14th Swiss Plant Molecular and Cell Biology Conference

March 9-11, 2005

Eurotel Victoria, Les Diablerets

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14th Swiss Plant Molecular and Cell Biology Conference
March 9 – 11 Eurotel Victoria, Les Diablerets

PROGRAMME

Wednesday 9th March (2005)

17h00-18h30 Welcome and registration

Dinner

Session I: **Light**
Chairperson: Michel Goldschmidt-Clermont (Geneva)

20h.00 Frédy **Barneche** (Geneva)
The nucleus-encoded At-TAB2 protein is involved in photosystem I and photosystem II protein synthesis in *Arabidopsis thaliana*

20h20 Claire **Bréhélin** (Neuchâtel)
Suborganelle proteome analysis reveals participation of Arabidopsis plastoglobules in chloroplast metabolic function

20h40 Paula Debora **Duek** (Lausanne)
The degradation of HFR1, a putative bHLH class transcription factor involved in light signalling, is regulated by phosphorylation and requires COP1

21h00 Michel **Goldschmidt-Clermont** (Geneva)
Raa1, a novel multifunctional factor involved in trans-splicing of group II introns in the chloroplast of *Chlamydomonas reinhardtii*

Thursday 10th March

Session II: **Responses to biotic and abiotic stress**
Chairperson: Felix Mauch (Fribourg)

08h00 Klaus **Apel** (Zürich)
Keynote address : "An attempt at learning more about the role(s) of reactive oxygen species (ROS) in plants"

08h40 Christophe **Laloi** (Zürich)
Distinct and antagonistic effects of different ROS on gene expression and cell death in *Arabidopsis thaliana*

09h00 Lucie **Dubugnon** (Lausanne)
Reactive lipids, jasmonates, and gene expression

09h20 Thierry **Genoud** (Fribourg)
Pathogen-induced changes in the plant defense signaling network: a systems biology approach

09h40 Georg **Felix** (Basle)
The receptor kinase FLS2 from Arabidopsis determines specificity of flagellin perception and interacts directly with the flg22-epitope

Coffee break

Session III: **Resistance to pathogens and insects**
Chairperson: Willy Gruissem (Zürich)

10h30 Thomas **Hohn** (Basle)
About caulimoviruses circumventing the rule

10h50 Philippe **Reymond** (Lausanne)
Transcriptional responses of *Arabidopsis thaliana* to chewing insects

11h10 Gabor **Jakab** (Neuchâtel)
BABA-induced resistance of grapevine: wine is not the only difference from Arabidopsis

11h30 Nabila **Yahiaoui** (Zürich)
Evolution of resistance specificity at the Pm3 locus of powdery mildew resistance in wheat

11h50 Christof **Sautter** (Zürich)
A Swiss field test with GM-wheat in 2004
Followed by a podium discussion on the field-test moratorium

Lunch, ski afternoon

17h00-18h45 **Poster session**

Dinner

Session IV: **Symbiosis**
Chairperson: Didier Reinhardt (Fribourg)

20h15 Uta **Paszkowski** (Geneva)
Genome-wide expression profiling and reverse genetics to define factors essential for the arbuscular mycorrhizal symbiosis in rice

20h35 Marcel **Bucher** (Zürich)
Molecular biology of phosphate transport in the arbuscular mycorrhizal symbiosis

20h55 Didier **Reinhardt** (Fribourg)
Petunia mutants defective in the arbuscular mycorrhizal symbiosis

Friday 11th March

Session V: **Transport**
Chairperson: Doris Rentsch (Berne)

08h00 Markus **Klein** (Zürich)
The MATE transporter TT12 is a vacuolar membrane protein mediating anthocyanin transport and controlling vacuolar integrity in the Arabidopsis seed coat endothelium

08h20 Markus **Geisler** (Zürich)
The TWISTED DWARF1 modulates auxin transport activities of ABC transporter, AtPGP1, by protein-protein interaction

Session VI: **Gene Expression**
Chairperson: Fred Meins (Basle)

08h40 Vivien **Exner** (Zürich)
Chromatin assembly factor subunits are required for CAF1-dependent and -independent functions during development

09h00 Margaret **Collinge** (Zürich)
The quest for orcs

09h20 Fred **Meins** (Basle)
Possible roles of Arabidopsis Dicer-like 3 (DCL3) genes in regulating the expression of retroelements and small gene families

Coffee Break

Session VII: **Biotechnology**
Chairperson: Michel Schneider (Geneva)

10h10 Pierre **Goloubinoff** (Lausanne)
Massive controlled expression of recombinant proteins in the moss *Physcomitrella patens* using a conditional heat-shock promotor

10h30 Emmanuel **Boutet** (Geneva)
The Swiss-Prot Plant Proteome Annotation Program (PPAP)

10h50 Yves **Poirier** (Lausanne)
Studying the carbon flux through peroxisomal ß-oxidation in plants and yeast using polyhydroxyalkanaotes

Session VIII: **Development**
Chairperson: Uta Paszkowski (Geneva)

11h10 Christoph **Ringli** (Zürich)
Mutations affecting pectin formation suppress the Arabidopsis cell-wall-formation mutant *Irx1*

11h30 Christian **Hardtke** (Lausanne)
Exploiting natural genetic variation in Arabidopsis to identify novel regulators of quantitative aspects of root development

11h50 Sam **Zeeman** (Berne)
Understanding how plants make and degrade semi-crystalline starch granules

12h10 Cris **Kuhlemeier** (Berne)
Models for phyllotaxis

12h30 *End of the meeting*

Abstracts of the oral communications
(in order of presentation)

Session I: Light

The nucleus-encoded At-TAB2 protein is involved in photosystem I and photosystem II protein synthesis in *Arabidopsis thaliana*

Barneche Frédy, Crèvecoeur Michèle and Jean-David Rochaix
Département de Biologie Moléculaire, Science III, Université de Genève, 30 Quai Ernest Ansermet, 1211 Genève

Several nucleus-encoded proteins targeted to the chloroplast have been identified in plants and algae that are necessary for the processing and/or the translation of mRNAs encoding proteins of the photosynthetic apparatus. In particular, a Chlamydomonas mutant strain affected in the *TAB2* nuclear gene is specifically deficient in the translation of *psaB* mRNA, encoding a photosystem I reaction center polypeptide. The *tab2* mutation drastically reduces the amount of *psaB* mRNA associated with polysomes and its translation. The TAB2 protein is localized in the chloroplast stroma and is part of a high molecular weight ribonucleoprotein complex comprising the *psaB* mRNA, as shown by co-immunoprecipitation of *psaB* RNA with the TAB2 protein. Moreover, gel-shift experiments demonstrated that the TAB2 protein is able to interact directly with the *psaB* 5'UTR *in vitro* via an unknown RNA-binding domain (Dauvillee et al., 2003. EMBO J., 22: 6378-6399).

Tab2 is conserved in land plants and cyanobacteria. This indicates a prokaryotic origin and raises questions on its function in plants. In contrast to Chlamydomonas, inactivation of *At-TAB2* in *Arabidopsis thaliana* has pleiotropic effects: in addition to the loss of photosystem I (PSI), the level of photosystem II (PSII) is severely reduced. The levels of the corresponding chloroplast mRNAs are not affected. Growth of the *at-tab2* mutant plants is severely affected and the plants display an albino phenotype with altered chloroplast structure. Immuno-blots and *in vivo* pulse-labelling experiments indicate that At-TAB2 is a chloroplast protein necessary for normal synthesis and/or accumulation of the PSI and PSII core proteins but not of subunits of other thylakoid complexes. The *At-TAB2* gene is induced by light during de-etiolation, and more specifically by blue light during early plant development. Taken together, these data suggest that this factor could have a wider role in photosystem biogenesis in land plants than previously expected from its analysis in Chlamydomonas.

Suborganelle proteome analysis reveals participation of Arabidopsis plastoglobules in chloroplast metabolic function

Pierre-Alexandre Vidi, Sacha Baginsky, Felix Kessler, **Claire Bréhélin**
Université de Neuchâtel, Institut de Botanique, Laboratoire de Physiologie Végétale, Emile Argand 11, Case Postale 2, CH-2007 Neuchâtel

Proteomics have emerged as a powerful tool to assign functions to cellular organelles. The chloroplast has been the target of several proteomics projects leading to the identification of new proteins and functions in major chloroplast compartments (stroma, envelope membranes, thylakoid membranes and lumen). Plastoglobules are stromal lipoprotein particles associated with thylakoid membranes and have been proposed to function as a reservoir of thylakoid

lipids. Here we present the protein composition of plastoglobules contributing a new suborganellar proteome to the inventory of the chloroplast. In addition to seven members of the plastid lipid associated protein (PAP)/fibrillin family, which we term plastoglobulins, we identify known metabolic proteins as bona fide plastoglobule components. Using immuno-blotting analysis, enzyme assays and transient expression of GFP-fusions, we demonstrate that significant fractions of the fructose-bisphophate aldolases and the allene oxide synthase specifically associate with plastoglobules. The data indicate that plastoglobules have a previously unrecognized role in chloroplast metabolism. Moreover, the presence of tocopherols (reported previously) and the tocopherol cyclase (reported here) suggests a role of plastoglobules in protection against photooxidation.

The degradation of HFR1, a putative bHLH class transcription factor involved in light signalling, is regulated by phosphorylation and requires COP1

Paula D. Duek, Mireille V. Elmer and Christian Fankhauser
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Plant development is influenced by light throughout their life cycle. In Arabidopsis, multiple photoreceptors including the UV-A/blue sensing cryptochromes (cry1-2) and the red/far-red responsive phytochromes (phyA-E) monitor the ambient light conditions. Light-regulated protein stability is a major control point of photomorphogenesis. The ubiquitin E3 ligase COP1 (constitutively photomorphogenic 1) regulates the stability of several light signalling components. HFR1 (long hypocotyl in far-red light) is a putative transcription factor with a bHLH domain acting downstream of both phyA and the cryptochromes. HFR1 is closely related to PIF1, PIF3 and PIF4 (phytochrome interacting factor 1, 3 and 4), but in contrast to the latter three there is no evidence for a direct interaction between HFR1 and the phytochromes. Here we show that the protein abundance of HFR1 is tightly controlled by light. HFR1 is an unstable phospho-protein, particularly in the dark. The proteasome and COP1 are required *in vivo* to degrade phosphorylated HFR1. In addition, HFR1 can interact with COP1, consistent with the idea of COP1 directly mediating HFR1 degradation. We identify a domain, conserved among several bHLH class proteins involved in light signalling, as a determinant of HFR1 stability. Our physiological experiments indicate that the control of HFR1 protein abundance is important for a normal de-etiolation response.

