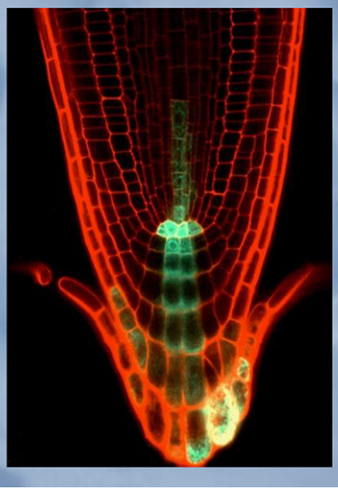
# Plant Growth Biology and Modeling 2011

Covering the latest advances in the biology, modeling and automated phenotyping of

**leaf and root development**

**Elche, September 19-21**



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# Plant Growth Biology and Modeling 2011 Workshop

**19-21 September 2011**

**Centro de Congresos "Ciutat d'Elx"**
**Elche, Spain**

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*Chair: José Luis Micol, Universidad Miguel Hernández, Elche, Spain*

9:00-9:50 José Luis Micol, Universidad Miguel Hernández, Spain
9:50-10:40 Scott Poethig, University of Pennsylvania, USA
10:40-11:10 Coffee break and poster viewing
11:10-12:40 Short presentations:
Eliezer Lifschitz, Technion, Israel
Carmen Fenoll, Universidad de Castilla-La Mancha, Spain
Enrique López-Juez, Royal Holloway, Univ. London, UK
Franck Ditengou, Institute of Biology II/Botany, Germany
Violaine Pinon, Utrecht University, The Netherlands
Gyung-Tae Kim, Dong-A University, South Korea
12:40-13:30 Sarah Hake, Plant Gene Expression Center, USA

13:30-15:00 Lunch

15:00-15:50 Dirk Inzé, VIB/Universiteit Gent, Belgium
15:50-16:40 Patrick Laufs, INRA, France
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17:10-18:00 Thomas Berleth, University of Toronto, Canada
18:00-20:30 Refreshments and poster viewing

**Tuesday, September 20th**

**Session 2: Modeling of plant development**

*Chair: Christine Granier, INRA, Montpellier, France*

9:00-9:50 Christine Granier, INRA, Montpellier, France
9:50-10:40 Przemyslaw Prusinkiewicz, University of Calgary, Canada
10:40-11:10 Coffee break and poster viewing
11:10-12:40 Short presentations:
Rahul Bhosale, VIB/Universiteit Gent, Belgium
Sedeer El-Showk, University of Helsinki, Finland
Séverine Lorrain, University of Lausanne, Switzerland
Eugenio G. Minguet, IBMCP, CSIC, Spain
Alexis Maizel, University of Heidelberg, Germany
Klaartje van Berkel, Utrecht University, The Netherlands
12:40-13:30 Elliot Meyerowitz, California Institute of Technology, USA

13:30-15:00 Lunch

15:00-15:50 Enrico Coen, John Innes Centre, UK

**Session 3: Root biology I**

*Chair: Malcolm Bennett, CPIB, Nottingham, UK*

15:50-16:40 Malcolm Bennett, CPIB, Nottingham, UK
16:40-17:10 Coffee break and poster viewing
17:10-18:00 Pierre Hilson, VIB/Universiteit Gent, Belgium
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20:30 Refreshments offered by the City Council (Restaurant at Parc Municipal)

**Wednesday, September 21st**

**Session 3: Root biology II**

*Chair: Malcolm Bennett, CPIB, Nottingham, UK*

9:00-9:50 Ben Scheres, Utrecht University, The Netherlands
9:50-10:40 Ykä Helariutta, University of Helsinki, Finland
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Ana I. Caño-Delgado, CRAG, CSIC-IRTA-UAB, Spain
Anthony Bishopp, University of Helsinki, Finland
Jamie Twycross/Daniela Dietrich, CPIB, UK
Jérôme Duclercq, VIB/Universiteit Gent, Belgium
Sigal Savaldi-Goldstein, Technion, Israel
George W. Bassel, University of Nottingham, UK
12:40-13:30 Philip Benfey, Duke University, USA
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13:40-15:30 Lunch

# Talks,
# oral communications
# and posters

**SESSION 1: LEAF BIOLOGY**



**SESSION 2: MODELING OF PLANT DEVELOPMENT**

**POSTERS**

# Abstracts

# SESSION 1:
# LEAF BIOLOGY

## Forward and reverse approaches to the genetic dissection of leaf development

Mollá-Morales, A., Sarmiento-Mañús, R., Ferrández-Ayela, A., Rubio-Díaz, S., Muñoz-Viana, R., Esteve-Bruna, D., Casanova-Sáez, R., Muñoz-Nortes, T., Wilson-Sánchez, D., González-Bayón, R., Jover-Gil, S., Candela, H., Pérez-Pérez, J.M., Ponce, M.R., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, 03202 Elche, Alicante, Spain

Plant leaves are the best solar panels ever built, and they also perform well as air purifiers and food factories. Leaves efficiently trap sunlight, remove carbon dioxide from the air, and are the ultimate source of most of the oxygen that we breathe and of the food that we eat. Understanding how a leaf is made is important for several reasons, which include gaining knowledge of the biology and evolution of a multicellular organ with no equivalents in the animal kingdom, as well as identifying —and eventually manipulating, to increase crop yield— the genetic, environmental, and hormonal cues that determine its final architecture and function.

To shed light on the making of plant leaves, we took a forward genetics approach to the saturation of the Arabidopsis genome with viable mutations causing abnormal leaf morphology. The identified mutations fell into 147 complementation groups. Using a high-throughput gene mapping method that we developed, we have already cloned 47 of these genes identified by mutation. The products of these genes participate in various developmental processes, such as polar cell expansion, transduction of hormonal signals, gene regulation, plastid biogenesis, and chromatin remodeling, among others. The broad spectrum of leaf morphological alterations that we identified is helping to dissect specific leaf developmental processes.

We are now combining traditional linkage analysis and next-generation sequencing techniques in order to positionally clone 40 non-allelic mutations already isolated in our laboratory, which affect leaf morphology. In addition, we have started to use clonal analysis to study essential, embryonic-lethal genes that are expressed in wild-type leaves.

We also aim at identifying genes involved in the development of an organ —the leaf— at a scale with no precedent in plants, and perhaps animals. The Ecker laboratory is producing 50,000 sequence-indexed homozygous T-DNA lines representing all the protein-coding genes in the Arabidopsis genome. We are searching this collection for leaf morphological aberrations, and have already identified 340 genes required for normal leaf development. These leaf mutants provide an opportunity to propose models and test hypotheses about how genes control plant development at the organ level.

## Heteroblasty, phase change, and miRNAs: genetic regulation of shoot maturation

Poethig, R.S., Willmann, M.R., and Yang, L.

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA

The vegetative morphology and physiology of the shoot change as it develops. To characterize the molecular basis of these changes, we profiled gene expression in shoot apices of different ages, and in leaves at successive positions within the rosette of early (*FRI flc-3*) and late-flowering (*FRI FLC*) genotypes of Arabidopsis. Six temporal programs of gene expression were identified. Three of these programs occur in all leaves (leaf maturation, leaf aging, leaf senescence), two involve changes in the identity of the shoot apex (vegetative phase change, floral induction), and one involves changes throughout the entire shoot (shoot aging). We have focused on the genetic regulation of the vegetative phase change program. Studies of a number of herbaceous species, as well as trees with distinctive juvenile and adult phases (e.g. *Acacia, Eucalyptus*), reveal that this transition is regulated by the microRNAs miR156 and miR157. miR156 and miR157 act by repressing the expression of SPL transcription factors. miR156 and miR157 are expressed at high levels during the juvenile phase and then decline in abundance, leading to an increase in SPL gene expression, and the production of adult traits. The decline in the expression of miR156 and miR157 is mediated by a factor or factors produced by leaf primordia. The nature of this factor will be discussed.

# The genetic control of shoot architecture: Florigen and the SFT/SP ratio link global regulatory and communication systems

Lifschitz, E.[1], Shalit, A.[1], Eviatar-Ribak, T.[1], and Eshed, Y.[2]

[1] Department of Biology, Technion, 32 000, Haifa, Israel
[2] Dept. of Plant Sciences, Weizmann Institute of Science, Rehovot, 76100, Israel

The origin and diversification of elaborated shoot systems in flowering plants was likely escorted by the co-evolution of long range communication systems that inform distant growth zones the overall developmental status. The understanding that such a system exists has emerged from our studies of *SFT*, the gene encoding the mobile florigen protein, and of *SP* its potent antagonist. Together, the two constitute the tomato equivalents of the Arabidopsis *FT* and *TFL1* which were studied strictly in relations to floral induction. Gene dosage analysis reveals that organ–specific **SFT/SP ratios**, as opposed to absolute levels, define the basic parameters of shoot architecture in tomato by regulating differential flowering responses of primary and secondary shoots. At the same time, and abiding by the same rules, the SFT/SP ratios regulate growth, termination and morphogenesis in all above ground organs (Lifschitz et al., 2006, Shalit et al., 2009).

Boosting flowering is thus only one facet of florigen in its function as a new systemic **growth hormone** which regulates growth and termination in all meristems. In addition, our model states that a mobile florigen, imported by a target organ, elevates local SFT/SP ratios and confers a switch from growth to termination, differentiation and maturation. Maturation in turn converts organs into donors of florigen which now reaches new growing zones, thus reporting the developmental state of its source. Florigen emerges as both the **regulator** and **'great communicator'** of the shoot system.

We will discuss A) Physical and kinetic evidence for the distribution of the mobile florigen in all aerial organs. B) Biochemical evidence for the vegetative function of florigen and for the antagonistic role by *SP*. C) The response of the tomato transcriptome and the auxin/cytokinin balance to manipulated SFT/SP ratios as reflected by deep RNA sequencing and hormone reporters respectively.

## Differentiation and ploidy levels of Stomatal Lineage Ground Cells during Arabidopsis cotyledon development

Triviño, M.M., Martín-Trillo, M., Mena, M., and Fenoll, C.

Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-la Mancha, Toledo 45071, Spain

Stomatal development in Arabidopsis occurs through a stereotyped inward spiral of asymmetric divisions initiated in a protodermal cell that produces up to three Stomatal Lineage Ground Cells (SLGCs) surrounding the stoma. During leaf and cotyledon expansion, SLGCs grow and increase their ploidy, constituting the majority of the epidermal pavement cells in the mature organs. Thus, leaf growth is closely dependent on SLGCs production and development. SLGCs contacting a meristemoid or a stoma never acquire meristemoid identity, although they can experience an asymmetric division and produce a satellite meristemoid that is always placed away from the primary stoma. Both the lineage-based development of stomatal lineages and this behavior of SGLCs effectively prevent the appearance of stomatal clusters and ensure correct stomatal spacing patterns.

MUTE is a bHLH transcription factor specifically expressed in the immediate stoma precursor cell in these lineages, the Late Meristemoid. MUTE is required for the differentiation of this meristemoid into a guard mother cell, which divides symmetrically to originate a guard cell pair that terminates the lineage. In severely hypomorphic *mute* mutants, lineages are halted prior to stomata formation, after producing a variable number of SLGCs that have a limited expansion. Although *MUTE* function in promoting guard mother cell identity is well established, little is known on how *MUTE* is related to SLGCs production and differentiation.

We developed a β-stradiol-inducible system that controls *MUTE* expression in its own promoter domain. Using this system in homozygous plants for the severe *mute-3* allele, we investigated how the late meristemoid and the SGLCs in the *mute-3* epidermis are affected by the timing of MUTE expression. We found that MUTE expression in the late meristemoid through cotyledon development is not only necessary for stomata formation, but that it also signals SLGCs to prevent guard mother cell fate and to enter the pavement cell differentiation pathway and it influences epidermal cell ploidy levels.

## Leaf initiation: lessons from a light trigger

López-Juez, E.[1], Dillon, E.[1], Hatzimasoura, E.[1], Doczi, R.[2], Magyar, Z.[1], Ditengou, F.[3], Palme, K.[3], and Bogre, L.[1]

[1] School of Biological Sciences, Royal Holloway, Univ. London, Egham, UK
[2] Agric. Res. Inst. Hungarian Acad. Sci., Martonvásár, Hungary
[3] Institute of Biology II, University of Freiburg, Freiburg, Germany

In dicotyledon angiosperms, the shoot apical meristem and very early leaf primordia of seedlings germinated in the dark are arrested until the first exposure to light (deetiolation). This light trigger allowed us to examine a natural mechanism of activation of meristem activity, and to observe very early events in leaf initiation. We carried out a whole transcriptome analysis of dissected shoot apices of Arabidopsis seedlings grown in the dark, or after first light exposure (Plant Cell 20: 947–968). Detailed examination of those data revealed, among others:

A state in the dark-grown shoot apex analogous to starvation, rapidly turned off in the light, preceding both growth and photosynthesis.

- The activation by light of a global cellular proliferation response, from either G1 or G2 stages, and a synchronous activation of ribosome biogenesis. Cell proliferation was accompanied by opposite changes in the levels of cell cycle-promoting E2FB and differentiation-promoting E2FC transcription factors.
- A state of the shoot apex of high auxin and ethylene response in the dark, rapidly but transiently disappearing in the light, to be replaced by a cytokinin response burst, coinciding with the time of highest cell proliferation and ribosome biogenesis.

Gene expression signatures suggested a mechanism of cessation of the high auxin response state in dark, involving both synthesis and transport components. We have recently also identified a MAP kinase signalling module controlling PIN protein expression and localization. A component of this module exhibited elevated expression in the dark-grown apex and was rapidly reduced in the light. Genetic ablation of this component indeed results in more rapid establishment of a PIN protein localization pattern at the apex in the light, and accelerated leaf initiation, consistent with a role for auxin accumulation or its removal in the repression (dark) or reactivation (light) of leaf initiation.

## Context-specific role of auxin in the control of leaf vascular tissue development

Ditengou, F.[1], Kneuper, I.[1], Teale, W.[1], Tsugeki, R.[6], and Palme, K.[1,2,3,4,5]

[1] Institute of Biology II/Botany, Freiburg, Germany
[2] Freiburg Institute of Advanced Studies (FRIAS), Freiburg, Germany
[3] Albert-Ludwigs-Universität, Freiburg, Germany
[4] Center of Biological Systems Analysis (ZBSA), Freiburg, Germany
[5] Center of Applied Biosciences (ZAB) University of Freiburg, Germany
[6] Department of Botany, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

So far, the best supported explanation for the mechanism of leaf vein patterning is the 'canalization hypothesis', which states that auxin transport through cells promotes their differentiation into veins thus increasing their capacity to transport auxin. Here we present evidence that auxin synthesis in specific cells in the developing leaf primordium controls the initiation and the architecture of the leaf vascular network. Expression pattern analysis of genes involved in IAA biosynthesis revealed a correlation between auxin synthesis and its transport. Our study reveals a close connection between auxin synthesis and its transport and provides basis for an adaptation of the canalization hypothesis dogma.

# Unravelling PLETHORA transcription factors function in the control of phyllotaxis in Arabidopsis

Pinon, V.[1], Prasad, K.[1,2], Grigg, S.P.[1], and Scheres, B.[1]

[1] Molecular Genetics, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[2] School of Biology Indian Institute of Science Education and Research, Thiruvananthapuram 695016, Kerala, India

In plants the shoot apical meristem, localized at the tip of the stem, is the source of all above ground postembryonic organs. The shoot apical meristem arises during embryogenesis and operates throughout the all life of the plant to maintain a self-renewed population of undifferentiated stem cells, and to generate different types of organs following a specific pattern.

Specification, organization and maintenance of meristems are genetically tightly regulated. However meristems are also flexible and dynamic structures, properties that allow the plant to build up its architecture depending on developmental constrains and environmental conditions. Therefore the control of organ formation and patterning are essential to understand plant morphology.

The auxin efflux facilitator PIN-FORMED1 (PIN1) is required for organ initiation through its postulated function in concentrating auxin at specific sites at the periphery of the meristem. Modeling of polar auxin transport has demonstrated that PIN1 is essential for the establishment of stable phyllotactic patterns. Mutants displaying phyllotaxis defects show irregular arrangement of organs, and are usually impaired in meristem size. Therefore polar auxin transport and meristem architecture are involved in the control of organ patterning.

