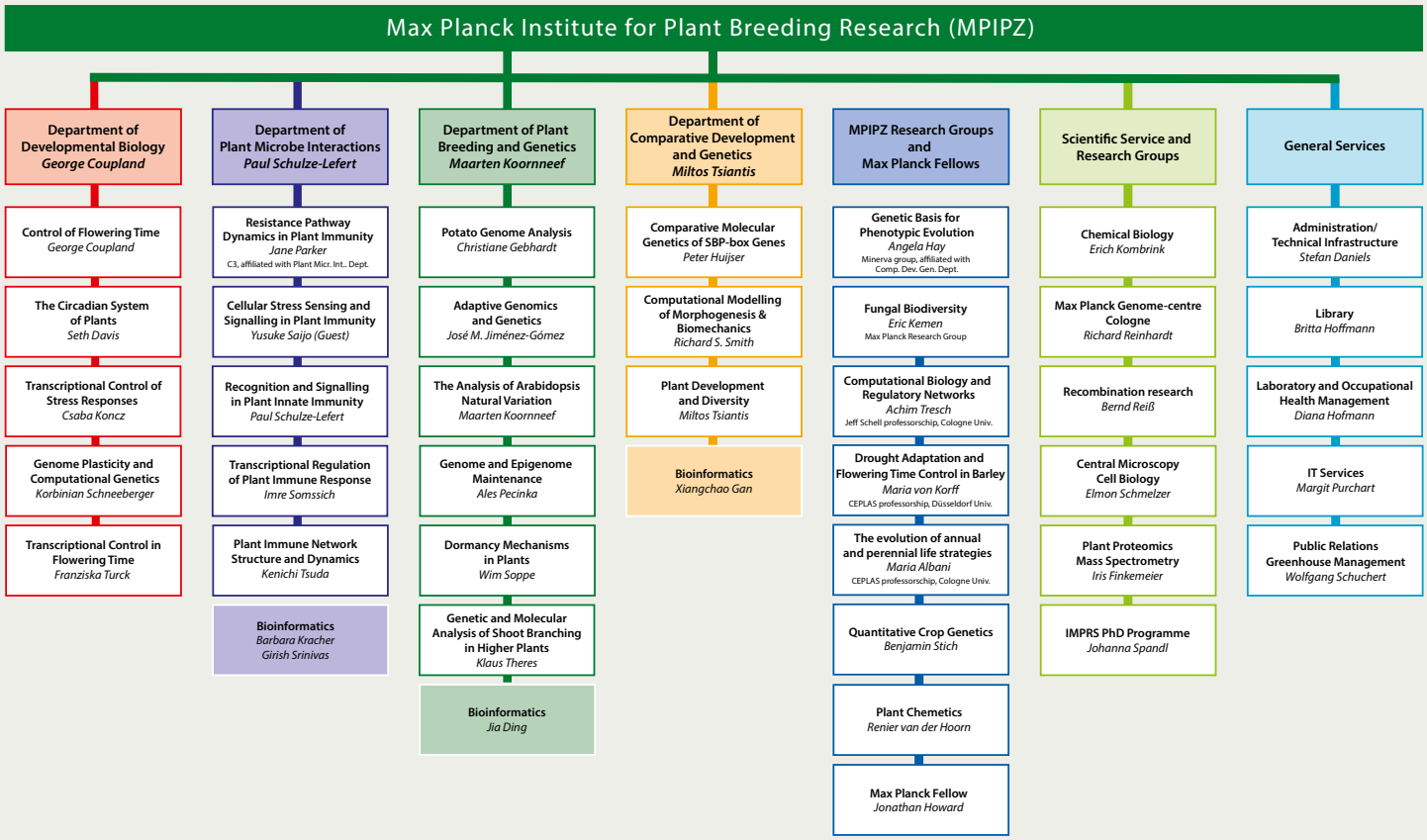
1936 – 1961	**Wilhelm Rudorf**
1961 – 1979	**Josef Straub**
1967 – 1978	**Wilhelm Menke**
1978 – 2000	**Jeff Schell**
1980 – 2009	**Heinz Saedler**
1983 – 2002	**Klaus Hahlbrock**
1985 – 2004	**Francesco Salamini**
2000 – present	**Paul Schulze-Lefert**
2001 – present	**George Coupland**
2004 – present	**Maarten Koornneef**
2012 – present	**Miltos Tsiantis**

Organization and governance of the Institute

The Institute comprises four science departments, five independent research groups, five scientific service groups, the greenhouse service group, an outreach department that presents plant science to the public and the press, and the administrative department, which includes the technical workshops and library.

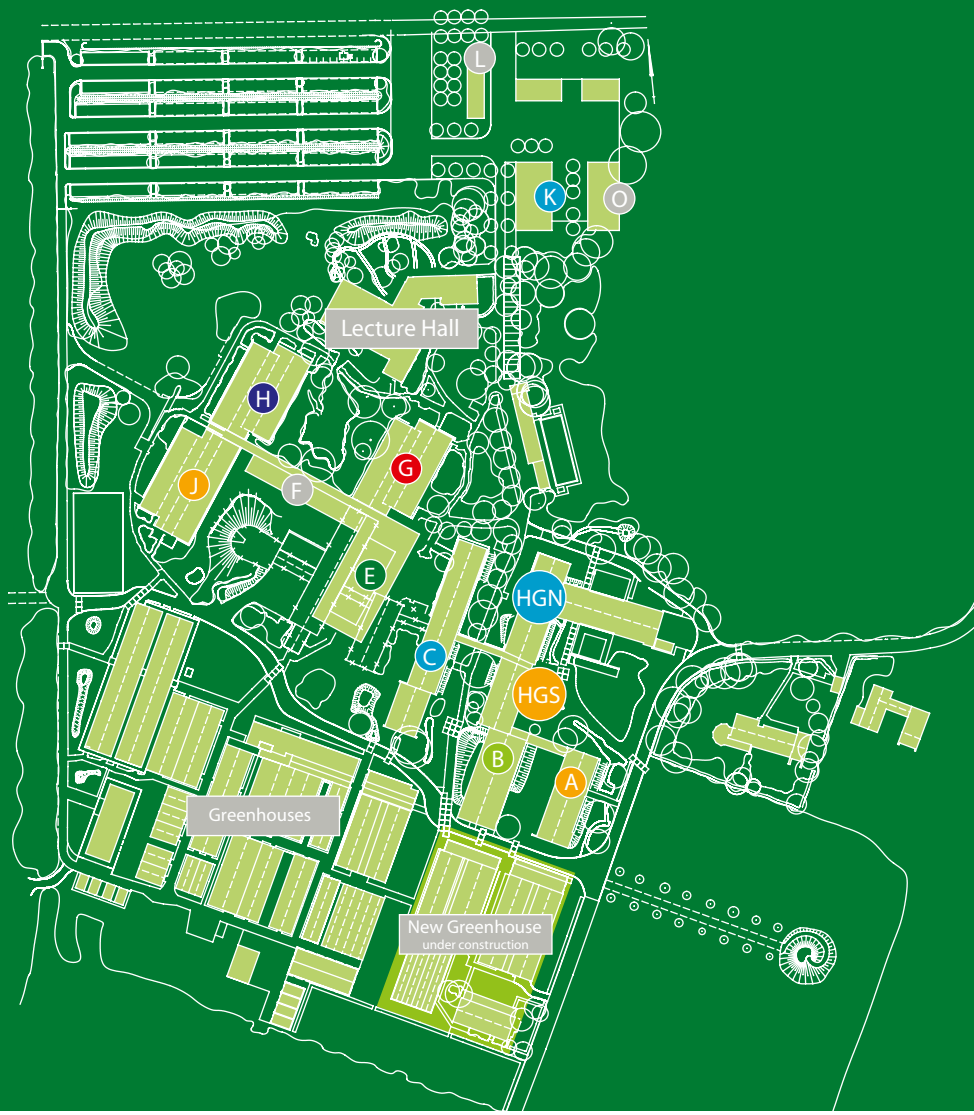
The Board of Directors is responsible for the management of the Institute. The Board is made up of the Directors of the four scientific departments and the Head of Administration. It meets once a month under the chairmanship of the Managing Director. The Board of Directors takes decisions on matters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

Each Director also heads a science department and is responsible for its research programme, budget and personnel. Each department comprises several research groups, which are led by scientists who are in turn responsible for the scientific programme, personnel and budget of their respective groups.



Present plan of MPIPZ

A	Department of Comparative Development and Genetics (Miltos Tsiantis)
B	Max Planck-Genome-centre Cologne (MP-GC), Computational Biology and Regulatory Networks (Achim Tresch)
C	Administration (purchase, book-keeping, personnel, International office)
E	Department of Plant Breeding and Genetics (Maarten Koornneef)
F	Link building with offices
G	Department of Plant Developmental Biology (George Coupland)
H	Department of Plant Microbe Interactions (Paul Schulze-Lefert)
HG N	Library, Guest rooms, SUSAN
HG S	Department of Comparative Development and Genetics (Miltos Tsiantis), SNG Quantitative Crop Genetics, Central Microscopy
J	Department of Comparative Development and Genetics (Miltos Tsiantis), under construction
K	Administration (mail, office supplies), Technical services, PR-AG
L	Entrance/Gate
O	Workshops



The service groups provide support in technical areas that underpin the work of the science 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 science department and the Head of the service group. Each of these committees is chaired by a Director.
The Head of Administration is responsible for managing the administration department, workshops, library and security. The administration department takes care of issues such as appointment contracts, budgetary matters, the building programme, the canteen, campus housing and maintenance of the Institute's 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 formed committee made up of the Board of Directors, one scientist from each department, the heads of service groups and leaders of independent research groups. This committee meets monthly to discuss topics raised by its members. Major issues discussed have included future scientific strategy - particularly the question of how to incorporate crop-plant research into the Institute, major investments in 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 organizations within the Institute's sphere of activity.

Research objectives and major emphases

Background and present status

"Can plant breeding be transformed into a rational, predictive science?" This is the question that motivates all research programmes at the Institute. We wish to determine how a detailed understanding of molecular mechanisms defined in model plant species can be used to predictively 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 has come largely from studies on model species, principally *Arabidopsis thaliana*. However, the deeper knowledge of the regulatory components and mechanisms controlling plant traits that has resulted has not yet had a sustained impact on plant breeding. The aim of all work at the Institute is to find ways of utilizing this knowledge to develop 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 direct transfer of useful 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 that control traits of agronomic importance. These programmes investigate seed dormancy, plant growth and plant architecture (Koornneef), plant-pathogen interactions and the plant microbiome (Schulze-Lefert), the control of flowering time (Coupland), and how biological forms develop and diversify (Tsiantis). All of these topics 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 and genomics has become an indispensable adjunct to quantitative genetics. Comparative studies of the function of similar genes in different species, combined with computational modelling, 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* and *Cardamine hirsuta)* 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 tools and techniques drawn from 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 the development of a multiscale understanding of selected plant traits.

Together, these approaches allow detailed questions to be posed: How many components contribute to a particular trait and how are their functions interrelated? 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 eliciting 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. Since 2010 the integrated Max Planck-Genome-centre Cologne (MP-GC) has been in operation on our campus. Providing state-of-the-art next-generation DNA- and RNA-sequencing services, it also includes a computing group for first-pass data analysis. Moreover, its advanced sequencing services are utilized by three additional Max Planck Institutes based in Cologne, Marburg and Bremen. The MP-GC consistently applies the very latest developments in genome/bioinformatics technologies and generates large-scale raw datasets for subsequent genome annotation of plant or microbial genomes and comparative genome analysis by individual researchers. In addition, by setting up a dedicated independent research group on computational biology and regulatory networks (led by Achim Tresch), the Institute has significantly augmented its resources in the area of bioinformatics and modelling. We have also greatly enhanced our technology platform in protein mass spectrometry by appointing Iris Finkemeier in 2013 to head a research and service group. This advanced technology enables highly sensitive detection of post-translational protein modifications, identification of components of regulatory protein complexes and compartment-specific proteomic profiling. In recent years we have made considerable investments in both confocal light and electron microscopy to visualize dynamic processes at subcellular scales, or at tissue/organ levels for computational modelling of morphogenesis. Continuous development of this complex technological infrastructure is vital for a rational understanding of plant traits. This infrastructure is heavily utilized 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 coordinated efforts to balance research in model systems and crop plants. We have begun to extend our research activities on the reference plant *Arabidopsis thaliana* to its relatives, including *Cardamine hirsuta* and *Arabis alpina*. This enables us to apply comparative approaches that are driven by evolutionary trait analysis in a phylogenetic framework to reveal mechanisms underlying interspecies trait variation and the origin of evolutionary novelties. In our efforts to explore how knowledge of fundamental traits and trait variation obtained in model plants can be transferred to crop plants, crop-related research across all departments is focused on barley. Additional crops used for transfer of insights gained from model plants are potato, tomato and oilseed rape. We believe that future trait modelling, based on the quantitative interactions of the relevant genetic components, will expedite the transition from empirical to rational, predictive plant breeding. Widening our research to take in evolutionary trait analysis with phylogenetically related reference plants also affords opportunities to understand the molecular basis of their ecological adaptations to different natural environments. An example of such an adaptation is given by the soil type and host genotype-dependent influence on the plant root-associated microbiome. Understanding the potential and constraints of plant adaptation to natural environments might ultimately impact on present-day agricultural management practices.

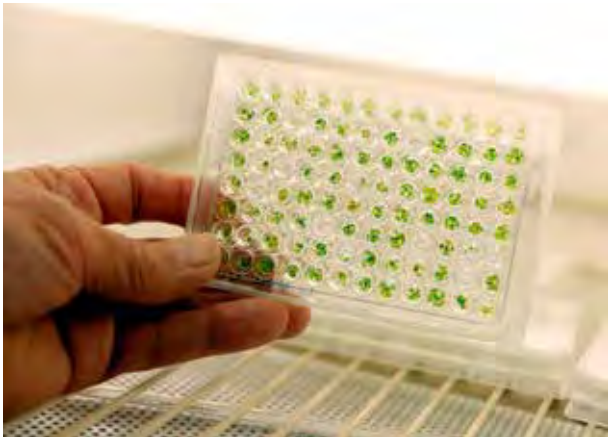
Co-operation and communication within the Institute and with regional universities

Interactions within the Institute

Numerous collaborative research projects are ongoing, involving individual research groups within a department or teams based in different departments. These collaborations play a vital part in testing new ideas at the interface between seemingly distinct plant processes or enable the dissection of complex traits using different methods. Both the International Max Planck Research School (IMPRS) and the Centre of Excellence on Plant Sciences (CEPLAS), a collaborative research and training venture with the universities of Düsseldorf and Cologne, provide incentives for joint PhD projects between research groups that have complementary skills and expertise. The mission of CEPLAS is to establish new paradigms to solve urgent problems in plant performance and production through exploitation of natural variation and biodiversity. A combination of evolutionary analysis and synthetic biology is being utilized in interdisciplinary research on four major themes: annual and perennial life histories, C4 photosynthesis, plant microbiota, and metabolic interactions.

Co-operation with Düsseldorf and Cologne Universities

Groups from the Institute take part in three Collaborative Research Centre (SFB) programmes with Cologne University, which are funded by the DFG (Deutsche Forschungsgemeinschaft). One of these focuses on Post-Translational Control of Protein Function (SFB 635). SFB 680 deals with the Molecular Basis of Evolutionary Innovation and SFB 670 (together with the Cologne University Medical Center) with Cell-Autonomous Immunity. In total, these programmes fund eleven research projects in the Institute.



The Institute's Graduate School is run in close collaboration with the regional universities in Cologne and Düsseldorf. The International Max Planck Research School (IMPRS) includes faculty from both the Institute and the two universities. It provides a forum for scientific communication, intensifies day-to-day contacts and collaborations between plant science groups in all three institutions, while the annual IMPRS retreats involving both faculty and students afford a comprehensive overview of all plant research activities.

Ph.D. programme and education

Providing high-quality education for young researchers is of particular concern to the MPIPZ. 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 with diverse scientific backgrounds from all over the world 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 encourage students to reflect on their position in a globalized world and 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 serve as valuable career assets. During the entire graduate programme each student receives scientific support from a Ph.D. Advisory Board, as well as general support from fellow students and scientists from neighbouring disciplines. Programmes that impart additional skills, such as training in the communication of science and preparation for an increasingly dynamic and flexible global job market equip graduates to meet other demands being made on the modern scientific community.

Kick-off meeting of the Cluster of Excellence on Plant Sciences (CEPLAS) at the MPIPZ in May 2013. Source: Wilhelm-Peter Schneider / HHU

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. programme at the Max-Planck Institute for Plant Breeding Research is internationally competitive, and attests to the MPIPZ's reputation as an institution that offers a first-rate Ph.D. education.

Department of Plant Developmental Biology

Director: George Coupland

Plant growth and development respond to a wide variety of environmental conditions. This versatility enables plants to succeed in diverse environments. To control these responses they continuously monitor external cues such as light, temperature and nutrient availability. 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 cues, allowing them to reproduce successfully in different locations. These effects have been exploited by plant breeders to maximize yields of crop plants in diverse environments.

Our studies employ molecular-genetic, biochemical and cell biology-based approaches in the model species *Arabidopsis thaliana* to investigate the roles of key regulatory pathways on flowering.

To speed up these approaches we develop new bioinformatic methods to exploit next-generation sequence data for the solution of genetic problems, and explore the genomes of plants showing diverse flowering responses. Our particular biological interests are the mechanisms by which seasonal changes in day length control flowering, the roles of systemic signalling and growth regulators in flowering, the importance of chromatin structure in controlling the transcription of flowering-time genes and the impact of abiotic stresses on 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 perennial and annual flowering plants diverged during evolution.

Control of flowering: Molecular mechanisms and phenotypic diversity

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. Flowering is also closely related to longevity and the reproductive strategies of different plant species. Annual plants live for less than one year, flower profusely and die after flowering. In contrast, perennials live for many years, and flower several times during their lives. We use a combination of genetics, molecular biology, cell biology and biochemistry to understand these flowering-related processes.

Mechanisms of flowering: *Arabidopsis thaliana* as a model

We use the powerful methodologies available for the model annual plant *A. thaliana* to identify mechanisms underlying flowering control. *A. thaliana* grown under summer day lengths flowers 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 *A. thaliana* found in the far North or at high altitude require vernalization (extended exposure to winter temperatures) before they will flower even under summer day lengths. We defined a regulatory pathway that allows *A. thaliana* to discriminate between long and short days, and to trigger flowering in response to the long-day signal. The difference in day length is perceived in the leaves through regulation of the *CONSTANS* gene and, in response to exposure to light late in the day, the level of *CONSTANS* mRNA rises and the protein is stabilized. We study the mechanisms conferring these two layers of regulation, which ensure that the CONSTANS protein accumulates specifically under long summer days and activates transcription of two closely related genes, *FT* and *TSF*. The FT protein then moves from the leaves to the shoot apical meristem through the vascular tissue. At the meristem FT triggers changes in gene expression so that

Arabis alpina plants flower after vernalization (left), whereas transgenic plants overexpressing microRNA156 never flower (right). Plants were grown for 8 weeks after germination, exposed to cold for 8 weeks and then returned to warm conditions for 12 weeks.

Figure 1: *Arabis alpina* plants

Convergent pathways responding to age and winter cold determine flowering time of perennial *Arabis alpina*

George Coupland

sets of transcription factors specifically associated with flowering are expressed. Using laser dissection microscopy we identified over 200 genes that are rapidly induced in the shoot meristem in response to FT during the floral transition. Related approaches led us to demonstrate that at the meristem FT increases levels of the growth regulator gibberellin, which activates a subsequent wave of transcription-factor activity at the transcriptional and post-translational levels. Our aim is to understand how these distinct processes induce the rapid, coordinated changes in growth and developmental identity required to convert the vegetative meristem into an inflorescence meristem.

Flowering of perennial *Arabis alpina*

We have developed *Arabis alpina,* a close relative of Arabidopsis, as a model system to study perennialism. *A. alpina* shows classical features of perennials. For example, it flowers repeatedly in episodes lasting for only a few weeks before reverting to vegetative growth, and only a small number of branches of the plant undergo the floral transition during a single episode. These effects are due to flowering being controlled by two repressive pathways, one conferring a requirement for winter cold and the other requiring that the plant reaches a certain age. We identified a mutant of *A. alpina* called *perpetual flowering 1 (pep1)* that does not alternate vegetative and reproductive development but flowers continuously through the summer and autumn, and in which many more shoots undergo the floral transition than in wild-type plants. The PEP1 protein is a transcription factor that represses flowering and is orthologous to the *A. thaliana* protein FLC. In wild-type *A. alpina* plants levels of PEP1 are transiently reduced by winter cold to allow flowering to occur, but increase again as temperatures rise in spring, ensuring that plants revert to vegetative growth. Surprisingly, we found that many populations of *A. alpina* contain naturally occurring mutant alleles of *PEP1,* and therefore contain seasonal and perpetual flowering perennials. Furthermore, exposure to low temperatures can induce flowering in *A. alpina* only if plants are at least 5-6 weeks old. Such a juvenility phase is characteristic of perennials and ensures that they produce sufficient meristems to be able to survive flowering and grow vegetatively afterwards. We found that this response is conferred by the microRNA156, the level of which falls as plants age. We are now exploiting populations made by crossing annual and perennial sister species in the *Arabis* genus to identify directly genes that contribute to the evolution of these different life strategies.

Selected publications

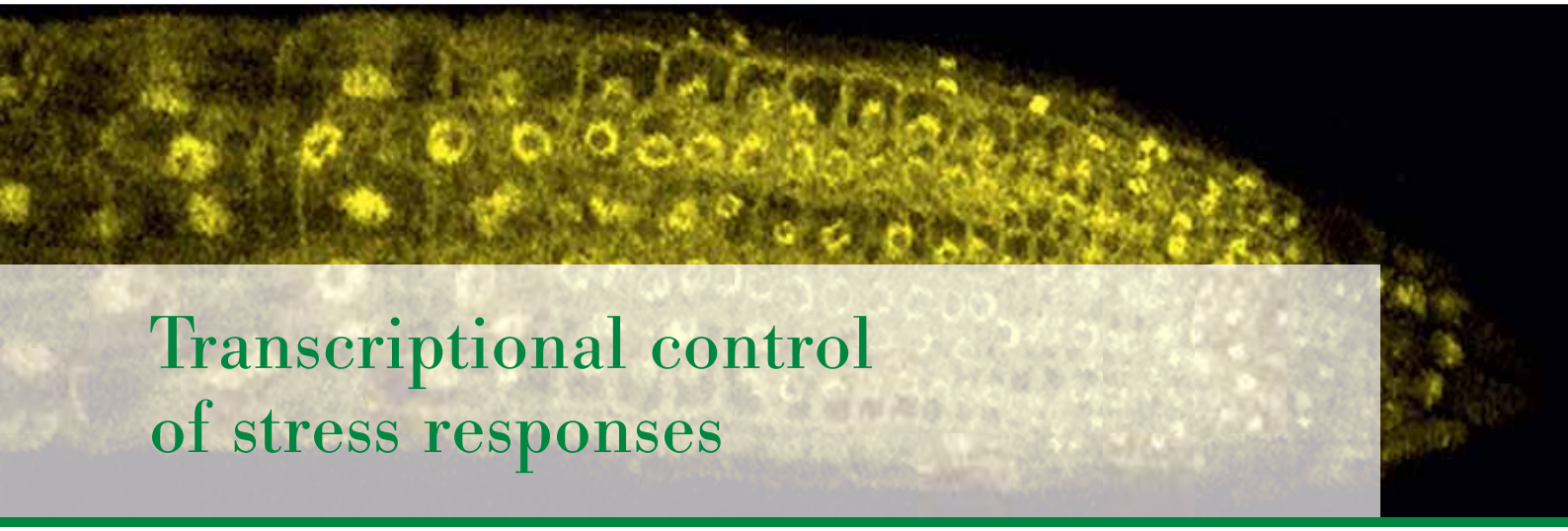
Bergonzi, S., Albani, M. C., Ver Loren van Themaat, E., Nordström, K.J.V., Wang, R., Schneeberger, K., Moerland, P.D. and Coupland, G. Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. Science 340, 1094 – 1097 (2013)

Albani, M.C., Castaings, L., Wötzel, S., Mateos, J.L., Wunder, J., Wang, R., Reymond, M. and Coupland, G. PEP1 of *Arabis alpina* is encoded by two overlapping genes that contribute to natural genetic variation in perennial flowering. PLoS Genetics 8 (12), e1003130. doi:10.1371/journal.pgen.1003130 (2012)

Porri, A., Torti, S., Romera-Branchat, M. and Coupland, G. Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development 139, 2198-2209 (2012)

Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. Plant Cell 24, 444-462 (2012)

Wang, R., Albani, M.C., Vincent, C., Bergonzi, S., Luan, M., Bai, Y., Kiefer, C., Castillo, R. and Coupland, G. Aa *TFL1* confers an age-dependent response to vernalization in perennial *Arabis alpina*. Plant Cell 23, 1307-1321 (2011)



Transcriptional control of stress responses

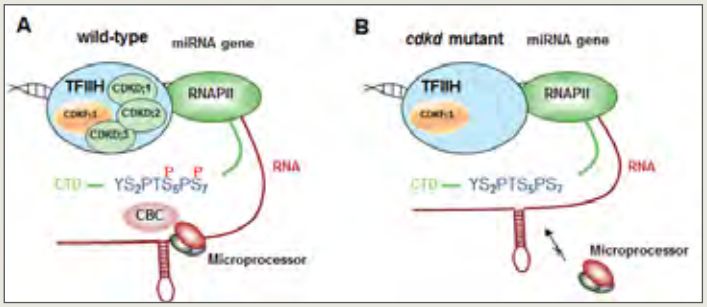
Our research aims at understanding the functional roles of RNA polymerase II (RNAPII)-associated regulatory factors, including the cyclin-dependent CDCD and CDKF kinase subunits of general transcription factor TFIIH, AMP-activated Snf1-related protein kinases (SnRK1s), and components of the spliceosome-activating NineTeen complex (NTC) in Arabidopsis. Study of molecular mechanisms controlling the activity and stability of these transcription regulatory complexes provides an insight into coordinate control of hormonal and developmental pathways governing plant responses to biotic and abiotic stress.

Regulation of RNAPII transcription by TFIIH kinase modules

Phosphorylation of conserved $Y_1S_2P_3T_4S_5P_6S_7$ repeats in the C-terminal domain of RNAPII largest subunit (RNAPII CTD) plays a central role in the regulation of transcription and co-transcriptional RNA processing. We found that the plant-specific master kinase CDKF;1 phosphorylates the CTD at S7 positions and mediates the activation of cell cycle kinase-activating CDKD kinases that control the initiation of transcription by phosphorylating the CTD at S5 positions. Inactivation of CDKF;1 causes defects in 3′-polyadenylation of pre-microRNAs and transcripts coding for regulators of biogenesis of small RNAs. This decreases the levels of precursor and mature small RNAs, and enhances the removal of introns carrying pre-microRNA stem-loops. Inactivation of all but the one allele required for viability inhibits CTD S5-phosphorylation, triggers the accumulation of uncapped precursor microRNAs and reduces the levels of mature micro-RNAs. The *cdkf* and *cdkd* mutations severely compromise normal control of the cell cycle, causing growth inhibition and dwarfism, and alter the regulation of hormone, light and stress signalling pathways.

Regulation of transcription in response to stress, hormone and metabolic signalling by SnRK1

SnRK1 kinases belong to the AMP-activated protein kinase (AMPK) family and modulate transcription according to changes in cellular energy homeostasis. AMPKs are found in association with histone acetylases of S(T)AGA transcription co-activators of RNAPII, and in Arabidopsis function as regulatory components of SCF (SKP1-CULLIN1-F-box protein) E3 ubiquitin ligases, mediating their recruitment



(A) CDKF;1 is required for activation of three functionally redundant CDKD kinases (D1, D2 and D3), which phosphorylate the S5 positions of $Y_1S_2P_3T_4S_5P_6S_7$ repeats of RNAPII CTD. Phosphorylated S5 residues in the CTD are recognized by the mRNA CAP-binding complex (CBC), which in turn recruits the Microprocessor that mediates the processing of mature microRNAs from stem-loops. (B) In *cdkf*;1 and *cdkd* mutants, phosphorylation of RNAPII CTD, and hence recruitment of CBC and Microprocessor to RNAPII CTD on microRNA transcripts, is compromised, which leads to inhibition of microRNA processing.

Figure 1: Regulation of microRNA processing by TFIIH-associated kinases

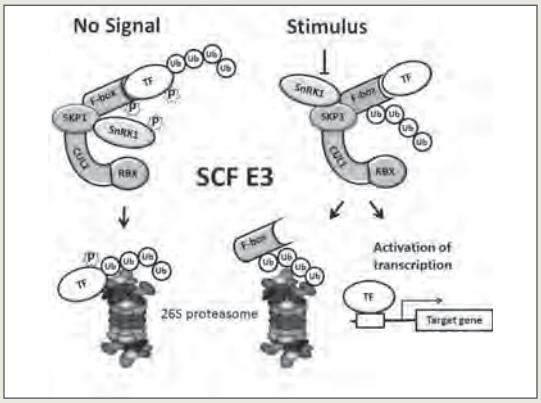
Understanding the functions of RNAPII regulatory modules provides insights into the coordinate control of signalling pathways and transcription

Csaba Koncz

to the proteasome. SCF complexes control ubiquitination and stability of important transcription factors and thereby modulate transcription in response to hormone and stress signalling. Therefore, our current studies focus on the definition of SCF-related regulatory roles, transcription targets, and regulatory interactions of RNAPII-associated SnRK1 kinase co-activator and SCF complexes in Arabidopsis.