Raa1, a novel multifunctional factor involved in trans-splicing of group II introns in the chloroplast of *Chlamydomonas reinhardtii*

Michel Goldschmidt-Clermont, Livia Merendino, Karl Perron, Michèle Rahire, Isabelle Howald and Jean-David Rochaix
Departments of Molecular Biology and of Plant Biology, University of Geneva, 30 quai E. Ansermet, 1211 Genève 4, Switzerland.

In the chloroplast of *Chlamydomonas reinhardtii*, there are two group II introns in the *psaA* gene. The three exons are widely scattered in the plastid genome and are transcribed as separate precursors that are then spliced *in trans*. The

maturation of *psaA* mRNA depends on the products of at least fourteen nuclear genes, including *Raa1, Raa2* and *Raa3*.

Two mutant alleles of *Raa1* (*raa1-L137H* and *raa1-HN31*) are blocked in *trans*-splicing of both split introns of *psaA*, while one allele (*raa1-314B*) is defective for *trans*-splicing of only the second split intron. The *Raa1* locus is rearranged in *raa1-314B*, but part of the gene is still expressed. Furthermore, C-terminal deletions of *Raa1* can rescue *raa1-314B*, but not *raa1-L137H*. Maturation of a small non-coding RNA (*tscA*) , which is a structural component of intron1, is defective in *raa1-L137H* and *raa1-HN31*. Thus different functional domains of Raa1 can be genetically separated. The C-terminal part is involved in *tscA* maturation and / or splicing of intron 1, and an N-proximal domain is required for splicing of intron 2.

The predicted Raa1 polypeptide (2103 residues) contains five degenerate repeats of a 38 amino acid sequence, which are related to the repeats in the chloroplast translation factor Tbc2, but Raa1 has no other similarity to known proteins. Raa1 is imported in the chloroplast where it is associated with a membrane fraction and is part of two complexes. The smaller complex also contains another factor required for splicing, Raa2. The size of the larger complex is reduced after treatment with RNase. Raa1 is thus a new type of factor involved in group II intron splicing and seems to be part of a large ribonucleoprotein complex.

Session II: Responses to biotic and abiotic stress

Keynote address "An attempt at learning more about the role(s) of reactive oxygen species (ROS) in plants"

Klaus Appel
Institute of Plant Sciences, ETH-Zürich, 8092 Zürich

The evolution of aerobic metabolic processes such as respiration and photosynthesis unavoidably lead to the production of reactive oxygen species (ROS) in mitochondria, chloroplasts and peroxisomes. A common feature among the different ROS types is their capacity to cause oxidative damage to e.g. proteins, nucleic acids and lipids. These cytotoxic properties explain the evolution of complex arrays of ROS scavengers. In plants chloroplasts and peroxisomes are the major sites of ROS production. Various abiotic stress conditions may limit the ability of a plant to use light energy for photosynthesis. Under such stress conditions hyper-reduction of the photosynthetic electron transport chain and photoinhibition of photosynthesis may occur even at moderate light intensities, often causing damages that have been interpreted as unavoidable consequences of injuries inflicted upon plants by toxic levels of ROS. However, at least for one of these ROS, singlet oxygen, this paradigm needs to be modified. Stress responses triggered by this ROS are not due to physicochemical damages but are caused by the activation of genetically determined stress response programs. We'll present data from our work that show that changes in singlet oxygen production resulting from metabolic disturbances are used by plants as a signal that activates acclimatory responses and helps the plant to cope with environmental changes.

Distinct and antagonistic effects of different ROS on gene expression and cell death in *Arabidopsis thaliana*

Christophe Laloi
Institute of Plant Sciences, Federal Institute of Technology Zürich, LFW, Universitätstrasse 2, CH-8092 Zürich, Switzerland

During abiotic stress conditions like high light exposure, different reactive oxygen species (ROS) are generated simultaneously in plants, making it difficult to determine the biological activity and mode of action for each of these ROS separately. Such a study requires finding conditions under which only one specific ROS is generated. In order to address this problem, we made use of the conditional *flu* mutant of *Arabidopsis thaliana* that makes it possible to generate singlet oxygen ($^1O_2$), a non-radical reactive oxygen species, in plastids in a controlled and noninvasive manner. Rapidly after the release of $^1O_2$, *flu* plants stop growing and initiate a cell death response. We have shown that $^1O_2$ is involved in activating distinct sets of early stress-response genes that are different from those activated by superoxide (O_2.-) / hydrogen peroxide (H_2O_2) during a treatment with paraquat, an herbicide that acts as a terminal oxidant of photosystem I. The up-regulation of genes selectively activated by O_2.-/H_2O_2 - but not by $^1O_2$- was strongly suppressed during a co-treatment of plants with DCMU or in plants over-expressing thylakoidal ascorbate peroxidase (tAPX). Surprisingly, the over-expression of tAPX in the *flu* mutant increased the extent of $^1O_2$-induced cell death and the up-regulation of genes selectively activated by $^1O_2$. These results suggest that O_2.-/H_2O_2 antagonize the $^1O_2$-mediated stress response and highlight the necessity of considering different ROS separately rather than as a whole.

Reactive lipids, jasmonates, and gene expression

Lucie Dubugnon, Laurent Mène-Saffrané, Robin Liechti and Edward E. Farmer. Gene Expression Laboratory, Plant Molecular Biology, Biology Building, University of Lausanne, CH-1015 Lausanne, Switzerland

Lipid oxygenation plays a central role in the plant immune system and numerous genetic studies have confirmed the importance of the canonical jasmonate pathway in plant defense. We now suspect the existence of a second, related pathway whereby reactive lipids can activate stress gene expression. Several of these lipids are jasmonates, but most are generated non-enzymatically and include malondialdehyde (MDA), a remarkable and ubiquitous lipid fragmentation product. We term these compounds 'reactive electrophile species' (RES), the current idea being that plants use RES, along with ROS, to monitor and respond to the severe stresses that cause non-enzymatic lipid oxidation. We are attemping to alter RES levels in both Arabidopsis and Saccharomyces in order to investigate their biogenesis and biological activity. In Arabidopsis, jasmonate pathway mutations eliminate cyclopentenone jasmonates and fad mutations eliminate many RES derived from tri-unsaturated fatty acids. In yeast, we can introduce substrates for MDA production into living cells The current results support the previously proposed RES limb of the jasmonate pathway (Stintzi et al. 2001) and are consistent with RES activity in and around wounded cells.

Pathogen-induced changes in the plant defense signaling network: a systems biology approach

Thierry Genoud[1,4], John Pufky[2], Beth A. Krizek[3], Patrick Bozo[1], Patrick Hurban[2], and Jean-Pierre Métraux[1]
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Salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) play a central role in the activation of plant defense responses during pathogen attack: We performed a global analysis of gene expression in *Arabidopsis* submitted to exogenous treatment with these three compounds (SA, JA, ET), as well as their combinations (SA+JA, SA+ET, JA+ET, SA+JA+ET). Genes have been grouped in regulons/clusters according to their expression profile. The resulting classes were used to build a model of the signaling network used by plant cells upon activation by the three hormones. The model has been inferred as a minimal Boolean/digital circuit and is represented in the form of a single network for computer simulation. Data mining was conducted to find transcription factors that undergo changes in expression during interaction with virulent pathogens. Among the putative factors, whose expression shows a reverse behavior during *Pseudomonas syringae* infection (up- instead of down-regulated), two genes coding for zinc-finger proteins (ZAT7, ZAT12) have been identified. They cluster in regulons with several genes involved in salt and drought resistance. Further analysis of the resistance of *Arabidopsis* plants containing mutations in ZAT7 and ZAT12 and of lines of transgenic plants constitutively over-expressing these genes suggests that the *Pseudomonas*-induced changes in ZATs expression increase its virulence.

The receptor kinase FLS2 from Arabidopsis determines specificity of flagellin perception and interacts directly with the flg22-epitope

Delphine Chinchilla, Zsuzsa Bauer, Martin Regenass, Thomas Boller and **Georg Felix**
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Flagellin, the main building block of the bacterial flagellum, acts as a pathogen associated molecular pattern (PAMP) triggering the innate immune response in animals and plants. In Arabidopsis, the leucine–rich repeat transmembrane receptor kinase FLS2 is essential for flagellin perception. Here, we demonstrate direct, specific interaction of the elicitor-active epitope flg22 with the FLS2 protein by chemical crosslinking and immunoprecipitation. Functionality of this receptor was further tested by heterologously expressing the AtFLS2 gene in cells of tomato. Perception of flg22 in tomato differs characteristically from that in Arabidopsis. Expression of AtFLS2 conferred an additional flg22-perception

system to the cells of tomato that showed all the properties characteristic for perception of this elicitor in Arabidopsis. In summary, these results show that FLS2 constitutes the flagellin receptor that determines the specificity of flagellin perception.

Session III: Resistance to pathogens and insects

About caulimoviruses circumventing the rule

Faiza Noreen, **Thomas Hohn** and Katja Richert-Poeggeler, Botanical Institute of the University of Basel.

Plant viruses that replicate via reverse transcription are grouped in the family of caulimoviridae. This include species with icosahedral and bacilliform capsids; they have a genome length of approximately 8000 basepairs, coding for reverse transcriptase, protease, an icosahedral or bacilliform capsid, movement and insect transmission functions and sometimes a transactivator, but in contrast to the mammalian retroviruses no integrase. Consequently caulimoviridae do not integrate obligatorily into the host chromatin but accumulate in the host nucleus as episomal minichromosomes.

Despite of this rule, DNA of several classes of caulimoviridae were recently detected in almost all types of plants. These are well distinguished from retrotransposons and occur in different stages of fossilation. From the stage of their genome and the degree of fragmentation, rearrangement and other mutations, one can conclude that the integration events occurred at different stages of plant evolution. Usually, the integrated forms are not productive, due to the mutations mentioned above, but also, because single integration events would not lead to templates that can produce RNA with redundant ends, as they are required for reverse transcription of true retroviruses.

Nevertheless, at least two types of endogenous caulimoviridae can produce infectious viruses, banana streak virus and petunia vein clearing virus, probably because their establishment in the host plant occurred relatively early. Caulimoviridae have a very high recombination rate due to frequent template switching during reverse transcription, and one can imagine, that, once active reverse transcriptase is produced, the whole genome can be assembled from incomplete and fragmented RNA. Rousing of active virus occurs upon various types of stress, such as repetitive wounding, callus culturing, etc.
In course of our studies with petunia vein clearing virus (PVCV) we found that interestingly tandem integration events had occurred. From a tandemly integrated caulimogenome, terminally repeated RNA can be easily produced, much like as from integrated retroviruses with their long terminal repeats. Such rousing occurs despite the fact that PVCV is integrated into heterochromatin, i.e. the pericentromeric regions and they are by default methylated and connected to inactive histones. PVCV occurs in some wild petunia and nearly all types of hybrid petunia.