PLETHORA (PLT) transcription factors regulate organ patterning partly through their control of PIN1 activity. *PLT3*, *PLT5* and *PLT7* genes are all expressed in the central zone of the meristem, suggesting also a role in stem cell maintenance. Indeed in the triple mutant *plt3, plt5, plt7* the size of the *WUSCHEL* expression domain, also called organizing centre, was two times smaller compared to wild-type. Moreover when *PLT3*, *PLT5* or *PLT7* were driven back into *plt3, plt5, plt7* under shoot specific promoters, only *SHOOTMERISTEMLESS* (*STM*) and *CLAVATA3* promoters could fully rescue the phyllotaxis defects of the triple mutant.

*STM* and *CLV3* are respectively expressed throughout the meristem except in organ primordia, and in the stem cell niche. *AINTEGUMENTA* (*ANT*) promoter could only partially rescue the mutant phenotype. *ANT* is expressed in organ primordia, where PIN1 function in organ initiation and patterning takes place.

Therefore PLETHORA transcription factors are involved in controlling the size of the stem cell niche at the shoot apical meristem. Also their expression in the centre of the meristem is required for organ positioning. To conclude PLT function in the control of phyllotaxis is complex and not limited to the regulation of PIN1 activity. It will be necessary and challenging to determine which event is first and maybe feedbacks on several processes. Further experiments using live imaging and also *in silico* modeling of process will allow us to establish the chain of events between PLT, PIN1 activity, organ positioning and meristem maintenance.

# Cell division influences shoot apical meristem maintenance and leaf development

Jun, S.E.[1], Hwang, J.[1], Yun, H.S.[1], Ha, C.M.[2], Okushima, Y.[3], Umeda, M.[3], and Kim, G.-T.[1]

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[2] Postech, Hyoja-dong, Nam-gu, Pohang, Gyungbuk 790-784, South Korea
[3] Nara Institute of Science and Technology, Takayama-cho, Ikoma, Nara 630-0101, Japan

Cell division and cell proliferation play key roles in proper organ formation and appropriate shape during plant development. In this study, we focused on the negative regulator of cell cycle, Kip-related proteins (KRPs) of *Arabidopsis* to understand complex cell division controls for shoot apical meristem (SAM) maintenance and organ development. Firstly, we have identified the expression patterns of KRPs in *Arabidopsis* with KRP-GUS fusion proteins under the control of their own promoters. As a result, we found that KRP1 and KRP3 were highly expressed in SAM and leaf primordia, whereas KRP6 and KRP7 were expressed weakly in the proximal side of leaf primordia. Secondly, we have characterized the developmental changes of transgenic plants overexpressing KRPs which are highly expressed in the vicinity of SAM and leaves. As a result, we found that inhibition of cell division caused remarkable size reduction of SAM and leaves with structural alterations. In addition, we found that CDKA activity might have a critical role in SAM maintenance through the control of *CLAVATA3* expression.

In summary, our study indicated that the regulation of cell division may directly affect SAM maintenance as well as organ size and shape. Taken together, we will discuss the roles of cell division during SAM and leaf development. And we will also discuss the effects of ploidy changes in SAM and leaf development.

## Patterning the maize leaf

Hake, S., Bolduc, N., O'Connor, D., Moon, J., and Lewis, M.

Plant Gene Expression Center, USDA-ARS and UC Berkeley, 800 Buchanan St, Albany, CA 94710, USA

The maize leaf is composed of two major tissues, a distal blade that tilts away from the stem and the more proximal sheath that tightly wraps around the stem. At the junction of blade and sheath, the ligule and auricles are found. The auricles act as a hinge to let the blade lean back and the ligule is a flap of tissue, preventing water from entering into the stem. Our goal is to understand how cells in a leaf primordium differentiate according to position and adopt specific cell types. We are using a number of maize mutants that affect the patterning in the leaf. *liguleless1*, *liguleless2* and *Liguleless narrow* mutants remove the ligule and auricle. The *knotted1* family of homeobox genes displace the ligule and auricle when misexpressed in leaves. Using a combination of genomics, genetics and cell biology, we are studying the targets of KN1 and how KN1 interacts with auxin signaling. We are following the expression of *liguleless* genes and other markers during the early stages of patterning.

## Leaf growth in a changing environment

Inzé, D., Claeys, H., and Skirycz, A.

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Environmental stress inhibits plant growth and causes large yield losses. There is a good understanding on how mature plants tolerate stress but the mechanisms that regulate plant growth under relatively mild stress are only poorly understood (Skirycz and Inze, 2010 Curr. Opin. Plant Biol). Our research is focusing on how plant growth and organ size is regulated under normal as well as drought stress conditions. Using a robotized plant growth and watering system, WIWAM, we could demonstrate that there is no obvious relationship between the ability of plants to cope with severe, often lethal, stress and their growth potential under mild drought stress conditions (Skirycz et al., 2011 Nature Biotech). This observation clearly highlighted that the more research is required to decipher how growth of plants is hampered by mild drought stress. To this end, we investigated the molecular mechanisms underlying growth inhibition in young proliferating leaves of the model plant Arabidopsis thaliana when subjected to mild osmotic stress. A detailed cellular analysis demonstrated that as soon as osmotic stress is sensed, cell cycle progression rapidly arrests, but cells are kept in an ambivalent state allowing a quick recovery ("pause") when conditions rapidly improve. Remarkably, cell cycle arrest coincides with an increase in 1-aminocyclopropane-1-carboxylate (ACC) levels and the activation of ethylene signaling. Careful studies showed that ethylene acts on cell cycle progression via inhibition of cyclin-dependent kinase A (CDKA) activity independently of EIN3 transcriptional control. When the stress persists, cells exit the mitotic cell cycle and initiate the differentiation process ("stop"), reflected by early endoreduplication onset, in a process independent of ethylene. Nonetheless, the potential to partially recover the reduced cell numbers remains thanks to the activity of meristemoids. Together, these data present a novel conceptual framework to understand how environmental stress reduces plant growth.

## When dissecting is shaping: roles of the *CUC* genes during leaf development

Laufs, P.

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Leaf margins show various levels of dissection/outgrowth such as lobes or serrations in simple leaves, or leaflets in compound leaves. Variations in the pattern of dissection contribute to a large extent to inter- and intra-species leaf shape diversity. Although much progress has been made during the last decade in deciphering the regulatory networks controlling leaf shaping, the basis of this tremendous diversity is not yet understood.

Here, we'll present the contribution of the *CUC* genes to leaf dissection in Arabidopsis and other Eudicots. These genes, which code for plant specific transcription factors of the NAC family, were initially identified for their role in organ separation and meristem promotion. We show that they have a conserved expression pattern at the leaf margins and that they are essential for all levels of marginal dissection/outgrowth.

We further analysed the role of the *CUC1*, *CUC2* and *CUC3* genes in the serration of Arabidopsis leaves. We show that in addition to *CUC2*, *CUC3* contributes to leaf dissection, though via a different mechanism. In contrast, *CUC1* is not involved in Arabidopsis leaf serration, though CUC1 can efficiently replace CUC2 when expressed at the leaf margin. Specific and overlapping roles of the *CUC* genes during leaf development will be interpreted in the light of the evolutive history of the *CUC* genes within the Brassicales.

## Control of Leaf Vascular Patterning

Berleth, T.[1], Scarpella, E.[2], Marcos, D.[1], Krogan, N.[3], Ckurshumova, W.[1], and Caragea, A.[1]

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[3] Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Feedback-regulated auxin flows have been implicated in a large number of plant patterning processes. Improved genetic dissection, experimental interference and enhanced visualization, integrated with computer simulations, lead towards increasingly precise predictions.

Auxin Response Factors have critical, partially overlapping functions in controlling the expression of AtPIN proteins and can be used as genetic tools to locally manipulate auxin signal transduction and auxin transport. We have used genetic and experimental interference tools as well as live visualization to dissect the formation of Arabidopsis leaf venation patterns. Loss of ARF5/MP function results in reduced vascularization, while a truncated version of ARF5/MP, missing the binding sites for Aux/IAA co-regulators, leads to increased leaf vascularization and altered leaf anatomy. Interestingly, expression of this transgene does not only trigger the transcription of auxin-responsive genes, but also the expression of a number of cell proliferation loci.

A crucial member of the AtPIN family of auxin efflux proteins, AtPIN1, is expressed prior to preprocambial and procambial cell fate markers in domains that become restricted toward sites of procambium formation. Subcellular AtPIN1 polarity indicates that auxin is directed to distinct “convergence points” in the epidermis, from where it defines the positions of major veins. Integrated polarities in all emerging veins indicate auxin drainage toward pre-existing veins, but veins display split polarities as they become connected at both ends. Live visualization of individual pre-procambial domains demonstrates the dynamic nature of the process selecting procambium cells.

# SESSION 2:
# MODELING OF
# PLANT DEVELOPMENT

## Standardization of protocols for high-throughput analyses of leaf growth: needs and limits

Granier, C., Massonnet, C., Fabre, J., Dapp, M., Tisné, S., Cookson, S., Radziejwoski, A., and Dauzat, M.

Laboratory of Plant Ecophysiology under Environmental Stresses, INRA, Montpellier, France

A major goal of the life sciences is to understand and model how molecular processes control phenotypes and their alteration in response to biotic or abiotic stresses. The study of *Arabidopsis thaliana* genomics is providing new insights into the understanding of these processes. The functional analysis of genes associated with these responses is made possible by the phenotypic analyses of mutants or natural genetic variants, high-throughput genetic mapping and large-scale analyses of gene expression. Ten years ago, an important bottleneck was the phenotypic analysis of the genetic variability, which requires simultaneous analysis of hundreds to thousands of plants. Automated platforms now exist in many labs and provide large quantities of micro-meteorological data, images and phenotypical data for the study of genotype x environment interaction effects on different plant processes. Standards and ontologies have been integrated when possible to share the datasets with scientific community and ensure that the data produced by specific groups can benefit the *Arabidopsis thaliana* community in analyses of which the purposes extend beyond the ones that have been published. In some cases, increasing the throughput of plant analyses, i.e. increasing the number of genotypes for example, leads to a simplification of leaf growth analyses, mainly due to technical constraints. On the other hand, increasing the throughput of plant analyses, allows the use of statistical tools for modeling. Examples will be presented that illustrate both the limits and the power of increasing the throughput in plant leaf growth analyses.

## Genetics and geometry of leaf development

Runions, A.[1], Tsiantis, M.[2], and Prusinkiewicz, P.[1]

[1] Department of Computer Science, University of Calgary, Canada
[2] Department of Plant Sciences, University of Oxford, UK

Leaves exhibit an astonishing diversity of shapes. Particularly striking is the variability that can be found among leaves of related species, cultivars, or individual plants. Such variability suggests that relatively small differences in the regulatory processes may be amplified into substantial differences in the developing patterns and forms. This contrast makes leaf development an intriguing subject of study.

Molecular-level analysis of leaves in selected model species, including Arabidopsis, tomato and pea, points to a common mechanism of leaf form development. The interplay between activators (auxin) and inhibitors (e.g., CUC2 protein) of margin outgrowth plays a fundamental role. We hypothesize that the dynamic distribution of the activated and inhibited regions results from a self-organizing process, which both controls and is controlled by the changing geometry of the leaf. Using computational models we show that different parameters of this process yield diverse forms including simple, lobed, and compound leaves.

## Residual uncontrolled variation in a tightly controlled gene expression experiment on individual Arabidopsis plants yields biologically relevant expression modules

Bhosale, R.[1,2], Hollunder, J.[1,2], Michoel, T.[1,2], Vuylsteke, M.[1,2], Goossens, A.[1,2], Hilson, P.[1,2], and Maere, S.[1,2]

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We re-analyzed a tightly controlled Arabidopsis gene expression experiment probing the reproducibility of leaf growth-related molecular phenotypes among individual plants of three Arabidopsis accessions cultivated in ten laboratories. In the original study, significant inter- and intra-laboratory expression variation was found. We show that, after controlling for lab-, ecotype- and lab x ecotype-effects, the residual expression variation among 41 individual plants still provides enough signal to discriminate biologically relevant expression modules. We show that, from a guilt-by-association perspective, subtle uncontrolled variations among individual plants are at least as informative as controlled experiments on pooled plants employing more severe, laborious and often unrealistic perturbations.

# Multi-level modelling of Vascular Morphogenesis in Arabidopsis

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We have recently described a novel regulatory network responsible for positioning an auxin signalling maximum through the central axis of the stele in the Arabidopsis root, resulting in the specification of protoxylem in the correct position. The network involves a mutually inhibitory feedback loop between the auxin and cytokinin which is capable of specifying the boundary between them and regulating the size of their respective signalling domains. The feedback is mediated by the PIN class of auxin efflux transporters and AHP6, an inhibitor of cytokinin signalling. (Bishopp & Help *et al.* Current Biology 2011).

In order to better understand the dynamics of this regulatory network, we have undertaken a modelling approach based on previous work using the Cellular Potts Model to simulate auxin dynamics in the Arabidopsis root (Grieneisen *et al*. Nature 2007). Formally modelling this network will allow us to examine how the various elements contribute to its remarkable robustness and identify those which are critical, as well as predicting whether unidentified elements may be required for this level of robustness.

Furthermore, we have not been able to experimentally determine whether this regulatory loop is sufficient to generate a pattern *de novo* or whether additional input, such as a hormonal bias communicated from the shoot, is necessary to establish the pattern which is then reinforced and maintained by this loop. The ability to generate patterning information *de novo* can be directly tested *in silico* using a virtual root section. If *de novo* pattern generation is possible, it will be intriguing to explore what factors contribute to various aspects of the vascular pattern, such as the number and orientation of the poles.

# Towards modeling of the shade avoidance response of A. thaliana hypocotyls

Lorrain, S.[1], Hersch, M.[2,3], Bergmann, S.[2,3], and Fankhauser, C.[1]

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Plants constantly monitor their environment and integrate different incoming parameters (such as temperature, light) to adapt to the surrounding conditions. This will ultimately optimize the light capture and plant development.

In the frame of a system biology project we plan to model plant growth in such a changing environment. We first focus on plant growth in response to changes in light quality during neighbour detection in the shade-intolerant plant *A.thaliana*. Such neighbouring is detected through a reduction in the red (R) to far-red (FR) ratio by the phytochrome photosensors and triggers the so-called Shade Avoidance Syndrome characterized by reallocation of energy resources to growth mechanisms in order to reach unfiltered sunlight. We use hypocotyl elongation as a read-out for SAS that combines easiness of study and numerous molecular data present in the literature. Especially reduction of the R/FR ratio triggers phytochrome B inactivation leading to auxin production through the Tryptophan Amoninotransferase (TAA1) as well as the stabilisation of Phytochromes Interacting Factors 4 and 5 (PIF), which are required for a full SAS. After prolonged exposure to shade a negative feedback loop limits the response of plants through formation of heterodimer between PIF4 and 5 and another transcription factor called HFR1 (long Hypocotyl in Far Red light).

New molecular data will be presented in this poster concerning the regulation of the neighbour detection as well as a computational model developed to better understand this regulation.

# SIMCHIP: Prediction of Transcription Factor DNA binding landscape

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A deep understanding of the dynamics of gene regulatory networks relies on the availability of models faithfully predicting the binding of transcription factors (TFs) to their DNA targets. Several experimental techniques are available to describe the preference of a given TF for specific sequences (i.e. SELEX), and to describe in vivo binding at the genomic scale under particular circumstances (i.e. ChIP-chip, ChIP-seq). Such information can be used to generate models allowing to predict TF binding, the identification of gene regulatory networks in non-model species, and the manipulation of agronomically important traits controlled by those TFs.

Several methods to evaluate TF binding models (BMs) quality are currently used to confront their prediction to a genome-wide experimental TF binding result. For these methods (Spearmann rank correlation or ROC-AUC), the BM generates a single value per bound area (as the score of the best binding site (BS) or the probability of occupancy). But no method is available that allows to compare the observed and the predicted binding profiles (i.e. the shape of the peaks). To fill in this gap, we have developed SIMCHIP, a bioinformatic tool that uses a BM to predict the TF binding landscape to any genomic region. SIMCHIP calculates the affinity of all TF BSs based on a Position Weight Matrix (PWM) describing the TF specificity and then uses a biophysical model to compute the occupancy of all simulated overlapping fragment covering a genomic region (mimicking the sonicated DNA from a ChIP-seq experiments). Then, the simulated binding landscapes are confronted to the experimental observations. This new method allows the comparison between different PWM for a specific TF, by assessing the performance of each model to predict the topology of the different in vivo binding regions.