Regulatory interactions of TFIIH and SnRK1 with NTC

Components of NTC are loaded onto RNAPII by their contacts to initiation, elongation and chromatin-modifying factors. Some Arabidopsis NTC subunit mutants are viable but show RNA splicing defects resulting in the accumulation of unprocessed introns and alternatively spliced forms of polyadenylated pre-mRNAs, so providing a tool for identification of pathway-specific NTC targets. Through its XAB1 subunit, NTC interacts with TFIIH, which acts also as a major regulator of transcription-coupled and general genome repair. The NTC subunit PRL1 is a substrate receptor of CUL4-DDB1 and PRP19 E3 ubiquitin ligases. A CUL4-DDB1-PRL1 complex is reported to control ubiquitination and proteasomal degradation of the SnRK1α subunit AKIN10, suggesting cross-talk between TFIIH, SnRK1 and NTC. Current studies focus on genome-wide compilation of splicing defects in the *prl1* NTC mutant, analysis of NTC interactions with TFIIH and SnRK1 co-activators, and identification of mechanisms by which NTC mutations affect the regulation of various signalling pathways.



SnRK1 is a component of SCF E3 ubiquitin ligases composed of SKP1, CUL1, RBX1 and substrate receptor F-box protein subunits. SnRK1 phosphorylation (P) of transcription factors (TFs) triggers their ubiquitination (Ubi) and proteasomal degradation, whereas signalling stimulus-dependent inactivation of SnRK1 leads to stabilization of TFs and transcriptional activation of specific target genes along with simultaneous degradation of F-box substrate receptors.

Figure 2: SnRK1 modulates SCF-mediated ubiquitination of transcription factors

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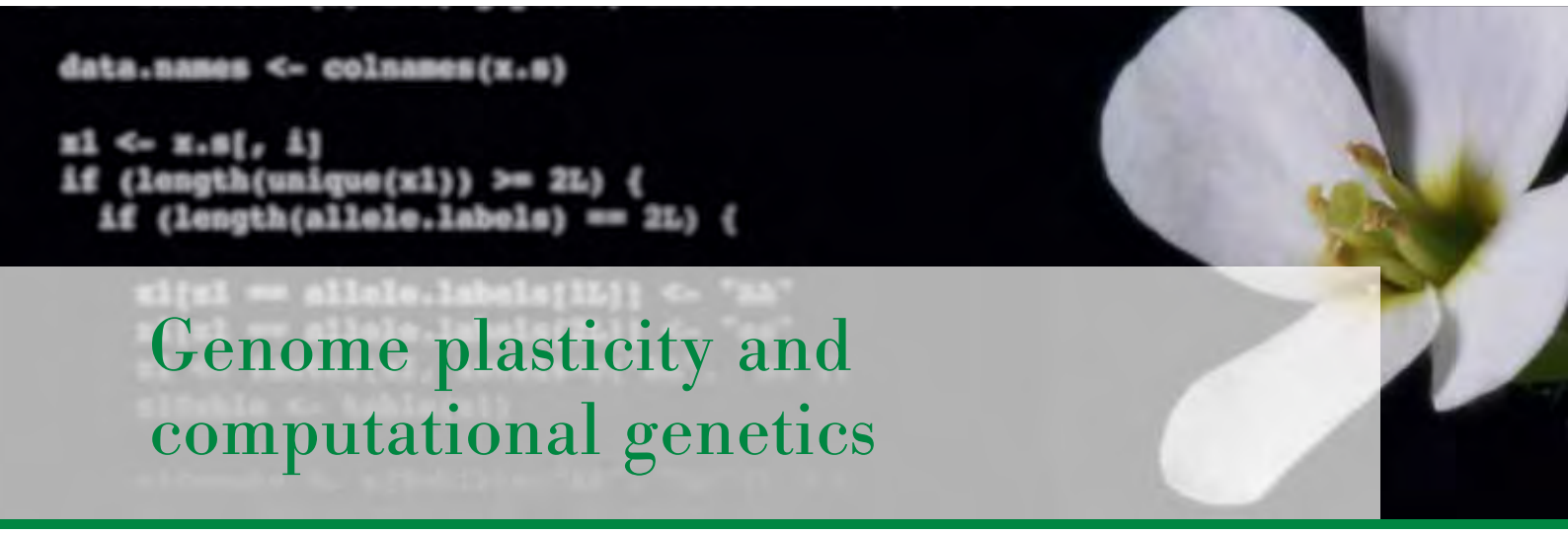


Genome plasticity and computational genetics

Introduction

Since the advent of next-generation sequencing (NGS) scientists all over the world have been using the new technology to assemble a steadily growing number of plant genomes. In addition to model species, the genomes of most plants that are eaten by humans, or are exploited for other purposes such as oil production, have already been sequenced. Clearly, the main challenge in genomics has now shifted from data generation to the reconstruction and interpretation of genomic sequences, and continuing development of efficient data-handling methods for the constantly evolving NGS technologies remains an urgent need. Our group's major focus is to advance NGS-based analyses by addressing questions that could not be resolved previously. But NGS can do more than simply assemble genome sequences. We use whole-genome sequencing-based methods to accelerate forward genetics by directly linking mutant phenotypes to the genetic changes that cause them.

Natural variation between and within plant species

We are performing *de novo* assembly of *Arabidopsis thaliana, Arabis alpina* and *Arabis montbretiana* genomes. In particular for *A. thaliana,* where all whole-genome studies so far have relied on the established reference sequence, additional *de novo* assemblies reveal an unexpected amount of genic variation. This allows us to study the increased level of segregation of recently duplicated genes as compared to unique genes, a phenomenon that was predicted by theoretical evolutionary models, but could not be investigated in natural populations hitherto.

Although they are closely related, *A. alpina* and *A. thaliana* form an interesting contrast. *A. thaliana* has an annual life cycle. *A. alpina* is a perennial plant and continues to grow for years. Both species share large parts of their genic content, but differ substantially at the level of gene expression. Following the principle of phylogenetic shadowing we are exploring the regulatory code that controls the pattern of circadian gene expression in these two species.

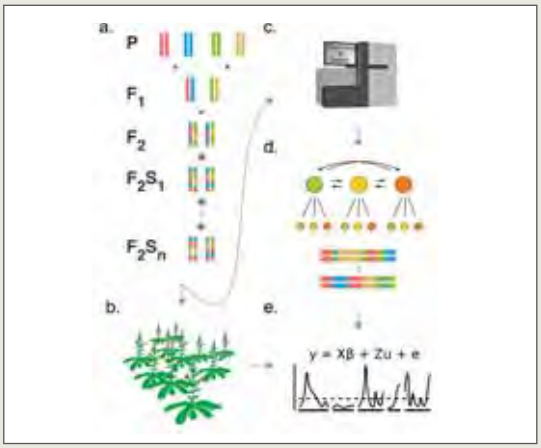
Computational genetics

Forward genetic screens have been fundamental to the success of *A. thaliana* as a model species. Such screens, however, involve tedious mapping and identification of mutagenized genes. Some years ago we significantly increased the efficiency of this approach by combining bulk segregant analysis with whole-genome sequencing. This method, commonly referred to as mapping-by-sequencing, relies on crossing the mutant with a genetically different individual to introduce molecular markers, which can result in undesired phenotypes that may mask the phenotypic differences caused by the mutation. To overcome this, we (together with F. Turck's group) have introduced mapping-by-sequencing based on F2 individuals descended from a cross between the mutant and its non-mutagenized progenitor. By combining this method with a novel, reference-free, genome comparison algorithm, we can now identify causal mutations without making use of any reference sequence.

Natural traits, such as flowering time, are generally controlled by more complex genetic architectures, which will not be deciphered by bulk segregant analysis. In order to facilitate the identification of genes involved in the control of such quantitative traits, we are developing

It has never been easier to fall for false positives than in this genomics era, in which generation of even large numbers of genomic datasets is cheap and easy

Korbinian Schneeberger



A. Construction of the AMPRIL population. Founder accessions were crossed to produce two hybrids, which were then crossed to each other. The resulting population is selfed for multiple generations. B. Each recombinant is phenotyped for a broad range of traits. C. The genome of each line is sequenced using next-generation sequencing. D. Hidden Markov Models are used to reconstruct the recombinant genomes. E. Advanced statistical models enable one to associate the phenotypes with the underlying genetic variations.

Figure 1

machine-learning methods for the reconstruction of recombined genomes, which enables us to use advanced statistical methods to link recombinant genotypes with their individual phenotypes. In collaboration with M. Koornneef, we are applying these methods to the complete Arabidopsis multi-parental-intercross (AMPRIL) population (see Figure 1).

The landscape of meiotic recombination

A key ingredient in linking phenotypes to their causal genetic variation is meiotic recombination. In order to understand the exact makeup of meiotic recombination, we are analysing the genomes of meiotic tetrads derived from Arabidopsis hybrids, which reveal the landscape of reciprocal exchange of genetic information during male meiosis. We will continue to study the relation of sequence divergence and meiotic recombination in isogenic as well as in hybrid meiosis.

Selected publications

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Transcriptional control of flowering time

Introduction: How plants make decisions in the absence of a brain

Life-altering decisions, such as whether or not to start a family, require careful consideration of the pros and cons, taking every possible angle and aspect into account. Like us, plants need to carefully "consider" when to abandon the relatively safe vegetative phase, during which mostly leaves are generated and energy is produced. Commitment to the reproductive phase requires the generation of the more vulnerable flowers and channelling of resources into seeds in order to provide a good start for the next generation.

In the absence of a brain or a nervous system, the complicated regulation of so-called floral integrator genes plays a crucial role in optimizing the timing of flowering. Integrator genes receive inputs from several regulatory pathways controlled by internal or external signals that encode information on variables such as developmental age, stress, light quality and quantity, temperature and, last but not least, day length. By definition, floral integrator genes are not expressed during the vegetative phase, but their induction upon reception of cues from upstream regulatory inputs commits the plants to flowering. In many plants, one of these integrator genes, *FLOWERING LOCUS T (FT),* functions to communicate the decision to flower from the leaves, where many signals are perceived and *FT* is transcribed, to the apical meristem, where the decision to flower is executed with the help of the FT protein. Transcriptional regulation of *FT* is fascinatingly complex and we have set ourselves the goal of dissecting the *cis*-regulatory code of this gene and studying the protein complexes involved in its control (see Figure 1).

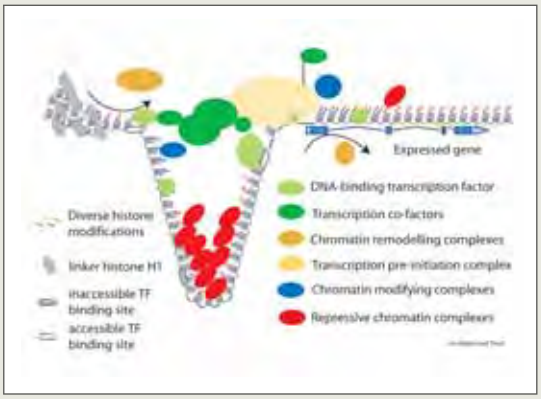
Gene expression is regulated by *trans*-acting factors, mainly proteins that bind to short sequence motifs present in the regulatory regions of target genes. All specific sequence motifs with a cognate binding partner constitute the *cis*-regulatory code of a gene. However, it is still relatively unclear how exactly the binding of a cognate factor influences the expression of a particular target gene, although several scenarios have been described. These scenarios include the recruitment of a second layer of regulatory proteins, which remodel chromatin structure. The term chromatin is a collective name given to DNA that is wrapped around histone protein complexes. This organized state is necessary to accommodate the DNA in the small space available in the nucleus but, by making the DNA inaccessible to binding factors and gene-transcribing complexes, it inhibits gene expression. In consequence, local loosening of the chromatin structure may facilitate expression of a gene, whereas enhancing its compaction may further repress it.

Deciphering the *cis*-regulatory code of *FT*

At *FT,* we started to map the important regions by creating series of transgenic plants that carried only selected parts of putative regulatory regions or in which selected candidate regulatory elements were mutated. These transgenic plants were mutant for the endogenous *FT* locus and therefore flowered late in long days unless the transgenes could reconstitute the natural regulation of the gene. Since the regions potentially involved in *FT* regulation are relatively large, we used the pattern of conservation of *FT* genes in related plants to guide our analysis. The rationale for this approach, which is termed phylogenetic shadow analysis, is that sequences that are important for regulation are more highly conserved than those without a functional role. Using this approach, we identified a regulatory enhancer that plays a crucial role in *FT's* response to stimulating long days, although it is located at a considerable distance from the transcription start site of the gene. We believe that this enhancer must be folded into

FLOWERING LOCUS T is the mastermind of the Arabidopsis decision to flower

Franziska Turck



A model of the interplay between cis-regulatory regions, trans-factors and chromatin complexes at the *FT* locus.

Figure 1

a three-dimensional structure to bring it into contact with the start site (see Figure 1). We have shown that chromatin-associated complexes play an important role in gene regulation because they govern access to cis-regulatory elements. In the future, we will take a more detailed look at the variation within these regulatory regions among *Arabidopsis thaliana* accessions that have been collected across the Northern hemisphere. We believe that this can teach us a lot about how gene regulation evolves to adapt plants to different climatic conditions.

Chromatin-associated complexes and their role in gene regulation

Chromatin-associated complexes are crucial for the proper regulation of *FT* and many other genes. One group of proteins is particularly important for the repression of key developmental genes, such as *FT,* at times when they are not needed. We are interested in learning more about these complexes, which are called Polycomb Repressive Complexes (PRCs). We exploit the strengths of the genetic model organism *A. thaliana* and perform mutant screens to identify new components that either belong to PRCs or interact with them. Genetic screening has been greatly facilitated by the availability of high-throughput sequencing approaches in the recent past. In the future, we hope to learn more about the interplay between *trans*-acting factors, the *cis*-regulatory code and chromatin-associated complexes.

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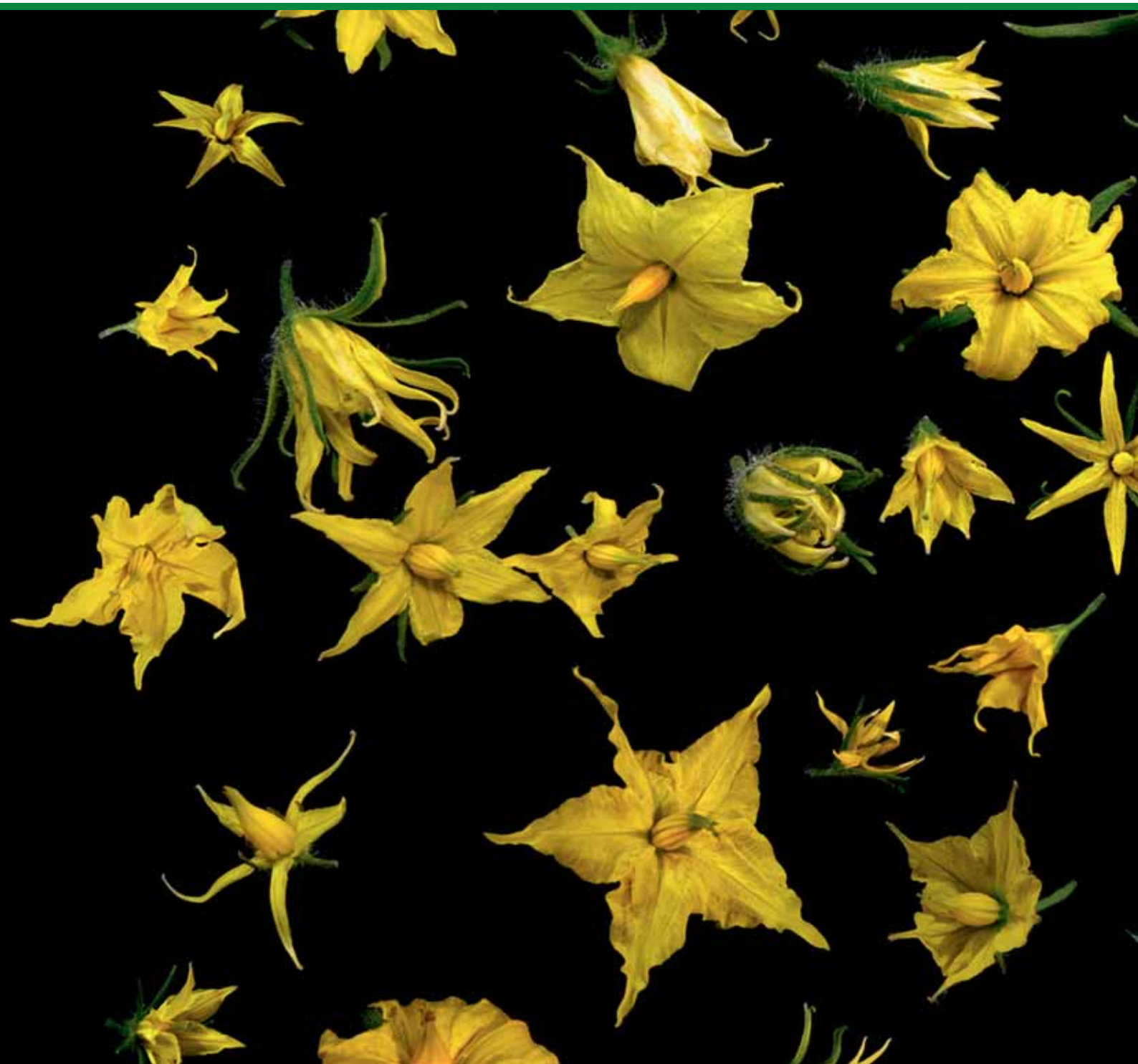
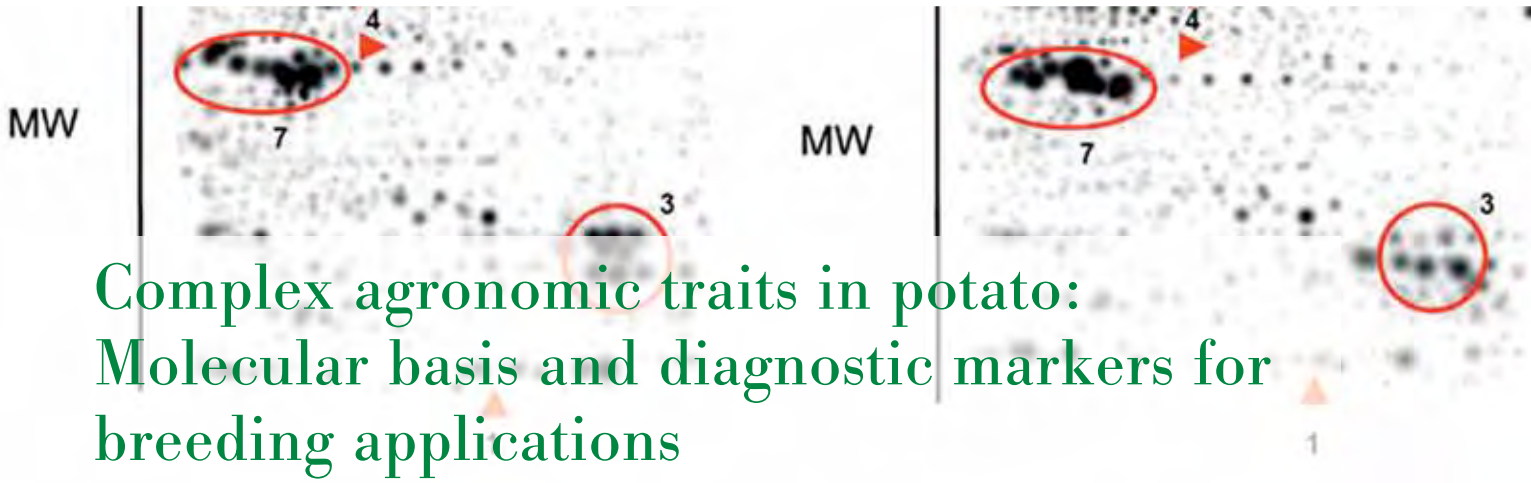


Department of Plant Breeding and Genetics

Director: Maarten Koornneef

The aim of plant geneticists is to unravel the nature and function of genes that underlie the phenotypic variation (biodiversity) in nature and among crop plants. Knowing which genes control specific processes in plants helps us to understand plant biology but it can also be used to improve the efficiency of plant breeding (precision breeding). The study of variation uses existing variation which can be found in nature (so-called natural variation) or makes use of induced mutations. The analysis of natural variation requires quantitative genetics, which can identify so-called Quantitative Trait Loci (QTL) using a combination of trait and molecular analyses.
The latter approach increasingly makes use of markers provided by new sequencing technologies. The objectives of our department are (i) to unravel the genetic and molecular basis of several developmental traits in plants – such as seed dormancy, stress tolerance and plant architecture – using molecular genetics, genomics and natural variation as tools, and (ii) to develop methods and materials that facilitate this type of studies.

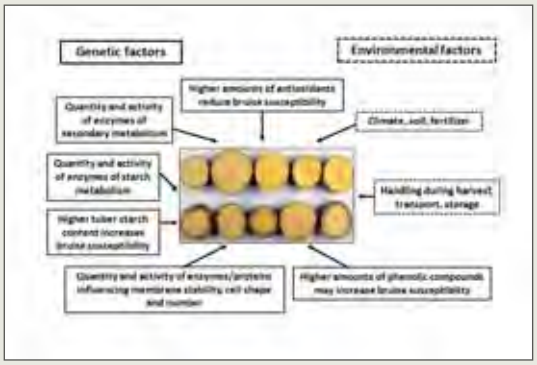


Complex agronomic traits in potato: Molecular basis and diagnostic markers for breeding applications

Worldwide the potato *(Solanum tuberosum)* is the most important crop species of the Solanaceae family, which includes about 200 tuber-bearing *Solanum* species native to Mexico, Middle and South America. The cultivated potato is tetraploid and highly heterozygous due to cross breeding. Genotypes are fixed by the vegetative propagation of tubers. Most traits relevant for variety development are complex, meaning that they are controlled by natural DNA variation at multiple loci and by environmental factors. The objectives of our research are (1) to elucidate the genetic basis of complex agronomic characters of potato at the molecular level, (2) to develop molecular diagnostic tools to assist the selection of improved cultivars and (3) to contribute to the understanding of structure, evolution, function and natural diversity of crop plant genomes. To reach our objectives, we exploit the natural variation amply present in *S. tuberosum,* adopting principles and approaches of human population genetics such as association mapping. Recently, comparative proteome and transcriptome profiling were used to discover novel functional candidate genes for controlling complex traits. Our outputs are (1) knowledge of genomic positions and identity of genes and their superior or inferior alleles that control agronomic characters and (2) diagnostic DNA-based markers that can be used for marker-assisted selection of superior cultivars (precision breeding).

Example: Tuber quality

Tuber quality is of primary importance in potato crop production. The tuber's susceptibility to enzymatic discoloration upon mechanical damage (bruising) is genotype dependent and controlled by numerous genetic and environmental factors (Figure 1). Genes and biochemical pathways involved in the formation of the dark coloured pigments in bruised tissues are mostly known, thus providing candidate genes for association mapping. In collaboration with six potato-breeding companies, a population of 205 varieties and breeding clones was evaluated in field trials for susceptibility to bruising on the one hand, and on the other for DNA polymorphisms in functional candidate genes. Among others, DNA variants in genes encoding polyphenoloxidase, the enzyme forming the pigments, were associated with increased or decreased bruising susceptibility (Urbany et al. 2011). By comparing the tuber proteomes of ten bruising resistant and ten susceptible varieties, we identified differential proteins that had not been connected with tuber bruising. DNA variation in one of the corresponding genes encoding a class III lipase was indeed associated with increased bruising susceptibility. The associations found between natural DNA variation and bruising susceptibility can be used to develop diagnostic markers for breeding applications.

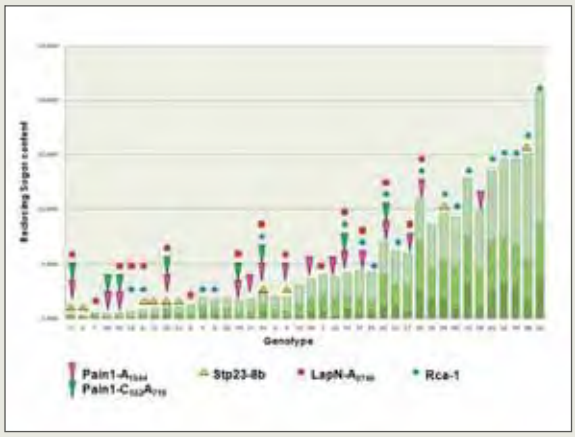


Susceptibility to bruising is highly relevant in variety development. The levels of dark pigments that accumulate under the tuber skin after mechanical impact depend on multiple genetic and environmental factors, some of which are listed. Tubers are shown 24 h after mechanical damage with a pendulum. Tubers in the upper and lower row are from bruising-resistant and -susceptible genotypes, respectively. (Reproduced from Gebhardt 2013)

Figure 1: Example of complex tuber quality trait

The combination of comparative proteomics or transcriptomics with association genetics leads to the discovery of novel candidate genes underlying complex traits in crop species

Christiane Gebhardt



Twenty good (No 1-20) and 20 bad (No 21-40) processing varieties are ranked according to the amount of reducing sugars in tubers before (dark green bars), after two weeks (green bars) and four weeks (light green bars) storage at 4°C. The lower the reducing sugar content, the higher is the tuber quality for processing. Presence of specific alleles of soluble acid invertase *(Pain-1),* plastidic starch phosphorylase *(Stp23 = PHO1a),* Rubisco activase *(Rca)* and leucine aminopeptidase *(LapN)* is indicated by the coloured symbols. The distribution of the alleles in the 40 varieties is skewed towards varieties with lower (Pain1-A_{1544}, Pain1-$C_{552}A_{718}$, Stp23-8b, LapN- A_{2746}, positive alleles) or higher (*Rca-1,* negative allele) reducing sugar content. The markers explain only part of the phenotypic variation, as there are low sugar varieties that have none of the positive marker alleles (No 3, 9, 15, 1, 16).

Figure 2: Illustration of associations between DNA polymorphisms in candidate genes and tuber content of reducing sugars.

The biochemical and molecular basis of starch and sugar metabolism has been extensively studied in plants. Tuber starch and sugar content served therefore as models for testing the candidate gene approach to identify genes controlling complex agronomic traits. Moreover high tuber content of the reducing sugars glucose and fructose negatively affects the quality of chips and French fries. Reducing-sugar content increases during low-temperature storage (cold sweetening). DNA variants in genes functional in starch-sugar interconversion were identified which are associated with lower reducing sugar content (Figure 2). The diagnostic value of candidate-gene markers associated with tuber starch and sugar content was tested in pilot experiments for marker-assisted selection (Li et al. 2013). Comparative protein profiling of cultivars with low and high capacity to accumulate reducing sugars uncovered novel candidate genes for controlling tuber starch and sugar content. A single nucleotide polymorphism (SNP) in a leucine aminopeptidase was strongly associated with decreased reducing sugar content (Fischer et al. 2013) (Figure 2).

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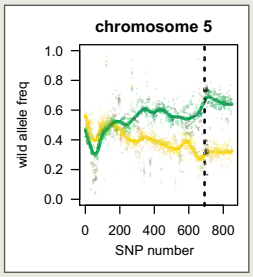
Adaptive genomics and genetics

Understanding the molecular basis of phenotypic variation in nature is a major goal in modern biology. Our group focuses on the application of genetics and bioinformatics to the study of plant natural variation, evolution and domestication.

Technological advances such as high-throughput sequencing (HTS), image analysis or automated phenotyping allow us to describe plants with unprecedented precision. We exploit these methods in various complementary directions: First, we develop and use automatic devices to monitor plants and their responses to the environment with high temporal and spatial resolution. Second, we use HTS and metabolomics to accurately describe differences in the molecular landscapes of diverse individuals in various conditions. Third, we develop computational pipelines that integrate and extract the useful information from these complex datasets. Finally, we devise experimental designs that take advantage of these novel technologies and protocols, and enable us to identify the individual genes, but also general patterns, that underlie phenotypic variation in plants at the molecular level.

Domestication

Our model system for studies of domestication is tomato. We use the wild tomato species from remote islands, deserts, high mountains or humid forests in South America and compare them to modern cultivars to identify the genetic changes involved in domestication. Examples of our research in these wild species include the combination of HTS and bulk segregant analysis to map genes involved in variation in flowering time (Figure 1), or the identification of genes affecting drought tolerance using bioinformatics. The new technologies are allowing us to detect differences between cultivars and wild accessions in traits that could not be studied before. For example, we use RNA-seq to characterize the global amount of stochastic noise in expression or alternative splicing, processes that are not understood even in well studied model systems. Moreover, automatic phenotyping allows us to monitor differences in circadian rhythms between tomato and its wild relatives. As the circadian clock is an essential regulator of physiology in all organisms, understanding its modification during domestication could have an enormous impact on the way we understand this process.



An F2 population derived from a cross between early and late flowering tomato accessions was generated and individuals were pooled according to their flowering time. RNA-seq was performed to detect SNPs and significant differences in allele frequencies between the pools were found at the bottom of chromosome 5. The vertical line shows the point at which frequency differences are highest.

Figure 1: Allele frequency in SNPs along chromosome 5 in tomato.