Universal degenerated primers reveal that many more types of caulimoviridae occur in petunia, either in addition to PVCV in petunia hybrida or in its absence in wild type petunia species. Some of these are related to PVCV, while others are related to other classes of caulimoviridae.

Transcriptional responses of *Arabidopsis thaliana* to chewing insects

Philippe Reymond
Department of Plant Molecular Biology, University of Lausanne

Transcriptional patterns elicited in response to insect attack are fashioned in response both to physical damage as well as to biological components displayed or released by the aggressor. Using a large-scale DNA microarray we characterized gene expression in damaged as well as in distal Arabidopsis leaves in response to to the specialist insect, Pieris rapae. Over one hundred insect-responsive genes potentially involved in defense were identified, including genes involved in pathogenesis-related response, indole glucosinolate metabolism, detoxification processes, or signal transduction. A large proportion of inducible genes was found to be regulated by the jasmonate pathway. Finally, the comparison of Arabidopis responses to larvae of the specialist *Pieris rapae* and the generalist insect *Spodoptera littoralis* revealed a remarkably similar transcript signature.

BABA-induced resistance of grapevine: wine is not the only difference from Arabidopsis

Gabor Jakab, Hamiduzzaman Mollah Md, Romain Dubresson, Sophie Marc-Martin, Michael Bel, Zhanna Kravchuk, Jean-Marc Neuhaus, Brigitte Mauch-Mani
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ß-aminobutyric acid (BABA), a non-protein amino acid is able to induce resistance in Arabidopsis plants through the priming of the salicylic acid (SA)- and abscisic acid (ABA)-dependent defence signalling pathways. BABA-primed Arabidopsis plants responded more efficiently to pathogen attack by a faster activation of defense-related gene expression and an earlier and stronger accumulation of callose around the penetration sites. BABA-induced resistance (BABA-IR) was also observed in grapevine against downy mildew (*Plasmopara viticola*). Treatments of the susceptible variety Chasselas with either benzothiadiazole (BTH, a SA analogue) or ABA, however, did not lead to protection while jasmonic acid (JA) treatment was able to induce resistance against *P. viticola*. Using of mutants, a successful approach in Arabidopsis research, to determine the importance of the different signalling pathways in BABA-IR was not feasible in grapevine. Therefore we have used alternative methods. Co-application of specific inhibitors, on one hand, suggested that callose deposition and defence mechanisms depending on products derived from the phenylpropanoid and the lipoxygenase pathways all contributed to the observed resistance following BABA treatment. Expression pattern of marker genes, on the other hand, indicated the priming of both SA- and JA-signalling pathways in BABA-treated Chasselas plants. These results indicate significant differences of BABA-IR in the model plant Arabidopsis and grapevine. The similarity of BABA- and JA-induced resistance against downy mildew, the potentiated expression pattern of JA-regulated genes in BABA-treated plants and the suppression of BABA-IR with ETYA treatment suggest an involvement of the JA pathway in BABA-IR of grapevine which leads to a primed deposition of callose and lignin around the infection sites. To determine the key components of BABA-IR in grapevine, application possibilities of gene silencing techniques were tested.

The efficacy and applicability of the different techniques of siRNA production (pHELLSGATE vectors, dsRNA producing vectors, U6 hairpin cloning systems) and delivery into grapevine (infiltration, gene gun, Agrobacterium) will be discussed.

Evolution of resistance specificity at the Pm3 locus of powdery mildew resistance in wheat

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There are 10 alleles (*Pm3a* to *j*) conferring powdery mildew race-specific resistance at the *Pm3* locus of hexaploid bread wheat (*Triticum aestivum* L.). The *Pm3b* gene, a member of the CC-NBS-LRR type of disease resistance genes was cloned from hexaploid wheat by map-based cloning. Haplotype studies in different wheat lines carrying known *Pm3* specificities (*Pm3a* to *j*) indicated a good conservation of the haplotype in the *Pm3* region. This led to the rapid isolation of additional candidate alleles from these wheat lines using a PCR-based strategy. The *Pm3* candidate alleles were tested in a transient transformation assay and were shown to be functional. These results indicated that the *Pm3* genes form a true allelic series encoded by a single member of a large gene family on wheat chromosome 1A. A pattern of conservation and non conservation of blocks of sequences between alleles indicated recombination or gene conversion events at the origin of alleles sequence divergence. In addition, two out of 7 functional alleles only differed by three point mutations. *Pm3* orthologous genes were further cloned from cultivated and wild wheat relatives. Sequence comparisons of all these genes shed light on the mechanisms leading to the generation of functional powdery mildew resistance genes in wheat species.

A Swiss field test with GM-wheat in 2004

Christof Sautter
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In a SNF-project we studied genetically modified Swiss wheat varieties for possibilities to improve fungal resistance to bunt diseases (Clausen et al. 2000). A comparison between greenhouse, a vegetation hall, and field showed different reactions of different wheat varieties to different environmental conditions. This is apparent in flavonoid content as an example for a secondary metabolite and for gene expression as seen with cross-hybridizing genes from a barley micro-array. These results confirmed that a field test with our GM wheat was required for final proof of concept. This test finally was performed during the season 2004. A number of safety measures prevented undesired spreading of the transgene. Bio-safety was monitored during the experiment and following. I will discuss scientific, legal, political and financial implications of this field test and compare our experiences to the worldwide steadily increasing agronomic application of GMO.
Reference: Clausen M, Kräuter R, Schachermayr, Potrykus I, Sautter C (2000) Antifungal activity of a virally encoded gene in transgenic wheat. Nature Biotechnology **18**, 446-449.

Session IV: Symbiosis

Genome-wide expression profiling and reverse genetics to define factors essential for the arbuscular mycorrhizal symbiosis in rice

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Amongst the plants that enter into the arbuscular mycorrhizal (AM) symbiosis rice currently represents the only plant whose genome has been completely sequenced. The Syngenta 51K rice Affymetrix GeneChip® was used to perform whole transcriptome analysis of mycorrhiza-regulated genes at the stage of an established symbiosis. The expression of the complete set of candidate genes was validated by real-time RT-PCR. Differential regulation upon mycorrhizal colonization for a total of 225 genes was confirmed. These genes provide an "index" of mycorrhiza-regulated genes in rice. The specificity of their regulation was analyzed in rice roots infected with pathogens and in rice roots grown at different phosphate regimes. Classes of genes were defined according to their expression profiles: Class I contains genes commonly regulated in response to colonization by symbiotic and pathogenic fungi, Class II consists of genes responding to enhanced phosphate availability and Class III comprises putative mycorrhiza-specific genes. Within Class III we identified a subset of genes for which transcripts were detected exclusively in mycorrhizal roots. In order to determine the conservation of mycorrhiza-regulated genes in mono- and dicotyledonous plants available protein or nucleotide sequences from dicotyledonous plants were blasted against the rice genome and searched for the overlap with the rice "index" of 225 genes. A total of 74 mycorrhiza-regulated dicot genes found homologs among the list of rice genes. For the assessment of the relevance of these genes during the development of the AM symbiosis we have established an assay that allows for the rapid geno- and phenotyping of transposon-tagged rice lines (compare poster: Toumi et al.).

Molecular biology of phosphate transport in the arbuscular mycorrhizal symbiosis

Marcel Bucher
Institute of Plant Sciences, ETH Zürich, Experimental Station Eschikon 33, 8315 Lindau

A functional symbiotic interaction between arbuscular mycorrhizal fungi and their host plants is based on signal recognition and transduction mechanisms which eventually lead to the induction of key genes involved in *e.g.* nutrient transfer. We have recently identified StPT3, a mycorrhiza-upregulated phosphate transporter (PT) from potato (Rausch et al., 2001) and subsequently demonstrated that the *StPT3* gene is specifically induced upon colonization of root cortex cells with fungi from the phylum *Glomeromycota* in a cell-autonomous way (Karandashov et al., 2004). Very recently, StPT4 and StPT5, two novel mycorrhiza-specific PTs from potato and their orthologs from tomato have been identified (Nagy et al., 2005). Molecular physiological analysis of symbiotic phosphate transport in a tomato PT gene knock-out mutant indicated

functional redundancy of two distinct phosphate uptake systems in solanaceous species, suggesting that different PT genes are co-regulated in the symbiosis. Recent data obtained using molecular approaches to decipher mycorrhiza signaling involved in symbiotic phosphate transport will be presented.

References:
Karandashov et al. (2004) Evolutionary conservation of phosphate transport in the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA, 101: 6285-6290
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Petunia mutants defective in the arbuscular mycorrhizal symbiosis

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The majority of plant species engage in the arbuscular mycorrhizal (AM) symbiosis with fungi from the phylum Glomeromycota. In this mutualistic association, the strictly biotrophic fungus improves the nutritional status of the plant in exchange for carbohydrates. We want to undestand how the development of the symbiotic partners is coordinated. Using the Petunia hybrida line W138 that harbors the high copy number transposable element dTPh1, we have isolated three mutants that are affected in the development of the AM symbiosis. The contact defective1 (con1) mutant is blocked at a very early stage, presumably before mutual recognition of the symbiotic partners. Two further mutants are affected at later stages, after the penetration of the root. In the arbuscule defective1 (arb1) mutant, the fungus can colonize the root, but the development of arbuscules is inhibited, resulting in the abortion of the fungus. In the bloated arbuscule1 (blo1) mutant, short bloated structures are formed in the place of the arbuscules. Furthermore, we show that Petunia strongly suppresses fungal colonization at elevated phosphate levels. This suppression is associated with developmental defects that resemble the limited colonization in the arb1 mutant. These results show that Petunia is a valid experimental model for the genetic dissection of mycorrhizal development, including the nutritional control of the symbiosis.