As a proof of concept, we have applied this method to two TFs with different DNA binding properties: *Arabidopsis thaliana* LEAFY, and

the human STAT1. In both cases we have been able to establish the better BM even improving published ones. Future predictive models for TFs in vivo binding will take additional considerations, such as DNA accessibility, epigenetic marks, or relationships between the different TFs bound to a particular region, thus improving the predictive power of this approach.

# Imaging plant growth in 4D at cell resolution in near physiological conditions using light sheet fluorescence microscopy

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Quantitative information on growing organs is required to better understand morphogenesis. Non invasive time-resolved imaging of intact, fully functional organisms allows studies of the dynamics involved in shaping complex organisms. Conventional and confocal fluorescence microscopy suffer from limitations when whole living organisms are imaged at the single-cell resolution. We applied light sheet-based fluorescence microscopy (LSFM) to overcome these limitations and study the dynamics of plant growth. In LSFM, a sheet of light is used to specifically illuminate a given plan of the specimen while the emitted light is collected at a perpendicular axis. Thus, only fluorophores close to the focal plane of the detection system are excited and contribute to the image. Intrinsically, only a fraction of the fluorophores suffer from photobleaching. We designed a special imaging chamber in which the plant is maintained vertically under controlled illumination with its leaves in the air and the root in the medium. We show that minimally invasive, multi-color, 3D imaging of live *Arabidopsis thaliana* samples can be achieved at organ, cellular and sub-cellular scales over periods of time ranging from seconds to days with minimal damage to the sample. LSFM opens several new avenues for studies of plant development. First, it allows for very little invasive, high resolution, time resolved imaging of plant growth as well as studies of protein or gene expression dynamics at various long to short time scales. Second, combined with segmentation and tracking methodology, it allows the extraction of quantitative information on growing organs, which is essential for our understanding and modeling of morphogenesis and growth patterns at the cellular resolution.

## Analysing polar auxin transport models in a new light

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Polar auxin transport (PAT) is driven by a complex feedback between auxin and the localisation of its efflux carriers, the PIN proteins. Because of this complexity, it is a popular subject for modeling studies. Two main classes of PAT simulation models can be distinguished. The first, flux-based models, proposes that flux over a membrane increases the PIN concentration at that membrane. The second, concentration-based models, suggests that PINs localise toward the neighbouring cell that contains most auxin. Both classes of models are able to explain part of the PIN polarities found in plants. Flux-based models generate vein formation patterns whereas concentration-based models generate phyllotaxis.

We developed a mathematical framework in which we compare and classify PAT models. We will go beyond the most obvious pattern formation properties (phyllotaxis/venation) and also study the models in terms of membrane bistability, cell polarity and ability of tissues to self-organise. We show that model assumptions at different levels of organisation (membrane, single cell and tissue) have consequences for the ultimate behaviour and that some of these consequences can be uncoupled from the proposed mechanism of PIN polarisation (with-the-flux or up-the-gradient).

We will also point out the limitations that current PAT models have with respect to the type of pattern formation they are able to generate: no model has yet shown both with-the-flux and up-the-gradient behaviour at the same time and for the same parameter conditions. We discuss the future of PAT models and try to propose a solution for this issue.

## Modeling Transcriptional Dynamics in the Shoot Apical Meristem: The Spatial Element

Meyerowitz, E.

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One open question in studies of the shoot apical meristem of Arabidopsis is that of the control of the spatial domains of gene expression in shoot apical meristems. Domains such as those of *CLAVATA3* expression in the central zone, and of *CLAVATA1* and *WUSCHEL* expression in the rib meristem, are tightly controlled in cell number and in position relative to the meristem boundaries and the domains of expression of other genes. How is it that a gene expression domain can remain constant, even though the cells of the domain divide and depart from the domain, and other cells enter the domain it by division of cells in neighboring regions? We have started exploring possible answers to this question by developing computational models of domain maintenance in the shoot meristem, involving the complex interactions of cytokinin signaling with elements of the CLAVATA signal transduction system. The models, which include multiple levels of feedback between the cytokinin and CLAVATA systems, have suggested experiments, and the results of the experiments have led to additions and changes to the models. We have now completed multiple iterations of modeling and experimentation, leading to refined models that have predicted new types of interactions between cytokinin perception, regulation of WUS and CLV1 by cytokinin, feedbacks of WUS on cytokinin perception, and cytokinin biosynthesis.

## From Genes to Shape

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Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities within an organism during its development. However, the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify anisotropic growth of multicellular tissues in 3D space at multiple scales. We have been addressing this problem using a combination of genetic, morphological, computational and imaging approaches in collaboration with Andrew Bangham (University of East Anglia) and Przemyslaw Prusinkiewicz (Calgary). The results provide new insights into how genes interact to specify orientations and rates of growth, leading to particular shapes. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

# SESSION 3:
# ROOT BIOLOGY

## Regulatory Networks in Root Development: an emerging story…

Middleton, A., Peret, B., French, A., Wells, D., Voss, U., Band, L., Jensen, O., Pridmore, T., King, J., and Bennett, M.J.

Centre for Plant Integrative Biology (CPIB), University of Nottingham, UK

The Centre for Plant Integrative Biology (CPIB) at the University of Nottingham is creating a *virtual root* model as an exemplar for using Integrative Systems Biology (ISB) to model multi-cellular systems. Our systems approach involves creating increasingly sophisticated multiscale models of plant development, populating them with experimental data, and then testing their predictive ability. Multiscale models integrate information about the gene regulatory networks and tissue geometries in which developmentally important signals, such as auxin, operate. I will describe how a multiscale modelling approach is proving essential to understand complex, multi-cellular experimental systems and obtain new biological insights.

My presentation will focus on *Arabidopsis* lateral root development. In *Arabidopsis* lateral roots (LRP) originate exclusively from pericycle cells located deep within the parental root, necessitating that new primordia emerge through several overlaying tissues (reviewed in Peret et al., 2009). LRP development is a highly regulated process involving the active participation of cells in both new lateral root primordia and the parental root (Swarup et al., 2008). This study revealed that auxin originating from the developing LRP acts as a local inductive signal which reprograms adjacent cells, causing cell separation, to facilitate organ emergence. Multiscale models have been developed that successfully simulate the spatial expression patterns of components of the lateral root emergence pathway. Our modelling approach reveals the importance of accurately capturing the non-linear topology of the gene regulatory networks (Middleton et al., 2010) and tissue geometries in which auxin signals.

Swarup et al. (2008). *Nature Cell Biology* 10: 625-628.
Peret et al. (2009). *Trends in Plant Science* 14: 399-408.
Middleton et al. (2010). *Bull. Math. Biol.* 72: 1383-1407.

# Secretory peptides regulate tropic growth and root development

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Growth and development are coordinated by an array of intercellular communications. Known plant signaling molecules include phytohormones and hormone peptides. Although both classes can be implicated in the same developmental processes, little is known about the interplay between phytohormone action and peptide signaling within the cellular microenvironment. We show that genes coding for small secretory peptides, designated GOLVEN (GLV), modulate the distribution of the phytohormone auxin. The deregulation of the GLV function impairs the formation of auxin gradients and alters the reorientation of shoots and roots after a gravity stimulus. Specifically, the GLV signal modulates the trafficking dynamics of the auxin efflux carrier PIN-FORMED2 involved in root tropic responses and meristem organization. These results link mechanistically the local action of secretory peptides with phytohormone transport.

A complementary research project will be introduced. We have assembled a high-content screening platform to study the cellular phenotypes of isolated plant cells. This system has been optimized to track the proliferation of isolated Arabidopsis cells with high-throughput and robotized protocols geared towards compound library screening.

# Integrated molecular circuits for stem cell activity in Arabidopsis roots

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Plant stem cells reside in niches and are maintained by short-range signals emanating from organizing centres. The Arabidopsis *PLETHORA* genes encode transcription factors required for root stem cell specification[1,2]. *PLT* expression is induced by the indolic hormone auxin, depends on auxin response factors and follows auxin accumulation patterns. The *PLT* gene clade extensively regulates expression of the PIN facilitators of polar auxin transport in the root and this contributes to a specific auxin transport route that maintains stem cells at the appropriate position[3]. We are addressing the properties of the PLT-PIN feedback loop by gene and protein network analysis and computational modelling. The emerging picture is one in which flexible feedback circuits translate auxin accumulation into region- and cell type specification patterns.

Stem cells and their daughters in the root display specific asymmetric divisions at fixed locations. We investigate how such divisions are spatially regulated. The SHORTROOT-SCARECROW transcription factor pathway plays a role in patterning the quiescent center and cortex/endodermis stem cells and provides mitotic potential to the stem cell daughters that form the proximal meristem. This activity involves the conserved RETINOBLASTOMA-RELATED (RBR) pocket protein[4], and we have established molecular links between the RBR pathway and SCARECROW action that form a feedback control system. In addition, RBR activity is modulated by auxin abundance, itself regulated through an intercellular distribution system, and by cell cycle progression. Formal analysis of this feedback circuit indicates that it acts as a bistable switch that ensures the occurrence of an asymmetric division at fixed positions. Our work illustrates how formative divisions that shape plant tissues can be robustly positioned by dynamic regulatory circuits that combine intracellular and extracellular loops.

1. Aida et al. (2004). *Cell* 119: 109-120.
2. Galinha et al. (2007). *Nature* 449: 1053-1057.
3. Grieneisen et al. (2007). *Nature* 449: 1008-1013.
4. Wildwater et al. (2005). *Cell* 123: 1337-1349.

## Analysis of cell signalling during vascular morphogenesis in Arabidopsis

Helariutta, Y., Bishopp, A., Ruzicka, K., Zhang, J., Yadav, S.R., Miyashima, S., Furuta, K., Honkanen, A., Immanen, J., El-Showk, S., Help, H., Lichtenberger, R., Ursache, R., and Sevilem, I.

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Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. During root primary development, xylem is specified as an axis of two vessel element cell files, centrally located metaxylem and peripherally located protoxylem. We have recently identified AHP6, an inhibitory pseudophosphotransfer protein for cytokinin signaling as a spatially specific regulator facilitating protoxylem specification (Mähönen et al. Science 311, 94). Subsequently, we have identified two regulatory interactions that regulate AHP6 and the xylem pattern. First, we have shown that cytokinin and auxin interact in a spatially specific manner during procambial development to specify the AHP6 pattern. Furthermore, in collaboration with the laboratories of Philip Benfey, Ji-Young Lee and Annelie Carlsbecker, we have shown that the miR165/6 species act non-cell autonomously to regulate the differential gene dosage of the class III HD-ZIP genes, and thus the AHP6 pattern during protoxylem and metaxylem development (Carlsbecker, Lee et al. Nature 465, 316). Finally, through identification of dominant mutations affecting callose biosynthesis, we have engineered a temporally and spatially controlled system to control plasmodesmatal trafficking during root procambial development. The mobility of the various signals is discussed based on the analysis with this system.

## Systems biology to unravel brassinosteroid-regulated vascular development

Caño-Delgado, A.I.

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My research group is focused on the understanding of Brassinosteroid-regulated plant development. While the majority of the BR signalling components identified to date are expressed ubiquitously in the plant, our research aims at the identification of novel BR signalling components that operate within a specific cellular context. A combination of biological and computational approaches has been taken to address our goal (see also posters on pages 88, 99, 110 and 148, presented in this conference).

We are particularly interested in the BRs control at the stem cell population and vascular organogenesis, where additional vascular-specific BR-receptors BRL1 and BRL3 are present. The results of our cell-type-specific biochemical and transcriptomic analyses towards the identification of novel BR regulators in the vascular tissues will be presented at the conference.

1. Ibañes et al. (2009). *Proc. Natl. Acad. Sci. USA* 106(32): 13630.
2. Caño-Delgado (2010). *Ann. Rev. Cell Dev. Biol.*
3. González-García et al. (2011). Development 22(6): 810-23.

## Hormonal control of vascular pattern in *Arabidopsis* roots

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In *Arabidopsis* roots, the vascular tissues are arranged in a bisymmetric pattern. One plane of symmetry runs through the xylem axis and another runs at 90 degrees to this through the two phloem poles. This bisymmetric cellular pattern is the readout from bisymmetric domains of high auxin and high cytokinin response; there is an auxin signaling maximum in the xylem axis, whilst the highest cytokinin signaling output is in the intervening procambial cells adjacent to this. These two hormonal domains act in a mutually inhibitory manner to generate sharp and precise boundaries for vascular patterning. High auxin signaling promotes the expression of *AHP6*, whilst high cytokinin signaling promotes the radialization of the auxin efflux carries PIN1, PIN3 and PIN7.

During the early stages of embryogenesis, the root pole is radially symmetric and bisymmetry is established following the influx of auxin from the cotyledons and mutants with alterations in cotyledon numbers show altered vascular patterns in the embryo axis. In growing *Arabidopsis* roots this vascular pattern is maintained through the top-down transport of auxin (via polar auxin transport and cytokinin (transported through the phloem). Together the asymmetric transport of these hormones and the mutually inhibitory interaction between them are able to specify root vascular pattern.

# Modulation of ABA response through dynamic regulation of receptor and phosphatase populations

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The recent discovery of the new family of PYR/PYL/RCAR abscisic acid (ABA) receptors allows for the first time the description of the entire signal transduction pathway from hormone perception to changes in gene transcription. In essence, ABA binds to these receptors, leading to binding and inactivation of phosphatases of the PP2C subfamily. In the absence of ABA, these phosphatases bind to and inactivate SnRK kinases, which are necessary for activation of transcription factors and ABA responsive transcription. A number of questions still remain unanswered, not least how the individual components of the pathway are regulated to achieve modulation of the ABA response.

Using a high temporal resolution transcriptomics dataset and experimentally determined dissociation constants for receptor, phosphatase and hormone interactions, we are developing computational models to investigate the effects that dynamic changes in receptor and phosphatase levels have on phosphatase inactivation. Figure 1a shows a (reduced) model of the interaction of the PYL5 receptor and HAB1 phosphatase in the presence of ABA which exemplifies our approach. The model is simulated with and without dynamic receptor and phosphatase regulation. In the model without dynamic regulation, total phosphatase and receptor concentrations are constant, whereas in the model with dynamic regulation, total receptor concentration decreases and total phosphatase concentration increases over time in line with our transcriptomics data. Preliminary results (Figure 1b) show that dynamic regulation of the receptor and phosphatase concentration causes a marked decrease in phosphatase activation, providing a potential mechanism for the plant to attenuate its response to ABA in the face of the continued presence of ABA.

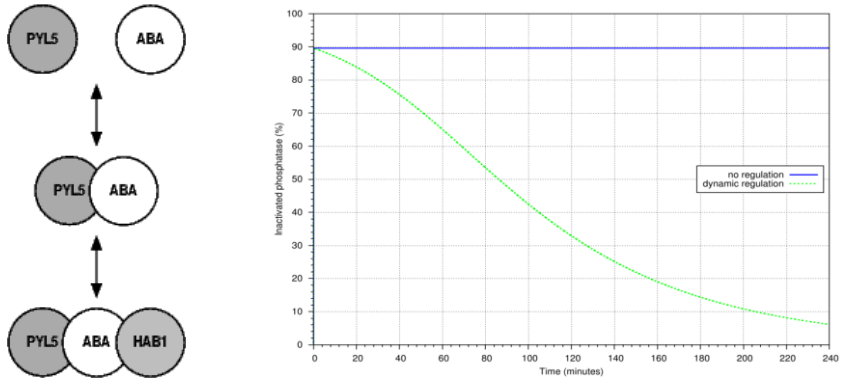


**Figure 1a** (left) shows a model of the interaction of ABA, PYL5 and HAB1. Each PYL5 receptor first binds a molecule of ABA to form a heterodimer, which then binds a HAB1 phosphatase to form a ternary complex. Bound in this complex, HAB1 is unable to regulate the phosphorylation of downstream kinases and an ABA response is initiated.