Adaptation

In addition to the effects of human selection, we are interested in how natural selection acts on wild plants. Our model for studies in adaptation is *Arabidopsis thaliana,* a plant with a worldwide distribution. In this species, we perform classic quantitative trait analyses to identify the genes responsible for natural variation in characters such as drought response or flowering time. One of the advantages of using Arabidopsis lies in the genomic and genet-

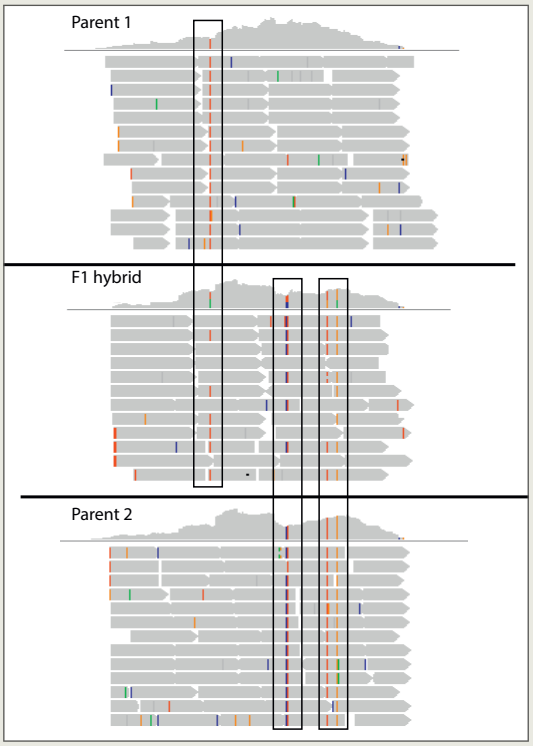
We are excited to live in a time in which technology enables us to precisely observe plant diversity at the molecular level

José Jiménez-Gómez

ic resources accumulated over the years. These resources permit us to combine classical approaches with novel bioinformatic algorithms and experimental designs. The identification of expression QTLs through quantification of allele-specific expression in F1 hybrids is one example. By performing RNA-seq in hybrids and calculating the relative contribution of each allele to the final concentration of each transcript we can estimate the mode of regulation for each gene (Figure 2). This is a major step towards understanding differences in expression between individuals, and has great potential for identifying the genes underlying natural phenotypic variation.

Characterization of selected genes

Both in tomato and Arabidopsis, it is fundamental for us to characterize in detail the genes that have been important for plant adaptation. One example is *FRIGIDA,* an important contributor to flowering-time variation among natural populations of Arabidopsis. We use a combination of molecular biology, population genetics and bioinformatics to understand the selective pressures acting on this and other selected loci. By this means, we hope to obtain a catalogue of genes and mutations that have been positively selected during evolution, and to draw conclusions about the general patterns that drive natural phenotypic variation in plants.



RNA-seq reads from two parents and their F1 hybrid aligned with the reference genome. Allele-specific expression is measured by comparing the number of reads overlapping SNPs in each parent and in the hybrid (highlighted in boxes).

Figure 2

Selected publications

Koenig*, D., Jiménez-Gómez*, J.M., Kimura, S., Fulop, D., Chitwood, D.H., Headland, L.R., Kumar, R., Covington, M.F., Kumar Devisetty, U., Tat, A.V., Tohge, T., Bolger, A., Schneeberger, K., Ossowski, S., Lanz, C., Xiong, G., Taylor-Teeples, M., Brady, S.M., Pauly, M., Weigel, D., Usadel, B., Fernie, A.R., Peng, J., Sinha N.R. and Maloof J.N. Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato. PNAS, doi:10.1073/pnas.1309606110 (2013) (* co-authors)

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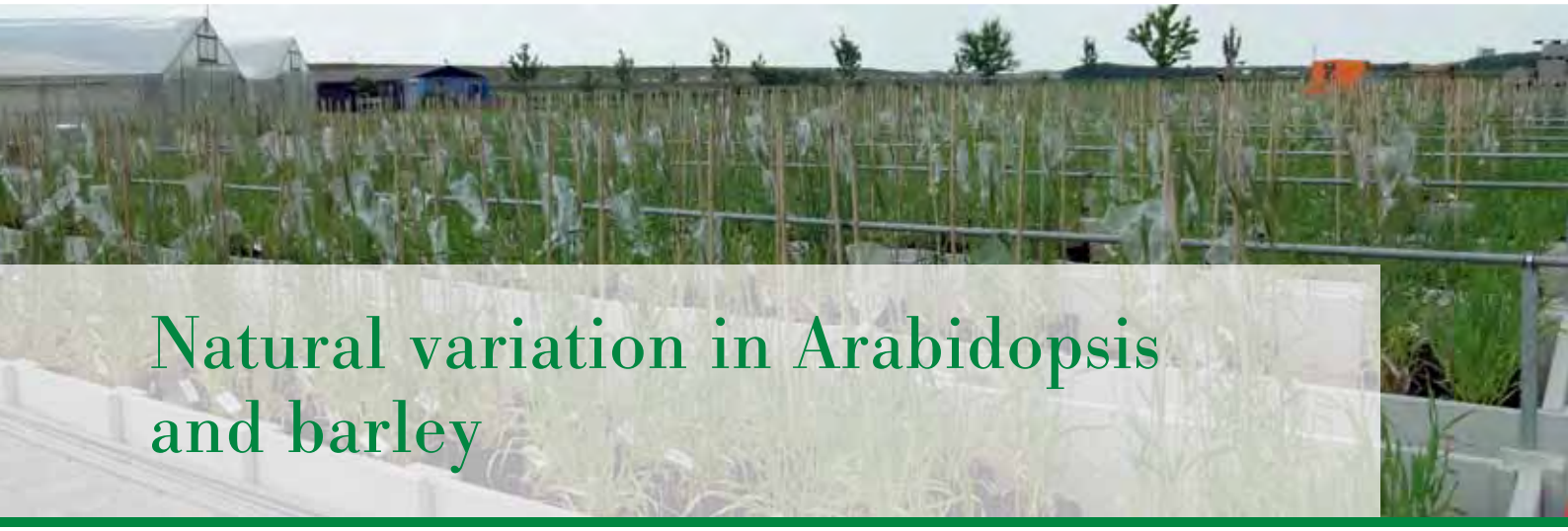

Natural variation in Arabidopsis and barley

Plants show a wide range of variation in their phenotypes, not only between species but also within a species. The latter can be used to uncover the genetic basis of these differences by genetic studies for which we increasingly use collections of wild accessions. However, in addition we also construct so-called multi-parent mapping populations. We have developed such populations based on crosses between Arabidopsis accessions collected from different parts of the world and therefore adapted to different climatic conditions. By intercrossing several such accessions and studying the progeny of these crosses, we can obtain novel combinations of genetic variants (alleles), which may result in novel phenotypes. The identification of the genetic and molecular bases of these phenotypes is a major aim of our research. One challenge is to identify the genes underlying Quantitative Trait Loci (QTL) in those cases where several QTL segregate and epistasis occurs, using Next Generation Sequencing. To achieve this goal we try to identify, in the progeny of our segregating populations, lines that segregate for single loci. In addition to the Arabidopsis Multi Parent Recombinant Inbred Line populations (AMPRIL) we have generated a multiparental population in barley, which was derived from a cultivated (and sequenced) *Hordeum vulgare* variety, three wild relatives *(H. spontaneum)* and one primitive cultivated form, *H. acrocriton*.

Natural variation for plant architecture in Arabidopsis

We demonstrated the usefulness of the AMPRIL population by analysing the branching pattern of Arabidopsis and by identifying genes that are important for this process. The effect of several of these loci depends on the action of flowering-time genes (epistasis). The cloning of one of these genes identified a new function of the MADS-box transcription factor AGL6 that was not

An Arabidopsis semi-dwarf accession and a related wild type. The semi-dwarf phenotype is due to a mutation in the GA20ox1 gene previously named GA5.
Such semi-dwarf Arabidopsis plants were found in populations from different parts of the world and may have a selective advantage under their native conditions.

Figure 1

New combinations of natural alleles at different loci can lead to novel phenotypes

Maarten Koornneef

previously known to be involved in this trait. We continue to study the role of this gene and its natural variants, and hope to identify additional loci and genes that show variation in nature. Another aspect of plant growth is the length of the plant, which shows considerable variation in nature. We discovered that most of the semi-dwarf accessions (Figure 1) have mutations in the GA20-oxidase 1 gene, which affects the biosynthesis of the plant hormone gibberellin. We obtained evidence that such natural mutants originate from independent mutations in different parts of the world because, at the gene level, they resemble wild-type alleles from the same region. Such semi-dwarf mutants appeared to have a selective advantage compared to taller plants in some populations, explaining why they are common in these populations. To investigate if hormone biosynthesis and hormone signalling variants occur in nature we have developed and applied various test systems to detect such variation.

Barley: natural variation for aspects of seed biology and plant architecture

Recombinant inbred lines derived from crosses between cultivated and wild barley were analysed for various developmental traits, such as growth rates and the formation of side shoots (tillers), as well as for seed-related traits such as seed dormancy and seed size, shape and colour. Subsequently Quantitative Trait Loci (QTLs) were identified for almost all traits, and some of these were followed up in experiments designed to refine their map positions and ultimately identify the underlying genes. It is expected that this will not only reveal genes that contributed to domestication but also tell us whether additional genetic improvement can be expected from the use of these wild relatives in plant breeding. In addition we are investigating the genetic basis of seed longevity using genetic approaches in combination with state-of-the-art 'omics'.

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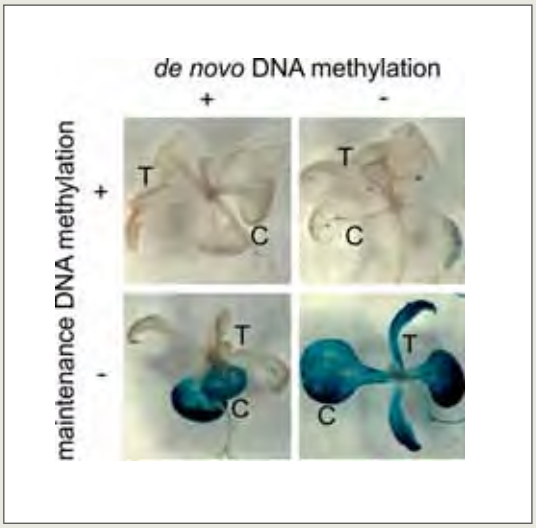
Genome and epigenome evolution

Extant plant genomes are the result of evolutionary processes during which DNA sequences have been added, removed, changed and corrected. We wish to understand these events at tithe molecular level and estimate their relative contributions to genome evolution. We specifically focus on the analysis of the effects of environmental factors such as solar UV-B radiation and heat on genome stability, the epigenetic control of repetitive DNA elements, the amplification of protein-coding genes using retrotransposon machineries, and characterization of the DNA damage repair system in plants.

Apical meristems are the hotspots of chromatin control in Arabidopsis

In plants, transposable elements are rendered inactive by transcriptional gene silencing, which is established by RNA-directed DNA methylation (RdDM) and maintained by repressive chromatin marks. We have identified a novel apical meristem-specific silencing function that reinforces silencing of transposons during early vegetative growth and is mediated by increased expression of silencing factors in the shoot apices. This function is implemented via the redundant action of both maintenance and RdDM silencing pathways (Figure 1). Such checkpoints are found in all apical meristems and ensure correct epigenetic inheritance during the transition from the vegetative to the reproductive phase and to the progeny.



Tissue-specific control of transcriptional gene silencing. The blue colour indicates activation of the reporter gene controlled by DNA methylation. While chemical interference with maintenance methylation is sufficient to activate the reporter gene in cotyledons (C), additional inactivation of de novo DNA methylation is required for bypassing the shoot apical meristem silencing center and for activation in true leaves (T)

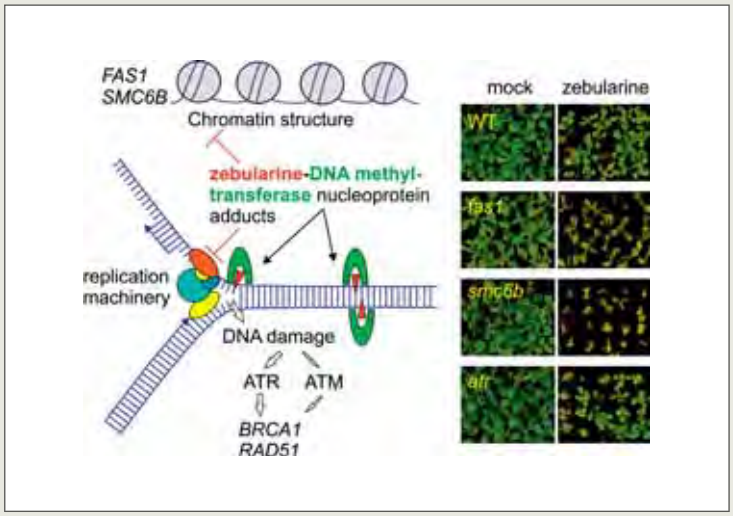
Figure 1

DNA damage repair of nucleoprotein adducts in plants

DNA damage repair is one of the essential functions required for genome stability. We use the DNA methylation inhibitor zebularine to study one specific type of genome damage. Zebularine is incorporated into DNA and forms a covalent complex with DNA methyltransferases, a nucleoprotein adduct which presents a physical barrier to the progression of DNA and RNA polymerases. However, it is currently not known how such adducts are repaired. We showed that they up-regulate DNA damage repair genes by activating the key signalling component ATAXIA TELANGIECTASIA MUTATED AND RAD3 RELATED (ATR) and to a smaller extent ATAXIA TELANGIECTASIA MUTATED (ATM) kinase-dependent signalling. The damage was

We aim to understand the mechanisms that add, remove, correct and control DNA sequences within plant genomes

Ales Pecinka



The model for DNA damage repair of zebularine-induced nucleoprotein adducts. The repair is ATR-dependent and the damaging effects are enhanced in mutants with disturbed chromatin.

Figure 2

G2-M phase specific and, together with the sensitivity of specific mutants to zebularine this pointed to a replication-coupled predominantly ATR-dependent mode of DNA damage repair by homologous recombination and base excision repair (Figure 2). Extreme sensitivity of *STRUCTURAL MAINTENANCE OF CHROMATIN 6B (SMC6B)* mutants to zebularine suggests the importance of the SMC5-SMC6 complex for repair of this type of damage by a so far unknown mechanism. Furthermore, the phenotype was aggravated in mutants with disturbed chromatin structure. Hence, we shed first light on the repair of nucleoprotein adducts in plants.

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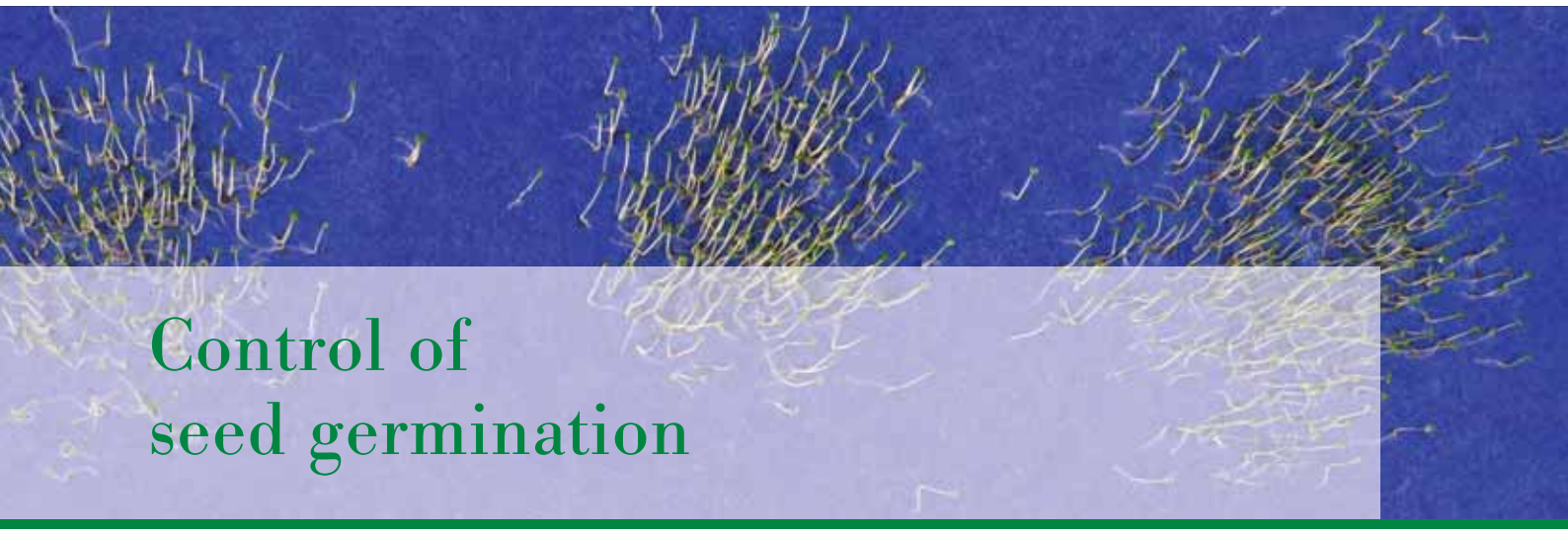


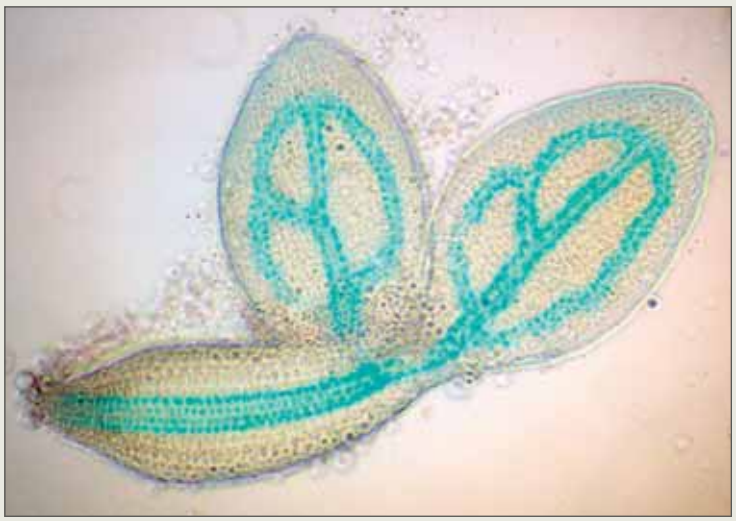
Control of seed germination

The timing of germination is controlled by seed dormancy and longevity. These traits are essential in the adaptation of plants to their environment, and constitute important agricultural properties that contribute to future food security. Seed dormancy is defined as the incapacity of a viable seed to germinate under (temporarily) favourable conditions and determines the moment at which a seed germinates. Seed longevity is operationally defined as seed viability after dry storage. Dormancy is induced during seed development and relieved by dry storage or imbibition at low temperatures. Genetic and physiological studies have identified an essential role for abscisic acid in the induction of dormancy during seed maturation, whereas gibberellins are required for germination. Our main research goal is to understand dormancy and longevity at the molecular level. We have discovered some of their basic components in the model plant Arabidopsis and started to explore their roles in crops and in the adaptation of plants to their environment.

Key regulators of dormancy

Our initial research was directed towards the identification of dormancy and longevity regulators by cloning and analysing quantitative trait loci and mutants that influence seed dormancy and longevity in Arabidopsis. This led to the isolation of several genes. Two of these turned out to be specific and key regulators of dormancy, whereas the others have more general roles in plant development.

The two key dormancy genes, *DELAY OF GERMINATION 1 (DOG1)* and *REDUCED DORMANCY 5 (RDO5),* represent our primary research focus. Present data suggest that DOG1 and RDO5 act in two separate pathways. Both genes have a seed-specific expression pattern (Figure 1), their mutants are non-dormant and do not show additional phenotypes. The DOG1 protein is conserved throughout the plant kingdom and accumulates during seed maturation. Its abundance in freshly harvested seeds strongly

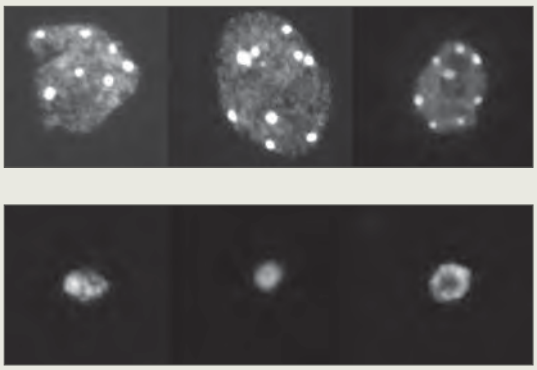


DOG1 promoter activity in a mature Arabidopsis embryo, visualized by GUS activity driven by the *DOG1* promoter.

Figure 1

The finding that DOG1 protein becomes modified during seed storage is an important step towards understanding dormancy release

Wim Soppe



Seed nuclei at the beginning of seed maturation (top panel) are bigger than at the end of seed maturation (bottom panel). All nuclei are stained with DAPI and shown at the same scale.

Figure 2

correlates with the duration of dormancy. We have recently shown that the DOG1 protein is modified during seed storage, leading to reduced functionality. This finding is an important step towards an understanding of the release of dormancy during seed storage.

Chromatin remodelling in seeds

Several of the dormancy genes that were cloned and studied in our research group play a role in chromatin remodelling. This aroused our interest in the nature and role of chromatin organization and epigenetic regulation in seeds. A detailed study, carried out in our lab, revealed a strong decrease in nuclear size during seed maturation in Arabidopsis, which occurs simultaneously with increased compaction of chromatin (Figure 2). Nuclei return to their original state during germination.
We plan to determine the relationships between the different dormancy genes and proteins and how they interact with environmental factors. This will lead to a model of the pathways and mechanisms that are involved in this process and should enable predictions and manipulations of seed dormancy levels in crop plants.

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Shoot branching in seed plants

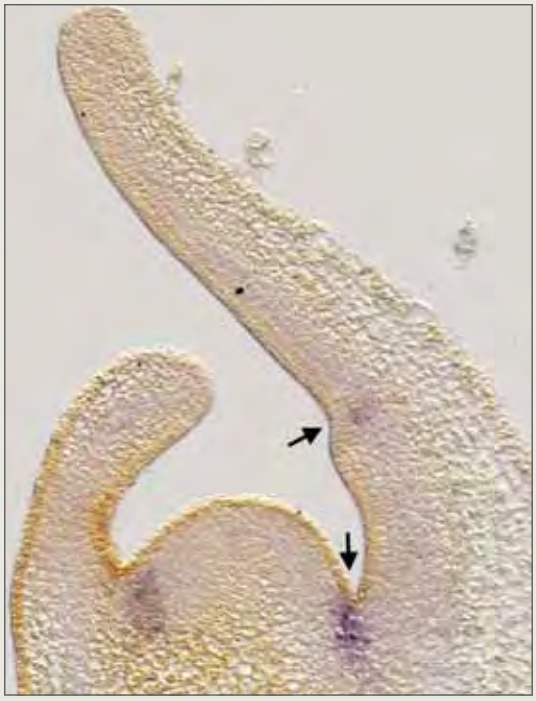
The aerial architecture of seed plants is determined to a large extent by the branching patterns of their shoots and the complexity of their leaves. Shoot branching is initiated by the formation of meristems in the axils of leaves, which develop into side-shoots. The aim of our work is to understand the molecular mechanisms that regulate axillary meristem (AM) formation. We are using Arabidopsis and tomato as model systems for our studies.

LATERAL SUPPRESSOR (LAS/Ls) expression is controlled by a 3' enhancer

The precise definition of organ boundaries is an essential step in the formation of new organs and in meristem initiation. *LAS/Ls* is expressed in a well-defined domain at the adaxial boundary of leaf primordia (Figure 1). To extend our understanding of how the highly specific transcript accumulation patterns at organ boundaries are established, we investigated the Arabidopsis *LAS* promoter. Our studies revealed that an essential enhancer element is situated about 3.2 kb downstream of the *LAS* open reading frame. This enhancer is sufficient to confer promoter specificity. Promoter-swapping experiments demonstrated that the *LAS* enhancer also has suppressor functions, largely overriding the activity of different 5´ promoters. Phylogenetic analyses suggest that *LAS* function and regulation are evolutionarily highly conserved.

Shoot branching and complex leaf development in tomato use homologous gene modules

Potato leaf (C), a key regulator of leaf dissection, was identified as the closest paralogue of the shoot branching regulator *Blind (Bl)*. Comparative genomics revealed that these two R2R3 MYB genes are orthologues of the Arabidopsis branching regulator *RAX1*. Expression studies



Pattern of *Ls* transcript accumulation (arrows) in a vegetative tomato shoot apex.

Figure 1

and complementation analyses indicate that these genes have undergone sub- or neofunctionalization due to promoter differentiation. *C* acts in a pathway that is independent of other leaf dissection regulators. Furthermore, the known leaf complexity regulator *Goblet (Gob)* is crucial for AM initiation and acts in parallel to *C* and *Bl*. Finally, RNA in situ hybridization revealed that the branching regulator *Ls* is also expressed in leaves (Figure 1). All four boundary genes, *C, Bl, Gob* and *Ls,* may act by suppressing growth, as indicated by the phenotypes of gain-of-function plants.

Complex leaf development and shoot branching rely on a conserved mechanism that regulates the morphogenetic competence of cells

Klaus Theres

Growth habit of a *trifoliate (tf-2)* tomato plant.

Figure 2

Trifoliate (Tf) modulates leaf and shoot architecture in tomato

The tomato gene *Tf* affects the formation of leaflets in the compound tomato leaf and the initiation of axillary meristems (Figure 2). *Tf* encodes a MYB transcription factor related to the Arabidopsis LOF1 and LOF2 proteins. *Tf* is expressed in the leaf margin, where leaflets are formed, and in the leaf axil, where axillary meristems initiate. During tomato ontogeny, expression of *Tf* in young leaf primordia increases, correlating with a rise in leaf dissection. Formation of leaflets and initiation of axillary meristems can be traced back to groups of pluripotent cells. Tf function is required to inhibit differentiation of these cells, thereby maintaining their morphogenetic competence. KNOTTED1-LIKE proteins, which are known regulators of tomato leaf dissection, require Tf activity to perform their functions in the basal part of the leaf. Similarly, the plant hormone auxin needs Tf activity to initiate formation of lateral leaflets.