Session V: Transport

The MATE transporter TT12 is a vacuolar membrane protein mediating anthocyanin transport and controlling vacuolar integrity in the Arabidopsis seed coat endothelium

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Phenolic compounds play a central role in the protection of plants against stress and are involved in processes such as pollination/insect attraction, seed dispersal, dormancy and longevity. In many cases, phenolic compounds produced by the plant are toxic to its own metabolism although they often accumulate at high concentrations. Therefore the plant cell has evolved efficient compartmentation strategies in order to protect the cytosol against their toxic effects. The plant central vacuole and the extracellular space are major destinations for secondary compounds. Biochemical, genetic and molecular analyses led to the elucidation of almost all major enzymatic steps and parts of the transcriptional regulatory network involved in the biosynthesis of phenolic compounds in model species such as *A. thaliana*. In contrast, with the exception of the genetic characterization of the *transparent testa 12 (tt12)* mutant in Arabidopsis no membrane protein involved in either the vacuolar or extracellular deposition of phenolic substances has been described (Debeaujon *et al.* 2001 Plant Cell 13, 853-871). Previous vacuolar transport studies suggest the presence of either directly energized ABC-type flavonoid pumps and/or secondary energized flavonoid/proton-antiporters. *TT12* encodes a protein with similarity to prokaryotic and eukaryotic secondary transporters with 12 transmembrane segments, belonging to the MATE (multidrug and toxic extrusion) family. This is the first plant MATE transporter whose potential role in flavonoid metabolism could be deduced from a mutant phenotype, i.e. an absence of proanthocyanidin deposition in the endothelium of seed integuments. The MATE family with more than 50 genes in the Arabidopsis genome has been discovered only recently. MATE homologs are absent in animals and functional data are still scarce. *TT12* is specifically expressed in the seed coat. A GFP-TT12 fusion which partially complements the *tt12* mutant phenotype localizes TT12 onto the plant vacuolar membrane after either transient or stable transformation. When expressed in *S. cerevisiae*, fluorescence of GFP-TT12 is detected mainly on the vacuolar membrane but also in other membranes. In order to establish a heterologous complementation system to study the function of TT12 and other MATE transports in flavonoid transport, growth-tests with wild-type yeasts and knockout mutants lacking several multidrug resistance transport systems were performed with respect for their sensitivity to different phenolic compounds including flavonoids and tannins. However, yeasts tolerated these compounds even at high concentrations. In the presence of MgATP, vesicles isolated from yeasts transformed with *TT12* exhibit time-dependent uptake of the model anthocyanin cyanidine 3-glucoside. Interestingly, immature seed coat integuments of *tt12* mutants have ‘disrupted’ vacuoles in confocal images after staining with fluorescent dyes which usually accumulate in the central vacuole. We conclude that TT12 is able to mediate vacuolar anthocyanin (and most

probably also proanthocyanidin) transport and that it is involved in vacuolar biogenesis in the Arabidopsis seed coat.

The TWISTED DWARF1 modulates auxin transport activities of ABC transporter, AtPGP1, by protein-protein interaction

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Previously, the immunophilin-like protein TWD1 (AtFKBP42) from Arabidopsis has been demonstrated to physically interact with the ABC transporters AtPGP1 and AtPGP19 (AtMDR1). Phenotypic analysis of atpgp1 atpgp19 and of twd1 mutant plants suggested a positive regulatory role of TWD1 on AtPGP1 and AtPGP19 transport activities. The later are thought to catalyze the primary export of the auxin indole-3-acetic acid (IAA).

In agreement, we here report that IAA export from twd1 protoplasts is even more reduced than in atpgp1 atpgp19 mutants. Moreover, polar auxin transport rates in hypocotyls are also further decreased in twd1 mutants compared to atpgp1 atpgp19 plants. As a result twd1 mutant roots reveal elevated levels of free IAA that might cause the strong developmental phenotype. While atpgp1 and atpgp19 mutants have been shown to perform hyperphoto- and hypergravitropic responses, roots and shoots of twd1 plants are agravitropic. Moreover, twd1 roots reveal different sensitivities toward auxin transport inhibitors, like NPA and TIBA.

Coexpression of TWD1 and AtPGP1 in yeast demonstrated that TWD1 inhibited AtPGP1-mediated auxin transport. This is surprising as the opposite was expected from the plant data. The interaction of both membrane proteins on the molecular level in yeast was verified and further analyzed by Bioluminescence Resonance Energy Transfer (BRET) upon co-expressing TWD1-rLuc and AtPGP1-YFP in yeast.

In summary, we provide molecular insights into the "twd1 syndrome" suggesting that TWD1 functions as regulator of auxin transport acivities via AtPGP1.

Session VI: Gene expression

Chromatin assembly factor subunits are required for CAF1-dependent and -independent functions during development

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Chromatin assembly factor CAF-1 facilitates the formation of nucleosomes on newly replicated DNA in vitro. However, the role of CAF-1 in development is poorly understood because mutants are not available in most multicellular model organisms. In *Arabidopsis thaliana*, FASCIATA1 and FASCIATA2 are two CAF-1 subunits, and *fasciata* mutants are viable in Arabidopsis, suggesting that CAF-1 is not absolutely required for cell division in plants. On the other side, there are five genes encoding MSI1-like proteins. Recently, one member of this family, MSI1, has been shown to form a complex with chromatin assembly complex

(CAC) subunits CAC1 and CAC2 of Arabidopsis, and this complex has nucleosome assembly activity in vitro (Kaya et al., 2001). Furthermore, there is genetic evidence that MSI1 is the third subunit of Arabidopsis CAF-1 also in vivo, a function that cannot be substituted by its homologs MSI2, 3, 4 or 5. All three Arabidopsis CAF-1 mutants affect the shoot apical meristem, but in addition CAF-1 is required to establish seedling architecture, plant size and trichome differentiation. These results reveal that the functions of FAS1, FAS2 and MSI1 are not restricted to the meristems but are also needed for control of genome replication at multiple steps of development.

The quest for orcs

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The Origin Recognition Complex (ORC) is a well conserved, six-subunit protein complex. In fungi and metazoans, the ORC has well characterized functions in DNA replication and heterochromatin-mediated silencing. Other functions for individual subunits were long suspected and are just now beginning to be defined. Where ORC action is best known, in DNA replication, the details of control and function, i.e. when different subunits bind during the cell cycle, how repeated origin firing is prevented, vary. We are interested in how ORC function and control has evolved with the demands of the plant lifestyle and development.

Genes, mostly single-copy, encoding proteins with similarity to all six ORC subunits occur in plants and the proteins interact in two-hybrid experiments. But we know neither whether all the functions defined in fungi and metazoa are also conserved in plants nor how ORC control and action has evolved in the much more distantly related plant kingdom. A mutant in the Arabidopsis gene for ORC subunit 2 (*orc2*, the first *orc* we found) has a zygotic-lethal phenotype, as might be expected for a protein involved in DNA replication. Unexpected was our observation that endosperm of *orc2* mutant seeds stopped dividing even before the embryo, and then began to endoreplicate. We are studying these enlarged nuclei in detail as they highlight differences in cell cycle control between embryo and endosperm in the developing seed.

To find out more about the other Arabidopsis ORC subunit homologues and to determine whether they might also act in a complex we are collecting and analysing more *orc* mutants from the insertion collections. So far, the phenotypes of these mutants show that one of the homologues may have evolved to have different functions in plants.

Possible roles of Arabidopsis Dicer-like 3 (DCL3) genes in regulating the expression of retroelements and small gene families

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Retroelements (RE) are major components of animal and plant genomes. RNA silencing has been proposed to help regulate RE activity, which is believed to be an important factor in genome evolution. Numerous 21-26 nt small RNAs

(smRNA) in the Arabidopsis genome have exact matches in REs with intact ORFs. Dicer and Dicer-like (DCL) proteins are at a key branch point in smRNA metabolism. Four *DCL* genes, *DCL1-DCL4*, have been found by protein domain-homology searches of the *Arabidopsis* genome. We used two well-characterized, *DCL1* loss-of-function mutants, *dcl3-1* and *dcl3-2* in combination with RNA profiling to examine the possible role of smRNAs in regulating RE activity. Studies by others had shown that *DCL1* is required for the production of microRNAs from stem-loop precursors and dsRNA. We show that *DCL3*, but not the other *DCL* genes, is required for production of 24-26 nt smRNAs representing the methylated SINE element *AtSN1*. It is also required for production of several smRNAs representing *AtCon*, a highly methylated 180 bp repeat, which is rich in *Athilia*-like REs and an integral component of the centromere. Southern analyses with methylation sensitive restriction enzymes showed that CpNpG sites in *AtCon* become progressively demethylated in successive generations of the deficient *dcl3-1* mutant. These results support the working hypothesis that DCL3 is part of an RNA-silencing pathway important for regulating RE activity and silencing of centromeric sequences. We also provide evidence that DCL3 could have a role in regulating expression of small gene families and the production of smRNAs representing these families.

Session VII: Biotechnology

Massive controlled expression of recombinant proteins in the moss *Physcomitrella patens* using a conditional heat-shock promotor

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The possibility to express controlled and elevated levels of endogenous or recombinant proteins in plant cells is an essential tool for plant biology and biotechnology. Here, the activity of the heat-inducible soybean Hsp17.3 promoter in the moss *Physcomitrella patens* was compared to that of the maize ubiquitin-1 gene and to that of the 35S promoter from Cauliflower Mosaic Virus, using beta-glucuronidase (GUS) and the F-actin binding fusion protein GFP-talin as reporter genes. GUS expression driven by the Hsp promoter was extremely low at 25°C and was induced more than 1000 fold in independent transformed lines. Levels of heat shock-induced GUS expression were proportional to the duration and to the temperature of induction enabling a fine tuning of the expression level. Repeated cycles of heat induction under non-damaging conditions for PSII activity accumulated GUS up to 2.3 % of the total soluble proteins, which is 40 times higher than GUS expression driven by the strong constitutive Ubi-1 maize promoter. In transgenic lines expressing the 35S-GFP-talin construct, F-actin labeling was restricted to fully differentiated cells. In contrast, a uniform labeling of F-actin structures were observed in every cells from heat-shock induced transgenic lines expressing the Hsp-GFP-talin construct. Remarkably, the natural compounds salicylic acid and a triterpen, celastrol, significantly induced GUS and GFP-talin expression from the Hsp17.3 promoter,allowing induction of recombinant proteins at 25oC. We conclude that the soybean Hsp17.3 promoter provides a highly reliable and versatile conditional promoter for the controlled

expression by mild heat or specific chemical treatment of recombinant proteins and reporter genes in the moss *Physcomitrella patens*.

The Swiss-Prot Plant Proteome Annotation Program (PPAP)

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Since 2002, the Swiss Institute of Bioinformatics (SIB), the European Bioinformatics Institute (EBI) and Protein Information Resource (PIR, Georgetown University, Washington) have joined forces to create and maintain the **Universal Protein Resource (UniProt)**[1].

Swiss-Prot, the **manually annotated** section of the UniProt Knowledgebase, was created in 1986. Since 1998, it is complemented by TrEMBL (translated EMBL), which is a computer-annotated section containing the translation of all the coding sequences deposited in the EMBL nucleotide sequence database[2]. Together, Swiss-Prot and TrEMBL give access to all protein sequences available.

While TrEMBL is highly redundant, Swiss-Prot curators **minimize database redundancy** by merging all data from different literature reports dealing with one given protein into a single entry. Comparison of several sequences helps in the detection of potential sequencing or frameshift errors. Moreover, alignment of paralogous and orthologous sequences from several species increases the detection of erroneous gene model predictions.

Every UniProt entries provide **cross-references to more than 60 external data collections** such as the underlying DNA sequence entries in the DDBJ/EMBL/GenBank nucleotide sequence databases, 2D PAGE and 3D protein structure databases, various protein domain and family characterisation databases, post-translational modification databases and species-specific data collections.