**Figure 1b** (right) shows the percentage of inactivated HAB1 (i.e. HAB1 bound in the ternary complex) over time. In the model without dynamic regulation of PYL5 and HAB1 (blue solid line), the percentage of inactivated HAB1 remains constant (i.e. there is a sustained ABA response). With dynamic regulation (green dashed line), the percentage of inactivated HAB1 decreases over time and the ABA response is attenuated.

## Auxin/cytokinin signaling network regulating lateral root organogenesis

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Root system is a dynamic organ formed along the plant whole lifetime as result of primary root growth and lateral root (LR) organogenesis. It performs the essential tasks of providing water, nutrients and physical support to the plants. The plasticity of a root system represents one mechanism by which plants overcome their inability to move towards nutrients.

The plant hormones auxin and cytokinin (CK) are key regulators of LR organogenesis acting in an antagonistic manner. During the last decades, both auxin and CK signaling pathways were intensively studied and their basic molecular principles were recognized. However, the molecular mechanism behind their crosstalk is poorly understood.

Using auxin/CK signaling mutants we demonstrated that perturbations in activity of these pathways lead to dramatic defects in auxin - CK regulated LR organogenesis. Based on the auxin - CK sensitivity and expression analysis (using microarray and Real-Time quantitative PCR), we have subdivided the B-type ARRs in two groups which modulate in an antagonistic manner the *AUX*/*IAA* genes involved in LR organogenesis. These data indicate that auxin and CK pathways converge during early LR organogenesis and underlie complex feedback loop mechanism that contribute to regulation of auxin response. Currently, we are building the computer model to access the complexity of cross-talk between auxin and CK during LR development.

## Understanding coordinated root growth through cell type-specific activity of brassinosteroids

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Multiple hormonal pathways, acting in a subset of cells, can promote or restrict organ growth. Our goal is to understand how information from distinct cell-types is integrated to achieve coordinated growth. Here, we focus on brassinosteroids (BRs) and ask how their spatial activity affects the root meristem and the expansion of cells in the elongation zone.

We have recently shown that BRs are required to maintain normal cell cycle activity and cell expansion of meristematic cells (Hacham et al, Development, 2011). We also showed that these two processes ensure the coherent gradient of cell progression, from the apical to the basal meristem. It was further demonstrated that restricted BR signaling in the root epidermis but not in the inner endodermis, quiescent center (QC) cells or stele cell files is sufficient to control root meristem size. Interestingly, expression of the QC and the stele-enriched MADS-BOX gene AGL42 can be modulated by BRI1 activity solely in the epidermis. Thus, our work further supports the importance of cell-cell communication as a mechanism for controlling meristem size.

Our studies in the elongation zone indicate that lateral communication is rather limited. Non-hair (atrichoblast) cells expressing BRI1 (in *bri1* background) confer unexpected sensitivity to salt and sucrose. Specifically, they undergo isotropic cell expansion accompanied by short root length, similar to mutants with cell-wall malfunction. This conditional swelling is a result of mechanical constrain generated by the non-growing neighboring cells. Our current experiments are aiming at building a model that explains how cell mechanics (wall properties) and chemical (hormonal) signals integrate to coordinate root growth.

# Use of cellular Morphodynamics and genome-wide networks to uncover mechanisms regulating cell shape change

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Cell expansion is an integral component of plant growth and development. The modulation of DELLA growth repressors through the hormone gibberellic acid (GA) represents a key step in the control of cell expansion. A connection between this repressor and the downstream molecular events driving cell expansion are however lacking.

Seed germination is an ideal system to uncover molecular mechanisms regulating cell expansion in plants. Following the release from dormancy, there is an induction of expansion that drives the completion of germination. This discrete commencement of cell expansion is a unique situation in the life cycle of a plant, allowing expansion-specific processes to be uncovered.

The analysis of high resolution whole mount confocal microscopy stacks with the image analysis software MorphoGraphX has enabled cell shape in germinating Arabidopsis embryos to be investigated. The 3D morphometric quantification of cell shape change in germinating wild-type Arabidopsis embryos has led to the identification of a subset of cells within the radicle-hypocotyl junction of the embryo, termed the collet, where the initial expansion events occur. This provides both the cellular target for expansion events in seeds, and represents the site where environmental signals are integrated by the seed to manifest germination.

A transcription factor that is specifically induced in the collet concurrently with these initial expansion events has been identified. This transcription factor is repressed by DELLA, induced by both GA and germination, and correlates spatially and temporally with the initial expansion events in early germination. Seeds lacking this transcription factor gene are hypersensitive to the inhibition of germination by GA limiting conditions, demonstrating a positive role in the promotion of GA-mediated cell expansion.

Genes upregulated specifically in the collet during germination have been identified independently. Collet-induced genes include a key GA synthesis gene and an expansin, both which are related to cell expansion. The binding site of the collet-specific transcription factor is present within botrh the promoters of these collet-induced genes. Bandshift and chromatin immunoprecipitation experiments are currently underway to establish whether there is direct binding of this transcription factor to these collet-induced promoters.

## Development rooted in interwoven networks

Benfey, P.N.

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Specification and maintenance of cell identity are central processes of development. In an effort to understand the regulatory networks that control cell identity, we have profiled all cell types and developmental stages within the Arabidopsis root. We are using experimental methods to identify networks functioning within different cell types and developmental stages under normal laboratory conditions and under abiotic and biotic stress. Lateral roots form as repeating units along the root primary axis, however the developmental mechanism regulating this process is unknown. We found that cyclic expression pulses of a reporter gene mark the position of future lateral roots by establishing prebranch sites and that prebranch site production is periodic. Microarray and promoter-luciferase studies revealed two sets of genes oscillating in opposite phases at the root tip. Genetic studies show that some oscillating transcriptional regulators are required for periodicity in lateral root formation. Finally, we are analyzing the dynamics of growth of physical root networks using novel non-invasive imaging methods and developing mathematical descriptors of root system architecture.

This work is supported by grants from the NIH, NSF and DARPA.

# POSTERS

## A search for mutations suppressing the morphological phenotype of an *argonaute1* allele

Aguilera, V., Micol-Ponce, R., Sánchez-García, A.B., Jover-Gil, S., Eren, Z., Isbilir, A., Erbasi, N., Micol, J.L., and Ponce, M.R.

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In *Arabidopsis thaliana*, the main component of the miRNA mediated silencing complex (RISC) is encoded by the *ARGONAUTE1* gene, whose *ago1* mutant alleles disturb many developmental processes and often cause lethality or sterility. With a view to identify genes functionally related to *AGO1*, we mutagenized with EMS seeds of the *ago1-52* mutant, which is moderately fertile and was isolated in our laboratory. The *ago1-52* recessive allele carries a G→A mutation that causes missplicing of an *AGO1* intron, which in turn partially deletes the PIWI domain of the AGO1 protein. We have screened ≈ 60.000 $M_2$ seeds, identifying 26 lines in which the phenotype caused by *ago1-52* is partially or almost completely supressed. We named these suppressor mutations *mas* (*morphology of argonaute1-52 suppressed*). All the putative *ago1-52 mas* double mutants were confirmed not to be revertants or pseudorevertants. We have mapped so far six of the suppressor genes (*MAS1-MAS6*), and positionally cloned four of them (*MAS1*, *MAS2*, *MAS3* and *MAS5*). The aberrant splicing of *AGO1* transcripts shown by the *ago1-52* single mutant was also observed in its double mutant combinations with *mas1-1*, *mas2-1*, *mas3-1* and *mas5-1*. We will present in this meeting the results of our screenings as well as the genetic and molecular characterization of the *MAS2* and *MAS5* genes.

## Hormonal profiling during adventitious root formation in carnation cuttings

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The rooting of stem cuttings is a common vegetative propagation practice in many ornamental species. In carnation (*Dianthus caryophyllus* L.), adventitious root formation (ARF) in the base of the cuttings depends on the basipetal auxin transport from the shoot (1). We present here a detailed histological analysis of the cellular changes occurring in the basal region of carnation cuttings in response to an exogenous application of auxin, which is known to induce ARF in this species (1,2). We found that the base of stem cuttings shows high activity of saccharolytic enzymes and low sugar content, which is consistent with a high energetic demand of growing tissues at the sites of ARF.

To unravel the regulatory processes underlying ARF in carnation cuttings, we studied the hormone profile in the base of the cuttings at different stages during the rooting process. We found that the levels of abscisic acid (ABA) and salicylic acid (SA) rise in the first stages of ARF in carnation cuttings treated with exogenous auxin while their cytokinin levels are reduced. Our results suggest a hormonal crosstalk that determines both the induction and growth of adventitious roots in carnation cuttings which, in turn, will help us to define novel strategies to ensure vegetative propagation of tissue-culture recalcitrant cultivars.

1. Garrido et al. (2002). Origin and basipetal transport of the IAA responsible for rooting of carnation cuttings. *Physiol. Plant.* 114: 303-12.

2. Agulló-Antón et al. (2011). Auxins or sugars: What makes the difference in the adventitious rooting of stored carnation cuttings? *J. Plant Growth Regul.* 30: 100-113.

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## Endocycles and organ size in carnation cultivars: new tools for breeding

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Carnation (*Dianthus caryophyllus* L.) is one of the most popular ornamental plants worldwide. High-quality carnation varieties are usually obtained by intraspecific hybridizations whose success depends on the ploidy levels of their parental species (1,2). Many desirable plant characteristics, such as size and adaptation under stress, are also related to the ploidy levels in different organs (3). To our knowledge, studies that correlate appropriate breeding characteristics with ploidy levels in carnation are missing.

In the work presented here, we characterized ploidy levels in different organs of seven carnation cultivars. Unlike the youngest leaves, other tissues display heterogeneity in nuclear DNA content resulting from different extent of endoreduplication. In addition, we quantified several morphological characteristics affecting cuttings and flowers. We found that both the endoreduplication levels and the proportion of endopolyploid cells were highly variable in petals from the different cultivars studied.

Our results indicate a positive correlation between endopolyploidy and petal size among the carnation cultivars studied. We believe this information should be considered in carnation breeding programs aimed to obtain new varieties with larger flowers. Our protocol for cell ploidy determination in carnation tissues could help during the breeding process.

1. Andersson-Kottö (1931). Interspecific crosses in the genus *Dianthus* . *Genetica* 13: 77-122.
2. Gatt et al. (1998). Yellow pinks: interspecific hybridization between *Dianthus plumarius* and related species with yellow flowers. *Scientia Horticulturae* 77: 207-218.

3. Levin (2002). The role of chromosomal change in plant evolution. Oxford University Press, New York, USA.

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# VVE: a simulation framework for discrete 2-manifold

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Modeling plant growth is a complex process that requires suitable software tools. Their construction and advancement of the underlying theories are still at an early research stage. Here, we present VVE, modeling software that extends and adapts to biological problems vertex-vertex systems, introduced previously in the context of geometric modeling.

The main data structure of VVE is the graph rotation system: a directed graph, associated with local editing operations, in which the neighborhood of each vertex is ordered in a cyclic manner. This structure proved convenient for representing discrete 2-manifolds and locally expressing their development. VVE is implemented as a C++ library and is written using current generic programming methods. This makes it possible to reuse existing code in C or C++, and to create modules for all aspects of the modeling process. The VVE software includes a number of modules, such as a cell-complex library, allowing for easy modeling of plant tissues as the cellular level (Fig. a), an embedded ODE solver for numerically solving systems of differential equations (e.g., reaction-diffusion systems) defined on growing discrete manifolds; and a cell-system library for simple specification of cell division using grammar-like rules (Fig. b). The VVE software also provides visualization and user interaction facilities, which rely on the well established, multi-platform, Qt toolkit and the OpenGL library.

VVE proved to be a useful tool for the modeling of growing tissues, and a step towards the development of a general modeling methodology for developing discrete 2-manifolds. It has been successfully used in a number of models: cell division in the leaf (1); stomata positioning in the leaf (2); trichome patterning (3); and also for data analysis, for example in MorphoGraphX (Smith's lab, University of Bern), in which it is used to work with surfaces.

1. Robinson et al. (2009). Cell division in the Arabidopsis leaf epidermis. Annual Meeting of the SEB 2009.

2. Robinson et al. (2011). Capturing the Dynamics of Stomatal Cell Specification in Growing Leaves. ICAR 2011.
3. Barbier de Reuille et al. (2007). Trichome patterning on growing tissue. FSPM 2007.

## Up and down and all around: PIN polarity regulation in Arabidopsis

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Cell polarity is one of the most basic and important processes for growth and development in eukaryotes. The mechanisms involved in cell polarity establishment and maintenance in model systems, like for example C. elegans and budding yeast, are well understood. In contrast, the mechanism(s) behind the establishment and maintenance of plant cell polarity are still largely enigmatic. Unfortunately, no homologs of the key players in cell polarity establishment and maintenance in other model systems are present in the Arabidopsis genome.

A good example of polarity in plants is represented by the process of polar auxin transport, where members of the PIN family are crucial for providing directionality to this transport. Most of these PIN proteins display a polar localization in specific cell types. The PIN2 protein displays two opposite polarities, to the upper side of lateral root cap and epidermis cells and to the lower side of cortex cells of the root tip.

To identify components of the cell polarity machinery in Arabidopsis a mutant screen was performed using PIN2 as a polarity marker. For the mutagenesis a plant line was used in which the pin2 mutant, that shows agravitropic root growth, has been rescued by a translational PIN2:GFP fusion. The screen identified several different classes of mutants that display a disturbed localization of PIN2. Moreover, these classes fit with the individual “steps” in a current model, which explains the polar localization of PINs. To genetically test this model, crosses between members of the different classes are currently being analyzed. Furthermore, several mutations are being mapped and the corresponding genes will be cloned.

## Use of whole-genome sequencing to identify point mutations that perturb leaf development in Arabidopsis

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To identify the *ANGULATA2* (*ANU2*), *ANU12*, *APICULATA7* (*API7*), *EROSA1* (*ERO1*), *SCABRA4* (*SCA4*) and *SCA5* genes, which are required for normal leaf development in *Arabidopsis thaliana*, we have sequenced the genomes of several double mutants using Illumina's HiSeq2000 system. Each double mutant is homozygous for two unlinked recessive mutations that perturb leaf development in an additive manner. The mutations were previously mapped to ~100 kb candidate regions using linkage analysis to molecular markers. By sequencing the genomes of double mutants we expect to identify two genes per sequenced genome, halving the cost of our study.

The genome of each double mutant (in the L*er* background) was sequenced at 50x coverage, with paired-end reads of 100 nucleotides. The reads were first aligned to the reference Columbia-0 genome, in order to identify changes between the genomes of Col-0 and the mutants in the candidate intervals. Distinction between causal mutations and L*er*/Col-0 polymorphisms was then made on the basis of the presence of the changes in one or more of the sequenced genomes. Our strategy has allowed us to assemble our own version of the L*er* genome and to identify several of the studied genes at the molecular level.

## Lectins and Lectin Receptor Kinases at the Cell Surfaces of *Arabidopsis thaliana*

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Lectins (Lecs) are widespread proteins in all kingdoms of life. They are able to bind mono- or oligo-saccharides in a reversible way and to decipher the information contained in these complex structures.

At the cell surfaces of *Arabidopsis thaliana*, lectins were found (1) as soluble proteins weakly bound to the cell wall, probed by proteomic studies (Irshad *et al.* 2008, BMC Plant Biol 8:94), (2) as chimeric proteins: an extracellular legume lectin domain is associated with a trans-membrane domain and a intracellular kinase domain to form a lectin receptor kinase (LecRK).

Recent studies have shown that receptor homo- and hetero-oligomerisation are essential events to regulate the signalling activity. The versatility of receptor combinations at the cell surface may explain how signalling specificity is maintained at the cytoplasmic level. Since cell wall allows cell-to-cell communication, it is then tempting to add Lecs into the play. Lecs could enlarge the variety of combinations of LecRK complexes and, in turn, the plant cell signalling capacity.

The current study focuses on the Lecs and the LecRKs of the legume-type lectin. Structural features of these proteins, sequence alignments of the lectin domains, phylogenetic trees, and gene expression analysis by exploring public repositories suggest the possibility that Lecs sharing domains with LecRKs might participate in the assembly of hetero-oligomeric receptors and in signalling processes.