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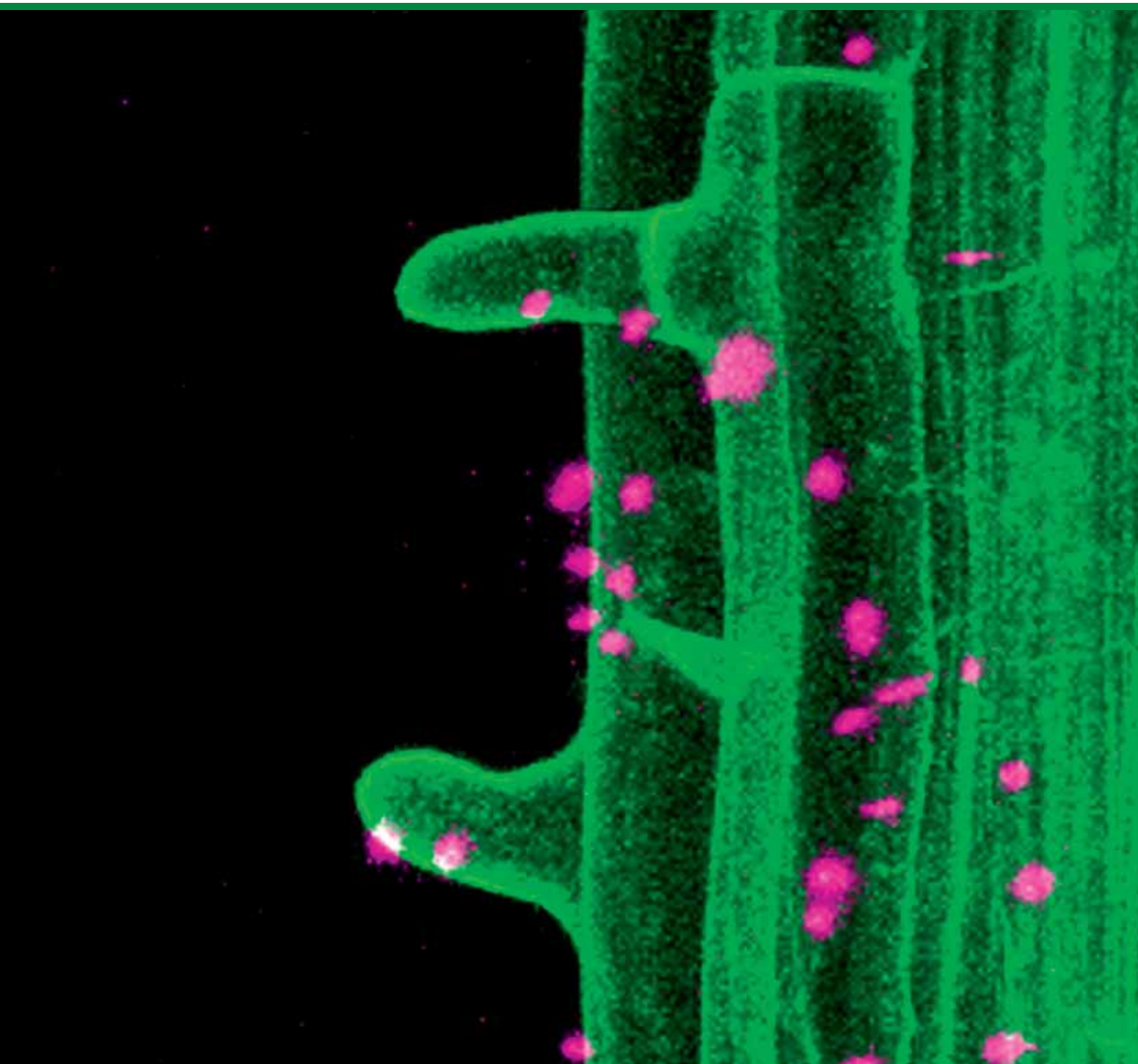


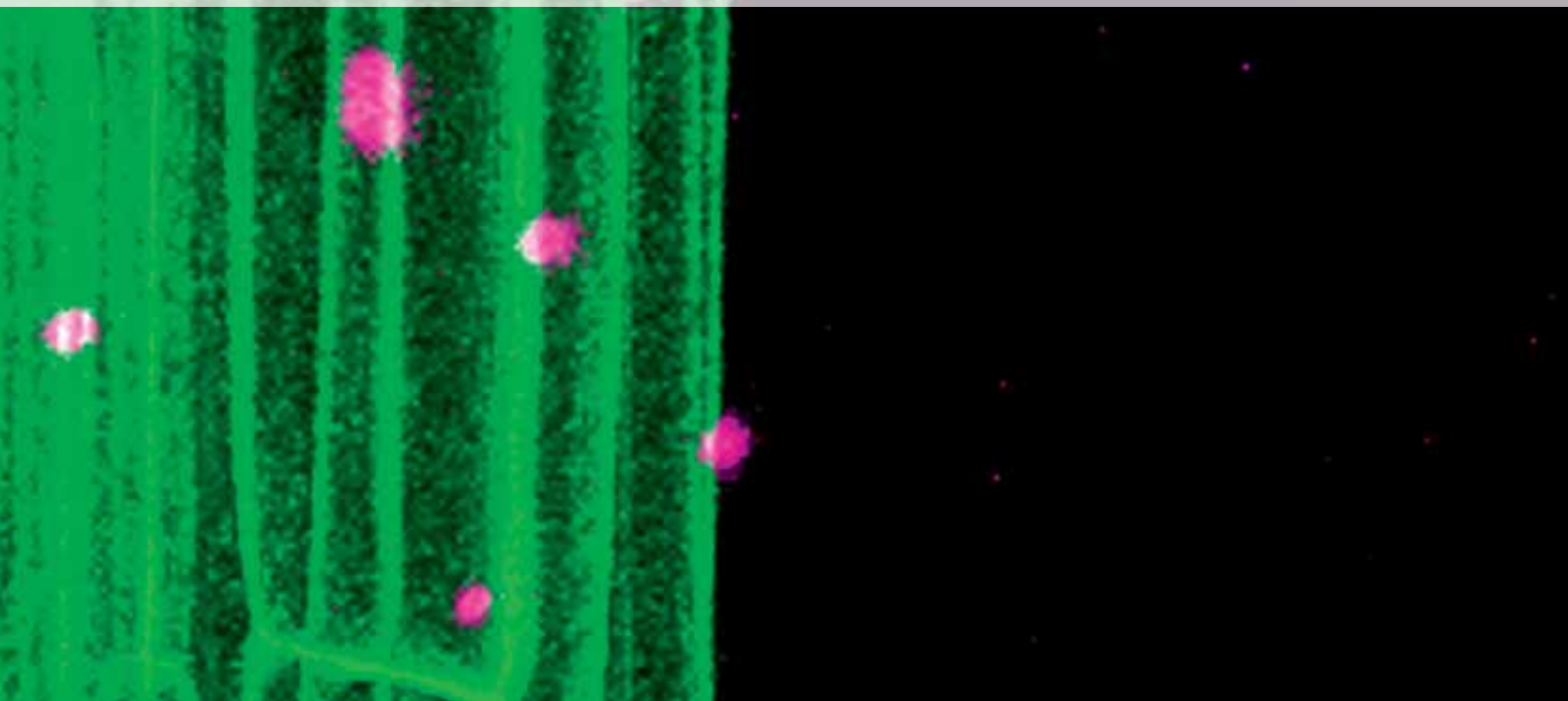
Department of Plant Microbe Interactions

Director: Paul Schulze-Lefert

Research in the Department of Plant-Microbe Interactions focuses on fundamental molecular processes underlying interactions between plants and microbes. The innate immune system of plants, mechanisms of microbial pathogenesis and functions of the plant microbiota have a central role in our discovery programme.

Although plants have evolved an elaborate innate immune system that ensures effective protection against most microbial pathogens, some intruders succeed in colonizing plants and causing disease. One goal of our research is to define a regulatory network of the plant immune system that has predictive power. This should provide insights into how components of the plant immune system can be modified to improve plant protection using molecular breeding techniques.

In nature, healthy plants host a staggering diversity of seemingly asymptomatic bacteria on and inside plant organs such as leaves and roots. This bacterial community is called the plant microbiota and provides diverse functions, including indirect protection against microbial pathogens. Members of the root microbiota serve additional host functions by acquiring nutrients for plant growth from soil. A deeper understanding of the establishment and functions of plant microbiota is needed for the development of rational probiotics to improve plant growth and crop health.

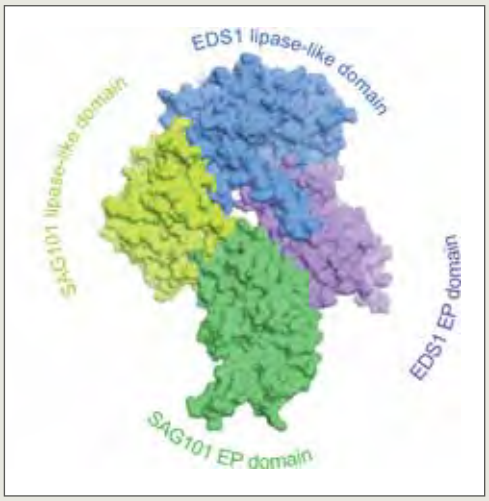


Resistance pathway dynamics in plant immunity

We are studying how plants regulate their innate immunity pathways. Co-evolution between infectious pathogens and their plant hosts determines the outcome of disease or resistance, often by setting defence thresholds and the level of transcriptional reprogramming. It is in the plant's interest to maintain tight control over its induced resistance programmes because there is a significant trade-off between immunity and growth. An intricate stress response network is in place to balance disease resistance with the need to respond effectively to other environmental challenges, such as drought, temperature or competition for space. Decision-making within this homeostatic framework is likely to be important for plant fitness in nature. We are interested in understanding fundamental processes governing resistance pathway dynamics and the evolutionary forces that shape plant adaptation to changing environments and pathogen pressures. For this, we study interactions between geographical accessions of the model plant *Arabidopsis thaliana* and a variety of biotrophic pathogenic strains that either cause disease (i.e. are not recognized by intracellular immune receptor systems) or trigger innate immune responses through receptor recognition and activation of antimicrobial pathways.

Molecular underpinnings of Arabidopsis post-infection immunity

Immunity to infectious biotrophic pathogens is governed by a large, polymorphic family of intracellular receptors (NLRs), members of which detect the physiological impact of particular pathogen effectors. Many questions remain about the nature and scope of receptor activation events and how receptors connect to a rather conserved set of downstream pathways. Our recent research has focused on a sub-family of NLRs called TNLs that converge on a set of immune regulatory complexes whose central component is the nucleocytoplasmic, lipase-like protein EDS1. We have started to identify molecular



The crystal structure of an *Arabidopsis* EDS1-SAG101 immune signalling complex provides important new insights into the regulation of disease resistance in plants.

Figure 1

Immune receptor pathways converge, in different ways, on the nuclear transcription machinery, for effective disease resistance.

Jane Parker

links between TNLs and EDS1, and have evidence for receptor signalling complexes operating inside nuclei to transcriptionally reprogram cells. We are in the process of purifying *in vivo* TNL receptor complexes and identifying functional partners by mass spectrometry. We are also applying chromatin-immunoprecipitation (Ch-IP) coupled to next-generation DNA sequencing and RNA expression profiling to trace dynamic changes in TNL receptor complexes on and off the chromatin during activation of defence responses. This should allow us to map transcription events that are decisive for cellular reprogramming. An important recent breakthrough complementing these studies was the determination, in collaboration with Karsten Niefind's group in the Institute of Biochemistry at the University of Cologne, of the crystal structure of a complex between EDS1 and one of its signalling partners, SAG101 (Figure 1). Structure-function analysis of EDS1-SAG101 complexes provides a new level of understanding of how such a central regulatory node links NLR receptors to immunity outputs.

Evolutionary forces shaping NLR maintenance and diversification

We are now attempting to fit the above molecular mechanisms into the context of NLR evolution. *Arabidopsis* accessions exhibit remarkable plasticity in coping with changing conditions and limiting NLR resistance pathway activation. Such plasticity is likely to facilitate the evolutionary diversification of NLR receptors. Together with Rubén Alcázar in the Department of Plant Breeding and Genetics at MPIPZ (now at Barcelona University) we are studying the processes underlying immune-related hybrid incompatibility (HI) in *Arabidopsis,* a phenomenon that arises in crosses between genetic accessions and is often caused by deleterious interactions between different allelic forms of immune-related genes, potentially driven by local adaptation (see title picture). By following the distribution of the causal variants in *Arabidopsis* populations we can piece together how their different evolutionary paths are determined.

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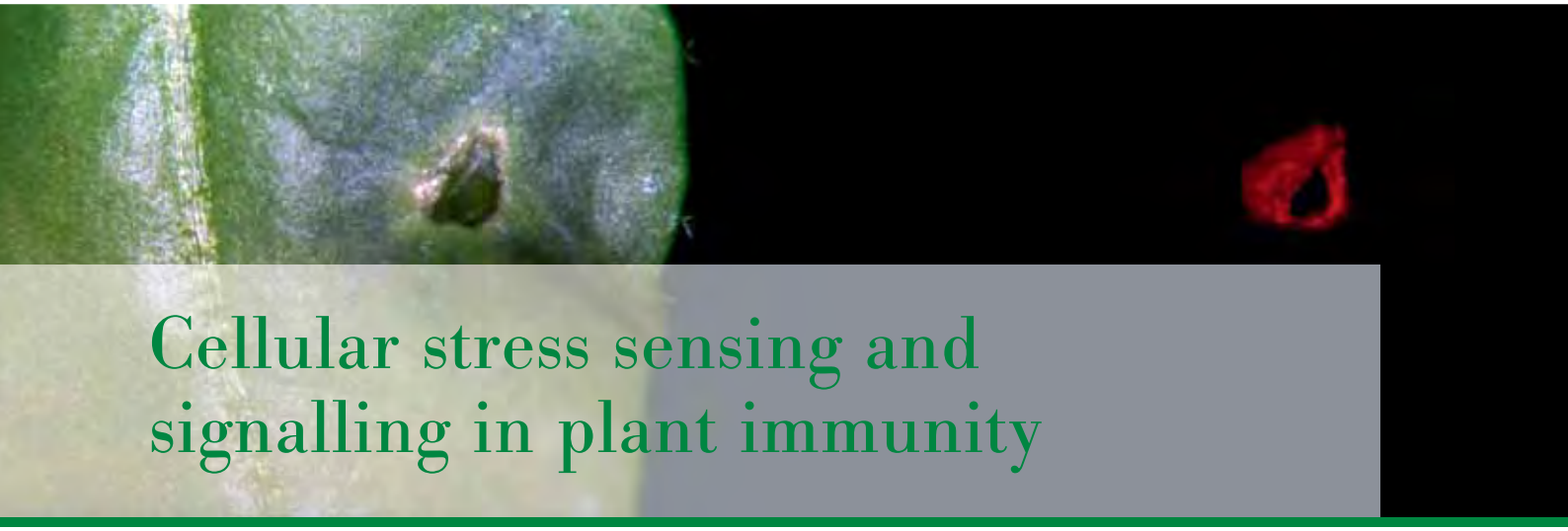


Cellular stress sensing and signalling in plant immunity

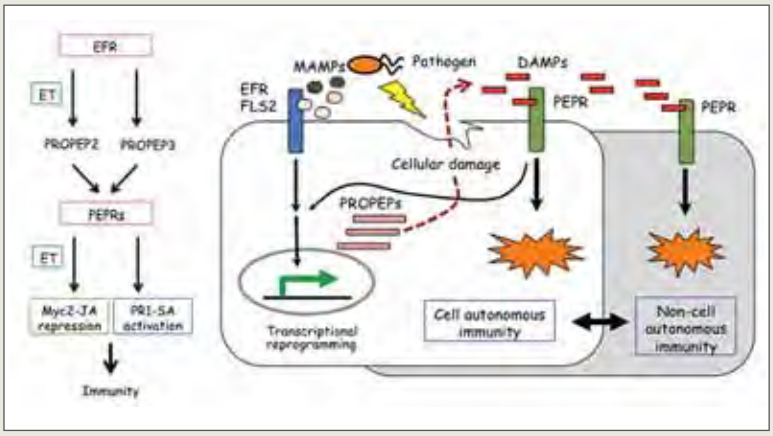
Plants recognize micro-organisms by cell-surface detection of molecular signatures typical of the microbes themselves, termed microbe-associated molecular patterns (MAMPs), or by sensing changes in their own cellular states upon microbial challenges (danger-associated molecular patterns or DAMPs). MAMPs include flagellin (flg22 epitope) and the elongation factor EF-Tu (elf18 epitope) of bacteria, which are perceived by the Leu-rich repeat (LRR) receptor kinases (RKs) FLS2 and EFR, respectively. DAMPs include the Pep epitope of endogenous PROPEP polypeptides that is perceived by the LRR-RKs PEPR1 and PEPR2.

In good agreement with the similarities in module structure between these pattern receptors, perception of different MAMPs/DAMPs triggers a stereotypic set of cellular outputs, and leads to an enhanced state of immunity that terminates or restricts the growth of potentially infectious microbes. This so-called pattern-triggered immunity (PTI) plays a critical role in protecting plants from numerous pathogenic microbes in the environment.

However, the molecular mechanisms that link MAMP/DAMP perception to effective activation of plant immunity remain poorly understood.

Signal integration between biotic and abiotic stress responses

In the reference plant *Arabidopsis thaliana,* PTI activation is accompanied by suppression of the synthesis of flavonoids that otherwise act as antioxidants and/or sunscreen pigments to protect plants from damage due to oxidative and light stress. In Serrano et al. (2012), we proved the functional significance of the MAMP-triggered repression of flavonoid accumulation. In *transparent testa4* plants that are devoid of flavonoids, we found a significant increase in flg22-triggered activation of pathogenesis-related (PR) genes and basal immunity to bacterial infection. This suggests that flavonoid repression has a positive influence on salicylate (SA)-based immunity in PTI. Moreover, our chemical screens identified nine small molecules that allow flavonoid accumulation in the presence of flg22. One



Our molecular genetic work suggests that MAMP receptors and DAMP receptors functionally interact with each other in a layered signalling network. Ethylene-dependent and -independent ligand generation upon MAMP signalling (left) seems to ensure robust engagement of the PEPR pathway during PTI, which would help plants to deal with perturbations of defence hormone signalling by pathogens.

Figure 1

How the plant immune system senses "danger signals" associated with the presence of microbes represents a central question in diverse plant-microbe interactions

Yusuke Saijo

of these compounds uncouples flavonoid repression and *PR* gene activation from the activation of reactive oxygen species, MAPKs, and callose deposition, which points to a close link between the two types of output in PTI. Together, our findings suggest that MAMP-induced repression of flavonoid accumulation plays a role in relieving the inhibitory effects of flavonoids on PTI activation.

Layered pattern receptor signalling in plant immunity

The ease and robustness of MAMP-induced flavonoid suppression in Arabidopsis seedlings has prompted us to use this readout in our genetic screens for MAMP hyporesponsive mutants. We have assembled a collection of *priority-in-sweet-life (psl)* mutants that allow the accumulation of anthocyanins, a subclass of flavonoids, in the presence of elf18 or flg22 (Saijo et al., 2009; Lu et al., 2009). In Tintor et al. (2013), we described *psl6* mutant plants that are impaired in both flg22- and elf18-induced outputs, including repression of anthocyanins. *PSL6* corresponds to *EIN2*, which encodes the master regulator of ethylene (ET) responses, pointing to a critical role for ET in PTI signalling. We confirmed that FLS2 expression is greatly reduced in *psl6/ein2* plants as previously described, but also found that expression of *EFR* (which encodes the EF-Tu receptor) and EFR accumulation remain largely unaffected. This allowed us to further investigate a role for ET in PTI, downstream of the MAMP receptor. Our discovery that *PROPEP2* is among the genes induced by elf18 in an ET-dependent manner led to the hypothesis that ET-dependent activation of *PROPEP2,* and thus that of the PEPR pathway, represents a critical mechanism by which ET contributes to PTI. In agreement with this idea, we showed that PEPRs are required for elf18-induced *PR1* activation and anti-bacterial immunity. This study thus provides compelling evidence that ET-dependent control of PTI signalling occurs in part through the PEPR pathway. However, we also showed ET-independent activation of *PROPEP3* in response to elf18, pointing to an ET-independent role for the PEPR pathway in EFR-triggered immunity. Hence both ET-dependent (via PROPEP2) and ET-independent (via PROPEP3) branches contribute to the activation of PEPR signalling downstream of EFR, which might ensure robust PEPR activation during PTI. Together with related studies by other labs, our findings confirm a previously suspected role for the PEPR pathway as an amplification mechanism in local PTI signalling. We now plan to decipher how the PEPR pathway acts in DAMP sensing and signalling.

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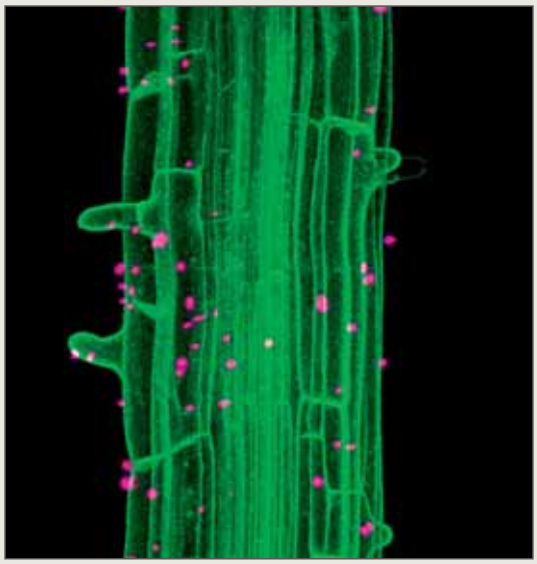
Plant innate immunity and the plant microbiome

Molecular research during the past decade has revealed the existence of an elaborate innate immune system in plants that recognizes 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 detect 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 is defined by the nucleotide-binding domain and leucine-rich repeat-containing protein family (NLR). Upon delivery of isolate-specific pathogen effectors into host cells, NLRs detect either the effector structures or their functions. Healthy plants in nature also host a huge diversity of commensalistic and mutualistic microorganisms, called the plant microbiota. How the innate immune system discriminates pathogenic from beneficial microbiota members is not known.



A gene encoding a fluorochrome (RFP, magenta-colored) was introduced into a rhizobacterium, and the resulting strain was used to inoculate gnotobiotic Arabidopsis plants in which the plasma membranes were labelled with another fluorochrome (YFP, green-colored). Bacterial colonization of the root surface was visualized by confocal laser scanning microscopy and is shown as a 3D projection.

Figure 1: Colonization of the Arabidopsis root surface by soil-derived rhizobacteria.

How NLR receptors initiate disease resistance signalling

The *Mla* locus in barley encodes allelic variants of NLRs with a coiled-coil (CC) domain at the N-terminus. Each of these MLA receptor variants detects a corresponding isolate-specific effector (AVRMLA) produced by the grass powdery mildew fungus *(Blumeria graminis f. sp. hordei)* and initiates powerful immune responses, linked to a host cell-death response, that terminate fungal pathogenesis. Barley MLA is fully functional in the model plant *Arabidopsis thaliana,* implying ~200 million years of evolutionary conservation of the underlying immune mechanism. Cytoplasmic and nuclear pools of MLA have been identified, and nucleocytoplasmic partitioning of the receptor appears to be needed to initiate a bifurcated signal transduction cascade upon AVRMLA detection: a host cell-death response in the cytoplasm and disease resistance signalling in the nucleus. The nuclear MLA pool initiates disease resistance signalling through direct interactions with WRKY and MYB transcription factors, but the cyto-

Understanding the innate immune system of plants and the plant microbiota is a key to the development of innovative ways to improve plant health and growth

Paul Schulze-Lefert

plasmic targets of activated MLA remain to be identified. Our structural and biochemical work revealed that a CC domain-dependent homodimerisation of the immune sensor is necessary for receptor function. The CC homodimer module defines a minimal functional unit, which is sufficient to activate host cell-death signalling. A complete mechanistic understanding of MLA-triggered immunity will aid future development of synthetic NLR-type receptors with novel pathogen-recognition specificities.

Structure and functions of the bacterial root microbiota

Remarkably little is understood about plant-microbe interactions that are, at first sight, 'asymptomatic'. These poorly studied plant microbiomes harbor an unknown reservoir of probiotic and plant-protective associations. We have applied bacterial 16S rRNA gene profiling to characterize and compare soil and root-inhabiting bacterial communities of *Arabidopsis thaliana*. Roots are preferentially colonized by soil-derived Proteobacteria, Bacteroidetes and Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. We have examined the evolutionary diversification of the bacterial root microbiota in relatives of *A. thaliana* and shown that microbiota diversification is driven by host species-specific ecological adaptation and host phylogenetic distance. In addition, this identified a core of only a few bacterial families, which is conserved over at least 30 Myr of evolutionary time and across biogeographical host specialization. We have isolated >60% of the root microbiota members from *A. thaliana* as pure bacterial cultures, permitting whole-genome sequencing and *in silico* analysis of the root microbiome. This makes it also feasible to test synthetic bacterial communities for contributions to plant growth and protective functions against pathogenic microbes in interactions with gnotobiotic *A. thaliana* plants. A deeper understanding of the organizational principles and biochemical activities of the root microbiota has potential for the development of rational probiotics that improve plant growth and health of crops.

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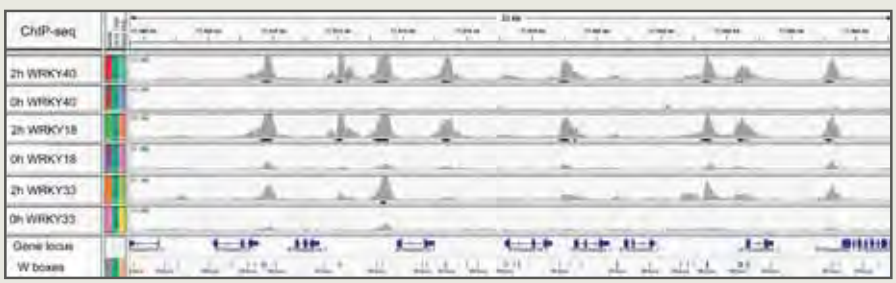
Transcriptional regulatory networks governing the plant immune response

Research over the past 20 years has uncovered many key molecular components essential in establishing a robust plant immune system. In general, dedicated surface receptors sense the presence of phytopathogens and transmit this information via an extensive signalling system to trigger a multitude of defence responses. Basically, one distinguishes between two branches of the plant innate immune system termed PTI (PAMP-Triggered Immunity) and ETI (Effector-Triggered Immunity). PTI is initiated by pathogen associated molecular patterns (PAMPs), conserved molecular features of various microbes. As a counter measure microorganisms secrete effector proteins into host cells that intercept and attenuate PTI. 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 temporal rather than qualitative, indicating that pathogens trigger a common or highly interconnected plant signalling network. A major consequence of the signalling process is massive transcriptional reprogramming within the host cell. The graded transcriptional responses associated with immunity however clearly indicate the existence of a complex regulatory circuitry comprised of transcriptional activators and repressors that fine-tune gene expression. Transcriptional reprogramming is mainly achieved by the action of transcription factors (TFs) that modulate the defence transcriptome. In particular, one TF family, designated WRKY, plays a broad and pivotal role in regulating host defences. The current challenge is to define how selective members of this TF family mechanistically fulfil their functions.

Projects

WRKY transcription factors as positive and negative regulators of plant immunity

Our research is centered on elucidating how Arabidopsis WRKY factors reprogram the host transcriptome upon pathogen challenge. Two kinds of interactions are being studied. 1) WRKY18 and WRKY40 act redundantly in negatively regulating host responses towards the powdery mildew *G. orontii,* while promoting ETI resistance to the bacterium *P. syringae* (M. Schön). 2) WRKY33 plays a key positive role in mediating resistance towards the necrotroph *B. cinerea* (Dr. R. Birkenbihl; S. Liu). Expression of all three WRKY factor genes is strongly induced by PAMPs but their selective functions in PTI remain unclear. Comparative expression studies revealed that hundreds of genes are transcriptionally affected by these three TFs. Transgenic lines expressing functional tagged versions of the respective WRKY TFs were used to define direct in vivo target genes on a global scale by means of chromatin im-

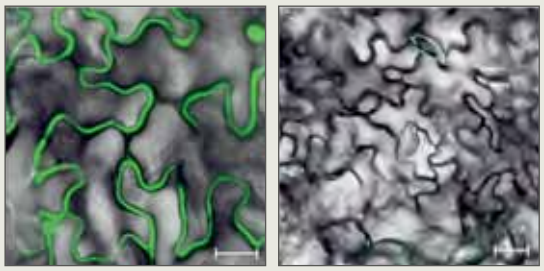


Plot showing PAMP-dependent binding of WRKY factors (peaks) to specific gene loci on Arabidopsis chromosome 4.

Figure 1

Unravelling the transcriptional networks engaged in the plant immune response remains a daunting task

Imre E. Somssich



Transgenic Arabidopsis plant expressing a gene tagged with GFP whose PAMP-dependent activation requires three intact WRKY factor binding sites (W boxes; left panel) within its promoter, compared to a similar plant expressing the same transgene but with the three W boxes mutated (right panel). Scale bars, 20 µm.

Figure 2

munoprecipitation followed by sequencing (ChIP-Seq, Fig. 1). These data are being evaluated and require substantial bioinformatics support (Dr. B. Kracher).

Transient transfection assays uncover pathogen-responsive cis-regulatory DNA elements

We employ a well-suited parsley protoplast system to rapidly identify DNA promoter elements of PAMP-responsive genes. For this, promoters derived from plant genes are fused to a reporter gene (i.e. GFP) and introduced into the protoplasts. Protoplasts are stimulated by addition of PAMPs to the media and induced expression levels are monitored. Numerous promoter derivatives can be tested simultaneously, allowing thereby rapid delineation of critical DNA motifs required for PAMP-induced expression. With this method we defined key WRKY factor binding sites (W box motifs) in two Arabidopsis gene promoters that are essential for strong transcriptional activation upon stimulation with PAMPs. Generation of transgenic Arabidopsis plants carrying such reporter constructs confirmed the validity of this approach (E. Logemann, Fig. 2).

Future challenges

WRKY factors act in a complex regulatory network involving both positive and negative feedback loops. Besides identifying their direct target genes we will need to uncover WRKY-associated co-regulators and define the combinatorial interactions between other TFs and chromatin-regulatory proteins, all of which determine proper temporal and spatial transcriptional outputs.