In 2000, Swiss-Prot initiated the **Plant Proteome Annotation Program (PPAP)**, a program focused on the annotation of plant-specific proteins and protein families. Our major effort is currently directed towards *Arabidopsis thaliana*, but the completion of the *Oryza sativa* genome sequence prompted us to start a new project specifically devoted to rice.

At the beginning of 2005 (release 45.5, 04-Jan-2005), Swiss-Prot contains 11804 plant entries, distributed in more than 2700 plant species. The three most represented plants are *Arabidopsis thaliana*, *Zea Mays* and *Oryza sativa* with 3096, 513 and 438 entries respectively.

Swiss-Prot homepage: http://www.expasy.org/sprot/
ExPASy homepage: http://www.expasy.org/
UniProt homepage: http://www.uniprot.org/

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Studying the carbon flux through peroxisomal ß-oxidation in plants and yeast using polyhydroxyalkanaotes

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Polyhydroxyalkanoates (PHA) are polyesters naturally synthesized by a wide variety of bacteria as a carbon reserve. These polymers have attracted considerable attention because of their plastic and elastomeric properties, making them a valuable source of biodegradable and renewable plastics. Our laboratory has worked in the past at looking at plants as vectors for the synthesis of these interesting polymers. More recently, we have demonstrated synthesis of PHA in the peroxisomes of both Saccharomyces cerevisiae and Pichia pastoris. In this system, PHA is synthesized through the polymerization of the 3-hydroxyacyl-CoA intermediates generated by the ß-oxidation of fatty acids. We are now using the synthesis of PHA in yeast and plant peroxisomes as a tool to study some fundamental aspects of the biochemistry of fatty acid metabolism in peroxisomes. For example, the relative contributions of various pathways involved in the degradation of fatty acids having either cis or trans unsaturated bonds at an even- or odd-numbered carbon can be analyzed. Furthermore, we are gaining some novel insight on the function of some poorly characterized peroxisomal proteins, such as a peroxisomal acyl-CoA thioesterase, on fatty acid metabolism.

Session VIII: Biotechnology

Mutations affecting pectin formation suppress the Arabidopsis cell wall formation mutant *lrx1*

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Plant growth requires a coordinated expansion of each cell and its surrounding cell wall. During the enlargement of the extracellular matrix, the biosynthesis of new cell wall material and its integration into the growing wall must be a tightly controlled process. Consequently, extracellular regulatory and/or signaling components are important to control cell wall growth.
LRX proteins are extracellular and consist of a leucine-rich repeat domain thought to be involved in protein-protein interaction, and a structural extensin domain important for anchoring of the protein in the extracellular matrix. Based on their structure, LRX proteins are good candidates for a regulatory- or signaling function during cell wall formation. In the past, *LRX1* of Arabidopsis was characterized and found to be important for cell wall formation in root hairs. *LRX1* is specifically expressed in root hairs and *lrx1* mutants develop root hairs that are short, branched, frequently collapsed, or form bulbous structures at the root hair base. Ultrastructural analysis revealed a strongly aberrant cell wall, indicating that LRX1 is indeed involved in cell wall formation.
To better understand the developmental process LRX1 is involved in, we have performed a suppressor screen on the *lrx1* mutant. After EMS-mutagenesis, several revertant *rol* (repressor of *lrx1*) lines displaying wild-type root hairs were

isolated. These *rol*/*lrx1* double mutants display a suppressed *lrx1* phenotype, suggesting that the *rol* loci might be involved in a process that is related to LRX1 function. The two recessive mutants *rol1* and *rol4* were found to be allelic and map-based cloning lead to the identification of point mutations in *RHM1*, one of three *RHM* genes encoding enzymes that interconvert UDP-glucose to UDP-rhamnose. As rhamnose is a major component of pectin, the two mutants are likely to be affected in the pectin structure. The suppression of the *lrx1* phenotype by a mutation that affects the pectin structure suggests that LRX1 might be involved in the formation of the pectic matrix. To support or reject this speculative hypothesis, we are currently comparing wild-type, *lrx1*, *rol1*/*lrx1*, and *rol4*/*lrx1* plants for changes in the polysaccharide composition and structure of the pectic matrix.

Exploiting natural genetic variation in Arabidopsis to identify novel regulators of quantitative aspects of root development

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To isolate novel factors that modulate quantitative aspects of root development and are responsible for intra-specific morphological variation, we investigated natural genetic variation in the model plant *Arabidopsis thaliana*. In a sample of 55 accessions, we observed a 2-3 fold variation in basic root system growth parameters, such as lateral root formation or primary root growth. Two accessions with slow-growing primary roots were investigated in further detail. Through various crosses to accessions with fast-growing primary roots, we were able to mendelize the major quantitative trait loci (QTL) responsible for slow growth. In map-based cloning approaches, we so far isolated one of these two loci, which we named *BREVIS RADIX (BRX)*. This locus is responsible for approximately 80% of the variance of the observed difference in root length between the short-rooted accession Uk-1 and the control line, Sav-0. BRX is a novel regulator of root growth and controls the extent of cell proliferation and elongation in the growth zone of the root tip. *BRX* is a member of a small group of highly conserved genes, the BRX gene family, which is only found in multicellular plants. Analyses of Arabidopsis single and double mutants suggest that *BRX* is the only gene of this family with a role in root development. Cross-complementation analyses involving various *BRX-like* genes suggest that this lack of redundancy is partly due to differential activity of BRX-like proteins as well as differential expression patterns. The BRX protein is nuclear localized and activates transcription in a heterologous yeast system. BRX family proteins contain two distinct highly conserved domains without previously characterized function. In a yeast two hybrid system, one of these domains mediates protein-protein interaction with another family of putative transcription factors. Thus the combined data indicate a role for BRX family proteins in transcriptional regulation. Further details on the genetics and biochemistry of this gene family will be reported.

Understanding how plants make and degrade semi-crystalline starch granules

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Carbohydrates produced through photosynthesis are stored primarily in the form of transitory starch, which is used to sustain metabolism when photosynthesis is not possible. This starch is remarkable substance composed of polymers of the simple sugar glucose. The major polymer is amylopectin, in which chains of glucose molecules are connected to one another by branch points and arranged in such a way that the end product, the granule, has a semi-crystalline structure. At least three enzymes are directly involved in the synthesis of amylopectin; starch synthases (which make component chains), branching enzymes (which create the branch points) and, surprisingly, debranching enzymes (which cut the branch points). The involvement of the latter class has been revealed by examples of debranching enzymes-deficient plants which accumulate phytoglycogen, an altered glucose polymer. Phytoglycogen has an unusually high degree of branching, which prevents the formation of the semi-crystalline granule. However, the exact function of debranching enzymes in determining amylopectin structure is not yet well defined. The aim of our research is to define precisely the how debranching enzymes are involved in the synthesis and degradation of starch. The Arabidopsis genome encodes four debranching enzymes. Using reverse genetics to knockout each gene we have created a full suite of single, double, and triple mutants together with the quadruple knockout. Our current analysis of the mutant phenotypes reveals functional specialisation for biosynthesis and degradation within the small gene family and illustrates the importance of starch metabolism in plant growth.

Models for phyllotaxis

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A major determinant of plant architecture is the arrangement of leaves and flowers around the stem, known as phyllotaxis. Spiral phyllotaxis is of particularinterest, because the divergence angle between successive leaves approaches the golden ratio of 137.5°, and the spiral arrangements are characterized by Fibonacci numbers.

Although mathematical models can recreate phyllotactic patterns, experimental support for them has largely been lacking. I will present experiments thatestablish the plant hormone auxin as a central regulator of phyllotaxis. These experiments provide the basis for biologically meaningful models that can guide the design of new experiments. Ultimately, models should account for all aspects of phyllotaxis, for its reiterative nature, regularity and stability, as well as for the transitions between phyllotactic systems.

Abstracts of the posters
(alphabetically according to the 1st author)

Chloroplast Protein Import: Studies on Toc Receptor GTPase Interactions

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To acquire functionality, chloroplasts import most of their constituent proteins from the cytosol. The cytosolically synthesized chloroplast proteins (precursor proteins) have a cleavable, amino-terminal transit sequence, specifying their targeting and translocation. The Toc complex (translocon at the outer membrane of chloroplasts) enables recognition of precursor proteins and their translocation across the outer envelope membrane. The Arabidopsis Toc-complex consists of three principle components: two homologous GTP-binding receptor proteins, Toc159 and Toc33 (known as Toc34 in pea), and the protein-import channel, Toc75 (for review see Kessler and Schnell, 2004; Jarvis and Robinson, 2004). Currently, two hypotheses for Toc-GTPase function are under investigation:

In the "targeting model" Toc159 functions as the primary precursor protein receptor docking onto Toc33 to hand down the precursor protein and insert it into the Toc75 channel (Hiltbrunner et al., 2001). In the alternative "motor model", however, precursors are first recognized by Toc33 and subsequently transferred to Toc159. Here, Toc159 functions as a GTP-dependent motor protein threading the precursors through the Toc75 channel (Schleiff et al., 2003). Despite the apparent contradictions in the models, the interaction of Toc33 and Toc159 via their GTP-binding domains (G-domains) is a key feature of both (Weibel et al., 2003).

We therefore analyze Arabidopsis Toc receptor GTPase interactions and dynamics using different assay systems based on protein complementation (e.g. Two Hybrid System, Split-Ubiquitin System). Using a Two Hybrid based random mutagenesis screen we identified Toc159 mutations affecting Toc33 binding. The loss-of-interaction phenotype has been confirmed using *in-vitro* pull down assays. Recent results on the effects of these mutations on Toc159 GTPase activity and the sequence of events in vivo will be presented.

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Characterization of the snowy cotyledon 1 mutant: the impact of chloroplast elongation factor G for plant vitality

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Chloroplasts are involved in different metabolic processes. Proper function of

chloroplasts is necessary for normal growth of plants. Several analyses of mutants gave indications, that development of chloroplasts may be different in cotyledons and true leaves. In aim to identify additional clues to this organ specificity, we screened for mutants exhibiting pale cotyledons and green true leaves. The isolated mutant was named *snowy cotyledon1 (sco1)* for its white appearance of cotyledons in the early seedling stage. The *sco1* mutant exhibits a point mutation in the chloroplast elongation factor G (EF-G) within the ribosome-binding region in front of the second GTP-binding effector domain necessary for binding to 70S ribosome. The analyses of this mutant gave evidence that proper chloroplast function, in the *sco1* mutant with a reduced chloroplast protein translation, is required for different aspects in plant life cycle like germination, development, and plant vitality but not senescence.