Finally, in addition to the legume-type lectins, many cell wall proteins (Jamet *et al.* 2008, Proteomics 8:893) share functional domains with extracellular region of receptor-like kinases such as LRR, curculin-type lectin, LysM, proline-rich, thaumatin, chitinase and DUF26 domains, thus enlarging the possibilities of hetero-oligomeric receptors.

## Positional cloning and molecular characterization of leaf mutants in *Arabidopsis thaliana*

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The leaves are multicellular organs that are important for light harvesting, photosynthesis and gas exchange. The shape, size and internal architecture of leaves are critical for these functions, but the genetic basis of their development is not completely understood.

To further our understanding of leaf morphogenesis in *Arabidopsis thaliana*, we are systematically fine mapping 41 mutants with abnormal leaves, using polymorphisms between the Landsberg *erecta* and Columbia-0 ecotypes. This strategy has allowed us to define short intervals for 23 mutations and to identify 3 of the genes under study: *ANGULATA10* (*ANU10*), *ANGUSTA1* (*ANG1*) and *APICULATA2* (*API2*).

A G-to-A transition in the third exon creates a premature stop codon that is predicted to truncate the ANU10 protein in the *anu10-1* mutant. Two insertional alleles failed to complement the *anu10-1* mutation, and we have been able to rescue the phenotype of *anu10-1* using a wild-type copy of the *ANU10* gene provided by a transgene. ANU10 is a protein of unknown function that is predicted to be chloroplast-localized. We have confirmed this subcellular localization using an *ANU10:GFP* translational fusion.

In addition, we identified a G-to-A transition in the 5'UTR region of *API2*, which encodes the RPL36aB protein of the large subunit of the cytoplasmic ribosome. We also demonstrated that *ang1-1* and *ang1-2* are new mutant alleles of *PIGGYBACK1* (*PGY1*), which encodes the ribosomal protein RPL10aB.

# Revealing the roles of the RETINOBLASTOMA-RELATED (RBR) protein in the Arabidopsis root stem cell niche with the help on an amiGO

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The *Arabidopsis* RETINOBLASTOMA-RELATED (RBR) protein has been proposed to be involved in coordinating cell cycle exit and the progression of the stem cell state towards differentiation. The indispensable activity of RBR in both male and female gametophyte development represents an obstacle for the detailed analysis of RBR functions in Arabidopsis development. We generated cell-type specific loss-of-function lines for RBR by designing an artificial microRNA that targets the 3'UTR of the *RBR* mRNA.

By expressing this amiRNA under specific promoters we were able to efficiently silence RBR transcripts in a tissue-specific manner, demonstrating that RBR function in the stem-cell niche is cell-autonomous. Another advantage of this tool is the possibility to complement the silencing lines with any *RBR* cDNA that lacks the 3'UTR region.

With the help of this **a**rtificial **mi**croRNA for **G**ene-silencing **O**vercome **(amiGO)** we plan to characterize the role of RBR in specific cell lineages in the Arabidopsis root meristem.

# A bHLH heterodimer specifies vascular stem cells during *Arabidopsis* embryogenesis

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During early embryogenesis, the vasculature and its stem cells are first specified, but how this process is genetically controlled remains poorly understood. Recently, we identified TARGET OF MONOPTEROS 5 (TMO5/bHLH32) as being expressed in the vascular stem cells in the embryo (1), but the *tmo5* mutant shows no phenotype. We have generated a *tmo5 tmo5-like1* double mutant, and found that it shows early division defects in vascular stem cells in the embryo and later develops a narrow, monarch vascular bundle in the root. This phenotype is strikingly similar to that of the *lonesome highway* (*lhw/bhlh157)* mutant (2). Using IP-MS and FRET-FLIM, we show that both TMO5 and TMO5-LIKE1 form a functional heterodimeric complex with LHW. Interestingly, co-localization of TMO5 and LHW is limited to a few distal vascular cells, potentially marking the stem cells. Elimination of TMO5, TMO5-LIKE1 and LHW activity in a *tmo5 tmo5-like1 lhw* triple mutant caused a dramatic reduction of vascular tissues, demonstrating the crucial role of this complex.

Conversely, overexpression of TMO5 or TMO5-LIKE1 resulted in an increased vascular bundle, both in embryo and root, and induced ectopic *WOODEN LEG* (3) expression in the root meristem, suggesting that TMO5 controls vascular stem cell identity. This is supported by the ectopic vascular proliferation in other plant tissues in TMO5 overexpression lines.

In conclusion, our data suggests that overlapping expression of TMO5 and LHW genes defines a population of cells in which the TMO5/LHW heterodimer promotes vascular stem cell identity.

1. Schlereth et al. (2010). *Nature* 464(7290): 913-6.
2. Ohashi-Ito et al. (2007). *Development* 134(16): 2959-68.
3. Mähönen et al. (2000). *Genes Dev.* 14(23): 2938-43.

## RBR interaction partners have different effects in the stem cell maintenance in the root meristem

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Retinoblastoma-related protein (RBR) is a protein involved in stem cell maintenance. It binds and regulates the transcriptional activity of E2F and SCR transcription factors. It also binds to many other proteins, and the role of each of those interactions for the stem cell maintenance is not known. We have generated a new mutant alele for RBR by artificial microRNA interference and called it *amiGO RBR*. This alele can be used for complementation analysis. We have produced a set of point mutations in RBR that disrupt specific interactions or in phosphorylation sites. We have analyzed the effect of those mutants in the stem cell phenotype in the root. With this set of data we have started construction a network of interactions related to functions to explain the RBR phenotypes in the stem cell maintenance.

## Application of time-lapse laser scanning to quantify circadian hyponastic movements of single Arabidopsis leaves – a novel phenotyping approach

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Optimal positioning of leaves to the incoming light is essential for plants to optimize light interception and primary production. In nature, plants often need to compete with neighbors for light and have developed adaptive mechanisms. Having detected neighbors, plants initiate a set of growth responses commonly referred to as the shade avoidance syndrome (SAS). The SAS is caused by a decreased red:far-red ratio (R/FR) of the incoming light compared to direct sunlight. Plants forming a rosette during the juvenile growth phase (such as *Arabidopsis thaliana*), which are threaded to be shaded, display an upward movement of leaves (increased petiole angle or hyponasty) and increased petiole elongation gated by the circadian clock.

Up to now, measurements of petiole angle have usually been done using a protractor or photogrammetric methods. These techniques are robust and easy to apply but time-consuming, rendering them not suitable for high-throughput studies. To overcome this obstacle, we propose time-lapse laser scanning using the Scanalyzer HTS (LemnaTec GmbH, Würselen, Germany).

Arabidopsis plants are grown in normal pots and scanned each 40-60 min during a period of 48-72 hours. The outputs of the laser scanner are stacks of height-scaled images. The principal image processing steps are: i) image conversion to 3D point clouds, ii) segmentation of point clouds to relate points to distinct plants, iii) selection of the basal plant point, iv) selection of the blade-petiole intersection for each leaf, v) fitting of a parametric surface that superimposes best with the point cloud.

As a result, leaf surfaces are depicted as parametric surfaces that are computed using a 3D leaf model. The principal model parameters

petiole angle and length are the SAS traits we are interested to measure. Here, we want to present our new phenotyping method, and demonstrate its applicability and robustness on a range of shade avoidance experiments with *Arabidopsis*.

## A new allele of *AUXIN RESISTANT6* uncovers a role for the SCF complex in Arabidopsis leaf vein specification

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To build an almost flat wild-type leaf lamina, the growth of its different tissue layers must be coordinated. Mutations affecting the genes involved in such process should produce uneven leaves. We are testing this hypothesis by studying a large collection of viable mutants which exhibit leaf incurvature. Following a positional approach, we identified the *incurvata13* (*icu13*) mutation as a novel allele of the *AUXIN RESISTANT6* (*AXR6*) gene, which encodes a core subunit of the SCF complex of E3 ubiquitin ligases. The *icu13* mutation causes mRNA missplicing and is predicted to truncate the C-terminal domain of the AXR6 protein. To study the spatial and temporal expression pattern of *AXR6*, we obtained transgenic plants carrying either the upstream regulatory sequences or the entire genomic region of *AXR6* fused to the β-glucuronidase gene or the green fluorescent protein gene, respectively. Both the *icu13* and *enhancer of tir1-1 auxin resistance* (*eta1*) alleles of *AXR6* exhibit hyponastic leaves, simpler venation patterns and reduced auxin responses. The expression levels of some markers of vascular development ($ATHB8_{pro}$*:GUS*), auxin perception (*DR5:GFP*), and auxin transport ($PIN1_{pro}$*:PIN1-GFP*) are reduced in *icu13* and *eta1*.

To ascertain the role of AXR6 in venation pattern formation, we analyzed the genetic interactions of *icu13* and *eta1* with mutations affecting other components of the SCF and auxin signaling pathways. Most double mutant combinations of *AXR6* mutant alleles and gain-of-function alleles of *Aux/IAA* genes exhibited additive phenotypes. Exceptions were the double mutants of *icu13* and *eta1* with *bodenlos* (*bdl*), whose phenotypes were synergistic. We hypothesize that the *icu13* vascular phenotype is caused by the stabilization of BODENLOS and the inactivation of its downstream target, MONOPTEROS.

# Revealing brassinosteroids vascular signaling through BRL3 receptor in Arabidopsis

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Brassinosteroids (BRs) are plant steroid hormones that regulate a wide range of plant developmental processes. BRI1, the main receptor, was previously described as a membrane leucine-rich-repeat-receptor-like kinase (LRR-RLK) which can directly bind BR-ligand, Brassinolide(BL)[1,2,3]. Upon BL perception, BRI1 receptor activates downstream signalling events that control cell division and growth in a variety of plant organs as in the primary root [4].

Two BRI1-like (BRL) receptors were shown to bind to BL with similar BRI1 binding affinity[5] and named BRL1 and BRL3. BRL1 and BRL3 receptors are specifically expressed in the vascular tissue where BRs are known to contribute in the vascular pattern formation[6] and control xylem/phloem differentiation ratios [5].

In order to understand the function of those specific receptors, we used a proteomic approach to identify novel signaling components acting together with BRL3 in vascular development.

Immunoprecipitation of the BRL3 receptor protein complex was done in stable plants expressing 35S:BRL3:GFP followed by trypsin digestion and LC-MS/MS. Remarkably, the expression pattern of these plants was predominantly specific of the vascular tissue, like the endogenous BRL3 protein. Bioinfomatic analysis rendered a list of promising interactors involved in the BRL3 signaling pathway. To confirm the real interactors we carried out FRET-FLIM analyisis, co-immunoprecipitation and genetic crossings of the *knock-out* mutants.

Validation and initial functional characterization of the most relevant interactors will be presented in the conference.

1. Li et al. (1997). *Cell* 90 (5): 929.
2. Wang et al. (2001). *Nature* 410 (6826): 380.
3. Kinoshita et al. (2005). *Nature* 433 (7022): 167.

4. Gonzalez-Garcia et al. (2011). *Development* 138 (5): 849.
5. Cano-Delgado et al. (2004). *Development* 131 (21): 5341.
6. Ibanes et al. (2009). *Proc. Natl. Acad. Sci. USA* 106 (32): 13630.

# Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN1-dependent acropetal auxin transport

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Nitric oxide (NO) is considered a key regulator of plant developmental processes and defense, although the mechanism and direct targets of NO action remain largely unknown. We used a phenotypic, cellular and genetic analysis in *Arabidopsis thaliana* to explore the role of NO in regulating primary root growth and auxin transport (1). Treatment with the NO donors SNAP, SNP and GSNO compared to NO depletion by cPTIO, reduces cell division affecting the distribution of mitotic cells and meristem size by reducing cell size and number. Interestingly, genetic backgrounds where the endogenous NO levels are enhanced (*cue1/nox1*) mirror this response, together with increased cell differentiation phenotypes. Due to the importance of auxin and its specific distribution pattern in regulating primary root growth, we analysed auxin dependent response after altering NO levels. Exogenous NO addition and those genetic overproducer mutants with higher NO content alter the expression of auxin response reporter $DR5_{pro}$*:GUS/GFP,* coincident with a reduction of auxin transport in the *cue1/nox1* mutant. Experiments with the auxin efflux carrier PIN1, after NO application and in the *cue1/nox1* background, confirmed the relevance of NO in the disappearance of PIN1 in a proteasome-independent pathway. Remarkably, the *cue1/nox1* mutant root phenotypes resemble those of *pin1* mutants. The use of both chemical treatments and mutants with altered NO levels, demonstrates that high levels of NO reduce auxin transport and response by a PIN1-dependent mechanism, and concomitantly root meristem activity is reduced.

1. Fernández-Marcos et al. (2011). *Proc. Natl. Acad. Sci. USA*, accepted.

## *TCU1* encodes a nucleoporin required for leaf development in Arabidopsis

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In a screening for Arabidopsis leaf mutants, we isolated *transcurvata1-1* (*tcu1-1*). The leaves of *tcu1-1* are folded downwards in a slightly asymmetrical manner relative to the midvein. The size of cotyledons is reduced in *tcu1-1,* but the length of hypocotyls, epidermal cells, petioles and root hairs are increased. *tcu1-1* is hypersensitive to the synthetic auxin 2,4-D and exhibits early flowering, bolting seven days earlier than L*er*. Some of these traits suggest that the photomorphogenic response is impaired in *tcu1-1*.

We positionally cloned the *TCU1* gene, which was found to encode a putative nucleoporin. We identified five *tcu1* insertional alleles and complemented the mutant phenotype of *tcu1-1* with a transgene carrying a wild-type copy of *TCU1*. No phenotypic effects were caused by *TCU1* overexpression in a L*er* genetic background. The *TCU1* gene was found expressed in most plant organs and its protein product is localized to the nuclear envelope.

After conducting Y2H experiments, we found interactions between TCU1 and some components of the protein ubiquitination SCF complex, the cytoskeleton and the nucleopore. The *tcu1-1* mutant did not exhibit alterations in the transport of mRNAs through the nuclear envelope, and contained more ubiquitin conjugates than its wild type. We analyzed the genetic interactions between *tcu1* alleles and alleles of genes encoding proteins involved in nucleocytoplasmic transport. Synergistic phenotypes were observed in the double mutant combinations of *TCU1* alleles and alleles of genes encoding other nucleoporins or importins.

## Auxin influx carriers control vascular patterning in the shoot of Arabidopsis

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In the shoot of Arabidopsis, the plant conducting tissues are arranged in a very ordered periodic radial pattern, giving rise to the vascular bundles. Brassinosteroids, plant steroid hormones, and auxin control distinct features of this pattern [1]. While brassinosteroids control the number of vascular bundles, auxin transport is involved in their periodic distribution.

It has been shown that proper efflux of auxin from cells plays a pivotal role in plant development. Indeed, polar localization of the auxin efflux carriers in the cell membrane enables the creation of auxin maxima. In the shoot of Arabidopsis, reduced auxin efflux leads to a disorganization of the vascular pattern [1], but the role of auxin influx carriers remains unclear. In phyllotaxis, auxin influx carriers have been proposed to act as pattern stabilizers, particularly in challenging environmental conditions [2,3]. Triple and quadruple influx mutants exhibit weaker and broader peaks of auxin in the shoot apical meristem, and a more irregular phyllotactic pattern [2]. From theoretical grounds, pattern stabilization mediated by influx carriers has been proposed to occur through auxin-induced influx carriers production [3].

Herein we address theoretically and computationally the role of auxin influx carriers in the periodic patterning of vascular bundles in the Arabidopsis shoot. Our modelling results unveil an unprecedented leading role of auxin influx carriers. We support our predictions with a complementary experimental approach using the shoot vascular phenotypes of influx mutants.

1. Ibañes et al. (2009). *Proc. Natl. Acad. Sci. USA* 106(32): 13630.
2. Bainbridge et al. (2008). *Genes Dev.* 22(6): 810-23.
3. Sahlin et al. (2009). *J. Theor. Biol.* 258(1): 60-70.

## Imbalanced growth as a system to reveal cell-wall remodeling and sensing mechanism

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Expanding cells require a controlled remodeling of their contacting cell wall. It is therefore thought that cells monitor and properly respond to signals emanating from the wall. Brassinosteroids (BRs) are key regulators of cell expansion but how exactly they remodel the cell wall is largely unknown. Here, we aim to identify components which are involved in BRI1 mediated cell-wall remodeling and in sensing cell-wall integrity.