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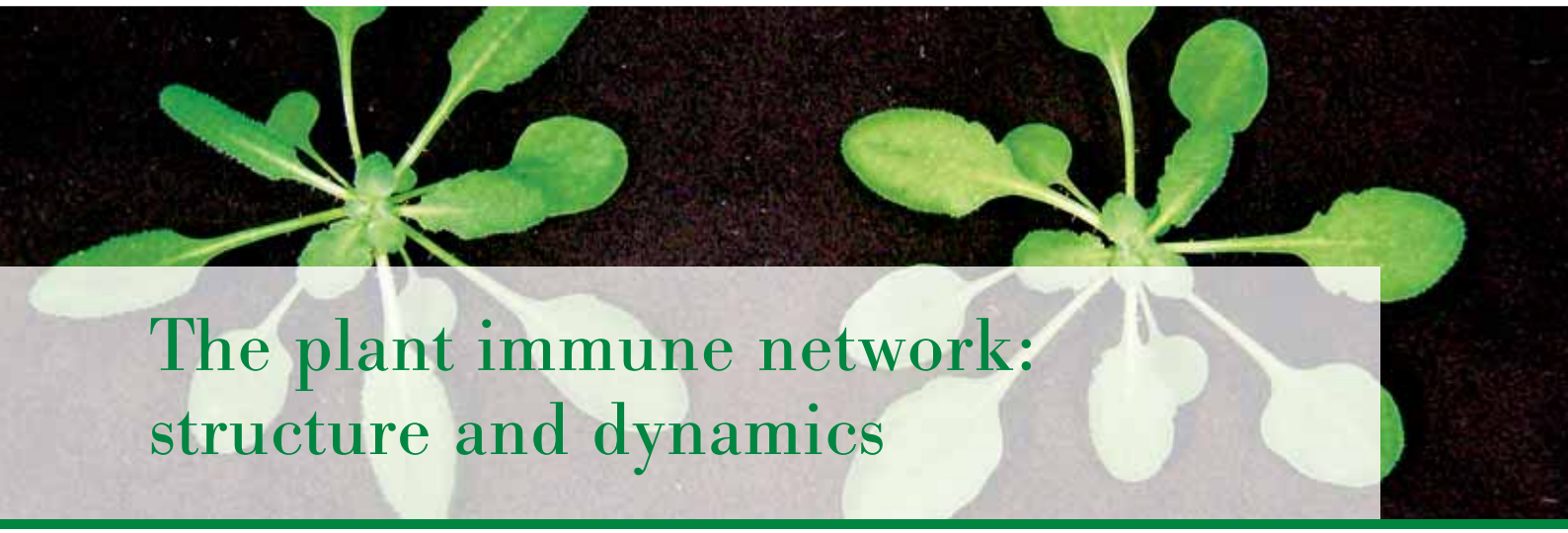
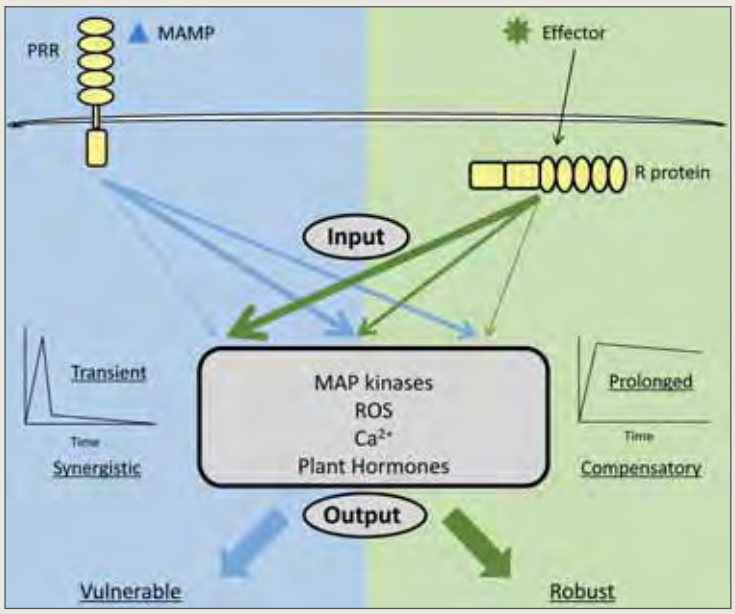


The plant immune network: structure and dynamics

The plant immune signalling network is complex, and how and why such complexity has arisen are important questions. Pathogens target components of the network to dampen immune responses. Therefore a simple network can be manipulated. Unwarranted immune responses have a negative impact on plant fitness. In nature, plants are beset by many microbes, and different immune responses are variably effective against different pathogens and impose different fitness costs. Thus, the spectra and strengths of immune responses must be appropriately tuned. Our major goal is to understand this complex immune network at the system and molecular level using Arabidopsis and the bacterial pathogen *Pseudomonas syringae*.

Building a dynamic model of the plant immune signalling network

Previous studies revealed that network components are extensively shared in different modes of immunity. How then do plants differentiate outputs using shared machinery? Characterization of the molecular processes that underpin the quantitative and dynamic nature of the immune signalling network is necessary to answer this question. The generation of time series of transcriptome data collected under multiple conditions is a powerful approach to disentangling complex biological processes, as it allows construction of dynamic gene regulatory networks. We are using next-generation sequencing technology for time-series transcriptome analysis, followed



Pathogen-derived molecules such as MAMPs and effectors are recognized by receptors, which triggers pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively. Signalling machinery employed in PTI and ETI is extensively shared, but is utilized differently in PTI and ETI, resulting in distinctive outputs.

Figure 1

We need systems approaches to understand the structure and dynamics of the plant innate immune network

Kenichi Tsuda

by network analysis, in order to characterize the dynamics of plant immune networks. In addition to defining the structure of the plant gene regulatory network, we are integrating transcriptome analysis of pathogens to construct a larger network that should help us to understand the complete web of plant-pathogen interactions.

Modulation of network properties by duration of MAPK activation

Our previous studies suggest that different types of plant immune responses share network components, but use them in different ways (Figure 1). During PTI, salicylic acid (SA) signalling, which regulates a major portion of the plant immune response, was required for the proper regulation of the vast majority of SA-responsive genes. However, during ETI, triggered by the effector AvrRpt2 (AvrRpt2-ETI), most SA-responsive genes were still as responsive in SA-deficient mutants as in the wild type, suggesting robust gene regulatory mechanisms during AvrRpt2-ETI. The activation of the two immune-related MAPKs, MPK3 and MPK6, persisted for several hours during AvrRpt2-ETI but less than one hour during PTI. Sustained MAPK activation was sufficient to confer SA-independent regulation of most SA-responsive genes. Furthermore, a double mutant deficient in both SA and MPK3 showed increased susceptibility during AvrRpt2-ETI although single mutations do not have much effect. These results indicate that the duration of the MAPK activation is a critical determinant for modulation of robustness of the immune signalling network and that the gene regulatory mechanism conferred by sustained MAPK activation is a source of robustness during ETI. Our aim is to unravel the molecular mechanism responsible for decoding information about the duration of MAPK activation and how plants regulate this parameter.

Mode of action of immune outputs for suppression of bacterial growth

Although much has been learned about pathogen recognition and plant signalling components, very little is known about which plant immune outputs affect bacterial activity and stop bacterial multiplication, or how they do so. We are now working to identify immune outputs that inhibit bacterial growth, and to elucidate the molecular basis of the suppression of bacterial activity.

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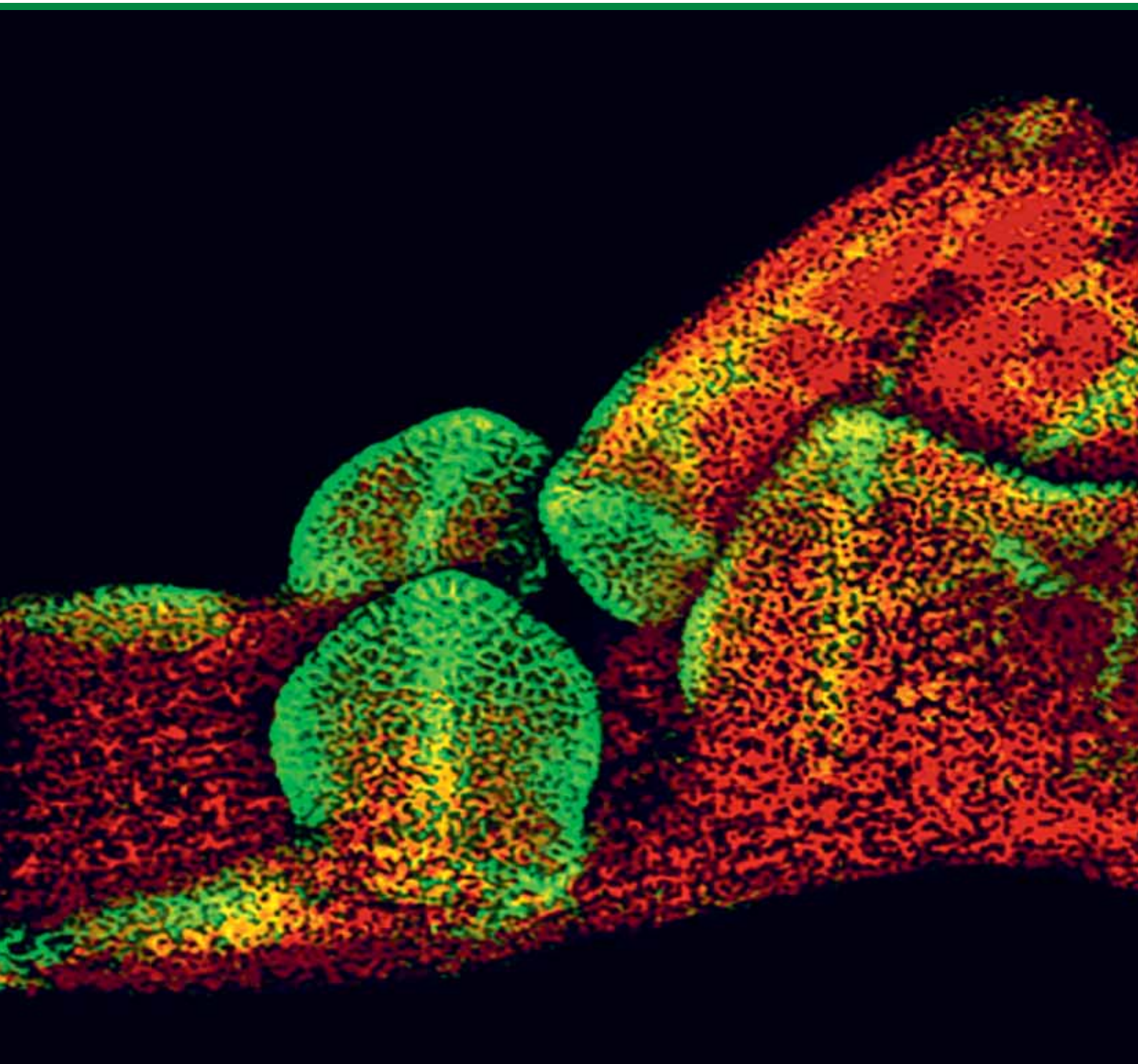
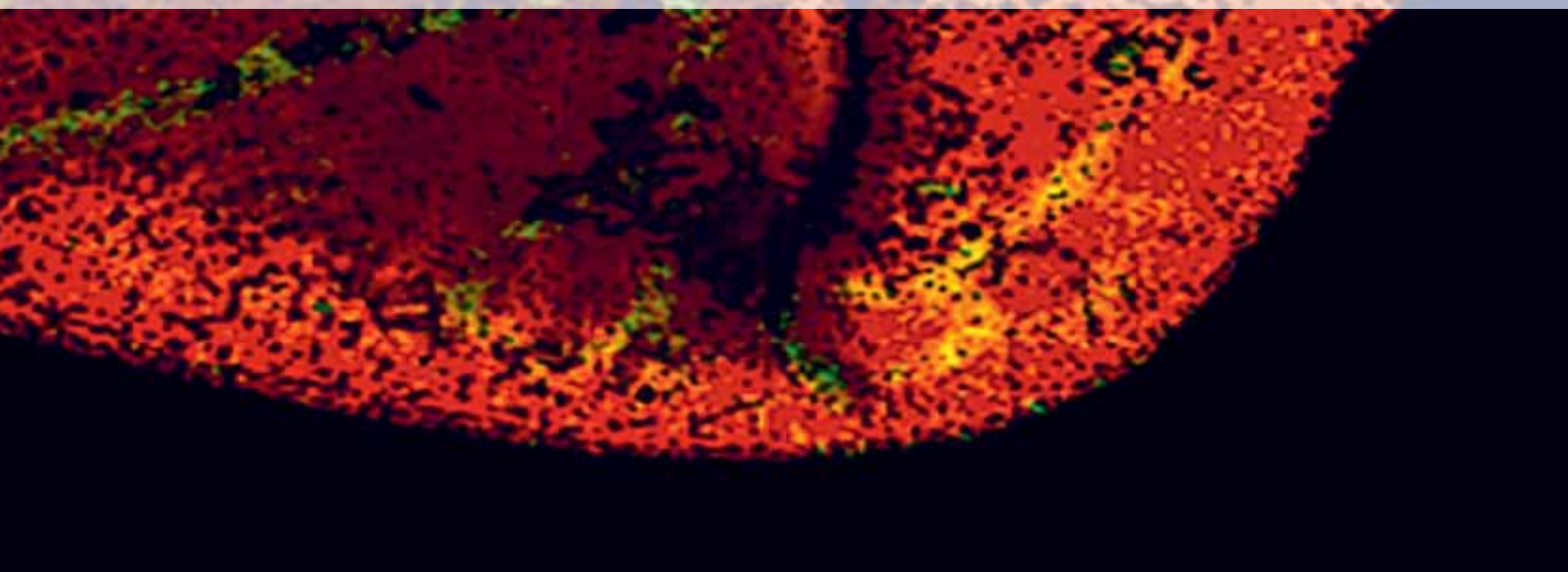


Department of Comparative Development and Genetics

Director: Miltos Tsiantis

Plants show considerable morphological variation in organ shape, size and number. Work in our department seeks to elucidate the basis for such variation. We address two fundamental questions: first, how do plants develop and grow into complex organisms starting from a fertilized egg and, second, how did plant form diversify through evolution? To answer these questions we work at the interface of developmental genetics, evolutionary biology and biomechanics. This combination of approaches helps us to identify genetic networks that underpin the generation of different aspects of morphology and understand how the balance between conservation and divergence of these networks generates diversity during evolution. However, one key challenge arises from the fact that an organism's form is determined by a cascade of processes that take place at different levels of organization, and yield the final form through complex feedback loops of genetic regulation, signalling, cell proliferation patterns, and tissue growth. Thus, it becomes increasingly difficult to conceptualize how the processes that influence growth and patterning are combined and integrated to produce organismal form. To resolve these issues and synthesize our biological findings, we use computational approaches to help reveal fundamental principles that govern the development and diversity of plant form. Our work is important for two reasons: By building a predictive framework that conceptualizes how biological forms develop and diversify, we attain a clearer understanding of the natural world and improve the knowledge base that underpins plant breeding.



Genetic basis of phenotypic evolution

We investigate the genetic basis of phenotypic differences between *Cardamine hirsuta* (an emerging model species) and its relative *Arabidopsis thaliana,* and explore the evolutionary processes underlying such differences. We focus on variation in petal number and explosive pod shatter, which are traits found in *C. hirsuta* but not in *A. thaliana,* and use parallel genetic studies to investigate the evolution of developmental processes controlling these traits.

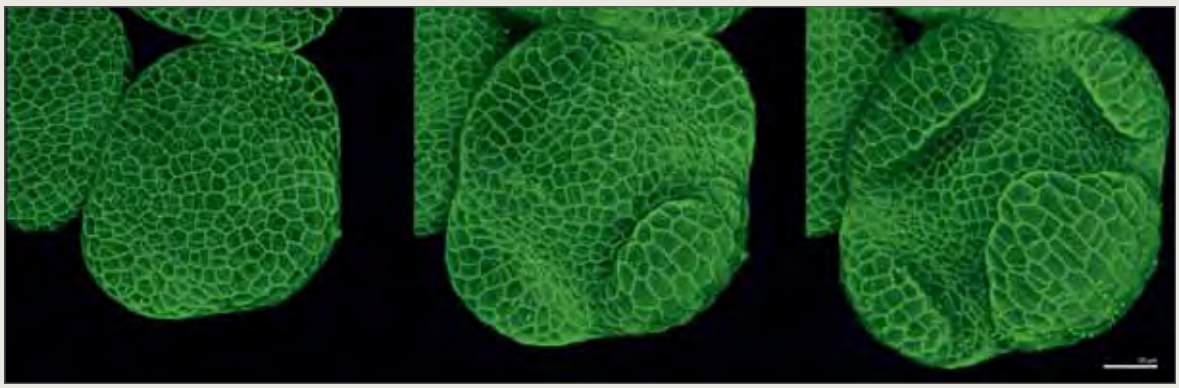
My group exploits the extensive experimental tools developed in *C. hirsuta* to ask how variation between *C. hirsuta* and *A. thaliana* in the organization of gene networks drives the evolution of fruit and floral structures. Our reasons for focusing on these morphologies are four-fold: first, these are rapidly evolving traits that can be compared between related but reproductively isolated species; second, the direction of evolutionary change in these morphologies is known; third, these traits probably have adaptive value for pollination and seed dispersal strategies and, finally, understanding these processes has potential translational value for Brassica crop improvement.

Petal number variation: Inter- and intraspecific variation

Petal number is constant in *A. thaliana* but variable in *C. hirsuta.* We find petal number variation not only between these two species but also within *C. hirsuta.* We capitalize on this inter- and intraspecific variation to address fundamental questions about evolutionary change: Are single gene changes sufficient to explain species-specific form? What are the precise genetic changes involved? How are genetic changes translated into phenotypic differences? Does inter- and intraspecific variation have the same genetic basis?

Explosive pod shatter: Genetics and mechanics of development

Explosive pod shatter in *C. hirsuta* is a ballistic seed dispersal mechanism that transfers stored mechanical energy from fruit tissues to the seeds, launching the seeds with such impetus that they disperse up to several metres away from the parent plant. In comparison, *A.*

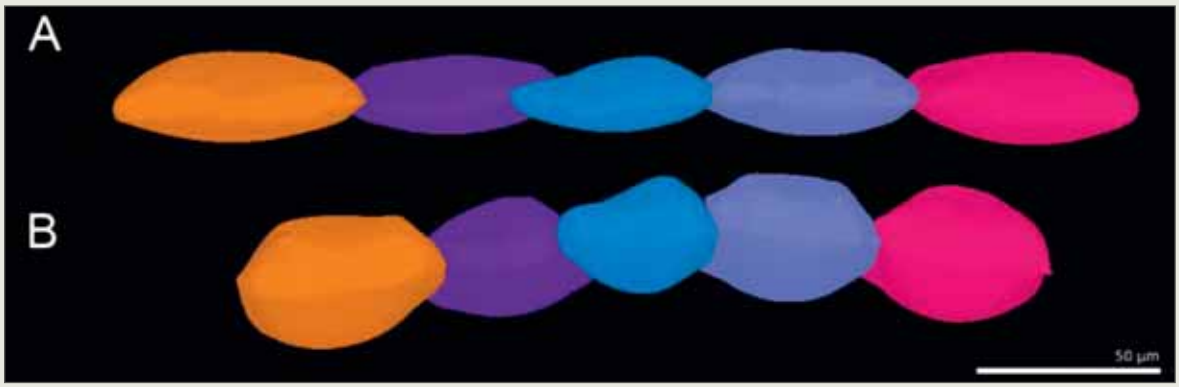


Here, we show three successive time points in the early development of a *C. hirsuta* flower. We use these images to track growth and gene expression dynamics in the space available for petal initiation between each sepal.

Figure 1: Time-lapse imaging of *C. hirsuta* floral organ growth

We use parallel genetic studies to investigate the evolution of developmental processes

Angela Hay



We model C. hirsuta fruit valves as mechanical bilayers that build elastic energy because tissue shrinkage in the outer layer is constrained by an inextensible inner layer. Here, we show outer layer cell shrinkage in the length dimension from pre- (A) to post-explosion (B).

Figure 2: Turgor-driven tissue shrinkage builds up energy for explosive pod shatter

thaliana pod shatter is non-explosive. Our aim is to understand how differences in fruit morphology evolved to provide these two species with dramatically different seed dispersal strategies.

We are taking a combined biological and computational approach to conceptualize how the interplay of genetic and mechanical regulation in the developing fruit is translated into explosive pod shatter. The outcome of this work will be predictive models of fruit development that capture the diverse seed dispersal strategies of *C. hirsuta* and *A. thaliana* in a quantitative fashion.

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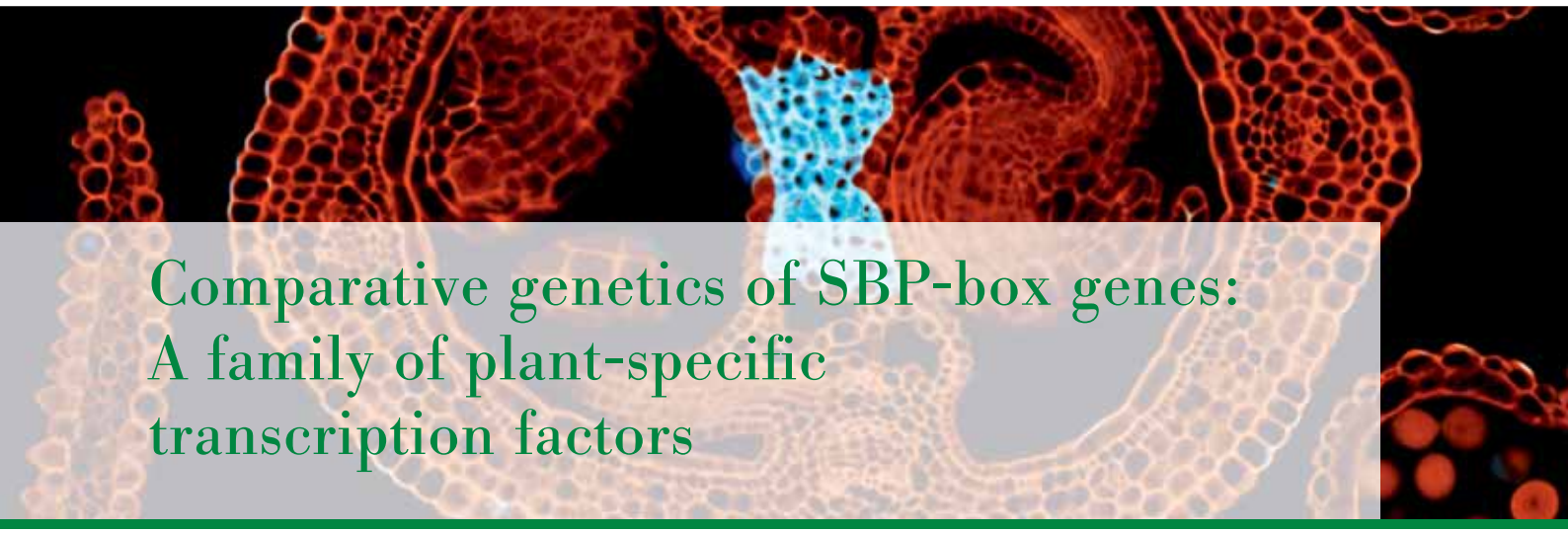
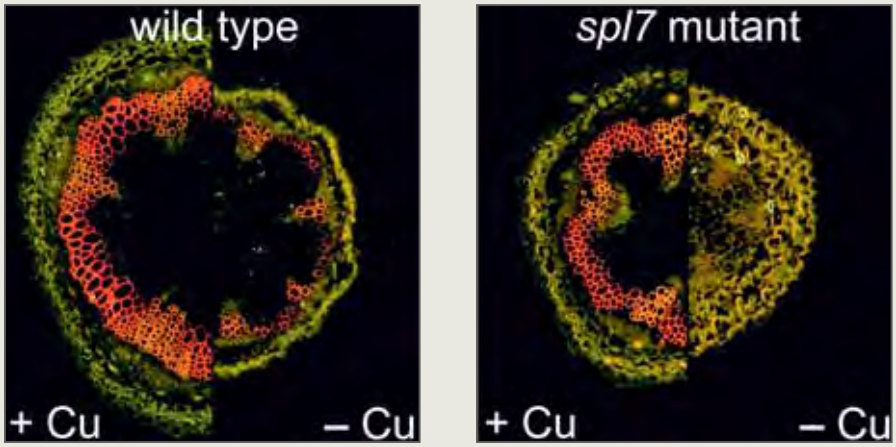


Comparative genetics of SBP-box genes: A family of plant-specific transcription factors

SBP-box genes encode plant-specific transcription factors that control differential gene expression as required for growth and development. Plants have evolved a remarkable ability to adapt their growth and development to varying environmental conditions. Whereas homeostatic responses to random fluctuations in, for instance, nutrient availability are required to maintain growth, developmental transitions generally rely on more gradual and lasting shifts in environmental variables like seasonally changing day length. However, limitations to the degree of phenotypic alteration are set by (epi)genetically determined mechanisms to ensure the ordered progression of development and successful reproduction. In the context of these mechanisms, SBP-box transcription factors play an important role as components of either environmentally triggered signalling cascades or endogenous genetic programmes.

SBP-box genes and copper homeostasis

Copper is a vital nutrient and plays an essential role in almost all aspects of plant physiology, including processes like photosynthesis and respiration. However, in nature plants are often confronted with copper-deficient soils, which also pose an agronomic problem as they result in reductions in growth rate, plant stability, fertility, seed set and yield. Whereas the electrochemical properties of its ions are beneficially exploited in various enzyme complexes, these properties rapidly become detrimental to cellular structures when copper is imported in excess. Consequently, plants maintain Cu levels within a tolerable range through homeostatic mechanisms which, in the flowering plant Arabidopsis, are partly orchestrated by the SBP-box transcription factor SPL7 (Figure 1). One obvious SPL7 function under Cu starvation conditions is to stimulate Cu uptake from the soil by activating high-affinity copper transporters localized in the plasma membranes of root cells. As part of a collaborative effort with German and US groups specialized in metal



Copper deficiency may result in weak stems and lodging in cereals, and the transcription factor SPL7 plays an important role in counteracting these symptoms. Lignification to strengthen cell walls, visualized (red coloration) with Mäule stain in these cross-sections through Arabidopsis stems, is virtually absent in the *spl7* mutant grown under copper-deficient (- Cu) conditions.

Figure 1

Comparative analyses of SBP-box genes will lead to a better understanding of the molecular genetic mechanisms driving plant diversification and ecological distribution

Peter Huijser

homeostasis, we recently showed that this response also involves the SPL7-dependent induction of the ferric reductases FRO5/FRO4, which reduce Cu(II) to Cu(I) as required for high-affinity copper uptake.
How SPL7 senses the cellular Cu status, and how its activity is modulated in organs as different as roots and shoots remains unclear. Current research in our group therefore concentrates on the interactions between SPL7 and other proteins.

SBP-box genes and sexual reproduction

Before they reproduce, flowering plants pass through a phase of vegetative growth during which nutrient uptake and photosynthetic capacities increase and reserves of assimilate are built up, which will later be mobilized to support seed development. To optimize the accumulation of such reserves, and ensure successful seed set at the end of the growing season, plants integrate changing environmental cues with developmental maturation in order to time the onset of reproductive growth. It is now well established that a particular subset of SPL genes, in combination with a suppressing regulatory microRNA, act as key determinants in enabling this transition process. More recently, however, we discovered that members of the same subset of microRNA-targeted SPL genes are functionally redeployed at later stages in the flowering process: here they act redundantly with the non-targeted SBP-box gene SPL8. Simultaneous loss-of-function of targeted SPL genes and SPL8 results in severely reduced fertility, owing to the failure of anthers to produce pollen and of gynoecia to form a transmitting tract to guide pollen tubes to the ovules for fertilization. If and how this function of microRNA-targeted SPL genes in tissue differentiation of reproductive organs relates to their role in the transition to the reproductive phase is unknown. Current research in our group is therefore devoted to the identification of target genes of SPL8 and microRNA-targeted SPL transcription factors.

Selected publications

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Bernal, M., Casero, D., Singh, V., Wilson, G.T., Grande, A., Yang, H., Dodani, S.C., Pelegrini, M., Huijser, P., Connolly, E.L., Merchant, S.S. and Krämer, U. Transcriptome sequencing identifies *SPL7*-regulated copper acquisition genes *FRO4/FRO5* and the copper dependence of iron homeostasis in Arabidopsis. Plant Cell 24, 738-761 (2012)

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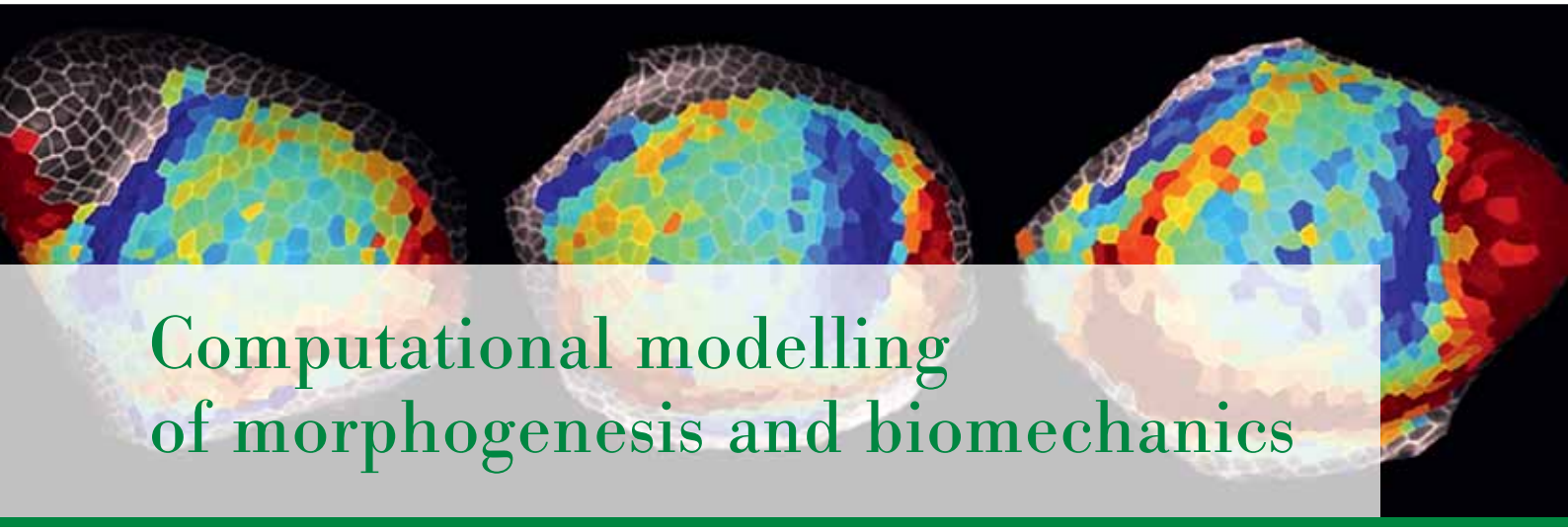
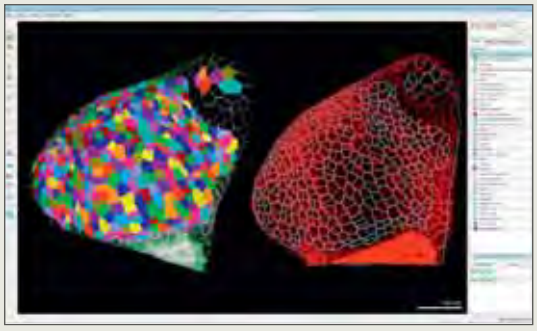


Computational modelling of morphogenesis and biomechanics

Our research uses mathematical and computer simulation techniques to investigate questions in plant development. Working in close collaboration with experimental biologists, we develop cellular-level simulation models of hormone signalling and patterning in plant tissue. These models involve a biochemical aspect - genes, proteins, hormones - combined with changing geometry, as cells divide and tissues grow. We are interested in the interaction between these two types of processes: how genes control physical properties of cells resulting in growth, and how the resulting change in geometry and physical forces feeds back on signalling and gene regulation. With this in mind, we are researching methods to quantify mechanical properties in plant tissues, to facilitate the construction of biophysically based simulation models of plant growth.