Chlorophyll breakdown: functional analysis of RCC reductase

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During leaf senescence chlorophyll is broken down to colorless nonfluorescent catabolites (NCCs) in a multi-step pathway. A key reaction that leads to the loss of green color is catalyzed by the joint action of two enzymes, PaO and RCC reductase (RCCR). RCCR is present exclusively in land plants and ESTs are known from more than 25 species. RCCR acts in a stereospecific manner, thereby forming one of two possible isomers of a colorless fluorescent breakdown intermediate (pFCC-1 or -2). This specificity is defined for a given species, thus i.e. RCCR from tomato (LeRCCR) produces pFCC-2, whereas Arabidopsis RCCR (AtRCCR) produces pFCC-1.

Using a PCR-based cloning strategy we produced chimeric RCCR proteins in *E. coli* that contain different portions of AtRCCR and LeRCCR, respectively. By analyzing the activity of these chimeric RCCRs we could show that replacement of a single phenylalanine (F218) to valine (present in LeRCCR) is sufficient to change the stereospecificity of AtRCCR.

RCCR is a single copy gene in Arabidopsis. Surprisingly, *rccr*-mutants, like *acd2-2*, that are devoid of RCCR activity produce downstream catabolites (FCCs and NCCs). This raises the question whether at all RCCR is involved in chlorophyll breakdown *in vivo*. To answer this, *acd2-2* was functionally complemented using RCCR constructs that exhibit both stereospecificities. HPLC analysis of the types of FCCs and NCCs that are produced during leaf senescence confirm the participation of RCCR during chlorophyll breakdown.

Female gametogenesis and early zygotic development in Arabidopsis revisited by cytogenetics tools

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Sexual reproduction in plants poses the fascinating problem of how two genetically identical gametes, the egg cell and the central cell are determined to take distinct post-fertilization fates, and how the two genetically identical zygotic

products undergo distinct differentiation. There is growing evidence in yeast and animals that the transcriptional activity of genes, and hence their expression during differentiation, is influenced by nuclear organization. In plants, the role of chromatin dynamics in (epi)genetic differentiation of the female gametes and the zygotes has been completely ignored so far. We are developing cytogenetic methods to analyse the 3-dimensional distribution and organization of the DNA within the nuclear space and the biochemical modifications of the chromatin, in the precursors of the gametic nuclei and the gametes, as well as in the developing zygotes. This work will serve as a basis to understand how changes in nuclear organization help to establish specific patterns of gene expression during sexual reproduction in plants.

Fitting two females and one male in a single room: nuclear organisation in the endosperm of Arabidopsis

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The seeds of flowering plants contain two fertilization products: the embryo, precursor of the seedling, and the embryo-nourishing tissue, the endosperm. The embryo results from a fusion between a sperm and an egg while the endosperm is the result of fusion between a sperm and the homo-diploid central cell. In most species, the embryo and endosperm have the same maternal and paternal alleles but the endosperm contains an extra maternally derived genome. The ploidy ratio of two maternal to one paternal genome in the endosperm is critical for normal seed development but deviation of this ratio is not always lethal. Chromosome manipulation, genetic experiments and interploidy crosses have offered several hypotheses to explain this phenomenon. These include genomic imprinting, a mechanism whereby epigenetic modification of certain alleles leads to parent-of-origin dependent expression, and a mechanism whereby the action of some gene products is dependent on gene dosage. So far, the role of higher-order chromatin structure in endosperm nuclei and, in particular, the arrangement of the parental genomes, have not been investigated. Here, we report our preliminary understanding of the nuclear organisation in triploid endosperm nuclei from *Arabidopsis* seeds: similarities and differences compared with the typical organisation in diploid sporophytic nuclei are presented. The overall objective of our work is to determine whether the parental genomes in endosperm nuclei possess a non-random arrangement and/or a specific topology that could underlie the parent-of-origin effects observed during seed development.

Post-translational modifications of histones in Arabidopsis thaliana – a proteomics approach towards understanding the histone code

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Histones are involved in packaging the DNA into chromatin and are highly conserved among all eukaryotes. Their N-terminal tails can be post-

translationally modified by acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation. Specific lysine residues are subject to acetylation, lysine and arginine can be mono-, di- and tri-methylated and serine residues phosphorylated. These modifications occur singly or in combination and have different influences on gene expression and therefore are thought to generate an epigenetic code. This histone code hypothesis suggests that the combinations of histone tail modifications represent a language that is read by other proteins and can be translated by chromatin-remodelling machines and transcription factors (Strahl and Allis, 2000; Turner, 2000). With a few exceptions, however, it is not known which specific histone modification patterns exist and how these are associated with active or repressed chromatin. To understand the histone code we are investigating histone modifications and histone composition in *Arabidopsis thaliana* by applying mass-spectrometry based technologies.

Histone modifications have been analysed primarily using chromatin immunoprecipitation (ChIP) with antibodies generated against specific modified histone amino acids. However this method cannot detect multiple and different modifications on the same protein and it cannot distinguish between histone variants. For example, it is known that methylation of histone H3 K9 is associated with heterochromatin, but it is not known whether other modifications are also required or can inhibit (e.g. acetylation) the formation of heterochromatin. To analyse histones we established a method to separate histones by combining reversed phase chromatography with 1D SDS-PAGE, followed by analysis using MALDI-TOF/TOF and LC-MS/MS. The aim is to characterize the combination of multiple post-translational modifications of histones in Arabidopsis.

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De-novo synthesis of maltose in cold-stressed Arabidopsis plants

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Low temperature is one of the most important abiotic stress factors affecting growth and development of plants in temperate zones. It slows down many bioenzymatic reactions and may affect cells by the formation of ice. Plants respond and adapt to cold stress by various biochemical and physiological changes which are known as cold acclimation, a genetically programmed adaptation mechanism which is taking place during low non-freezing temperature.

Compatible solutes such as sugars, polyols, amino acids, quarternary ammonium compounds as well as certain proteins are synthesized by many organisms ranging from bacteria to plants in response to abiotic stress. They may contribute to the stabilization of cell compounds and the regulation of the cell osmotic potential. The disaccharide maltose a-glc-(1,4)-glc has been shown recently to act as a compatible solute during cold stress by protecting membranes and the photosynthetic electron transport chain in *Arabidopsis thaliana*. It was suggested that maltose is produced from starch degradation by a-amylase activity and accumulates during cold stress in the chloroplast stroma

(Kaplan and Guy, 2004; Kaplan et al., 2004).
We found increasing maltose concentrations in Arabidopsis leaves up to 2 days after cold treatment at 1°C. This maltose increase was transient during the first 1 to 3 days after starting cold treatment. Interestingly, maltose production was only found in plants which were grown under short day conditions (8h) and not under long day conditions (16h) prior to cold treatment. Maltose production was light dependent. No maltose was produced under cold conditions in darkness and glucose and fructose concentrations were quite low compared to control plants at 22°C. In photosynthetic pulse chase experiments with 14CO2 before cold treatment, we found increasing levels of 14C-labeled maltose already after two hours of cold treatment. Control plants growing at 22°C after 14C-treatment produced labeled glucose, fructose, and sucrose but no maltose. Additionally "maltose synthase" activity with α-glucose-1-phosphate as substrate (Schilling, 1982) was detected in crude protein extracts of cold-treated plants of Columbia and Landsberg as well as in two mutants which are deficient in starch synthesis (*pgm*-mutant) or starch degradation (*sex1-3*-mutant). Based on these results, we postulate the cold-induced maltose accumulation to proceed by *de novo* synthesis rather than starch degradation.

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Arabidopsis Mutants Enhanced in RNA Silencing

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In spite of extensive effort, previous genetic and molecular studies had identified only five plant genes required for RNA silencing. To identify additional silencing-related genes, we screened M2 populations of *Arabidopsis* ecotype "Columbia 0" for EMS mutants with enhanced RNA silencing (*ESI*) of a green fluorescent protein (GFP) reporter gene. Fifteen recessive mutants showing high GFP expression in cotyledons, but no GFP expression in true leaves fell into 5 complementation groups (*ESI1-ESI5*).

The *esi1* and *esi2* mutants studied in detail showed some variability in growth rate, but were otherwise normal in appearance. Nuclear run-on transcription experiments and the presence of GFP siRNAs verified that *esi1* and *esi2* mutants chosen for study exhibit RNA silencing and suggested that both mutants act upstream of siRNA production. We did not detect marked effects of *esi1* and *esi2* on either susceptibility to virus infection or virus-induced gene silencing (VIGS) as judged from inoculations of mutants, the high-GFP expressing wild-type line, and a silent GFP reporter line with *Cucumber mosaic virus* (CMV) and with RNA representing the genome of a recombinant *Turnip crinkle virus* (TCV) with the coat protein ORF replaced by a GFP-phytoene desaturase transcriptional fusion (TCV GFP-PDS CP). The CMV 2b protein has been shown to suppress RNA silencing and is believed to act in the cell nucleus. Our most interesting finding was that GFP silencing was not suppressed in *esi2* mutants infected with CMV. These results, and the fact a GFP reporter gene is constitutively silenced in *esi2*, lead us to propose that ESI2 is part of host

silencing-suppression pathway, which is activated by the CMV 2b protein.

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AtATM3 is involved in heavy metal resistance

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AtATM3 is a mitochondrial protein, probably involved in the maturation of Fe/S cluster protein, belonging to the large ABC transporter family of *Arabidopsis thaliana*. From sub-cDNA microarray experiments, *AtATM3* was found to be up-regulated by cadmium or lead in the roots. Interestingly, AtATM3-overexpressing yeast and plants were enhanced in cadmium resistance, whereas the *atatm3* mutant was more sensitive to cadmium than the corresponding wild type. Since previous reports often showed that the cytosolic glutathione level is positively correlated with heavy metal resistance, we measured non-protein thiols (NPSH) in the mutant plants. Surprisingly, we found that *atatm3* contained more NPSH than wild type under normal conditions. AtATM3-overexpressing plants were not different under normal conditions, but contained less NPSH than wild type under Cd(II) treatment conditions. These results suggest that AtATM3 might play a role in the regulation of cellular NPSH level. Gene expression studies supported this possibility. Indeed, under conditions that reduce expression of *GSH1* (γ-glutamylcysteine synthase) or inhibit glutathione synthesis, expression of *AtATM3* increased, whereas *GSH1* expression increased under Cd(II) stress and in *atatm3*. Since glutathione is required for supplying Fe/S cluster from mitochondria to the cytosol in yeast, AtATM3 is also possibly involved in glutathione transport across the mitochondrial membrane in the form of GS-conjugated Fe/S cluster. In conclusion, our data show that AtATM3 contributes to cadmium resistance, but the mechanism of action remains still unclear.