We have previously restricted the expression of the BR receptor BRI1 to the non-hair (atrichoblast) cell files in the *bri1* background and established bri1;GL2-BRI1-GFP transgenic lines (Hacham et al, 2011). Interestingly, atrichoblast cells expressing BRI1 undergo isotropic cell expansion and have short root when grown in media supplemented with salt and sugar. The meristematic cell number in bri1;GL2-BRI1-GFP lines remained unaffected under these conditions. The swelling response is not observed in lines that overexpress BRI1 in all cells. Because conditional swelling and inhibition of root growth is also a characteristic of different cell wall mutants, we hypothesize that uncontrolled modification of the cell wall by BRI1 results in cell wall stress response.

To test this hypothesis, I conducted a forward genetic screen and isolated numerous suppressors with longer roots in the presence of salt or sugar. These mutants are currently being characterized.

## Identification of novel genes involving in phloem cell determination in Arabidopsis

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Phloem is the important tissue to transport photosynthesized organic nutrient and various signal molecules, like mRNA and plant hormones. In phloem development, the *ALTERED PHLOEM DEVELOPMENT (APL)* gene was previously identified as an essential gene for phloem cell specification in Arabidopsis. *APL* encodes a MYB-CC like transcription factor and is specifically expressed in both sieve elements (SE) and companion cells (CC) of phloem. However, it is still unclear how *APL* expression pattern is established and restricted in SE and CC during both embryogenesis and post-embryonic morphogenesis. To identify the novel genes involved in *APL* expression and/or phloem cell determination, we started to perform new genetic screening using the *APL* promoter::reporter line. Here we report some isolated mutants that have abnormal expression of *APL*.

## MAB4-dependent auxin transport acts as an auxin-sink in organ formation

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PIN-FORMED1 (PIN1)-dependent polar auxin transport is essential to organ formation. Previous studies on PIN1 localization in meristem uncovered a two-step process in organ formation. (1st) PIN1 polarity converges toward organ initiation sites and auxin accumulates there widely in the L1 layer. (2nd) PIN1 is basally polarized in the future vein cells and auxin gradually sinks from the L1 layer. We have identified NPH3-like proteins, MACCHI-BOU 4 (MAB4) and its homologs MAB4/ENP/NPY1-LIKE1 (MEL1) and MEL2, involved in polar auxin transport in organogenesis. To elucidate the function of *MAB4* family genes, we analyzed PIN1 localization in pin-like inflorescence of *mab4 mel1 mel2* triple mutants. In the mutant, PIN1 polarity converged normally, but followed basal shift of PIN1 polarity did not occur. In addition, an auxin response marker *DR5rev::GFP* was expressed overall in the L1 layer of peripheral region of the mutant meristem, whereas in the wild type *DR5rev::GFP* was expressed widely in the L1 layer of the incipient primordia and then its expression sank from the L1 layer. These results suggest that *MAB4* family genes establish a downward auxin flow by shifting PIN1 polarity. Meanwhile, the transition from the 1st step to 2nd step can be triggered by auxin accumulation and signaling. Considering that *MAB4* family genes are key factors in the transition, it is likely that *MAB4* family genes are auxin-inducible. To confirm the possibility, we examined the relation between auxin signaling and *MAB4* family genes. Our results indicate that *MAB4* family genes function downstream of auxin signaling. This time, we will propose a model of auxin-dependent organ formation based on these results.

## Hierarchy of hormone action controlling apical hook development in Arabidopsis

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The apical hook develops in the upper part of the hypocotyl when seeds buried in the soil germinate, and serves to protect cotyledons and the shoot apical meristem from possible damage caused by pushing through the soil. The curvature is formed through differential cell growth that occurs at the two opposite sides of the hypocotyl, and it is established by a gradient of auxin activity and refined by the coordinated action of auxin and ethylene. Here we show that gibberellins (GAs) promote hook development through the transcriptional regulation of several genes of the ethylene and auxin pathways in Arabidopsis. The level of GA activity determines the speed of hook formation and the extent of the curvature during the formation phase independently of ethylene, probably by modulating auxin transport and response through HLS1, PIN3, and PIN7. Moreover, GAs cooperate with ethylene in preventing hook opening, in part through the induction of ethylene production mediated by ACS5/ETO2 and ACS8.

## Meristemoid cell division and leaf growth under standard and stress conditions

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In a fixed environment, the final size of plant organs, such as leaves, is constant, implying that organ growth is tightly controlled by genetic factors. The two effector systems, cell division and cell expansion, contribute to the final organ size. After emergence from the shoot apical meristem, the leaf primordium grows mainly through cell proliferation. This phase of growth is progressively replaced, in a distal-proximal manner, by a period of cell expansion associated, in Arabidopsis, with an alternative mode of cell cycle activity, namely, endoreduplication. Although most of the cells start to differentiate during that period, some, called dispersed meristemoids, still undergo several round of division. These cells will form specific cell types such as stomatal guard cells or vascular cells.

The contribution of the meristemoid cells to leaf growth and development seems to be important as increasing division of these cells by decreasing the expression of the peapod genes leads to the production of larger, more dome shape leaves (1). Furthermore, these meristemoids cells have also been implicated in cell number recovery upon osmotic stress treatment suggesting a role in stress response (2).

In order to study the contribution of the division of the meristemoids cells in the regulation of leaf development under standard and stress conditions, we initiated a detailed characterisation of the amiRNA PPD and PPD overexpressing lines by means of cellular kinematic analysis and genome wide transcript analysis. The behavior of these lines under several stresses was also assessed.

1. White (2006). PEAPOD regulates lamina size and curvature in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 103: 35.
2. Skirycz et al. (2011). Pause-and-Stop: The Effects of Osmotic Stress on Cell Proliferation during Early Leaf Development in Arabidopsis and a Role for Ethylene Signaling in Cell Cycle Arrest. *Plant Cell*.

## The *RUGOSA2* nuclear gene encodes an mTERF-related protein involved in organelle gene expression and leaf morphogenesis in Arabidopsis

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Little is known about the mechanisms that control transcription of the mitochondrial and chloroplastic genomes and their interplay within plant cells. We positionally cloned the Arabidopsis *RUGOSA2* (*RUG2*) gene, which encodes a protein dual-targeted to mitochondria and chloroplasts, and homologous to the metazoan mitochondrial transcription termination factors (mTERF). In the loss-of-function *rug2* mutants, most organs were pale and showed reduced growth, and the leaves exhibited both green and pale sectors, the latter containing sparsely packed mesophyll cells. Chloroplast and mitochondrion development were strongly perturbed in the *rug2-1* mutant, particularly in pale leaf sectors, in which chloroplasts were abnormally shaped and reduced in number impairing photoautotrophic growth. As expected from the pleiotropic phenotypes caused by its loss-of-function alleles, the *RUG2* gene was ubiquitously expressed. In a microarray analysis of the mitochondrial and chloroplastic genomes, 56 genes were differentially expressed between *rug2-1* and the wild type: most mitochondrial genes were down-regulated while the majority of the chloroplastic genes were up-regulated. Quantitative RT-PCR analyses showed that the *rug2-1* mutation specifically increases expression of the *RpoTp* nuclear gene, which encodes chloroplastic RNA polymerase. Therefore, the *RUG2* nuclear gene seems to be crucial for the maintenance of the correct levels of transcripts in the mitochondria and chloroplasts, which is essential for optimized functions of these organelles and proper plant development. Our results highlight the complexity of the functional interaction between these two organelles and the nucleus.

## Brassinosteroid-regulated root stele dynamics

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Brassinosteroids (BRs) are plant steroid hormones crucial for plant cell growth and development. BR signaling is required during vascular differentiation in Zinnia[1] and *Arabidopsis thaliana* (Arabidopsis) shoot[2,3]. A recent study has shown that BRs play an important role in controling meristem size during root growth[4]. However, how vascular-cell differentiation is regulated by BRs in the root is still unknown.

We have first characterized BRs vascular phenotypes in the primary root of Arabidopsis. Next, to decipher the spatio-temporal transcriptional response of BRs we have used a transcriptomic-based approach coupled to Fluorescence-Activated Cell Sorting (FACS) technology in the vascular tissues of the Arabidopsis root[5].

Gene expression profile during BR-signaling in the stele, the p*WOL*::GFP (WOODEN LEG), expressed in the vascular cylinder and pericycle, was used as a reporter for the sorting of plants treated with Brassinolide (BL) at different times. In addition, high-resolution response of the stele trascriptome upon BL treatment was obtained by further separating the stele in four different cell types using the transcriptional reporters p*AtHB15*::YFP, J0121, p*BRL1*::GFP and p*BRL3*::GFP. These reporters are expressed in provascular cells at the meristematic zone, xylem-pole pericycle, phloem-pole pericycle and procambium cells in the elongation and differentiation zones of the root, respectively.

Enrichment analysis using the GiTools software[6], a framework for analyzing and visualizing genomic data and further bioinformatics analyses led us to identify a number of cell type-specific BL-responsive genes involved in vascular development. Experimental data towards the construction of a transcriptional network in the stele regulated by BRs,

together with an updated status on the functional characterization of our most promising candidates will be presented at the conference.

1. Fukuda et al. (1994). *Int. J. Dev. Biol.* 38: 287-99.
2. Caño-Delgado et al. (2004). *Development* 123 (21): 5341-5351.
3. Ibañes et al. (2009). *Proc. Natl. Acad. Sci. USA* 106(32): 13630-5.
4. Gonzalez Garcia et al. (2011). *Development* 138(5): 849-59.
5. Birnbaum and Benfey (2004). *Curr. Opin. Plant Biol.* 7(5): 582-588.
6. Perez-Llamas and Lopez-Bigas (2011). PLoS One 6(5): e19541.

## Transcript profiling of protoxylem identity formation, differentiation and root procambial patterning in Arabidopsis

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The root vasculature consists of xylem, phloem and intervening pluripotent procambial cells that arise from stem cells within the root meristem. The genetic processes that control the formation of these different cell types are poorly understood. Previously we have shown that both auxin and cytokinin are required during root procambial patterning and vascular development in Arabidopsis (Mähönen *et al.* Science 2006, Bishopp & Help *et al.* Current Biology 2011, Bishopp *et al.* Current Biology 2011). An inhibitory interaction between these two hormones controls the cellular pattern within the vasculature. In addition to promoting procambial cell identity and proliferation, a domain of high cytokinin signalling positions and maintains an auxin signalling maximum in the xylem axis. In turn, the auxin maximum promotes the expression of a negative regulator of cytokinin signalling, creating a sharp boundary between the two domains; auxin signalling in the xylem axis also promotes xylem identity and protoxylem differentiation.

When roots are grown on media containing the auxin transport inhibitor NPA the auxin maximum is erased, resulting in the loss of protoxylem. Both *AHP6* and *VND7* (an early and a late protoxylem identity gene, respectively) are directly auxin upregulated. *AHP6* is misexpressed upon auxin inhibitor treatments and is inhibited by exogenous cytokinin treatments. NPA does not seem to affect *VND7*, but cytokinin erases its expression. In addition, *AHP6* and *VND7* respond slightly differently in treatments combining NPA and cytokinin, suggesting a complex tissue and root zone specific regulatory network.

When cytokinin is depleted from the vascular tissues of NPA treated roots via the induction of cytokinin oxidase, the auxin maximum returns to the stele. This is accompanied by a rapidly elevated expression of *AHP6* followed later by a dramatic increase in *VND7* expression and by formation of ectopic protoxylem cell files in the vasculature. Based on these findings I used the inducible experimental set up and conducted a multi time point array experiment to identify genes that control

protoxylem identity. By comparing the genes up- and down-regulated at the various time points, I aim to elucidate the genetic network responsible for determining protoxylem identity. In addition, I have cross referred genes found in the abovementioned setup to an array experiment in which protoxylem enriched roots were treated with auxin. By doing these array comparisons, I aim to find genes that not only affect protoxylem identity, but do so in response to auxin signalling and cytokinin depletion.

# Modeling Auxin Redistribution During Phototropism in etiolated Arabidopsis Hypocotyls

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Being sessile organisms, plants posses various mechanisms to react to different and changing environmental stimuli. One of these mechanisms allows plants to adjust their growth direction to the direction of incoming blue light. This phototropic response involves sensing of light by photoreceptors, here mainly the membrane-associated proteins phot1 and phot2, redirection of the flux of the hormone auxin, as well as other downstream signaling events. Although these key players in phototropism in *Arabidopsis thaliana* are known, detailed means of interaction remain hidden.

The redirection of auxin fluxes is of primary interest. Following the Cholodny Went hypothesis, it is commonly accepted that an accumulation of auxin on the shaded side is a necessary prerequisite for the bending reaction observed in phototropism. Still, little is known about the process leading to this auxin redistribution. To elucidate possible mechanisms, we investigate changes in localization of auxin efflux facilitators of the PIN family that are supposed to play a predominant role in polar auxin transport. The changes in localization of PIN3 are to be monitored using state of the art microscopy, complemented by quantitative modeling of resulting auxin fluxes. To this end, an ordinary differential equation based compartmental model representation is chosen where both, cells and surrounding apoplastic space, are explicitly considered. In this model auxin fluxes are a result of auxin diffusion in the apoplast, active transport predominantly in form of efflux out of the cells as well as pH-dependent diffusion driven auxin influx. As modeling domain a hypocotyl cross section is used and the vasculature is considered as auxin source while auxin diffusion towards neighboring cross sections as well as intracellular degradation play the role of an auxin sink.

This study is focused on capturing early events of redistribution.

## SCHIZORIZA regulates cell fate specification

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Asymmetric cell division is a fundamental mechanism for the generation of cellular diversity and patterns in multicellular organisms. In plants, where cell migration does not occur, regulation of asymmetric cell division is particularly important. The Arabidopsis root meristem, is laid down during embryogenesis. Its organization is derived from strict asymmetric cell divisions of different stem cells. The root stem cell niche specification and maintenance is controlled by SHORTROOT (SHR)/ SCARECROW (SCR) and PLETHORA (PLT) transcription factors[1,2,3]. Recently, we identified a novel mechanism of cell fate separation in plants that involves the SCHIZORIZA (SCZ) transcription factor[4]. SCZ acts both cell-autonomously to specify cortical cell identity and non-cell-autonomously to separate cell fates in surrounding layers. In addition, SCZ is implicated in stem cell formation[5] and we have evidence indicating SCZ acts in parallel with SHR/SCR to specify the root stem cell niche. We initiated a scz mutant suppressor screen to identify functional targets. In addition, we introduce tissue specific markers in the scz scr double mutant to determine tissue identities and further investigate SCZ involvement in root niche specification during embryogenesis. Finally, transcriptional profiling will be undertaken to identify downstream effectors of SCZ. Together these approaches will allow us to gain insight in the mechanism of SCZ action.

1. Sabatini et al. (2003). *Genes Dev.* 17: 354-358.
2. Aida et al. (2004). *Cell* 119: 109-120.
3. Galinha et al. (2007). *Nature* 449: 1053-1057.
4. ten Hove et al. (2010). *Current Biol.* 20: 452-457.
5. Pernas et al. (2010). *Current Biol.* 20: 818,823.

## amiRNAs targeting groups of transcription factor-encoding paralogs

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Our understanding of the function of individual Arabidopsis genes is obscured by the existence of gene families that include redundant members, a common occurrence in plant genomes. In fact, there is an expanding list of single null mutants not exhibiting any mutant phenotype. In addition, examples are known of double and even triple combinations of non-allelic, loss-of-function mutations affecting paralogous genes that cause no visible phenotypes.

The masking effects of gene redundancy can be overcome with new technologies based on gene silencing, such as artificial microRNAs (amiRNAs). Since gene redundancy is a problem often found in the study of transcription factor families, we are obtaining transgenic Arabidopsis lines expressing amiRNAs designed to repress groups of 2 to 6 paralogous genes encoding transcription factors, in the framework of the TRANSPLANTA (Consolider-Ingenio 2010 CSD2007-00057) consortium.