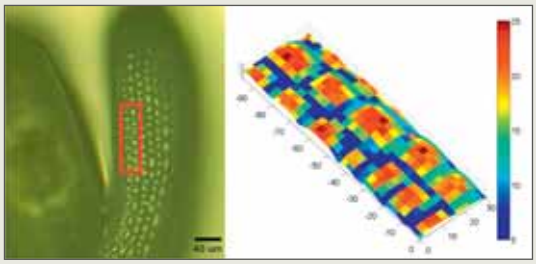
MorphoGraphX

Much of our research involves the precise tracking of cell shape changes, resulting either from growth or elastic deformation. Since plant cells are immobile, much information about morphogenesis can be obtained by looking at shape changes in the surface layer of cells. However, in many plant organs the surface layer of cells is not flat. To address this problem, we have developed specialized software called MorphoGraphX for the quantification of curved surface layers of cells (Figure 1). Working somewhere between 2 and 3D, MorphoGraphX is able to turn 3D confocal image stacks into curved surface images, which are then processed with algorithms we have adapted for this purpose. We are now extending our software for full 3D cell segmentation, fluorescence quantification, shape analysis, and other image processing problems as our research demands.



Screen-shot of MorphoGraphX showing the segmentation of a tomato shoot apex at successive time points.

Figure 1



Cellular Force Microscopy on a mature Arabidopsis embryo. Color indicates stiffness in N/m. Work done in collaboration with the G. Bassel lab, Birmingham.

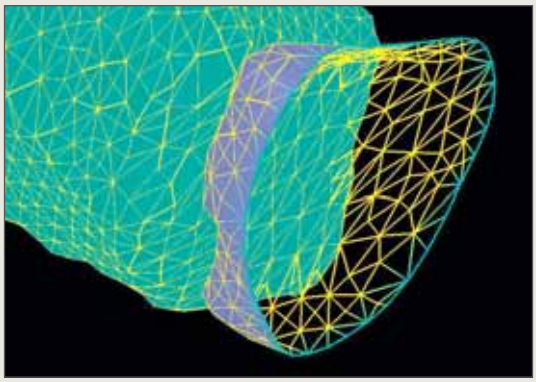
Figure 2

Cellular Force Microscopy

Cellular force microscopy (CFM) is a new computer-controlled micro-indentation technique (Routier-Kierzkowska et al. 2012) that we have developed to measure the stiffness of biological samples (Figure 2). It is able to handle much higher forces and displacements than the current atomic force microscopy (AFM) systems. The long, slender probe and fine tip also enable the system to be used as a computerized micro-ablation tool. We can customize both the software and hardware configuration,

We use mathematical and computer simulation techniques to investigate questions in plant development

Richard Smith



Cutaway view of a cortical cell from an *Arabidopsis* embryo used in a finite-element model of cell expansion. Our software allows us to import cell shape information taken from microscopy images directly into simulation models.

Figure 3

allowing us to combine the setup with confocal microscopy, and to adapt the system to new experimental requirements.

The physical basis of morphogenesis

We use several systems to investigate the mechanical changes in cells that are required to create form, and to model the interaction between genes and shape change.

In collaboration with the D. Weijers lab in Wageningen, we study cell division patterning during early embryo development in Arabidopsis. The stereotypical division patterns and relatively low numbers of cells allow development to be followed at the cellular level in exquisite detail. In the mature embryo, a binary growth switch occurs as the seed takes the decision to break dormancy. This makes it an ideal system for examining the relationship between the induction of growth-promoting gene expression and organ morphogenesis, which we pursue in collaboration with the G. Bassel lab in Birmingham. We also collaborate within the department with the A. Hay and M. Tsiantis groups, looking at the genetic control of leaf initiation and the biomechanics of seed dispersal in *Cardamine hirsuta*. We test our hypotheses by using 3D spatial simulation models, developed in collaboration with the P. Prusinkiewicz lab in Calgary, that are based on cell shape information extracted from sample tissue using MorphoGraphX. These data are being used to feed physically based finite-element (FEM) simulation models (Figure 3) that we are using to explore the regulation of cell expansion and morphogenesis in a geometrically and mechanically realistic environment. Our goal is to move one step closer to a true virtual plant tissue.

Selected publications

Vogler, H., Draeger, C, Weber, A., Felekis, D, Eichenberger, C., Routier-Kierzkowskai, A.L., Ringli, C., Nelson, B., Smith, R.S. and Grossniklaus, U. The pollen tube: a soft shell with a hard core. Plant Journal 73, 617-627 (2013)

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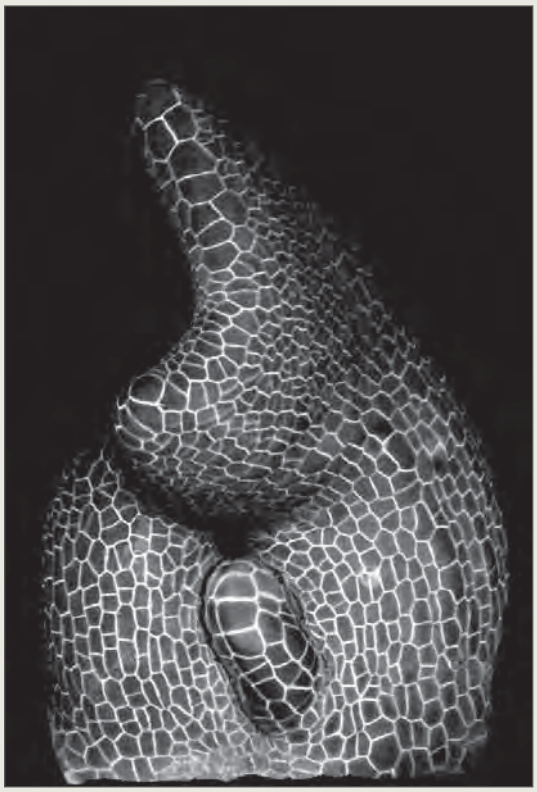
Bayer, E.M., Smith, R.S., Mandel, T., Nakayama, N., Sauer, M., Prusinkiewicz, P. and Kuhlemeier, C. Integration of transport-based models for phyllotaxis and midvein formation. Genes and Development, 23,373-384 (2009)

Plant development and diversity

We seek to address two fundamental questions in biology: how do biological forms develop and what is the basis for their diversity? To address these questions, we first aim to elucidate how genotypes are translated into organismal forms through the process of morphogenesis. Secondly, we seek to conceptualize how the balance between conservation and divergence in morphogenetic regulatory networks yield different organismal forms during evolution. We approach these problems using genetics, while also employing biological imaging, genomics and computational modelling. We believe that working at the interface of these areas will allow us to attain a predictive understanding of how biological forms develop and diversify (Figure 1).



Confocal laser scanning micrograph of the aerial part of a *Cardamine hirsuta* seedling stained with propidium iodide staining and visualized using MorphoGraphX software.

Figure 1

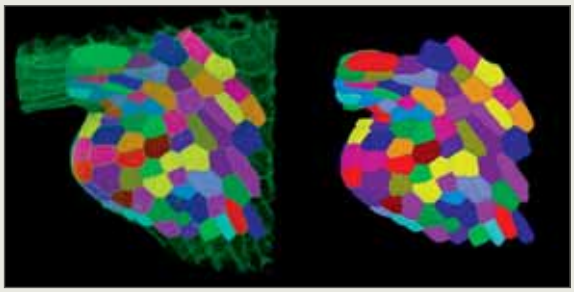
Our research programme is empowered by the use of *Cardamine hirsuta* (hairy bittercress), a common weed we developed as a model system for studies in the evolution of development. *C. hirsuta* is related to the reference plant *Arabidopsis thaliana* (thale cress) and, like *A. thaliana,* is amenable to both forward and reverse genetics approaches, including efficient transgenesis. However, *C. hirsuta* and *A. thaliana* differ in key morphological traits, including leaf shape, shoot branching, floral structure and fruit development, so comparative studies between these two species can greatly enrich our knowledge of the molecular mechanisms that drive the evolution of form. The analysis of both induced and natural variation within this comparative framework, coupled with broader, phylogenetically informed studies across seed plants, will help us to understand the genetic basis for evolutionary change. We are pursuing the following interrelated strands of research.

Morphogenesis and the control of form

Can we conceptualize morphogenesis in a predictive fashion? The form of an organism is determined by a cascade of developmental processes that take place at different levels of organization and yield the final form through complex feedback loops of genetic regulation, signalling and tissue growth. We aim to delineate such interactions and develop predictive models that conceptualize the process of development. Examples of the processes we study are leaf morphogenesis and patterning, and cell fate delimitation during embryo, shoot and root development (Figure 2).

We seek to understand the genetic basis for morphological evolution

Miltos Tsiantis



Segmentation of a developing *C. hirsuta* leaflet obtained using MophoGraphX software. The leaflet was imaged with the confocal laser scanning microscope after staining with propidium iodide as shown in the left panel. Colours indicate segmented cells. The scale bar indicates 40 microns.

Figure 2

The mechanistic basis for morphological diversity

Is morphological diversity between species generated by a large number of genetic differences of small effect, or by a few genetic changes with large effects? Are a handful of key genes responsible for the evolution of multiple morphological traits? How are genes that drive diversification positioned within genetic regulatory networks that influence form? Which specific genes have changed to produce the vast degree of morphological diversity seen in nature? And how do these genes change e.g. are mutations that result in stable morphological change more likely to lie in coding or regulatory segments of genes, do they behave in a dominant or recessive fashion, are they already present in populations or does their sudden appearance generate diversity?

Paths of evolutionary change

How repeatable is evolution? Does diversity in the same types of genes or pathways underlie variation in the same trait in different instances? Does inter- and intra-specific variation in morphology of the same traits arise via equivalent morphogenetic avenues? How prevalent is the role of positive selection in sculpting diverse plant forms and can the agents of selection be identified?

Selected publications

Dello Ioio, R., Galinha, C., Fletcher, A.G., Grigg, S.P., Molnar, A., Willemsen, V., Schered, B., Sabatini, S., Baulcombe, D., Maini, P.K. and Tsiantis, M. A PHABULOSA/Cytokinin Feedback Loop Controls Root Growth in Arabidopsis. Current Biology 22, 1699-1704 (2012)

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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 five independent research groups supply expertise in the evolution of obligate parasitism and the phyllosphere microbiome (Eric Kemen), plant chemetics (Renier van der Hoorn), computational biology and regulatory networks (Achim Tresch), photoperiodic control of flowering in barley (Maria von Korff), as well as the evolution of annual and perennial life strategies (Maria Albani). Maria von Korff's group was set up together with the University of Düsseldorf and is associated with a Junior Professorship at that university. The groups led by Maria Albani and Achim Tresch were established together with the University of Cologne and are associated with a Junior Professorship and a W2 professorship at that university, respectively.

Service groups are also independent of the departments and are headed by tenured scientists who perform research tasks, in addition to service duties which they carry out in collaboration with groups inside and outside the Institute. Erich Kombrink's group applies chemical biology approaches by screening libraries of compounds for small molecules that block or activate specific cellular processes. These are then used to isolate their plant targets. Elmon Schmelzer's group manages our imaging facilities comprising a wide variety of confocal light and electron microscopic instrumentation, Iris Finkemeier's group provides services in advanced protein mass spectrometry analysis and in plant proteomics, and Bernd Reiss' group explores recombination pathways in plants. Richard Reinhardt heads the aforementioned Max Planck-Genome-centre (MP-GC), a core facility providing cutting-edge technologies in next-generation DNA- and RNA sequence analysis.

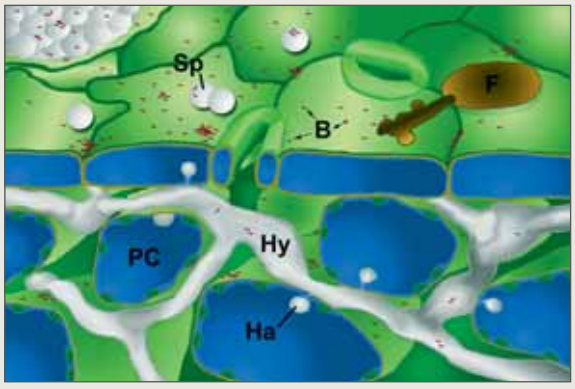
Evolution of obligate parasitism and biodiversity

New plant diseases emerge regularly and present a constant threat to food security. One major class of plant pathogens that causes massive epidemics on crops and wild plant species are the so-called obligate biotrophs. The biotrophic lifestyle requires keeping the host alive for days or weeks so as to maximize the number of spores produced. Specialized infection strategies are therefore essential. The crucial organ that mediates close host-parasite interactions for nutrient uptake and signal exchange is the haustorium. The close and intimate interaction with host organisms required by the biotrophic lifestyle significantly restricts the host range of the individual isolate. Nevertheless, biotrophy has evolved several times independently within the eukaryotes - at least twice in both fungi (powdery mildews and rust fungi) and oomycetes (downy mildews and white rusts). Numerous obligate biotrophic pathogens cause broad-spectrum suppression of innate immunity, thus facilitating the growth of other, opportunistic pathogens.

To understand the lifestyle, diversity and evolution of biotrophic pathogens, one must apply a combination of molecular, evolutionary and ecological approaches to obtain insights into how these parasites have evolved in close association with host plants and assorted pathogenic and non-pathogenic organisms. We therefore address two main questions: (1) How does an obligate biotroph maintain the ability to colonize new hosts while still being highly specialized? (2) What evolutionary pressures drive the build-up and maintenance of a complex biotrophic community?

Colonization of new hosts

Genomic sequencing of different white rust isolates (*Albugo* sp.) has revealed several polymorphic and heterozygous loci even within selfing *A. laibachii* isolates. To understand how these loci are maintained it is crucial to understand the life-cycle of *Albugo* sp., as well as infection strategies that might select for certain genotypes. A great variety of spore morphology has evolved in pathogenic fungi and oomycetes. Major differences exist between asexual spores with shorter lifetimes but high production rates and durable spores like oospores resulting from sexual recombination. It has been reported that durable spores are more likely to infect roots, while asexual spores predominantly infect leaves. We hypothesize that sexual recombination results in genotypes that are less well adapted to leaf infection than to root infection. Our goal



Hyphae (Hy) of the obligate biotroph pathogen *Albugo laibachii* span the intercellular space of its host plant *Arabidopsis thaliana*. Haustoria (Ha) have been formed inside the plant cells (PC). Following successful infection, spores (Sp) are released by rupturing the cuticle. Bacteria (B) are in close proximity to intercellular hyphae and attached to spores on the leaf surface. Fungal (F) growth and growth of other oomycetes is strongly promoted following A. *laibachii*-mediated suppression of the host plant's immune system.

Figure 1: A biotroph-facilitated microbial community (schematic view).

To understand and predict pathogen epidemics in the wild, it is crucial to dissect the complex microbial networks of which these organisms are a part

Eric Kemen

is to investigate the organ specificity of sexual and asexual stages with a view to understanding if and how organ specificity after sexual recombination influences genetic diversity.

Complex biotrophic communities

It has been hypothesized that intimate and frequent contacts, in combination with the ability to evade host defences, are essential for pathogens to colonize new hosts (van Baarlen 2007). The broad-spectrum suppression of plant innate immunity typically induced by the obligate biotrophic pathogen and primary colonizer promotes both. It permits elements of the microbiome to enter into close and lasting contact with non-host plants, but also allows these associated organisms to reproduce on the new pre-colonized host and therefore increases the likelihood that successful defence-evading genotypes are produced. This higher potential for foreign vectors to become pathogenic to the new host should tend to increase competition, which is presumably not in the interests of the broad-spectrum suppressor. It is therefore likely that other benefits to the primary pathogen follow from allowing multi-species co-habitation on the host.

Dissecting the microbial community in such pathogen-mediated niches is crucial to defining species networks that allow multiple organisms from different kingdoms to co-exist. These networks may serve to enhance the survival and fitness of their members, e.g., by manipulating the host environment to increase availability of nutrients or provide essential metabolites. We are using a combination of high-throughput sequencing techniques to identify species diversity and interaction capability. Collecting a broad range of different samples from numerous locations enables a search for network components that are essential for pathogenicity within associated communities. Connecting the underlying mechanisms is fundamental to understanding how diversity is generated in natural ecosystems. Therefore, we are isolating and purifying identified microorganisms for additional whole-genome sequencing. We are particularly interested in genes that are exchanged between organisms via horizontal gene transfer. The goal is to probe the stability of these complex communities, to dissect the mechanisms that enable organisms to co-exist on a living plant and identify the evolutionary forces that lead to co-existence based on pathogenicity.

Selected publications

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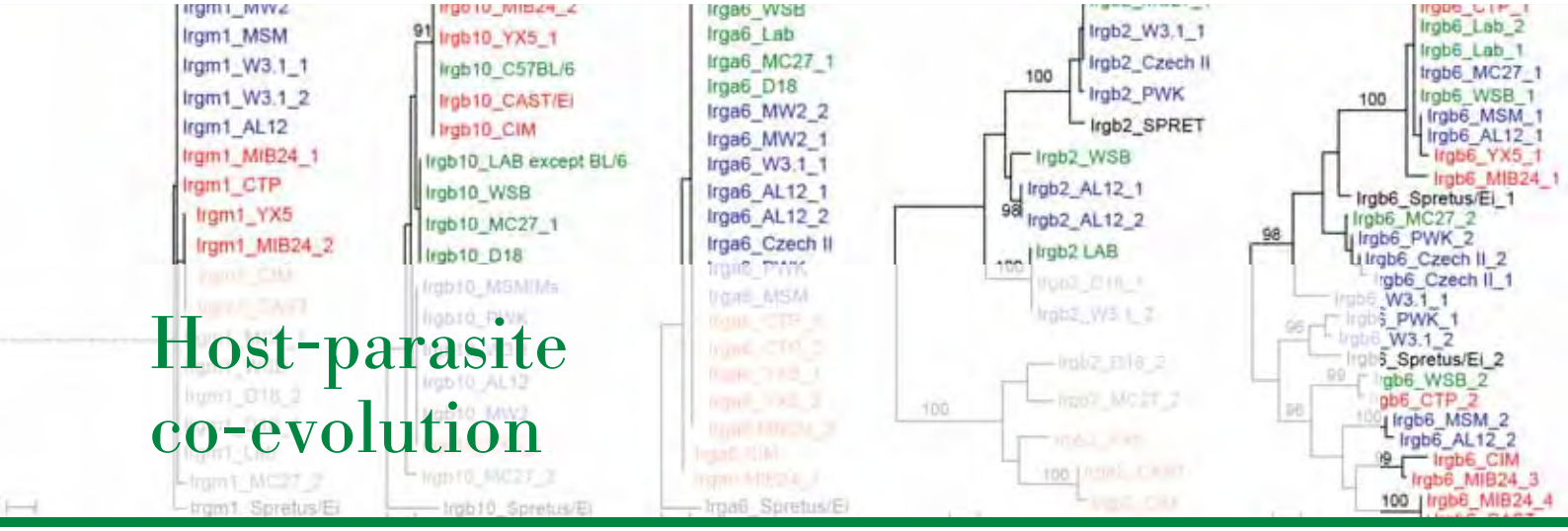
Kemen, E., Kemen, A.C., Rafiqi, M., Hempel, U., Mendgen, K., Hahn, M. and Voegele, R.T. Identification of a protein from rust fungi transferred from haustoria into infected plant cells. Mol Plant Microbe Interact. 18(11), 1130-9 (2005)

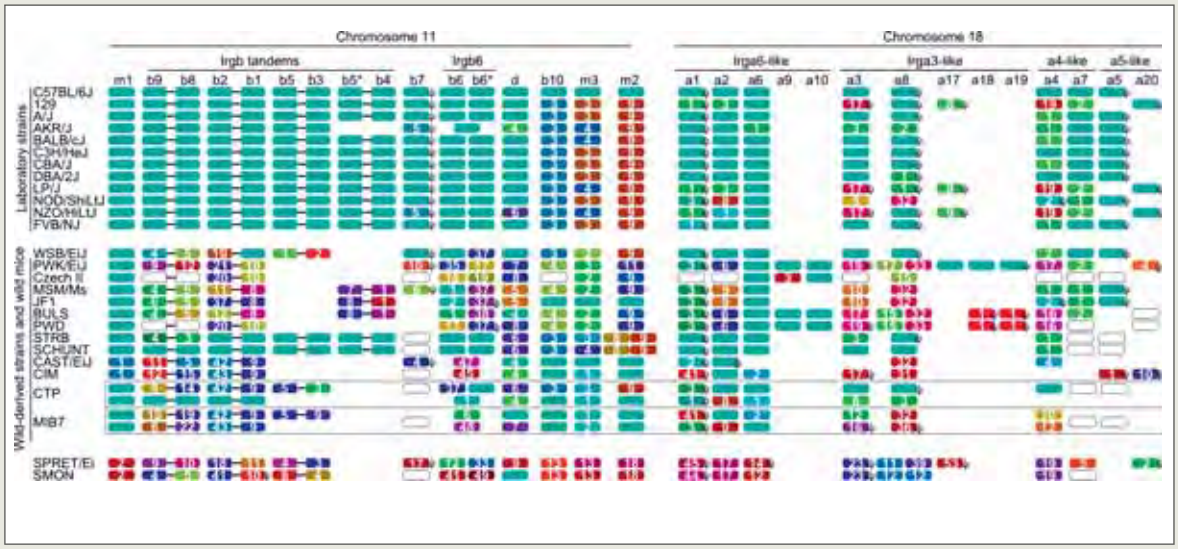


Host-parasite co-evolution

Host resistance and parasite virulence travel together through evolutionary time in a dynamic and often unstable equilibrium, each imposing unpredictable and intense selective pressures on the other. As a result, host-parasite relationships become remarkably specialized. General mechanisms of host immunity are now well understood, but for the parasite it is the details that matter, generating refined mechanisms of co-adaptation specific for each host-parasite complex. Our project is directed towards understanding an evolutionarily dynamic host-pathogen interaction in the wild in an animal example that has striking commonalities with plant disease resistance phenomena.

Toxoplasma gondii and the house mouse

T. gondii is a ubiquitous protozoan parasite. The sexual phase occurs in cats only; the infected cat sheds millions of environmentally resistant oocysts in the feces. These are infectious via the intestinal tract when inadvertently eaten by foraging animals. Infected animals either die of the infection, eliminate the infection by immunity, or find a middle way in which the organism forms cysts in brain or muscle. These persist until the infected animal is eaten by another cat. Because the domestic cat is so extremely abundant relative to all other cats put together, most evolutionarily significant hosts for *T. gondii* are now typical prey for domestic cats, such as small rodents and some birds. Among small rodent prey species, the house mouse is probably one of the more prominent world-wide and therefore likely to be an evolutionarily significant *T. gondii* transmitter. By evolutionarily significant, I mean that the

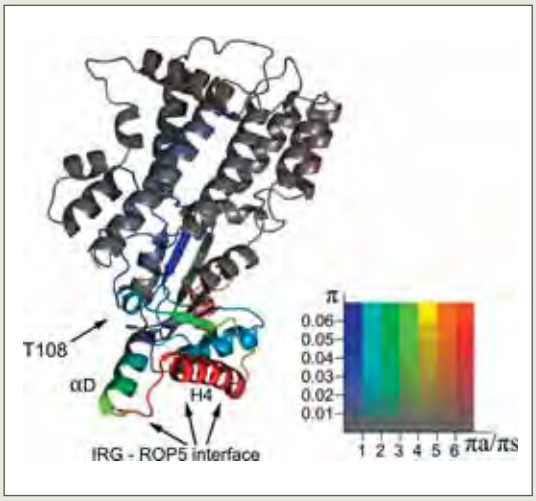


Each block represents a gene product; numbers of amino-acid differences from the inbred C57BL/6 laboratory strain are indicated. Colour similarity is related to phylogenetic distance between the sequences. See Lilue et al 2013 for details.

Figure 1: Polymorphism of IRG proteins in laboratory and wild mice.

Host-pathogen interactions are responsible for some of the strongest selective forces in nature, yet the relationships between pathogens and their hosts are often hard to disentangle in the natural environment.

Jonathan Howard



The highly polymorphic surface of Irgb2 that interacts with the polymorphic face of a *T. gondii* virulence protein is shown in heat colours to indicate the intensity of recent selection (Lilue et al, 2013)

Figure 2: Ribbon structure model of the IRG resistance protein Irgb2.

transmission of *T. gondii* from cat to cat would be significantly retarded if mice were to develop a highly efficient resistance mechanism that prevented encystment. A similar argument would apply with greater or lesser force depending on the proportional contribution of each prey species to *T. gondii* transmission. Wild mice are important because of their global sympatry with cats, but their importance is reduced by the puzzling low frequency with which they carry *T. gondii* cysts, around 1% while many evolutionarily irrelevant domestic animals including humans are infected at levels above 30%. It is certainly open to question whether the low frequency of infection in wild mice is due to extremely efficient resistance.

Our project is to understand the resistance of wild mice against sympatric *T. gondii* strains. The key resistance mechanism is based on a family of GTPases (IRG genes) whose genetic behaviour has strong analogies to plant R-genes. They are tightly clustered in the genome, multiple genes showing signs of dynamic behaviour, both cis and trans gene conversion, several pseudogenes etc. But above all, we recently showed that a remarkable polymorphism in wild mice (see Figure) correlates with the ability to resist certain strains of *T. gondii* (Lilue et al, 2013). However to show that there is a co-evolutionary situation it is essential to be able to correlate wild genotypes with wild infections, and this is the heart of our project. At the MPIPZ we take advantage of the excellent bioinformatics as well as the deep understanding of R-gene polymorphism and function.

Selected publications

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Hunn, JP, Feng, CG, Sher, A, Howard, JC. (2011) The Immunity-Related GTPases in Mammals – a fast Evolving Cell-Autonomous Resistance System against Intracellular Pathogens. Mammalian Genome, 22(1-2):43-54. Epub 2010 Oct 30. PMID: 21052678

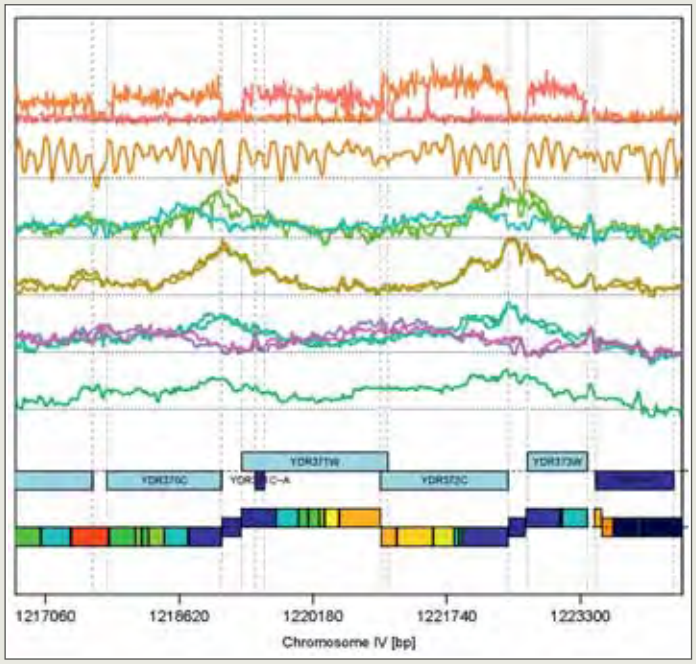
Tobias Steinfeldt1, Stephanie Könen-Waisman1, Lan Tong1§, Nikolaus Pawlowski1, Tobias Lamkemeyer1, L. David Sibley2, Julia P. Hunn1, Jonathan C. Howard1*(2010) Phosphorylation of IRG resistance proteins is an evasion strategy for virulent *T. gondii* strains. PLoS Biology 8(12): e1000576. doi:10.1371/journal.pbio.1000576

Computational biology of transcriptional regulation

On our way to a mechanistic understanding of the cell, we need to analyse and interpret huge amounts of multi-dimensional, incomplete and noisy data. We are developing and applying statistical techniques which are able to cope with these challenges. Our primary research target is the regulation of RNA transcription. In a joint effort with experimentalists, we impose well-defined perturbations on the cell's RNA transcription and degradation machinery, and collect large-scale protein-binding and mRNA expression data. These are analysed using dynamic models to shed light on the processes that together determine steady-state RNA levels. Ultimately, we hope to provide a comprehensive and quantitative model of RNA transcription.