Apomixis Technology Development

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Apomixis is asexual reproduction through seed and is thought to have evolved from the deregulation of sexual developmental processes. Key elements of apomixis are apomeiosis (the avoidance of meiosis to produce an unreduced egg) and parthenogenesis (the autonomous development of an embryo). Within the nucellus tissue, a cell is identified that undergoes meiosis, resulting in the production of (among others) a reduced egg cell. The nucellus tissue, therefore, is an obvious candidate tissue-type in which to mis-express genes that may result the avoidance of meiosis. In sexual reproduction the egg cell initiates

embryogenesis upon its fusion with a sperm cell. The egg, therefore, is an obvious candidate for the targeted mis-expression of genes that may allow parthenogenesis. Genes can be conditionally expressed in the nucellus and the egg cell using an activator construct that provides cell-specific expression of the chimeric transcription factor XVE and a tagging construct, which is able to trans-activate genes adjacent to a lexA-binding site.

Tagged genes will be mis-expressed in these cell-types conditionally and screened for elements of apomeiosis or parthenogenesis. In apomeiotic screens tagged genes will be rescued in plants showing sterility or lethality, by collecting viable seed from non-induced sectors of a plant. In parthenogenetic screens a conditional male sterile mutant is used to identify mutants the show elements of seed development in the absence of fertilization. Screening for deregulated gene expression in the nucellus and the egg will identify candidate genes that will serve as probes to study the evolution of altered reproductive processes in apomictic species.

Functional Proteomics: Towards a system view of plastid differentiation and functions

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During plant development, tissue specific programs control the differentiation of progenitor plastids into functionally specialized organelles, e.g. chloroplasts etioplasts amyloplasts or chromoplasts. The mechanisms that regulate these differentiation processes are still poorly understood. In addition little information is available about the proteomes and metabolic capacities of specialized plastids. We have performed a large scale analysis of the proteomes from Arabidopsis chloroplasts, rice etioplasts and the undifferentiated proplastids from a tobacco BY2 cell culture [1, 2]. Together, we have identified more than 800 plastid proteins. Many of the proteins can be assigned to known protein complexes and metabolic pathways, but more than 30% of the proteins have unknown functions and many are not predicted to localize to plastids. Parallel RNA profiling revealed a pathway-dependent correlation between transcript and relative protein abundance suggesting gene regulation at different levels [2]. An extensive proteome analysis of the light-induced development of dark-grown rice etioplasts to mature chloroplasts identified 407 different etioplast proteins. In addition to proteins with known functions most of which are active in the characteristic heterotrophic plastid metabolism and gene expression, almost 18% of the proteins have unknown functions. Structure function predictions using various HMM libraries together with phylogenetic analyses revealed novel protein functions that were not previously ascribed to the plastid compartment. Quantitative analysis of protein expression and posttranslational modifications at various time points after illumination revealed an initial up-regulation of proteins two hours after the onset of illumination. The phosphorylation status of plastid RNA-binding proteins increases rapidly suggesting the light-dependent activation of a protein kinase. Phosphorylation of RNPs is thought to alter their RNA binding properties, which is important for RNA stability and regulation of gene expression. Our data suggest that the initial events of light-dependent chloroplast development are associated with a stabilization of mRNAs that

encode for photosynthetic proteins.

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Interplay between 3 epigenetic marks: DNA, H3K9 and H3K27 methylation

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Both DNA methylation and post-translational histone modifications contribute to gene silencing, but the mechanistic relationship between these epigenetic marks is still unclear.

Histone H3 lysine 9 dimethylation (H3m2K9) and CpG DNA methylation mark heterochromatin in many eukaryotes including Arabidopsis. Interplay between these two epigenetic modifications has been reported. Complete removal of CpG methylation in the DNA methyltransferase 1 mutant (*met1*) results in a loss of H3m2K9 in heterochromatin indicating that CpG methylation directs H3K9 dimethylation. Also, CpNpG DNA methylation is dependent on H3m2K9 since it is reduced in the histone H3 K9-specific methyltransferase mutant *Kryptonite* (*Kyp*). Both DNA methylation and H3m2K9 patterns are affected in the SWI/SNF chromatin remodeling mutant *ddm1*.

Methylation of histone H3 lysine 27 (H3K27) is also associated with gene silencing in several organisms. H3K27 methylation is involved in polycomb group proteins-mediated silencing of homeotic genes, and it marks the X inactive chromosome in mammals. In Arabidopsis so far it has only been reported that H3K27 (di)methylation is correlated with inactivation of the FLC locus occurring during the vernalization process.

Like all lysine residues, K27 can be mono, di or trimethylated. Our goal is to elucidate whether/how H3K27 modifications are dependent on DNA and/or H3m2K9 methylation. We are using immunocytology and chromatin immunoprecipitation (ChIP) methods to analyze H3K27 methylation patterns at different genomic loci in wild type, *met1*, *kyp* and *ddm1* plants.

AtGAT1, a high affinity GABA transporter from *Arabidopsis thaliana*

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4-Aminobutyric acid (GABA) is well known as inhibitory neurotransmitter, paracrine signal and growth factor in vertebrates and is a significant component of the free amino acid pool in most prokaryortic and eukaryotic organisms. In contrast very little is known about the function of GABA in higher plants where it

is discussed to be involved in pH regulation, nitrogen storage, development and pathogen defense. In Arabidopsis flowers, a GABA concentration gradient has recently been shown to play a role in pollen tube growth and guidance.

A variety of stress conditions stimulate the accumulation of GABA in plant tissues. While GABA accumulation has been shown to be mediated via increased synthesis, it remains unclear if changes in intracellular or intercellular transport contribute to changes in GABA concentrations. Yeast complementation experiments showed that several members of the ATF-superfamily of amino acid transporters from *Arabidopsis thaliana* transport GABA among other substrates. However, all transporters studied previously transport GABA with low affinity (Km 4-12 mM), indicating that GABA is not transported by these proteins *in planta*. The Arabidopsis genome contains two genes with high homology to these low-affinity GABA transporters. We cloned one of them and could show that it codes for a high-affinity GABA transporter (AtGAT1). The apparent affinities determined by measuring uptake of radiolabelled GABA in *Saccharomyces cerevisiae* expressing AtGAT1 and using two electrode voltage clamp experiments with *Xenopus laevis* oocytes gave comparable results with Km values of 14 µM (*S. cerevisiae*) and 43 µM (*Xenopus oocytes*), respectively. AtGAT1 has a considerable selectivity for omega-amino acids with a chain length of four to eight carbon atoms. In contrast to the previously described transporters with low affinity for GABA, AtGAT1 is not able to transport proline, choline or glycine betaine. A detailed analysis of the expression pattern together with transgenic plants with altered expression levels of *AtGAT1* will help revealing the physiological function of GABA transport in plants.

Permeable cuticle (pec) mutants of Arabidopsis

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The aerial portions of plants are covered with a continuous extracellular layer of hydrophobic material, the cuticle, that plays an important role in protecting these organisms from water and solute loss, UV irradiation, frost damage, as well as pathogen and insect attack. The cuticle consists of two major components, cutin and wax. Cutin, a structural polymer, is in many species composed of interesterified hydroxy and epoxy-hydroxy fatty acids. In Arabidopsis, however, cutin monomers consist of a high proportion of C16 and C18 dicarboxylic acids, with octadeca-cis-6, cis-9-diene-1,18-dioate being the main component.

In order to study the molecular aspects of the biosynthesis and the functions of cutin in Arabidopsis a mutant screen has been developed to detect Arabidopsis plants with an increased permeability of the cuticle. 7 permeable cuticle (*pec*) mutants have been isolated that have a characteristic pattern of organ- and development-specific alterations in cuticle permeability as well as an altered ultrastructure of the cuticular membrane. Similar to cutinase-expressing transgenic Arabidopsis plants, *pec* mutants exhibit increases in the resistance to *Botrytis cinerea*. Some of the *pec* mutants show also occasional fusions between rosette leaves. The features identify *pec* mutants as a new group of cuticle mutants in Arabidopsis. One of the mutants of particular interest is *pec3* because of its organ fusions and strong resistance to *Botrytis*. Mapping results towards the cloning of *PEC3* will be presented.

Orchestrated methylation pattern of chromatin determines transcription level of the endogenous Petunia vein clearing (pararetro) virus (ePVCV-1) in *Petunia hybrida*

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The location of the integrated PVCV-1 sequences within the pericentromeric region of the chromatin provides for a first indication that they, like many types of retrotransposons, are part of silent heterochromatin. We compared two different hybrid petunia species (Rdc and W138) for various epigenetic markers. The latter host is interesting because of its increased rate of retrotransposition. EPVCV-1 sequences are methylated at CG, CNG and CNN sequences. Astonishingly, however, was the finding that in both cases the methylation rate in the intergenomic region containing the promoter was relatively low. This might indicate a special ability of the viral promoter region to avoid complete inactivation by methylation. While little differences of overall methylation of ePVCV-1 were observed for the two hosts analyzed, differences in histone modification were obvious. While in Rdc histones covering the ePVCV-1 coding region were methylated at lysine 9 of histone 3 9 (H3K9), a flag for heterochromatin, in W138 only about half of these histones were of the H3K9- and the other half of the H3K4-type, representative for euchromatin. Interestingly and in accordance with the DNA methylation data, the H3K4/H3K9 ratio was relatively high for the promoter region. The higher H3K4/H3K9 ratio in W138 correlates with the better ePVCV-1 inducibility in this host.

Furthermore, siRNAs of three different size classes (presumably 24, 22 and 21 nt) could be detected in both *P. hybrida* (RdC and W138). The produced signals were weaker in W138 than in RdC. This is in good agreement with our observations regarding the different levels of transcription of ePVCV-1 sequences in these two cultivars.

Genetic Dissection of the Arabidopsis Epigenome

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Phenotypic changes of Arabidopsis thaliana DNA methyltransferase1 (met1) mutants are generally stated as resulting from aberrant gene expression due to the loss of DNA methylation. Although several examples of DNA methylation changes at endogenous genes have been characterized, herein referred to as epialleles, the extent of epialleles across the entire Arabidopsis genome effecting plant development remains unclear. The primary objective of this project is to characterize genetically mapped epialleles significantly controlling phenotypic variation. A recombinant inbred line (epiRIL) population is being generated and phenotyped from selection of 96 MET1 homozygous F2 individuals resultant from the cross of a homozygous met1-3 mutant to its wild type parent, Columbia. An epiRIL DNA methylation map will be created and correlated with the segregation of trait variation within the population. DNA methylation mapping will be based on bisulfite treatment of genomic DNA followed by hybridization to oligoarrays to determine cytosine to thymine single nucleotide polymorphisms for linkage analysis. Detecting significant epiallele effects on phenotypic variation will be completed using quantitative trait loci detection methodologies, previously

established for identifying genomic regions controlling phenotypic variation of quantitative traits. This study will improve the understanding between DNA methylation changes impacting epigenetic regulation during plant development and adaptation to abiotic stresses.