Following the design principles available at http://wmd.weigelworld.org, we have generated 344 transgenes expressing an amiRNA precursor, 292 of which have already been transformed into Arabidopsis plants. Three well-known transcription factor-encoding genes with easily visible loss-of-function phenotypes were chosen as controls: *GLABRA1*, *AGAMOUS* and *PRODUCTION OF ANTHOCYANIN PIGMENT 1*. We have also used as a control a group of 3 genes involved in the patterning of trichomes. In most, but not all cases the transgenic plants obtained exhibited the phenotype expected from downregulation of the target genes.

## Genetic and physiological dissection of plant root architectural responses to nutrient stress

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Plants actively adjust their root system architecture (RSA) to environmental cues, such as the availability of mineral nutrients in the soil. These root growth responses provide a quantitative output of the sensing and signalling machinery underlying plant adaptation to nutrient stress.

We explored the natural genetic variation of RSA responses by growing *A. thaliana* accessions in a multitude of nutrient deficiency conditions. Deficiency for potassium (K) and phosphate (P) had the highest impact on RSA. Root staining showed that growth arrest of main and lateral roots was due to cell death within the apical meristems. Subsequent QTL analysis identified several loci in the Arabidopsis genome that determine the RSA phenotype in low K. Complementary forward and reverse genetics strategies are now adopted to identify the crucial genes.

Furthermore, we found evidence for crosstalk between K and P sensing/signalling pathways. Iron availability proves to be a key factor since lowering external [Fe(III)] releases the inhibition of main root and lateral root elongation caused by K or P deficiency. In addition, microscopy and pharmacological treatment indicate the involvement of ethylene and ROS signalling. We propose an integrative working model to guide future research in this area.

# Cytokinin-facilitated Proteolysis of ARABIDOPSIS RESPONSE REGULATOR2 Regulates Signaling Output in Two-component Circuitry

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Cytokinins propagate signals via multiple phosphorelays in a mechanism similar to bacterial two-component systems. In *Arabidopsis*, signal outputs are generally determined by the activation state of a subset of transcription factors termed type-B ARRs (*Arabidopsis* response regulators); however, their regulatory mechanisms are largely unknown. In this study, we demonstrate that the proteolysis of ARR2, a type-B ARR, modulates cytokinin signaling outputs. ARR2-HA is rapidly degraded by cytokinin treatment, but other type-B ARRs, such as ARR1-HA, ARR10-HA, ARR12-HA, and ARR18-HA, are not. ARR2 degradation is mediated by the 26S proteasome pathway and requires cytokinin-induced phosphorylation of the Asp80 residue in the receiver domain. Through mutational analysis of amino acid residues in the receiver domain, we found that substitution of Lys 90 with Gly inhibits ARR2 degradation induced by *t*-zeatin. *ARR2*$^{K90G}$*-HA* expression in transgenic *Arabidopsis* conferred enhanced cytokinin sensitivity in various developmental processes, including primary root elongation, callus induction, leaf senescence, and hypocotyl growth. ARR2$^{K90G}$-HA increased the expression of type-A *ARR*s, which known as primary cytokinin-responsive genes and indicators of signaling output in two-component circuits. Expression of *ARR2*$^{K90G}$*-HA* from the nascent promoter in the *arr2-4* knockout mutant also increased cytokinin sensitivity. Clearly, cytokinin-induced degradation of ARR2 is a potential desensitization mechanism for cytokinins and is required for the fine-tuning of the output intensities of developmental signals in *Arabidopsis*.

## Investigating the interplay between carbon partitioning and plant growth in *Arabidopsis thaliana* rosettes

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Photosynthesis and partitioning of the assimilated carbon into components of central carbon metabolism are essential to enable plant growth. Individual processes of central carbon metabolism are well understood, but there is little information on the dynamics of metabolic fluxes in growing tissues. In growing leaves photosynthetic carbon fixation and import of carbon as sucrose occur simultaneously. To get a better understanding of plant growth we are investigating carbon partitioning and fluxes in developing leaves of *Arabidopsis thaliana*.

We study carbon partitioning by applying radioactively labeled carbon dioxide ($^{14}CO_2$) to whole Arabidopsis rosettes or individual leaves. Labeling the whole plant allows us to investigate temporal changes in carbon partitioning, while labeling of individual leaves allows the analysis of carbon export from source to sink tissues. Our results indicate that carbon partitioning is, like leaf growth itself, a very dynamic process. Transport from mature tissues to growing tissues and partitioning into individual compounds within the tissue change over the diurnal cycle. In Arabidopsis plants with altered carbon metabolism (low starch levels or no starch), we observe major differences in the export from mature to growing tissue, but also major differences in the partitioning into plant compounds. Moreover, partitioning in a growing leaf varies for carbon assimilated by the leaf itself and for carbon imported as sucrose.

Ultimately, we will integrate these metabolic fluxes with growth patterns for developing leaves. This will help to understand the interplay between carbon metabolism and growth and to allow modeling of growth processes in leaves.

## Characterisation of promoter regulatory elements driving tissue specific BRL1 and BRL3 expression in Arabidopsis root

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Brassinosteroids (BRs), the steroid hormones of plants, have been shown to play important roles in promoting cell expansion and vascular development. Unlike their animal counterparts, BRs are perceived at the plasma membrane by direct BRI1 binding, a leucine-rich-repeat (LRR) receptor-like kinase (RLK), which acts in concert with BAK1/SERK3, a related LRR-RLK, to transduce BRs signals into the cytoplasm. Downstream signalling events of BRI1 receptor are among the best characterized signalling pathways in plants.

In addition to the BRI1 receptor, two BRI1-homologue proteins, the BRL1 and the BRL3 (BRASSINOSTEROID RECEPTOR LIKE 1 and 3 respectively), were identified that also bind BRs with high affinity. Unlike BRI1, which is ubiquitously expressed, these receptors are specifically expressed in the vasculature. BRL1 shows highest expression in the columella cells of the root tip and in vascular initials at the meristematic region. BRL3 is preferentially expressed in the two protophloem cell files at the elongation zone and in the quiescent center of Arabidopsis seedlings.

Compared to the characterized role of BRI1 in vascular development, the precise role of BRL1 and BRL3 receptors in these cells remain to be identified. In this study, we intend to define regulatory regions within BRL1 an BRL3 promoters, important for their expression. To do this several promoter deletion constructs fused to a GUS reporter gene have been engineered and transformed into Col-0. Expression analysis was done in 6-day-old Arabidopsis seedlings and resulting expression pattern was related to *in silico* predicted promoter elements. Finally, this should lead us to the identification of possible transcriptions factors driving BRL1 and BRL3 expression and in general to understand how these two receptors contribute to vascular development in the Arabidopsis root.

## Gibberellins control microtubule orientation and cell growth through the DELLA- prefoldin interaction

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Cortical microtubules (CMTs) are essential for plant morphogenesis. Their arrangement during plant cell growth and division determines the final shape of plant cells and therefore the architecture of a plant. In growing cells, CMTs are ordered mostly perpendicular to the cell's elongation axis. The arrangement of CMTs is subject to spatial and temporal regulation, but the precise mechanism that underlies CMT dynamics is not well understood.

Among the phytohormones that promote cell elongation, gibberellins (GAs) are known to regulate CMT arrangement too. GA mutants display disorganized CMTs rendering smaller cells and a dwarf plant. GA application is known to provoke an alignment of CMTs. Given that DELLA proteins mediate the regulation of cell expansion by GAs, we wondered whether DELLAs would also be involved in the regulation of CMT arrangement in growing cells, and if so, through which mechanism.

In a yeast two-hybrid screening for DELLA interactors, we found that the DELLA protein GAI interacts with two subunits of the prefoldin complex (PFD), a highly conserved cytosolic co-chaperone that mediates folding of tubulin. This interaction also occurs in plant cells, as we demonstrated by Bimolecular Fluorescence Complementation in agroinfiltrated *N. benthamiana* leaves and by co-immunoprecipitation. Interestingly, DELLA-PFD interaction promotes the translocation of PFD to the nucleus in a GA-dependent manner. The phenotype of PFD loss-of-function mutants mimicks that of GA deficiency, suggesting that the interaction with DELLAs impairs PFD function. We confirmed this hypothesis through gel filtration assays that show how the accumulation of DELLAs prevents the formation of properly folded $\alpha$/ß-tubulin heterodimers, and hence microtubule growth and their correct arrangement.

DELLA proteins have been recently reported to oscillate in a circadian fashion coinciding with plant growth rhythm, so these new results point to DELLAs as coordinators of cell growth at different levels: the time for growth, the execution of cell expansion, and the establishment of growth direction.

## Dynamic interplay between auxin and PLETHORAs orchestrates root growth

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Growth is an outcome of a dynamic interplay of cell division, expansion and differentiation. In the root meristem, the phytohormone auxin seems to affect all these processes. It is believed that a high concentration of auxin is required to maintain cell proliferation in the root meristem, and as auxin concentration decreases cells proceed with cell expansion and differentiation. However, it is well established that auxin is required for cell differentiation as well. This raises the question how auxin can promote the two opposing processes, cell division and differentiation, within the same organ. Our results show that in the root meristem auxin promotes cell division in the presence of PLETHORA (PLT) transcription factors, and in the absence of PLTs, auxin is required for differentiation. Auxin slowly induces PLT transcription in the proximal meristem but does not dictate PLT gradient shape. High auxin and PLT levels independently prevent cells from entering cell expansion. Therefore, after the PLT levels drop, it depends on auxin concentration how much cell can expand before finalizing the differentiation process. We show that the elongation zone (EZ) exists only because of the growth dynamics; meristem is constantly producing cells for differentiation, and as it takes constant time to finalize the differentiation process, the size of the EZ depends on the cell production and expansion rate. Together our data reveal a dynamic interplay between auxin and PLTs to determine whether cells will remain in mitotic state or whether they enter cell expansion and differentiation, the instrumental decision for organ growth.

## New insights on lateral root formation: Role of the cell cycle F-box SKP2B

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Plants are sessile organisms that have to adapt their growth to different environmental clues. Plant root system can grow almost indefinitely due to their ability to branch new organs (lateral roots) throughout their entire life. Lateral roots are formed at regular intervals along the main root by recurrent and auxin dependent specification of founder cells in the basal meristem. We show that *SKP2B*, a cell cycle F-box, represses lateral root formation. At present, the best molecular marker to follow and study this specification and lateral root formation is the DR5:GUS marker, which reflects the maxima auxin response. Here we present a new, reliable and specific marker, *SKP2B*:GUS, to study lateral root development from the specification of the founder cells to the emergence of the lateral organ. One advantage of this marker is that it responds to internal-genetic clues, epigenetic marks and also to external stimuli. Using a *SKP2B*–specific expression profiling in roots, via cell sorting of GFP-protoplasting and mutant analyses, we have identified new genes involved in lateral root development.

## Characterization of the Arabidopsis *MAS5* gene

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We screened 37.000 $M_2$ seeds derived from a second-site mutagenesis of the *ago1-52* line, which carries a hypomorphic and viable recessive allele of the *ARGONAUTE1* (*AGO1*) gene of *Arabidopsis thaliana* in a L*er* genetic background. We isolated in this way 17 mutants exhibiting suppression of the morphological phenotype of *ago1-52*, which we named *mas* (*morphology of argonaute1-52 suppressed*).

For the positional cloning of the *mas5-1* mutation, we first obtained $F_2$ mapping populations by crossing the *ago1-52 mas5-1* double mutant either to *ago1-25* or *ago1-27* single mutants, both of which carry a hypomorphic allele of *AGO1* in a Col-0 genetic background. Iterative linkage analysis to molecular markers allowed us to define a 445 kb candidate interval encompassing 122 genes. In silico analyses of these genes and the subsequent sequencing of some of them allowed us to identify a G to A transition —that would result in the substitution of a lysine by glutamic acid— in a gene involved in an essential step in the maturation of pre-mRNA.

Whereas null alleles of *MAS5* are embryonic lethal, our results suggest that *mas5-1* is a dominant allele, probably of gain of function. We will continue our molecular and genetic characterization of *MAS5* to unravel its functional relationship with *AGO1*.

## The role of symplastic connection during Arabidopsis root development

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Intercellular communication is a critical event in the development of multicellular organisms. Plant cells are connected through plasmodesmata (PD), membrane-lined channels that allow symplastic movement of molecules between cells. However, virtually nothing is known about the role of PD-mediated signaling during plant morphogenesis. It has been reported that callose deposition at PD affects cell-to-cell signaling. We have recently determined the dominant mutations in DVA2 gene which encodes a member of the glycosyl transferase family lead to over accumulation of PD localized callose and the enhanced callose accumulation is accompanied with a reduced PD aperture. Combined these gain-of-function mutations in DVA2 gene to a vector system that allows cell type specific and inducible control of expression of the transgene, we established the molecular tools which enable us to block the symplastic communication from each cell type in inducible manner. Using this new tool, we demonstrated that several cell signaling events are symplastically transmitted in root meristem. Also, using this new tool, we start to reveal the novel symplastic communications that regulate pattern formation during Arabidopsis root development.

## MONOPTEROS controls ground tissue stem cell specification during *Arabidopsis* embryogenesis

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The transcription factor MONOPTEROS (MP) specifies the root meristem founder cell during embryogenesis and is required for root initiation (1). In addition to this, we observed aberrant division of the ground tissue stem cells in *mp* mutants. In a microarray where MP was specifically inhibited in the vascular and ground tissue stem cells in the globular stage embryo, 145 genes were significantly down regulated. Expression analysis of 45 of these genes showed that three candidate MP target genes are specifically expressed in the ground tissue stem cells of the globular stage embryo. These genes are down regulated in the embryo as a result of MP inhibition, and together with the division defects of ground tissue stem cells in *mp* embryos, this result strongly suggests that MP specifies ground tissue stem cells.

*SHORTROOT (SHR)* is a member of the GRAS family of transcription factors that is involved in ground tissue specification (2). However, we observed that the earliest division defects in *shr* embryos occur at heart stage, when the stem cells are already specified. These results implicate that MP could indeed be an important factor controlling this specification event. Moreover, *SHR* and its targets *SCARECROW (SCR)* and *MAGPIE (MGP),* as well as several *SHR* homologues*,* are significantly down regulated in the microarray where MP is inhibited in the globular stage embryo.

We are currently investigating our hypothesis that *SHR* is a MP target gene that redundantly acts with other GRAS family genes to specify ground tissue stem cells. Moreover, we examine if the novel putative MP target genes that are expressed in the ground tissue stem cells contribute to the specification of these cells. In conclusion, our data suggest that MP is the first factor known to be required for ground tissue stem cell specification and might reveal MP target genes involved in this process.

1. Hamann et al. (2002). *Genes Dev.* 16: 1610-1615.
2. Helariutta et al. (2000). *Cell* 101: 555-567.

## Cytokinin inhibition is mediated by *AHP6* during lateral root development in *Arabidopsis thaliana*

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In *Arabidopsis thaliana*, lateral roots initiate from cell divisions of xylem- associated pericycle cells. Lateral root primordia formation is regulated antagonistically by the phytohormones cytokinin and auxin. It is known that cytokinin has an inhibitory effect on early patterning events of lateral root formation (Laplaze *et al.* 2007). However, the molecular players involved in cytokinin repression are still unknown. Previously, it was shown that AHP6 acts as a cytokinin inhibitor during vascular tissue formation in the *Arabidopsis* root (Mähönen *et al.* 2006). In this communication, data will be presented demonstrating that AHP6 also functions as a cytokinin repressor in early stages of lateral root development.

Laplaze et al. (2007). *The Plant Cell* 19: 3889.
Mähönen et al. (2006). *Science* 311: 94.

## Clonal analysis of the post-embryonic function of embryo-lethal genes in *Arabidopsis thaliana*

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Several hundred genes in the *Arabidopsis thaliana* genome are necessary for embryonic and gametophytic development as inferred from the lethality of their loss-of-function mutations. Despite many embryo-lethal genes are normally expressed throughout all stages of plant development, the corresponding mutants die at early stages, preventing the study of their post-embryonic functions using conventional methods. Clonal analysis techniques provide an effective solution to this problem by uncovering the effects of embryo-lethal mutations in sectors of mutant cells within an otherwise normal adult plant.