Dynamic transcriptome analysis

We have developed dynamic transcriptome analysis (DTA), an experimental bioinformatics method which allows us to measure RNA synthesis and degradation rates on an absolute and genome-wide scale. We have shown that RNA synthesis and degradation are coupled, such that they reciprocally compensate for global changes and thus stabilize RNA levels. We have also identified an enzyme which establishes the feedback loop between these two processes. Using DTA, we monitored the yeast response to salt stress and found that such a coupling also exists for dynamically induced stress-specific transcripts. Our studies of periodic gene expression during the cell cycle revealed that periodic transcription is intimately linked to delayed changes in RNA degradation,



De novo annotation of genomic states (the composition of the transcription complex) from genome-wide DNA-binding data (top lines) using a bidirectional hidden Markov Model. Input data are, from top to bottom: strand-specific wild-type RNA levels (salmon color for the Watson strand and orange for the Crick strand), occupancy maps of nucleosomes, 3 capping factors, 2 initiation factors, and 4 C-terminal domain isoforms of Polymerase II. Inferred genomic states are shown as colored boxes in the lowest track, one color for each state, where expressed states coding for Watson strand/Crick strand/no transcription are positioned above/under/on the axis. For comparison, literature-based transcriptome annotation is shown in the 2nd track from the bottom.

Figure 1

Mathematical modelling of RNA transcription and degradation will lead to a deeper understanding of gene regulation, one of the most fundamental processes of life

Achim Tresch

which lead to a sharpened, timely, yet highly efficient expression response. We have also modelled the process of RNA degradation by the exosome at single-nucleotide resolution.

Unsupervised detection of chromatin and transcription states

We intend to improve the statistical models of RNA regulation by dissecting the processes of RNA transcription and degradation into their elementary steps and pinpointing the relevant factors that intervene at the different stages of transcription and degradation. In particular, we will perform integrative modelling of data on epigenetic modifications, chromatin structure and protein-DNA interactions to discover the "grammar" of transcription. We will also search for regulators of RNA decay, and investigate how they couple RNA synthesis and degradation.

Reconstruction of gene regulatory networks

We are developing probabilistic graphical models that allow us to infer transcription factor- and microRNA activity, and even transcription factor interactions, from gene expression data. In particular, we have improved the theory of nested-effects models and implemented very efficient algorithms for parameter estimation in this model class. This enables us to reconstruct networks involved in gene regulation, such as the signalling network that links the Mediator multi-protein complex and its interaction partners.

Time-lapse image analysis

Time-lapse fluorescence microscopy has become one of the most powerful tools in molecular biology. Thousands or even millions of single cells must be automatically identified, tracked in time, and characterized phenotypically in order to draw valid conclusions. We are developing algorithms and software that will help experimentalists to interpret their data.

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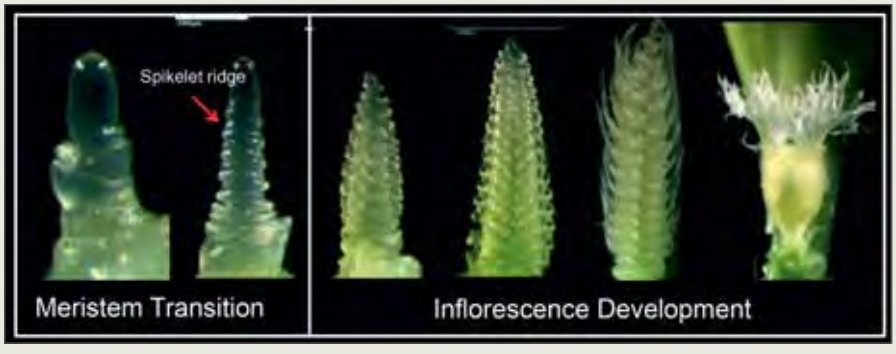
Photoperiodic control of flowering in barley

The control of flowering is central to reproductive success in plants, and has a major impact on grain yield in crop species. Flowers are sensitive to abiotic stresses, and flowering needs to be induced at the optimal time of the year to maximize seed set. Understanding the genetic control of shoot and inflorescence development thus has great potential for crop improvement. As the molecular mechanisms underlying developmental processes remain largely unknown in important monocotyledonous crop plants, our group has focused its work on the genetic and molecular basis of reproductive meristem development in barley.

Photoperiod control of flowering

Seasonal changes in photoperiod are major developmental cues in barley. The photoperiod flowering pathway channels inputs from light, day length, and the circadian clock to promote the floral transition. Plants measure time with the circadian clock, which is an internal timekeeper with a period of about 24 h. This clock allows plants to coordinate their metabolism and development with predicted daily and seasonal changes in the environment. In Arabidopsis, the circadian oscillator consists of transcription factors which are interconnected in negative feedback loops. We are interested in understanding how domestication and modern plant breeding have changed the circadian clock and thus photoperiod response and adaptation. Fast flowering under long days in wild barley is controlled by the major photoperiod response gene *Ppd-H1*, a barley orthologue of the Pseudo Response Regulator genes that are involved in the circadian clock in Arabidopsis. A natural mutation in the conserved CCT domain of *Ppd-H1* prevalent in spring barley causes a reduced response to long days and was selected for adaptation to long growing seasons in Northern latitudes. We have shown that circadian clock genes are structurally conserved between barley and Arabidopsis, but independent duplications/deletions of clock genes have occurred throughout the evolution of eudicots and monocots. Differences in both structure and expression of clock genes between barley and Arabidopsis suggest that the clock differs between eudicots and monocots. We have shown that the natural mutation in *Ppd-H1* delays flowering time, but does not change the circadian clock, suggesting clock-dependent and independent functions of this gene. We also found that natural variation at *Ppd-H1* primarily affected inflorescence development and, to a lesser extent, the transition from a vegetative to a reproductive meristem (Figure 1).



Developing shoot apical meristem (SAM) in barley. Different developmental phases can be distinguished based on morphological changes in the SAM.

Figure 1

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

Maria von Korff Schmising

Field trial of a barley recombinant-inbred-line population derived from a cross between the Syrian landrace Arta and the Australian cultivar Keel in Syria in 2011. The population is segregating for growth type as determined by variation at *Vrn-H1* and *Vrn-H2*.

Figure 2

We have recently identified and characterized the circadian clock orthologues *HvELF3* and *HvLUX1,* which correspond to the early maturity loci eam8 and eam10, respectively. These mutations alter clock function and cause photoperiod insensitivity and early flowering by up-regulating *Ppd-H1* under short day conditions in barley. Increased expression of *Ppd-H1* in *eam8* and *eam10* suggested a conserved function for *ELF3* and *LUX,* as its primary targets in Arabidopsis are related members of the PRR family. Interestingly, mutations in *HvELF3* were used to breed barley varieties adapted to short growing seasons, although these lines are severely compromised in clock function. In contrast to Arabidopsis clock mutants, the barley clock mutants are not strongly affected in photomorphogenesis, growth or primary assimilation under control conditions. We are currently testing the performance of these clock mutants under dry field conditions, in cooperation in partners in the Palestinian Territories.

We have already shown that variation in flowering time is the strongest determinant of yield in stress-prone, dry environments. A large field study in Syria conducted in cooperation with the International Center for Agricultural Research in the Dry Areas (ICARDA) revealed that the vernalisation genes *Vrn-H1* and *Vrn-H2* have pleiotropic effects on growth, spike and plant architecture, and yield stability in dry Mediterranean environments (Figure 2). Under changing climate conditions, shorter winters and early summer drought, the fast flowering, vernalisation-insensitive genotype outperformed the traditional Syrian genotype which is vernalisation sensitive.

Selected publications

Campoli, C., Pankin, A., Casao, C.M., Davis, S.J. and von Korff, M. HvLUX1 is a candidate gene underlying the *early maturity 10* locus in barley: phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytologist, doi: 10.1111/nph.12346 (2013)

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Faure, S., Turner, A.S., Gruszka, D., Christodoulou, V., Davis, S.J., von Korff, M.* and Laurie, D.A. Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley *(Hordeum vulgare)* to short growing seasons. Proceedings of the National Academy of Sciences 109 (21), 8328-8333, doi: 10.1073/pnas.1120496109 (2012) *Co-corresponding author

Campoli, C., Drosse, B., Searle, I., Coupland, G. und von Korff, M. Functional characterization of HvCO1, the barley *(Hordeum vulgare)* flowering time ortholog of CONSTANS. Plant Journal 69, 868–880 (2012)

The evolution of annual and perennial life strategies

Plants that live for a year or less follow the annual life strategy; others have adopted the perennial life strategy and can live for many years. Many plant families contain both annual and perennial species, indicating that the annual and perennial life strategies have been selected several times during evolution. Annual species are thought to be derived from perennial ancestors, but the molecular mechanisms underlying this evolutionary change are not understood. Our group is interested in understanding plant life-history evolution at the molecular level.

In our studies we perform comparisons between closely related, annual and perennial Brassicaceae species, but also use diverged perennials such as the wild strawberry *Fragaria vesca*. We have previously shown that differences in the expression patterns of a flowering-time gene contribute to differences in life strategy between the annual *Arabidopsis thaliana* and the perennial *Arabis alpina*. FLOWERING LOCUS C (FLC) is a key floral repressor in *A. thaliana* that regulates flowering in response to prolonged exposure to cold - a process called vernalisation. Its orthologue in *A. alpina,* PERPETUAL FLOWERING 1 (PEP1) ensures that plants require vernalisation to flower, but in addition contributes to the perennial life strategy. PEP1 in *A. alpina* also displays natural variation for flowering in response to vernalisation and perennial traits. Accessions that carry non-functional PEP1 alleles do not require vernalisation to flower and flower continuously.

A. alpina mutant with inflorescence phenotype (left) compared to wild type (right)

Figure 1

We wish to elucidate the mechanisms that lead to the adoption of the annual or perennial life strategies

Maria Albani

The *pep1* mutants and accessions that carry non-functional PEP1 alleles are still perennials, suggesting that there are additional mechanisms that regulate the perennial growth habit besides PEP1. To identify genes that contribute to the perennial growth habit of *A. alpina* we performed enhancer and suppressor mutagenesis screens, focusing on mutants that show additional phenotypes such as inflorescence and branching defects (Figure 1). Identification of the causal mutations should enhance our understanding of the perennial growth habit in *A. alpina* at the molecular level. To test whether identified genes contribute to life history evolution, comparisons will be performed with *A. thaliana* and other Brassicaceae species. Additional studies on *F. vesca* will also show whether already identified mechanisms are evolutionarily conserved in other families such as the Rosaceae.

Finally, one further characteristic of perennials is that they undergo secondary growth and are able to propagate asexually using special organs such as the runners in strawberry. We are interested to know how these vegetative traits are coordinated with flowering within the perennial life cycle of *A. alpina* and other perennials.

Selected publications

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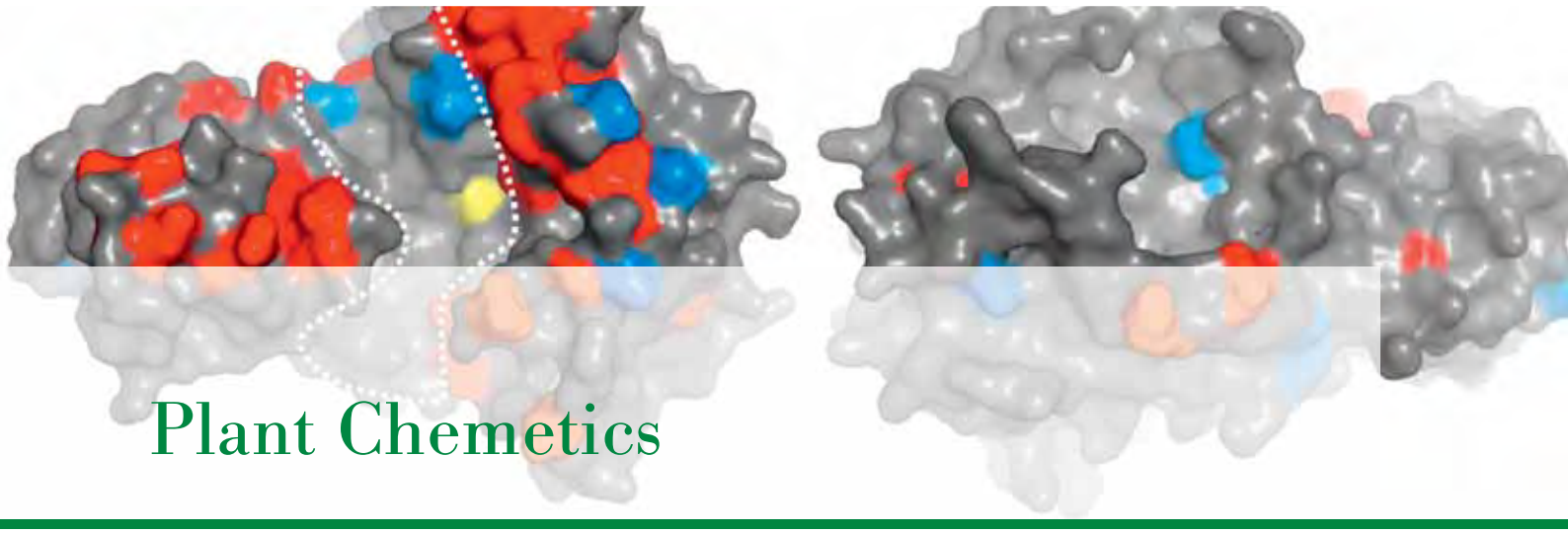
Koskela, E.A., Mouhu, K., Albani, M.C., Kurokura, T., Rantanen, M., Sargent, D.J., Battey, N.H., Coupland, G., Elomaa, P. and Hytönen, T. TERMINAL FLOWER1 is the switch between short-day and long-day flowering responses in the wild strawberry, *Fragaria vesca*. Plant Physiology 159, 1043-1054 (2012)

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

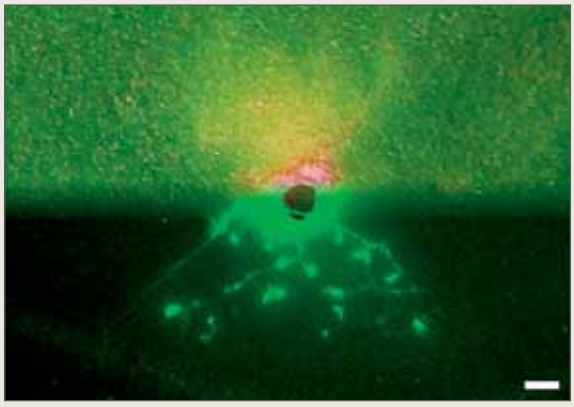


Plant Chemetics

The Plant Chemetics lab investigates plant-pathogen interactions using state-of-the-art chemical proteomics to reveal how protein functions are manipulated by pathogens during infection. Indeed, the lab has pioneered the development and application of activity-based protein profiling (ABPP) in plants. ABPP involves the labelling of proteomes, tissues or whole organisms with chemical probes - small molecules that react with the active sites of enzymes in an activity-dependent manner. Labelling is covalent and irreversible, which facilitates detection and identification by protein gel electrophoresis and mass spectrometry, respectively. We have introduced probes for over 3000 plant proteins, including proteases, lipases, kinases, acyltransferases, GSTs and glycosidases. The probes and protocols employed by the lab are unique in the world and are internationally recognized. This is reflected in the constant stream of visiting scientists who come to investigate protein activities in various experimental settings.

Several important discoveries have been made by studying the active proteome. We discovered that the activity of the proteasome is post-translationally up-regulated during the immune response (Gu et al., Plant J. 2010), and suppressed by inhibitors produced by pathogenic *Pseudomonas syringae* bacteria (Kolodziejek et al., Plant Physiol. 2011). Intriguingly, we found that Pseudomonas uses these proteasome inhibitors to facilitate wound entry and colonization of tissue adjacent to the wound site by moving through the xylem tissue (Misas-Villamil et al., PloS Pathogens, Figure 1). Using probes for vacuolar processing enzymes (VPEs) we discovered that VPE activity is up-regulated during infection by the oomycete *Hyloperonospora arabidopsidis*. Importantly, this pathogen is less effective on *vpe* mutant plants, suggesting that VPE activity is beneficial for the pathogen (Misas-Villamil et al., Plant J. 2013). This role is surprisingly distinct from the roles described for VPEs in programmed cell death.

We also determined that tomato secretes papain-like proteases during immune signalling and showed that these are inhibited by unrelated proteins secreted by various tomato pathogens. These targeted proteases contribute to basal immunity and are under diversifying selection in wild tomato, such that variant amino acids interfere with protease-inhibitor interactions (Shabab et al., Plant

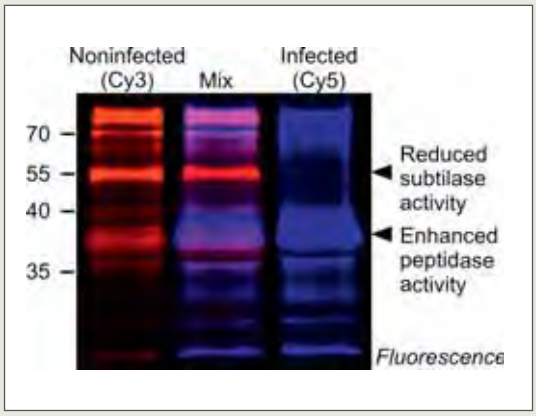


Bacteria spread from the wound infection site through the xylem and colonize the leaf apoplast in adjacent tissue by producing proteasome inhibitor SylA. Top: bright field; bottom, GFP fluorescence; scale bar 1mm.

Figure 1: **Colonization from wounding site by GFP-expressing *Pseudomonas syringae*.**

The functional proteome dictates the phenotype

Renier van der Hoorn



Dynamics of serine hydrolase activities in the apoplast upon bacterial infection. Secreted proteomes were isolated from *Nicotiana benthamiana* plants infected with *Pseudomonas syringae* and from uninfected controls. Active serine hydrolases were labelled with minitagged fluorophosphonate probes, and fluorescent Cy3 and Cy5 tags, respectively, were attached to the two labelled proteomes using click chemistry. The two proteomes were then mixed, fractionated on protein gels and detected by fluorescence scanning. The gel reveals suppression of subtilase activities and induction of peptidase activities upon infection.

Figure 2

Cell 2008, Hörger et al., Plos Genetics 2013). Using an ATP-based probe we have detected receptor-like kinases, calcium-dependent kinases and MAP kinases (Villamor et al., Mol. Cell. Proteomics 2013). Probes designed to tag a specific Pseudomonas protease revealed how it is exported from the bacterium and activated in the host plant cell (Lu et al., Chem. & Biol. 2013). Other probes target a novel class of serine proteases and catalytic residues of glutathione transferases (Gu et al., Chem. Biol. 2013).

Future challenges

We continue to expand ABPP using new probes, and will exploit label-free quantitative proteomics to further study host manipulation by Pseudomonas. We have detected suppression of the activities of several secreted hydrolases during infection with *Pseudomonas syringae* (Figure 2), and are pursuing these projects further to understand how microbes manipulate the hydrolytic environment of the apoplast.

Selected publications

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Misas-Villamil, J.C., Toenges, G., Kolodziejek, I., Sadaghiani, A.M., Kaschani, F., Colby, T., Bogyo, M., and Van der Hoorn, R.A.L. Activity profiling of vacuolar processing enzymes reveals a role for VPE during oomycete infection. Plant J. 73, 689-700 (2013)

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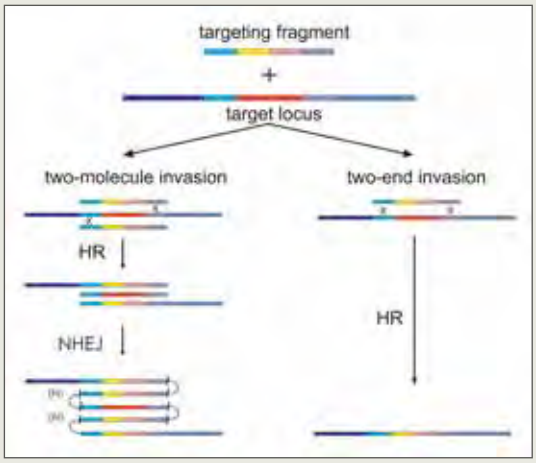
DNA damage repair and gene targeting in plants

Genomes are constantly being challenged by environmental and intrinsic threats. Constant and inevitable exposure to chemical and physical insults damages DNA, and such damage causes mutations, and cancer in humans. Organisms have therefore developed intricate and complex mechanisms for DNA repair. These are also important for biotechnological applications, in particular in the context of how transformed DNA integrates into the genome. This can occur at random positions in the genome, or be targeted precisely to a predetermined position. The corresponding recombination mechanisms are non-homologous end-joining (NHEJ) and homologous recombination (HR) respectively. Both are vital in the repair of double-strand breaks (DSBs), the most lethal form of DNA damage. NHEJ is a fast but an imprecise repair process that simply joins a broken end to any other free end available, while HR is precise and repair is guided by a DNA template present somewhere else in the cell. Gene targeting is based on HR and the precise integration of a DNA fragment in the genome is entirely dependent on this pathway. DNA recombination in *Arabidopsis thaliana* is relatively well understood, with the genes and mechanisms involved being quite similar to those in animals. In particular, as in most animal cells, gene targeting is inefficient in Arabidopsis. In contrast, gene targeting is highly efficient in the moss *Physcomitrella patens*. We use these two organisms in our efforts to understand the aspects of recombination in *P. patens* that lead to high gene targeting efficiencies and transfer this capacity to crop plants.

A novel gene targeting pathway in *P. patens*

In a variant of gene targeting, gene replacement, the modified gene integrates into the genome in such a way as to replace the resident sequences. The specific recombination mechanisms differ between organisms, but all have in common the fact that a single fragment integrates



The scheme shows the two-molecule invasion pathway that leads to complex integrations on the left. The two-end invasion pathway leading to precise replacement is shown on the right. The targeting fragment is shown above the target locus. The regions of homology are colour-coded in cyan (5`) and light blue (3`), the neighbouring genomic regions in dark blue (5`) and magenta (3`). The replaced portion is in red and the genes replacing it are in yellow and pink. Abbreviations: NHEJ, non-homologous end-joining; HR, homologous recombination; the "x" denotes crossover recombination, the "~"recombination by NHEJ.

Figure 1: The two-molecule in comparison to the two-end invasion pathway

as an entity. In *P. patens,* two independent molecules, as opposed to the two ends of one and the same fragment, initiate homology-directed DNA integration. HR and strand-exchange then produces a double-strand break (DSB) that ends with two targeting fragments linked to the genome. This DSB is then repaired by HR or NHEJ and produces recombination products that are hybrids formed by both processes. Although it is generally accepted that genomic DSB repair and gene targeting use identical recombination mechanisms, our data suggest that this is not the case, at least for *P. patens*. As expected, RAD51, an

The moss *Physcomitrella patens* is a goldmine for DNA recombination research

Bernd Reiss

important recombinase with a central role in HR is essential for gene targeting. However, the importance of RAD51 in *P. patens* must extend beyond its recombinase function, and it seems that as yet unknown features of the gene or its protein product may be crucial for the high efficiency of gene targeting in *P. patens*.

The DNA damage response and DSB repair in *P. patens*

The DNA damage response is under the control of ATM and ATR, and DSB repair may occur either by HR or NHEJ. Analysis of mutants in these pathways shows that regulation of the DNA damage response in *P. patens* has unique features and differs substantially from that seen in *A. thaliana*, yeast and mammals. Also in contrast to *A. thaliana* and mammals, NHEJ does not seem to play any role in DNA damage repair, but HR does. This is shown by mutations affecting NHEJ and HR. A mutant in the KU70/KU80 complex, which has an important function in classical NHEJ, is not affected in DNA damage repair while one in RAD51, which has an essential function in HR, is. These data suggest that *P. patens* has developed a unique recombination apparatus that differs from that in other organisms in both the regulation of DNA damage repair and in the pathways used for repair. This, together with the high-efficiency gene targeting mediated by the system, implies that *P. patens* has the potential to reveal novel recombination mechanisms, help us to understand the biology of gene targeting, and perhaps enable us to achieve similar targeting efficacies in other organisms.

Gene targeting in barley

Barley is an important crop plant and a model for more complex cereals, such as wheat. Gene targeting in barley would be a valuable tool, but it is not known how feasible this technology is in cereals. We have analysed gene targeting and shown that the efficiencies achieved with conventional methods, but also techniques like positive-negative selection (a breakthrough technology in mammals and rice), are too low to be useful. However, DSB induction is extremely efficient and generation of stably inherited targeted gene modifications is perfectly possible. Therefore the gene targeting problem in barley can probably be solved with modern DSB induction technology.

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Chemical Biology

Small molecules are powerful tools with which to dissect and understand biological systems. This is evident from the frequent use of potent and selective inhibitors of enzymes, or other biological processes such as transcription, translation or protein degradation. However, in contrast to animal systems, which greatly benefit from drug research, the systematic development of novel bioactive small molecules as research tools for plant systems is a largely underexplored research area. This is surprising, since small bioactive molecules have great potential as new tools for dissecting diverse biological processes. In particular, when small molecules are integrated into genetic strategies (the mix that defines "chemical genetics"), they can help to circumvent problems inherent in classical, forward genetics - such as lethality, pleiotropy or redundancy of gene functions - because they can be applied in a conditional, dose-dependent and reversible manner. There are now clear examples of important, fundamental discoveries originating from plant chemical genetics that demonstrate the power of this experimental approach.

Chemical genetics of plant signalling pathways

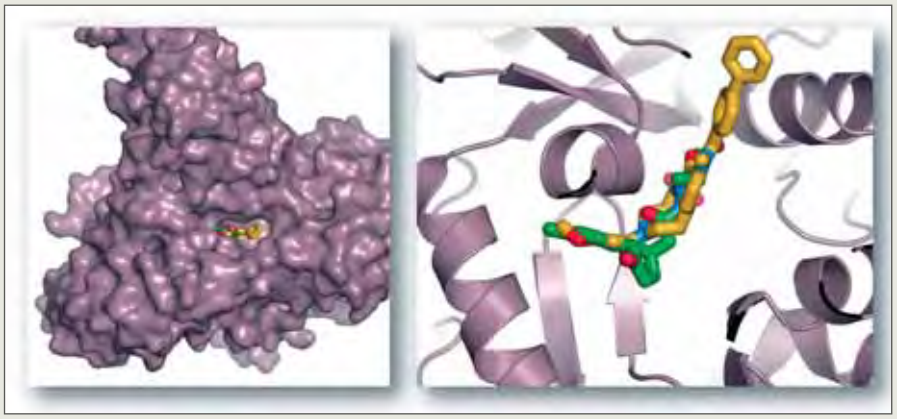
To fully exploit the potential of novel bioactive small molecules for discovery, we have established high-throughput screening procedures for a variety of biological responses using a miniaturized format with *Arabidopsis thaliana* plants growing in 96-well microplates and diverse chemical libraries comprising natural and synthetic compounds. This screening platform is not only used for our own research projects but also provided to other research groups at the Institute, and the multiple use of common resources has facilitated the identification of compounds that selectively modulate particular responses. Typically, a successful chemical screening campaign comprises three equally important steps: (1) establishment of a reliable, robust and quantitative readout, which allows one to distinguish between potent and less potent compounds, (2) critical evaluation of a compound's activity in diverse bioassays to establish selectivity, and (3) elucidation of a compound's mode of action and, ideally, identification of its molecular target. We have applied this approach to a number of different phenotypes that are related to plant immunity, such as expression of specific reporter genes representing different signalling pathways (e.g. mediated by jasmonic acid or salicylic acid) or defence-related reactions (e.g. increase in cellular calcium concentrations, production of reactive oxygen species or nitric oxide). Such comparative screening, in combination with the use of genetic resources and in-depth biochemical analyses, has led to the identification of a novel and highly selective inhibitor of jasmonate signalling, named jarin-1 (Figure 1). Clearly, this compound provides a useful tool for the creation of conditional jasmonate mutants in plant species that are genetically intractable. Thus, chemical genetics allows the identification of new regulatory components of biological processes or signalling networks that are not amenable to mutant analysis.

The yeast three-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 molecular mechanisms of signal perception and transduction. Of the different possible strategies for target identification, we chose to establish the yeast three-hybrid technology in our laboratory, which allows direct functional cloning of proteins that interact with synthetic hybrid ligands in vivo. The development

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

Erich Kombrink



Structure of the protein target of jasmonate response inhibitor 1 (jarin-1). The left panel shows the whole protein with the inhibitor inserted into the substrate-binding pocket. The enlarged view (right panel) of the substrate-binding pocket clearly shows that the inhibitor (yellow) overlaps with the natural substrate, jasmonic acid (green), and thereby prevents its binding and conversion to jasmonoyl-isoleucine.

Figure 1

of this new technology for plant systems serves two important functions: (1) it provides a general experimental platform for target identification of compounds that originate from chemical genetic screens, and (2) it enables one to identify primary targets of different signal molecules mediating plant defence, such as new jasmonic acid or salicylic acid derivatives, whose modes of action are still unknown. We use several cDNA libraries, synthesized from various organs, tissues or plants that have been subjected to different kinds of treatments, to systematically scan the whole expressed genome (proteome) of *A. thaliana* for such targets, thereby providing a system-wide overview of the corresponding binding proteins. We expect that unravelling the biochemical functions of these proteins will provide exciting insights into the mechanistic details of how signalling networks operate.