PKS4 and light dependant inhibition of gravitropism

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PKS4 is a protein homologous of PKS1 in Arabidopsis. Phenotypic analysis of pks4 nul and PKS4OE lines shows that PKS4 is involved inhibition of gravitropism in red and far red lights. Inhibition of gravitropism in red and far red is known to be phytochrome mediated. PKS4 ability to interact with phyA and phyB, combined to genetic interaction analysis clearly suggest that PKS4 acts downstream these two phytochromes. How PKS4 mediates this response is still under investigation. However, PKS4 interest resides in its rather specific involvement in phytochrome mediated gravity responses.

Biochemical and Molecular Characterization of a Putative Raffinose Translocator in Chloroplasts of Ajuga reptans and *Arabidosis thaliana*

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Ajuga reptans L. accumulates the trisaccharide raffinose (α-Gal-Suc) as well as longer members of the raffinose family of oligosacchrides (RFOs) during development and under cold conditions (Bachmann et al., 1994). We found earlier that raffinose is localized partially in the chloroplasts of *Ajuga* leaves. In this study, we have adressed the question if raffinose permeates through the inner chloroplast envelope by a translocator molecule. We developed methods to isolate intact chloroplasts from *Ajuga* leaf protoplasts and to perform uptake experiments with 3H-raffinose. We used the silicone oil centrifugation method to separate the chloroplasts from the uptake medium. Preliminary results indicate that there is indeed a raffinose uptake system operating at the inner chloroplast membrane after cold acclimation. Further, we have some evidence that raffinose accumulation is an active process in *Ajuga* chloroplasts.

Using the same methods, we have obtained evidence that, also in sterile-grown *Arabidopsis thaliana* L. plants, an active uptake of raffinose in chloroplasts takes place. These findings allow us to work with *Arabidopsis* as a model system to find a putative raffinose translocator (PRafT) gene. Five candidate genes from *Arabidopsis* are under investigation; they have been predicted to be located in the inner chloroplast envelope membrane (Ferro et al., 2002). To test their predicted localization, we have performed expression studies with PRafT-GFP fusion constructs. Our results indicate that four of the candidate proteins are indeed located in the chloroplast membrane.

Because raffinose is discussed as a cryoprotectant, we checked the susceptibility of PRafT knock-out mutants to cold treatment (1°C). Two kock-out lines from different candidate genes showed a cold sensitive phenotype.

To test if these two candidate genes are raffinose translocators we are currently performing expression studies in the invertase-lacking *Saccharomyces cerevisiae* yeast mutant SUSY7.

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On auxin, HD-ZIP class III transcription factors and mechanical stimuli in the development of the vascular system

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In *Arabidopsis thaliana* provascular strands are first formed in the embryo during the transition phase from the globular to the heart stage. During the vegetative and the reproductive phase, vascular strands are formed in newly developing leaves and floral organs and are connected to the existing vascular system. Thus the regulation of the vascular development includes a continuous growing of already existing vascular elements and de novo synthesis of the vasculature in newly forming organs, as well as a coordinated connection of vascular strands throughout the plant body. Primary xylem and phloem originate from procambium cells, which derive from apical meristems. The plant hormone auxin and several transcription factors of the HD-ZIPIII family have been described to regulate early leaf vein patterning and xylem development. Mutants that are deficient in the development of continuous vascular strands have been discovered and several of them have defects in auxin signalling or auxin transport. In addition, plants with mutations in class III HD-ZIP transcription factors are defective in vascular pattern formation and differentiation and may be key regulators of early differentiation steps. Besides hormones and transcription factors, mechanical signals are discussed to be involved in differentiation processes. Xylem cell walls pass through several steps of development ending with the formation of a secondary cell wall and the deposition of lignin to make vessel elements water-resistant. Mechanical stimuli, such as touch, wind or insect attack induce the expression of several genes also found to be upregulated in secondary xylem formation.

In our study we have made a detailed histological analysis of the vasculature of the inflorescence stem of two auxin transport mutants (pin1 and pinoid) and of the auxin signalling mutant monopteros. The expression of several HD-ZIPIII class transcription factors has been investigated in these mutants. Furthermore, we make use of a transcription activation system to drive AtHB8 expression with heterologous promoters in the auxin mutants and in WT plants. We also have constructed a system that allows applying mechanical forces on plant tissues. Using histological methods combined with microarray technology we investigate on the function of mechanical forces in xylem formation. We could show that Arabidopsis plants induce secondary cell wall formation and lignin deposition in xylem and fiber cells at an earlier time point, when they were mechanically stimulated.

A novel pathway of vitamin B6 biosynthesis in *A. thaliana*

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Vitamin B6 is an essential nutrient in all organisms. The vitamin can act as a coenzyme for numerous metabolic enzymes and has recently been shown to be a potent antioxidant. Plants and microorganisms have a de novo biosynthesis pathway but animals must obtain it from dietary sources. Despite of the ability of plants and microorganisms to make the compound, studies of vitamin B6 biosynthesis have been mainly restricted to Escherichia coli, where it is derived from deoxyxylulose 5'-phosphate (DXP, an intermediate in the non-mevalonate pathway of isoprenoid biosynthesis) and 4-phosphohydroxy-L-threonine (4-PHT). Recently, based on genetic evidence in fungi, it appears that a novel pathway is in place that involves two genes (PDX1 and PDX2) neither of which is homologous to any of those involved in the E. coli pathway. Furthermore, it seems that only a small subset of eubacteria utilize the "E. coli" type pathway while all others use the alternative pathway. We have initialized a detailed biochemical investigation of PDX1 and PDX2 from the Gram-positive bacterium Bacillus subtilis. We show that the proteins are part of the same complex, functioning as a glutamine amidotransferase, with PDX2 as the glutaminase domain and PDX1 as the acceptor domain and have begun to unravel the chemistry behind the pathway. We are now in the process of applying the knowledge accumulated in the bacterial model to a plant model. We have identified three PDX1 and one PDX2 homologue, respectively in A. thaliana. The functionality of the homologues has been elucidated from complementation studies in yeast and the subcellular localization of the pathway has been deciphered. Our studies indicate that vitamin B6 biosynthesis in plants appears to be independent of DXP. We also show that knocking out the single PDX2 gene is lethal for the plant.

Towards assigning function to mycorrhiza regulated genes in rice

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The arbuscular mycorrhizal (AM) symbiosis is the most ancient and widespread symbiotic interaction plants engage in. This association is mutually beneficial resulting in a nutrient exchange between both symbionts: the fungus enhances the phosphate nutrition of the plant, which provides it with carbohydrates.

Genetics and genomics of the AM symbiosis have been partially studied in dicotyledonous plants, mainly in legumes. At present there is no information available on forward genetic screens or large scale expression studies using monocotyledonous plants.

The availability of unique resources in rice (*Oriza sativa*) makes it an attractive plant to combine genome-wide assessment of transcriptional changes with functional analyses via reverse genetics. Among the hosts of mycorrhizal fungi rice represents the only plant species for which the genome has been sequenced. Using the SYNGENTA whole genome Affymetrix Chip we previously

identified a set of 225 genes differentially regulated upon colonization by AM fungi that were subsequently validated by real-time-RT-PCR. Transposon insertions into 51 out of the 225 candidate genes were *in silico* identified by blasting the corresponding gene sequence against the Rice Tos17 Insertion Mutants Database (http://tos.nias.affrc.go.jp/~miyao/pub/tos17/index.html.en). An assay for rapid genotyping and phenotypic analysis has been established. We expect a subset of the lines to display a mycorrhizal phenotype, which might ultimately enable us to assign functions to the corresponding genes.

Endogenous trans-Acting siRNAs Regulate the Accumulation of Arabidopsis mRNAs[1]

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Post-transcriptonal gene silencing (PTGS) is a sequence specific mRNA degradation process guided by complementary non coding small RNAs termed siRNAs (small interfering RNAs) incorporated into a protein complex termed RISC (for RNA-Induced Silencing Complex). Among PTGS inducers are sense transgenes (S-PTGS), transgenes with inverted repeats of the same gene (IR-PTGS)and viruses.
Observations that all Arabidopsis thaliana mutants impaired in S-PTGS exhibit leaf development abnormalities, has lead to the hypothesis that certain genes implied in leaf development are regulated by a PTGS-like phenomenon in wild-type plants.

Comparison of the transcriptomes of a wild-type plant and of two mutants impaired in S-PTGS allowed the identification of a new class of endogenous non-coding regulatory RNAs, corresponding to siRNAs. These siRNAs are produced from the At2g27400 gene. We partially characterized the pathway of gene regulation by these endogenous siRNAs. As for microRNAs, an other class of small endogenous non-coding RNAs, DCL1, HEN1 and HYL1 proteins are required for production of these endogenous siRNAs, and AGO1 is essential for cleavage of siRNAs targeted mRNAs. In addition, the production of these siRNAs requires the activities of RDR6, an RNA-directed RNA polymerase and of SGS3, a protein with unknown function. RDR6 seems to use At2g27400 RNA as a template for the synthesis of a long double-stranded RNA which is subsequently cleaved by DCL1 in multiple siRNAs of about 21 nucleotides. We have shown that these siRNAs are able to direct the cleavage of complementary mRNAs which encode proteins with unknown functions. The identification of this silencing pathway adds yet another dimension to posttranscriptional mRNA regulation in plants.
Work realised in: Laboratoire de Physiologie de la differenciation vegetale, USTL, 59650 Villeneuve d'Ascq.

Host and non-host pathogens elicit different jasmonate/ethylene responses in Arabidopsis

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Arabidopsis does not support the growth and asexual reproduction of the barley pathogen, *Blumeria graminis* f. sp. *hordei* Bgh). A majority of germlings fail to penetrate the epidermal cell wall and papillae. To gain additional insight into this interaction, we determined whether the salicylic acid (SA) or jasmonate (JA)/ethylene (ET) defence pathways played a role in blocking barley powdery mildew infections. Only the eds1 mutant and NahG transgenics supported a modest increase in penetration success by the barley powdery mildew. We also compared the global gene expression patterns of Arabidopsis inoculated with the non-host barley powdery mildew to those inoculated with a virulent, host powdery mildew, *Erysiphe cichoracearum*. Genes repressed by inoculations with non-host and host powdery mildews relative to non-inoculated control plants accounted for two-thirds of the differentially expressed genes. A majority of these genes encoded components of photosynthesis and general metabolism. Consistent with this observation, Arabidopsis growth was inhibited following inoculation with Bgh, suggesting a shift in resource allocation from growth to defence. A number of defence-associated genes were induced during both interactions. These genes likely are components of basal defence responses, which do not effectively block host powdery mildew infections. In addition, genes encoding defensins, anti-microbial peptides whose expression is under the control of the JA/ET signalling pathway, were induced exclusively by non-host pathogens. Ectopic activation of JA/ET signalling protected Arabidopsis against two biotrophic host pathogens. Taken together, these data suggest that biotrophic host pathogens must either suppress or fail to elicit the JA/ET signal transduction pathway.

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