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Schlicht, M. and Kombrink, E. The role of nitric oxide in the interaction of *Arabidopsis thaliana* with the biotrophic fungi, *Golovinomyces orontii* and *Erysiphe pisi*. Frontiers in Plant Science 4, 351 (2013)

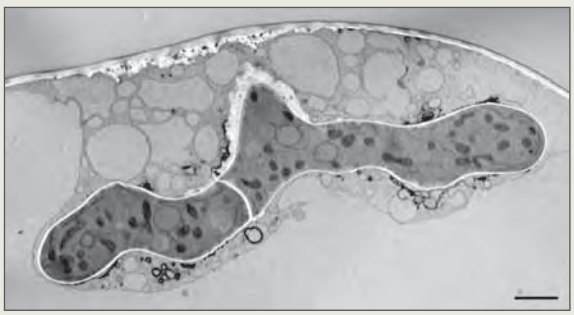
Central Microscopy (CeMic)

The rapid development of new microscopic imaging methods over the last 20 years has revolutionized our ability to directly visualize processes in living organisms. We are now able to watch plants at work, at levels spanning the full range from whole organisms to organs, tissues, cells and organelles, down to protein complexes and single proteins. The green fluorescent protein (GFP) and its growing family of relatives have had a remarkable impact on the development of sophisticated microscope systems for fluorescence imaging of living cells. Continuous advances in fluorophore design, molecular biological tools and imaging technology are enabling new discoveries to be made in many fields of biological research. To meet the growing demands on microscopic imaging, the Institute has made substantial investments over the last decade to provide adequate instrumentation. In our Institute the entire spectrum of imaging technologies is available. This includes up-to-date light microscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The service group Central Microscopy (CeMic) manages imaging technologies and the necessary equipment at the MPIPZ. Besides providing a complete service, CeMic also trains and supervises researchers in the operation of the instruments. In addition, CeMic consults with and advises researchers on questions concerning methodology and image interpretation, and provides practical courses. Furthermore, CeMic performs in-depth collaborative research on cell biology projects.

Services

Live cell imaging

Fluorescent protein tagging technology facilitates the analysis of protein function by enabling the visualisation of a wide range of processes, from protein expression, subcellular localisation and dynamics to topographic mapping of protein interactions. Thus, fluorescent protein tagging and *in vivo* imaging by confocal laser scanning microscopy have become standard technologies for many researchers in our Institute and are applied in a multitude of projects in all four departments, including studies on the control of flowering time, side branching and plant morphogenesis, and the regulation of plant-microbe interactions. An important aspect of confocal laser scanning microscopy is the ability to monitor protein-protein interactions in vivo by the measurement of fluorescence resonance energy transfer (FRET) using acceptor photobleaching (APB) and fluorescence lifetime imaging (FLIM).



TEM micrograph of the early biotrophic infection stage of *Colletotrichum higginsianum*, showing a fungal hypha developing inside an *Arabidopsis thaliana* leaf epidermal cell. The ultrahigh resolution of TEM enables visualisation of dramatic modifications in the structure of the host cytoplasm, such as the formation of numerous small vacuoles, deposits of callose cell-wall material, and novel structures called 'interfacial bodies' formed between the fungal cell wall and the plant plasma membrane. Scale bar: 2 µm.

Figure 1

Observing and analysing plant cells at work – amazing and exciting again and again

Elmon Schmelzer

Electron microscopy

For scanning electron microscopy (SEM) in the Institute, we have an instrument which employs a field-emission gun providing high resolution and high magnification even at low voltage, which is very advantageous for biological samples that are sensitive to electron beam damage. Fast handling of samples can be achieved using a linked cryo-preparation and transfer system. The instrument is widely used for detailed three-dimensional analysis of all kinds of morphological phenotypes.

Transmission electron microcopy (TEM) offers the highest possible magnification and resolution, and can provide information on cells, organelles and smaller cell constituents down to individual molecules. The resolving power of TEM makes it the ultimate tool for identifying subtle changes in the fine structure of the cytoplasm or the morphology of the cell wall. In combination with immunogold cytochemistry, TEM is also used to precisely localize specific gene products within their subcellular microenvironment, which provides important clues to gene function. The application of high-pressure freezing and freeze-substitution greatly improves the preservation of biological samples during preparation for TEM analysis by capturing specimens in a condition that is as close to their native state as possible.

Research

Our research projects are usually conducted in collaboration with groups at the Institute, but we also have external collaborations. Among the numerous studies employing the entire spectrum of imaging technologies, we have had a long-standing, fruitful collaboration with Richard O'Connell's group in the Department of Plant-Microbe Interactions. In this project, live-cell imaging with confocal laser scanning microscopy was combined with high-resolution SEM and TEM to analyse interactions between the hemibiotrophic fungus *Colletotrichum higginsianum* and its host plant Arabidopsis. As with many other phytopathogens, *C. higginsianum* secretes an array of small effector proteins that facilitate effective colonization by manipulating host metabolism. Using fluorescent protein tagging and TEM-immunogold labelling, we were able to localize effectors to specific compartments at the host-pathogen interface.

Selected publications

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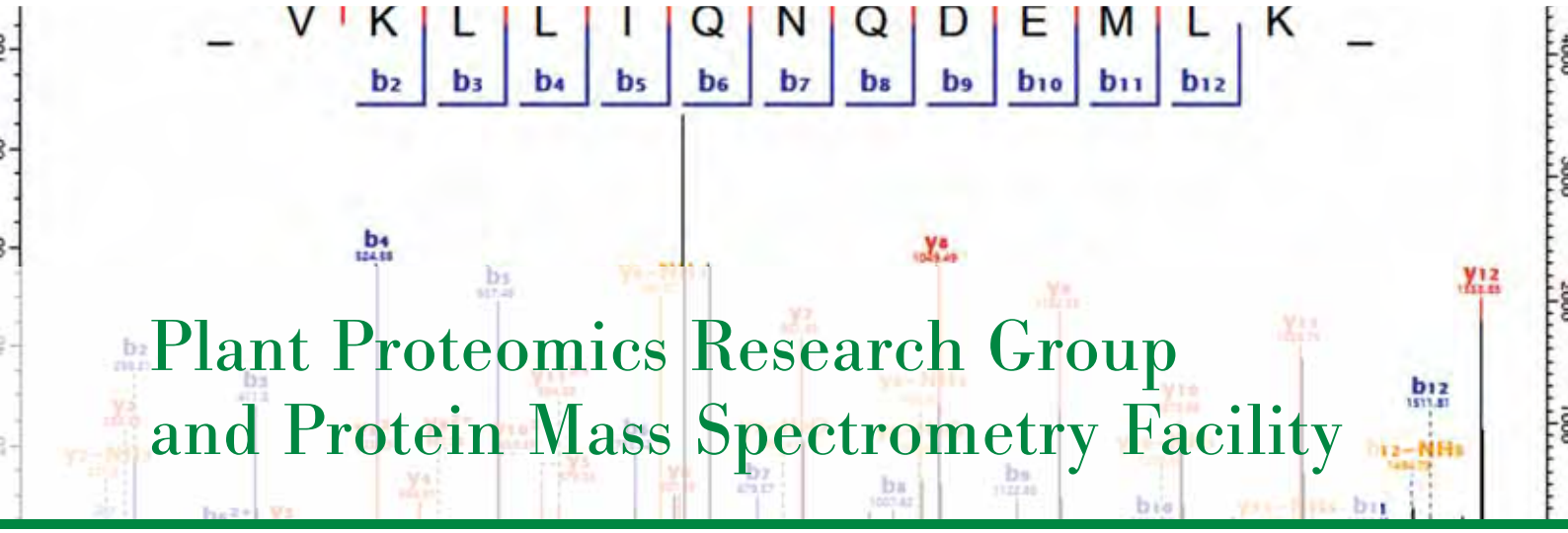
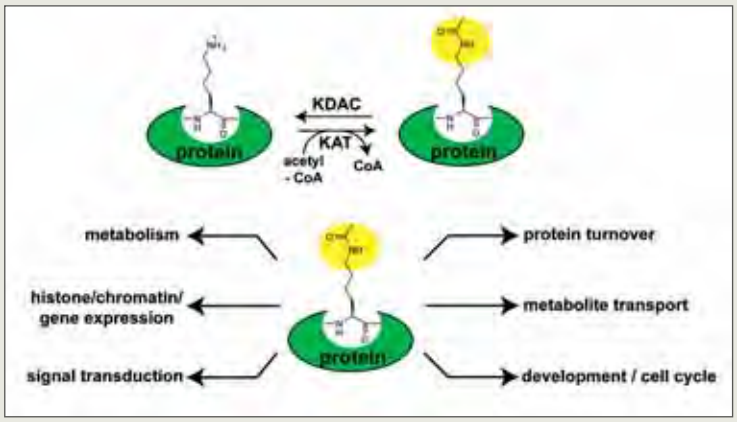


Plant Proteomics Research Group and Protein Mass Spectrometry Facility

In December 2013, my Emmy Noether group (funded by the Deutsche Forschungsgemeinschaft) moved from Ludwig-Maximilians-University in Munich to the MPIPZ, where I now head the new Plant Proteomics Service and Research Group. I took over from Jürgen Schmidt, the former head of the mass spectrometry group, who retired in October 2013. For more than a decade, Jürgen Schmidt and Thomas Colby had developed the Mass Spectrometry Facility at the MPIPZ into a widely used technical platform for the Institute's research groups.

Current research projects: Exploring the role of lysine acetylation in the regulation of cellular functions

Research in my group is concerned with the mechanisms plants have evolved to coordinate and regulate organellar functions and metabolism under adverse environmental conditions. We have a strong focus on organellar proteomics and the role of lysine acetylation in the regulation of plant metabolism and stress signalling. Reversible acetylation of the ε-amino group of lysine has recently emerged as a major post-translational modification of proteins, which controls many important cellular functions beyond its known significance for histone modification (Figure 1). Acetylation of lysine residues in proteins is regulated by the activities of acetyltransferases and deacetylases. Both our own recent results in Arabidopsis and research in animal systems have shown that lysine acetylation acts both as an on/off switch for enzyme activities and as a signal for cargo transport in several cellular processes that involve changes in energy metabolism, signalling cascades and cytoskeleton dynamics (Figure 2). The aim of our research is to uncover the function and importance of lysine acetylation in the regulation of plant metabolic pathways and signalling processes involved in the plant stress response. Recently, we mapped the Arabidopsis acetylome and identified lysine-acetylated sites in organellar and cytosolic proteins of diverse functional classes in *Arabidopsis thaliana*. We have also developed a new isotope-labelling and immune-enrichment-based method for relative quantification of lysine acetylation sites in plant proteins.



Lysine acetylation is a reversible post-translational modification of proteins catalysed by lysine acetyltransferases (KATs) and deacetylases (KDACs). The acetylated lysine is no longer positively charged, which can affect protein-protein interactions or enzymatic activities of proteins. Proteins with diverse functions and subcellular localizations have been identified as lysine acetylated in plants and other organisms (from Finkemeier and Schwarzer, Biospektrum, 2013, modified).

Figure 1

Quantitative proteomics is a key technology for dissecting and understanding complex plant cellular functions

Iris Finkemeier

Proteomics service

Besides our expertise in protein lysine acetylation and other post-translational modifications such as phosphorylation, we have also a lot of experience in the analysis of complex protein mixtures and whole proteomes, as well as in the quantitative analysis of protein co-immunoprecipitates, using heavy isotope labels, as well as label-free LC-MS/MS. Hence, we can offer a comprehensive proteomics service to departments and independent research groups at the Institute.

Plant proteomics methods we wish to establish at the MPIPZ in the next five years

Enormous advances have been made in liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics in recent years. Gel-free LC-MS/MS-based approaches now allow the identification and quantification of hundreds or several thousands of proteins in a single LC run, which takes 1 – 4 h depending on the length of the reverse-phase column used to fractionate the complex peptide mixture. These gel-free approaches can often be used in place of more time-consuming gel-based assays. There are two main research directions in LC-MS-based proteomics which will need to be developed further at the MPIPZ in the future.

(i) Discovery proteomics aims to identify and quantify a large proportion of a given proteome. Thus, it is the method of choice for a priori characterization of proteomes, including mapping of post-translational modifications, and quantification of relative protein abundances. The latest generation of instruments offers improved dynamic range and scanning rates, so that smaller proteomes (up to about 10,000 proteins) can be almost fully covered and quantified in single 4-h runs.

(ii) Targeted proteomics allows accurate and specific quantification of proteins with a high dynamic range down to low attomolar concentrations, albeit only for a limited number of proteins. Such single or multiple reaction monitoring assays (SRM/MRM) still require a lot of effort in development and optimization. However, once established they allow fast and highly accurate quantification of selected target proteins in the same sample. If one chooses to repeatedly measure a particular set of target proteins, the initial effort will be easily outweighed, when compared to the effort necessary to generate the same quantitative data with Western blots.

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The Max Planck-Genome-centre Cologne

The **M**ax **P**lanck-**G**enome-centre **C**ologne (MP-GC) was initiated as a core facility for **N**ext-**G**eneration **S**equencing (NGS) by a consortium of four Max Planck Institutes based in Bremen, Marburg and Cologne. The centre is located at the MPIPZ in Cologne and routine operations started in October 2010. Besides sequencing systems for raw data generation, the centre includes a core computing group, dedicated to achieving the scientific goals of the participating institutes by providing non-standard bioinformatics tools, advanced data assemblies and also "finished" genomes. In addition, the MP-GC continuously applies the most recent developments in genome/bioinformatics technologies according to the scientific needs of the participating institutes of the Max Planck Society.

Since the centre began to operate, approximately 190 scientists from nearly 90 research groups have requested MP-GC services and more than 800 NGS projects have been successfully completed. At present, the MP-GC provides sequencing services on four different NGS platforms. Most frequently requested are the Illumina (HiSeq 2500 and MiSeq systems) and Roche-454 (454 FLX+ and Junior systems) platforms, while the Ion Torrent system is used less frequently. In October 2013, the fourth platform, equipped with the PacBio RS II system (based on single molecule sequencing technology), was started. The PacBio system is able to generate long sequence reads (~10 kb) and record base modifications.

Due to the large amount of data generated on these NGS platforms, the MP-GC computing facility is equipped with high-performance servers, an infrastructure for data management including an Isilon storage system and a LOT-5 tape-based data archive system. System administration for all computer equipment is organized by an IT manager.

After sequence generation, routine bioinformatics analyses are organized in automated pipelines, while follow-up analyses are restricted to exceptional cases, due to limitations in the availability of bioinformatics personnel. Beyond that, the MP-GC offers individual training in bioinformatics tools for scientists with less experience in sequence analysis. In addition, the MP-GC is organizing workshops together with the CLC (once a year), as well as regular "Friday lessons" during university terms, to increase the knowledge base in bioinformatics of our associated scientists. Furthermore, the MP-GC includes a robotics section, which offers a range of instruments that allow high-throughput sample processing.

Green reads map in the forward (5' to 3') direction and red reads map in the reverse direction. The gap in this assembly could easily be bridged by PacBio reads.

Figure 1: **Mapping of Illumina reads to a Pacific Biosciences assembly.**

Generation and assembly of sequencing data is the first step in the elucidation and analysis of complex systems

Richard Reinhardt

Green reads map in forward (5' to 3') direction and red reads map in the reverse direction. Clonal reads lead to uneven coverage.

Figure 1: Mapping of Illumina reads to a Pacific Biosciences assembly.

A major feature that distinguishes the MP-GC from commercial sequencing providers is the planning and processing of any sequencing project according to the individual researcher's requirements. Specific methods are developed or modified for critical input material, different library preparation protocols are tested and, because every sample is bar-coded, small-scale sequencing is possible on the different sequencing platforms. MPI scientists are welcome to visit the MP-GC in order to learn the methods needed to prepare samples for sequencing and use the infrastructure of the MP-GC for the realization of their research projects.
For further information, please visit our website (http://mpgc.mpipz.mpg.de)

Selected publications

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International Max Planck Research School

Olof Persson
Johanna Spandl

The International Max Planck Research School (IMPRS) on "The molecular basis of plant development and environmental interactions", which was organized by the MPI for Plant Breeding Research and the University of Cologne, was first funded in 2001.
In 2012 the IMPRS applied for a six-year extension (2014-2019) and after a thorough scientific evaluation of the programme the extension was approved.
The new funding period includes an additional university partner – the Heinrich Heine University, Düsseldorf, and the IMPRS has been given a new focus to reflect the increased interest in the Institute in plant evolution and trait diversification. Consequently, the name was changed to IMPRS for "Understanding complex plant traits using computational and evolutionary approaches".

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. Annual retreats at which IMPRS students present their work, and close supervision by thesis committees, seek to ensure a high standard of doctoral training within the programme.
By September 2013, 91 doctoral students had graduated from the IMPRS programme and 33 students are still pursuing their doctoral studies within the IMPRS. Another 13 students joined the IMPRS in October 2013.

Postdoc and PhD Initiatives

PIM – Postdoc Initiative at the MPIPZ

The PostDoc Initiative at the MPIPZ (PIM) is open to all postdocs. Its main aim is to offer information and arrange courses intended to further the career development of the young scientists working at MPIPZ. It was founded in November 2007 and provides a platform to promote postdocs' interests, education and networking. We receive full support from the MPIPZ, which allows us to sustain a range of activities.

PIM regularly provides classes in statistics, project management and scientific writing. We try to meet the needs of our members by designing our programme in accordance with specific requests. Inviting and hosting speakers at Institute seminars is another activity that broadens our scientific horizons and allows us to establish collaborations with labs of interest. PIM also organizes well attended international conferences like the Next Generation Plant Science and Next Generation Sequencing meetings held in 2012 and 2010, respectively. Besides scientific activities, PIM regularly arranges social events to promote integration of newcomers and motivate participation.

PhD representatives at the MPIPZ

Once a year the PhD students at the MPIPZ elect representatives to act as spokespersons for the PhD community. Each of the four departments elects one representative, which ensures close interaction within and between departments. One additional representative is recruited from the PhD students working at the University of Cologne. This group of students is at the interface between the PhD community, the administration and the directors of the Institute, and is charged with bringing issues of concern to the PhD students to their attention. Communication between the PhD representatives of different institutes within the Max Planck Society is strongly supported by the PhDnet, the association of all PhD students in the Max Planck Society.

Within the past two years several improvements for PhD students have been put into place, mainly regarding equality of payment between stipend holders and students working on a contract. One big achievement was the successful implementation of a health-care subsidy that was set up by the Central Administration of the MPG. In addition, a contracting system between PhD student and the Thesis Advisory Committee consisting of supervisor and co-supervisor was set up to guarantee that every PhD student receives quality mentoring. By signing this contract, all parties agree upon fixed annual meetings for short progress reports and discussions on future directions of the thesis project.

Another highlight was the Career Day that took place on the MPIPZ campus in April 2012. Several invited speakers from academia and industry offered insights into their own careers, which can serve as examples for the future prospects of PhD holders in the field of biological sciences. As highlighted by these examples, the PhD representatives are actively working as a team to promote the interests of the PhD community at the MPIPZ.

WissenschaftsScheune, Press and Public Relations

Heinz Saedler
Wolfgang Schuchert

The WissenschaftsScheune, a Public Relations Project: History

The Institute started intensive public relations work in 1990, in response to its first field trial with transgenic plants in Germany. A Demonstration Garden displaying important agricultural and horticultural plants was established. Thus the public at large, and especially young people, now had the opportunity to learn about the research activities in the Institute and current developments related to plant sciences and agriculture in a broad societal context. Classes from secondary schools in the region were the most frequent visitors. In 1996, together with the University of Cologne, the project KölnPUB was initiated at MPIPZ to introduce molecular experiments to pupils and teachers. In 2006 the WissenschaftsScheune (Science Barn) was set up at MPIPZ. The Verein der Freunde und Förderer des Max-Planck-Instituts für Pflanzenzüchtungsforschung (Association of Friends of the MPIPZ) supervises the activities in the Science Barn on our farm and the neighboring Demonstration Garden.

The project

The aim of the WissenschaftsScheune is to spark curiosity about and arouse interest in plant science. It gives the public an opportunity to experience many aspects of plant science - from basic research to agriculture, from the DNA molecule to cultivation of crop varieties and their wild progenitors. Its facilities are the Science Barn itself, a building (500 m^2) on the farm that adjoins the Institute, with exhibitions and interactive displays documenting several fields of plant science, and the 6000-m^2 Demonstration Garden. In the garden more than 100 agricultural and horticultural crops (modern varieties and wild forms) are cultivated on small plots. Posters provide basic information about each plant for visitors. The layout therefore allows indoor and outdoor activities to be combined. Barn and Garden are integrated into an agricultural park, the Landschaftspark Belvedere, which was the result of a competition in the context of the Strukturförderprogramm Regionale 2010 initiated by the Cologne City Council and the State of NRW, together with MPIPZ.

The program

The WissenschaftsScheune offers special programs for primary schools, secondary schools and adults. Some 20 interactive stations are available at present. The main topics covered are domestication and evolution, flower development, biodiversity, renewable resources, plant pests and diseases, gene technology, Mendel's laws as the basis for classical breeding, and photosynthesis. The brochure series "WiS Begierig" and three films are available, which can be used to orient school classes prior to visits or encourage critical reflection afterwards. We use various media and tools designed for the different target groups. Here are some examples: While playing the treasure-hunt game "On the Amerindian Trail" children learn about the origins of some of our crop plants, and experience stages in the domestication of maize, beans, tomatoes and potatoes. In the case of wheat domestication for instance, the visitor can follow the evolution of wheat on a single plot which demonstrates how successive rounds of hybridization between tetraploid and diploid species gave rise to hexaploid bread wheat. The major differences between wild forms and domesticated varieties (mechanics of seed dispersal and kernel size) and the short-stem varieties that resulted from the Green Revolution are highlighted. The section for advanced high-school students on "Tools of evolution" begins by challenging them to decode a DNA sequence reading "mutations are rare". Selection is illustrated in the garden using charts that ask players to identify the organ altered

in a particular cultivated form relative to its wild progenitor, using examples from the domestication of some Brassicaceae. The genetic basis can then be discussed. For younger children simple experiments like DNA isolation from fruits or vegetables using kitchen tools, making glue from starch, dyeing with plant colours or determination of pests and diseases are offered. Additionally, special events like birthday celebrations, holiday events for children, a Girls' Day and Children's University are part of the program.

Planning your visit to the WissenschaftsScheune

The best time for a visit to the WissenschaftsScheune is from May until October. Each guide can look after a group of up to 12 individuals. Larger groups will be divided. Our team consists of specialists from different disciplines (research, agriculture, education). Visits can now be booked online on our new homepage. Normally a visit to the WissenschaftsScheune lasts for up to 3 hours, deals with two selected plant topics and includes an introduction to the MPG and the research of the Institute for adults.

Impact

Over the two-year period from July 2011 to June 2013 roughly 2000 people visited the WissenschaftsScheune and the MPIPZ. Most of these were schoolchildren and students, but various interest groups and representatives of business and politics have also been among the attendance.
Nine brochures in the series "WiS Begierig" each devoted to a single thematic area, and a new homepage (www.wissenchaftsscheune.de) with extensive information for the different target groups, are now available. In a new project in cooperation with the University of Cologne the WissenschaftsScheune is being incorporated into the education of school biology teachers. In addition, excursions to our Demonstration Garden are now part of the curriculum for student teachers at the University's Botanical Institute. The WissenschaftsScheune is also involved in the Annual Conference on Teacher Education organized by the Association of the German Chemical Industry (Fonds der Chemischen Industrie NRW). For the summer of 2014 a presentation at the State Garden Show (Landesgartenschau NRW 2014) is planned.

Further outreach activities

Regarding general PR activities the Institute cooperates with various committees and organizations. It is represented in relevant working 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). We are also actively involved in training courses for project leaders and biosafety representatives arranged by the University of Cologne.
On the Institute's homepage press releases documenting scientific highlights are listed chronologically, so that journalists can use these for reports.
Special events like the weekly TATA-Bar get-togethers organized by our PhD students, the Summer Party or sporting events such as the Institute Run promote internal communication among staff and students, and contribute to a pleasant working atmosphere.
One highlight was the Open Day in summer 2012. Around 800 visitors participated, talked with scientists and learned more about research on plant sciences.

IT Engineering, IT Support and Bioinformatic Platform

Margit Purchart

The Scientific User Support Administration Networking (SUSAN) group underwent a major reorganization in mid-2010, creating new staff structures and new service models and now offering new services. Apart from the Head of IT, SUSAN is currently staffed by four qualified IT specialists/system engineers, four student assistants and one trainee. Three trainees (in IT system integration) completed their apprenticeships successfully in 2011 and 2012.
The IT infrastructure of the whole Institute includes the connecting network, servers, storage capacity, desktops and notebooks, printers, video conference system, emergency call system, mobile phones and the core services, i.e. mail services, file services, back-up services, database systems, conference services and Web services. The bioinformatic platforms comprise a computer cluster with ca. 500 processor cores controlled by a job scheduler, and ca. 1.1 petabyte of disc space on a highly scalable storage system, as well as a small server and storage system for database applications and other scientific software that need a certain dedicated system environment. The whole infrastructure has been modified to achieve more performance and a higher degree of homogenization. This was achieved by hardware replacement, virtualization and redesign. The scientists can choose their PCs, laptops, tablets etc. from a well assorted shopping basket. Printers are leased and include a hardware service. Different operating systems and applications are available in order to meet the varying needs of different scientists.

A user helpdesk resolves users' daily IT problems, maintains user accounts, offers IT training sessions for users and provides advice concerning new desktops, laptops, mobile phones and data encryption, etc. For travelling scientists, special travel notebooks compatible with the legal restrictions in different countries are available. There are also some IT services for visiting scientists. Demand for conference services e.g. Web and video conferences is on the rise. A new ticketing system was installed in autumn 2011 and ca. 5.000 service and support requests had been processed within two years.
SUSAN also takes care of connecting scientific lab devices to desktops or to the network. Furthermore there is some support for the Genome-centre.
Collaboration with the Institute's bioinformaticians is very close and activities are discussed and planned in monthly steering committee meetings.

Library

Britta Hoffmann

The MPIPZ Library provides literature, electronic media and information services to the Institute's scientific staff and guests. The collection is focused on the fields of research covered by the departments and research groups at the Institute.

The MPIPZ Library, together with the Max Planck Society's Max Planck Digital Library, provides a selection of the numerous electronic journals now available, in addition to other scientific information resources like e-books and databases. The Institute spends 0.9 % of its budget on these services. A committee of scientists advises the Library on issues related to research needs.

In 2013 the Library's holdings comprised approximately 23,000 printed journal volumes and 5500 monographs. The printed holdings can be searched in the online catalogue. At present about 30,000 journals are available electronically, most of which were licensed by the Max Planck Society. The Electronic Journals Library offers an entry-point to all licensed and free e-journals. In addition to this the Library can obtain all literature that is not available on site, both electronically and in hard copy. Users can order such material by filling out a form available via the intranet, which forwards the data to a database.

Furthermore the Library collects all institute publications for institutional archiving of research output on the publication repository maintained by the Max Planck Society (MPG.PuRe) and provides all publications as PDFs on the web site with one year delay for copyright reasons.

Technical equipment

The number of PC workstations in the library has increased to fourteen and there are now eight additional workplaces for private laptops. There are also two separate rooms with PCs available for temporary staff.

Greenhouse Management

Wolfgang Schuchert

The greenhouse team cultivates model plants for scientific experiments in close cooperation with the scientists. The staff comprises nine gardeners (two part-time), two assistants and one horticultural apprentice, and it handles around 80 culture orders per week. Its main tasks are the preparation of soil, pricking out, transplantation, watering, plant propagation, plant protection measures, seed harvesting and control of growing conditions.

The net area under glass at present is limited to 420 m^2 cooled and 1200 m^2 uncooled (which includes 500 m^2 of space temporarily rented from the Horticultural Experimental Station of the NRW Chamber of Agriculture in Cologne-Auweiler). In two Saran and two transparency houses, plants as Arabis, tomato, potato and cereals can be grown under more natural conditions over a prolonged vegetation period. Twenty-one cold frames (15 m^2 each) and a field with automatic watering (800 m^2) are used for small outdoor experiments. The number of growth chambers has also been increased further. For the new department nine new Hettich/Elbanton chambers are already available now and five new Reftech walk-in chambers are on order. Moreover, five walk-in Bronson chambers have been installed in recent years.

For cultivation in trays and pots, 200 cubic meters of standardized soil substratum are needed per year. In accordance with the strict legal requirements regarding decontamination, steam sterilization of transgenic Arabidopsis plants, seeds and soil material, and the cleaning of planting trays after experiments, is now carried out in a separate new building. Other materials which have come into contact with transgenic plants are inactivated by the use of two modern Systec autoclaves.

A green group (a landscape gardener, an agrarian engineer and three assistants) looks after the green areas attached to the Institute, including the demonstration garden, and prepares and conducts field trials. The group also takes care of the disposal of waste paper, repair and maintenance of agricultural implements and winter maintenance.

Contact & Directions

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How to get to the MPIPZ

By car

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

Highway A1 (south):
Take the Lövenich exit (# 103), turn right at crossroads, follow Aachener Straße, direction Köln-Zentrum. After about 1 km turn right towards 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.

If you are using your navigation system, please note that to reach the Institute from the right direction you must take the approach via "Militärring" and "Gregor Mendel Ring". Only this route allows you to turn on Belvederestraße to reach the Institute.

By train

Arrival at Cologne main train station (Köln Hauptbahnhof)

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

By plane

Cologne/Bonn Airport:
Take S-Bahn S13 or train (Regionalbahn RE8) to Cologne main station (Köln Hauptbahnhof). Proceed as described above.

Düsseldorf Airport:
Take S-Bahn S7 to Düsseldorf main station (Düsseldorf Hauptbahnhof), then train (IC, ICE, RE, RB) to Cologne main station. Proceed as described above.

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

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