We have selected 35 embryo-lethal mutants of *Arabidopsis thaliana* for clonal analysis experiments, with a focus on their effects on leaf development. For the induction of mutant sectors in adult plants, we are using two different approaches: one based on the X-ray irradiation of so-called 'cell autonomy' lines (CAUT; Furner *et al*., 2008), and another based on the site-specific excision of transgenes mediated by Cre recombinase (Heidstra *et al*., 2004).

Furner et al. (2008). CAUT lines: a novel resource for studies of cell autonomy in Arabidopsis*. Plant J.* 53: 645-660.

Heidstra et al. (2004). Mosaic analyses using marked activation and deletion clones dissect *Arabidopsis* SCARECROW action in asymmetric cell division. *Genes Dev.* 18: 1964-1969.

## Functional analysis of the Arabidopsis RER gene family

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The Arabidopsis genome contains six paralogs of *RETICULATA* (*RE*), which we named as *RETICULATA-RELATED* (*RER*). We identified null alleles for five *RER* genes, which cause a green reticulation on a paler leaf lamina because of a strong reduction in cell number in the interveinal mesophyll. We characterized the expression patterns of *RE*, *RER1* and *RER3* using transcriptional fusions of their upstream regulatory sequences to the β-glucuronidase (GUS) gene. Although GUS staining was shown only in leaf perivascular cells, functional RER3-GFP protein was visualized in chloroplast membranes of all leaf cells. In addition, we compared mRNA expression profiles of *re* and *rer3* leaves. Several hundred genes were similarly missexpressed in both mutants. The metabolomic profiles of *re* and *rer3* vegetative leaves revealed that several key amino acid biosynthetic pathways are altered. We found that the reticulated leaf phenotype of *re* and *rer3* correlates with an accumulation of reactive oxygen species (ROS), on a light intensity- and photoperiod-dependent manner.

We obtained all the double mutant combinations of the available null *rer* alleles. A synergistic phenotype was shown only in the *re rer1* and the *rer5 rer6* double mutants. Overexpression of *RER1* (but not *RER3*) partially rescued leaf reticulation in *re* mutants, confirming the functional redundancy between the *RE* and *RER1* paralogs. Additive phenotypes were exhibited by the double mutant combinations of either *re* or *rer3* and previously described mutations that perturb palisade mesophyll development, such as *sca3*, *cue1* or *ven.*

Our results indicate that the RER proteins localized at the chloroplast membrane and are required in the transduction of a morphogenetic signal that controls from the chloroplast the proliferation and/or the differentiation of interveinal palisade mesophyll cells.

# *Arabidopsis* PLETHORA transcription factors control phyllotaxis

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Plants generate lateral organs at apical meristems, dynamic stem cell containing structures situated at tips of growing shoots, where organs are positioned in regular patterns in a process know as phyllotaxis. Overall, seed plants display a striking diversity of phyllotactic patterns, and individual plants can alter their pattern through different life phases or under different environmental conditions. Mutational analysis and computer modelling have linked the transport and accumulation of the phytohormone auxin with phyllotaxis, and further have demonstrated that changing the size of the apical meristem can affect phyllotaxis. However, as mutations which disrupt auxin transport impede organ formation, and changing meristem size generally leads to random phyllotaxis, non-pleiotropic regulators of phyllotaxis have remained obscure. Recent work in the Scheres lab has demonstrated that proteins of the PLETHORA (PLT) family of AP2-domain containing transcription factors control phyllotaxis. Multiple *plt* mutant combinations cause metastable switches between the regular phyllotactic patterns observed through the *Arabidopsis* life stages, and further can mimic patterns observed in other species as well as those predicted by computer models when polar auxin transport (PAT) dynamics are altered. PLT proteins act in part via transcriptional control of the *PINFORMED1* gene, which encodes an auxin efflux carrier and therefore contributes to PAT throughout shoot tissues. In summary, we have shown that PLT proteins, first identified for their role in controlling root meristem function by repression of differentiation pathways, are integral components of an auxin-based phyllotaxis system in the shoot. A comparison of PLT action in the shoot and root contexts is the current focus of our investigation.

## Effect of cold on PSII efficiency in Brassicaceae

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One of the first physiological factors that is affected if plants are exposed to unfavourable conditions is photosynthesis. Photosynthetic efficiency is a good indicator for stresses in plants and has already been shown to be affected in response to cold stress. We will try to correlate changes in photosynthesis efficiency to growth performance. The main crop plant species that we will test are *Brassica* (*oleracea* or *rapa*). We will use QTL mapping to elucidate those genetic factors that are involved in the effects of cold on PSII efficiency. An automated phenotyping platform for chlorophyll fluorescence, growth rate and leaf pigmentation will be used to screen a population with a sufficient number of individuals for QTL detection. This platform allows us to measure each plant several times per day in a highly controlled environment increasing the precision of the phenotyping and thereby the QTL analysis. To facilitate the selection of candidate genes, association mapping will be carried out on *Arabidopsis thaliana* grown under similar conditions. The results will provide additional insight into the genetics of photosynthesis and, specifically, those of cold adaptation.

So far we have tested different varieties of Brassica for their response in PSII efficiency to different cold treatments with individual measurements using a FluorCam7. We could identify changes in PSII efficiency between the control condition and different cold treatments as well as subtle differences between the genotypes.

## Functional characterization of nuclear genes encoding chloroplast proteins in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* chloroplast genome sequence was published in 1999. Its size is 154.5 kb and contains 133 genes, 88 of which encode proteins. Nuclear and chloroplast genome expression is precisely coordinated in order to respond to the metabolic needs of plant cells, as well as to establish the developmental program of these organelles. Bioinformatic and proteomic studies indicate that the chloroplast contains around 3000 proteins. However, just a few of them apparently participate in the control of transcription and translation within the organelle. We are taking a reverse genetics approach to the identification of nuclear genes encoding chloroplastic proteins involved in the flow of genetic information in this organelle. For this purpose, we have screened in several publicly available collections for T-DNA insertional alleles of 74 nuclear genes encoding putative chloroplast proteins, with the aim of finding loss-of-function mutations causing a morphological phenotype. We are conducting the genetic and phenotypic characterization of more than twenty mutants, some of them isolated and described for the first time. We have selected for more detailed studies four of them carrying T-DNA alleles of genes that encode chloroplast ribosomal proteins. These mutants are smaller than the wild type and show light green pigmentation and altered chloroplasts. One of them is affected in a protein that contains domains that are exclusive of chloroplast ribosomal proteins and is a component of the small subunit of the chloroplastic ribosome. A role for this protein in regulating translation has been suggested in *Chlamydomonas reinhardtii*. We will present at the meeting the preliminary characterization of these mutants, that we named *crd* (*defective chloroplast ribosome*).

## Interaction of light with cytokinin in regulating the elongation of the Arabidopsis hypocotyl

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Light and cytokinin signaling are intertwined at several levels, and the nature of their interactions is a matter of intense research. Low photosynthetic photon flux density (PPFD) is reportedly a signal for shade avoidance response of which hypocotyl elongation is an integral part. Here, we present data on the involvement of cytokinin in the hypocotyl response to low PPFD. When Arabidopsis seedlings were cultivated at decreased (~5-35 $\mu mol.m^{-2}.s^{-1}$) PPFD, hypocotyl elongation was stimulated by increased cytokinin even without modulating the action of other hormones. Mutant and transgenic plant analysis revealed that the cytokinin-dependent growth response relies on the activity of the cytokinin receptors AHK3 and AHK2, and the downstream signaling involves both type-B ARR and CRF transcription factors. Conditional depletion of endogenous cytokinin and interference with cytokinin signaling partly inhibited the standard hypocotyl elongation caused by low PPFD. Profiling of the hypocotyl proteome revealed some of the molecular events underlying the cytokinin-dependent response. Next, we examined cytokinin-dependent hypocotyl response in light of particular photomorphogenic wavelengths in wild-type and photoreceptor mutants. Stimulation of hypocotyl elongation comparable to that found in white light of low PPFD was seen only in far-red light and was phytochrome A-dependent. We conclude that cytokinin-dependent activation of the canonical two-component signaling pathway via AHK3 and AHK2 participates in the regulation of hypocotyl elongation in the shade avoidance response triggered by low PPFD. Further, our data reveal a novel interaction between cytokinin and phytochrome A signaling.

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## Generation of Spatial Patterns through Polarity Switching

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The mechanisms that generate dynamic spatial patterns within proliferating tissues are poorly understood, largely because of difficulties in unravelling interactions between cell specification, polarity, division, and growth. Here we address this problem for stomatal spacing in the Arabidopsis leaf epidermis. By using time-lapse confocal imaging to track lineages and gene activities over extended periods we were able to capture the dynamics of the system.

We show that stomatal precursor identity depends on the maintenance of the SPCH (SPEECHLESS) transcription factor in one of the two daughter cells through a sequence of divisions. The observed lineages tended to internalise the SPCH-expressing daughter and thus reduce contact with other precursors. We used modelling to investigate mechanisms of control stomata lineages can be re-created if SPCH maintenance is controlled by a polarity factor positioned away from new division walls.

We propose that BASL (BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE) is acting as the polarity determinant and displaces the new division wall. We validated the model by predicting the location of BASL and the resulting cellular arrangements over multiple divisions. Comparing the model to tracked BASL-expressing lineages showed a good match. Thus, complex patterning dynamics can be accounted for by the interplay of cell specification, division and polarity in a growing tissue.

## Genetic and molecular analysis of Arabidopsis mTERF genes

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After obtaining the sequence of the nuclear genome of *Arabidopsis thaliana* and rice, assigning functions to the newly identified genes is one of the main objectives of the scientific community. The mitochondrial transcription termination factors (mTERF) gene family, originally identified and characterized in humans and afterward in other metazoans, was recently discovered in plants, including Arabidopsis. The mTERF proteins studied so far in vertebrates are involved in the control of mitochondrial transcription initiation, termination, translation and mtDNA replication, and have been classified in four subfamilies (MTERF1-4). Some *mTERF* genes are essential for life, since loss of their function proves lethal very early in mice development. Although the number of *mTERF* genes in the genomes of different plant species is higher than in animals, very little is known about their functions. We are conducting a reverse genetics approach in Arabidopsis to elucidate the role of *mTERF* genes in plant development and abiotic stress tolerance. With this aim, we have initiated a molecular and genetic analysis of Arabidopsis mutants homozygous for T-DNA insertional alleles of some *mTERF* genes. These mutants exhibit pleiotropic phenotypes, including reduced fresh and dry weight, slow growth, and poor pigmentation due to decreased chlorophyll levels and the presence of abnormal chloroplasts. Germination of our *mterf* mutants is less sensitive than the wild type to the presence of $Na^+$, $Cl^-$ and $K^+$ ions as well as to the osmotic stress caused by mannitol, which might be due to their reduced sensitivity to abscisic acid (ABA). To identify genetic interactions between *mterf* mutations, we have obtained double mutants affected in *mTERF* genes. Our advances in the characterization of these genes will contribute to elucidate *mTERF* function in plants.

# A new insight into plant cell and tissue mechanics with Cellular Force Microscopy (CFM)

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In order to understand the physical basis of plant morphogenesis, mechanical properties has to be assessed at the single cell level. Although classical micro-indentation methods are designed for local mechanical measurements, they are limited both in resolution and level of automation. On the other hand, nano-indentation techniques with atomic force microscopy (AFM) use too low forces ($10^{-9}$N) to stretch the cell wall and measure its elastic properties.

This technical gap is filled by Cellular Force Microscopy (CFM), a new micro-indentation method dedicated to study plant cells mechanics, *in vivo* and *in situ*. CFM combines high resolution with a large range of forces ($10^{-6}$ N to $10^{-3}$N) particularly suited to assess plant cell primary walls mechanical properties. The system versatility and automation combined with a dedicated data analysis software enables to produce reliable 3D maps of cells topology and stiffness. CFM applications span from non-invasive raster scans to measure sample stiffness, up to puncture of walls in single cells using very high stresses. Simulations showed that, using the right indentation depth, stiffness measurements reflected mostly cell wall elasticity as well as its state of mechanical stress.

To validate the method, we used onion epidermis as a tissue monolayer. Experimental results revealed non-intuitive mechanical behavior of plant tissues at the sub-cellular level. CFM results can be reproduced in FEM simulations by considering detailed cell geometry. Our results reveal the mechanical subtlety of plant tissues, even in the case of a supposedly simple monolayer. They also show the importance of controlling the osmotic conditions during micro-indentation experiments, as well as crucial role of the choice of indentation depth and realistic simulations in order to provide a useful data interpretation.

## Role of RNA processing in protoxylem cell fate

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Plant hormones, cytokinin, as well as auxin, have been established as important regulators of Arabidopsis vascular cell fate. Protein AHP6, a member of cytokinin transduction cascade, has been demonstrated to play a critical role during protoxylem development. In order to put AHP6 protein in a broader molecular context, we made a genetic screen, using *AHP6p:GFP* as a marker of cytokinin activity in protoxylem tissue.

We isolated mutant px1, which shows a decreased *AHP6p:GFP* activity, accompanied with aberrant protoxylem formation. In addition, *px1* displays pleiotropic phenotype, including defective gravitropism, abnormal cotyledonal development and an altered sensitivity to auxins and auxin efflux inhibitors. *PX1* codes for a weak allele of an embryonic lethal gene (EMB) which is, based on homology to known Drosophila modules, possibly involved in splicing.

In order to elucidate the molecular function of PX1 complex, we performed a tandem affinity purification (TAP) experiment. We identified two RNA processing proteins and a regulator of ubiquitin degradation pathway. In addition, in order to find EMB downstream targets, we are sequencing the *px1* transcriptome, thus exploring the importance of alternative splicing during protoxylem formation.

## Genetic and molecular analysis of the *Arabidopsis thaliana MAS2* gene

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In eukaryotes, small non-coding RNAs mediate transcriptional and post-transcriptional gene silencing by binding to ARGONAUTE (AGO) proteins to form RNA-Induced Silencing Complexes (RISCs). AGO1 is the main component of the miRNA pathway in Arabidopsis. We had previously isolated *ago1-52*, a hypomorphic allele of *AGO1*. We isolated suppressor mutations that were named *mas* (*morphology of argonaute1-52 suppressed*) after an EMS second-site mutagenesis of *ago1-52* plants.

Positional cloning of the *MAS2* gene revealed that it is a single copy Arabidopsis gene that encodes a protein of unknown function and conserved among plants and animals. The *mas2-1* point mutation causes an alanine-to-threonine substitution at a highly conserved position of the MAS2 protein and has no visible phenotype by its own. A T-DNA insertion that disrupts *MAS2* (*mas2-2*) causes embryonic lethality. We are obtaining transgenic plants expressing an artificial microRNA targeting *MAS2*.

Overexpression of the wild-type allele of *MAS2* in *ago1-52* background suppressed the mutant phenotype of *ago1-52*. We have also obtained constructs that showed the ubiquitous expression of *MAS2* and the nuclear localization of its protein product, as already known for its animal´s orthologs.

## PLETHORA: understanding a complex meristem regulatory network

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Unlike animals, plants generate tissues and organs from two apical meristems throughout lifetime. The maintenance of these meristems requires coordination between the loss of stem cells through differentiation and the replacement of these cells through division. PLETHORA (PLT) transcription factors have distinct expression patterns and function redundantly, both in maintenance and positioning of root, shoot, and lateral meristems.

Our main goal is to unravel the gene regulatory network controlled by the PLT genes. For that, we generated inducible overexpression lines for PLT1, 2, 3, 4, 5 and 7. The transcriptome profiling analysis indicates that many functional modules are coordinately regulated as a response of the overexpression of the PLT genes. Interestingly, there is an extensive overlap in target regulation between all the PLT family members, though particular responses provide a unique profile for each PLT gene. Remarkably, the pattern expression for the different set of targets revealed a spatial-temporal component in the PLT response. Here, we provide a first glimpse of the mechanism of action for this family of master regulators in both meristems.

# A qualitative continuous model of cellular auxin and brassinosteroid signaling and their crosstalk

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Hormone pathway interactions are crucial in shaping plant development, such as the synergism between the auxin and brassinosteroid pathways in cell elongation. The perception machinery for both hormones has been characterized in detail, revealing a network of feedback loops. The complexity of this network, combined with a shortage of kinetic data, renders its quantitative analysis virtually impossible at present. Prediction of developmental consequences is further complicated by the impact of accessory, cell type-specific factors. As a first step towards overcoming these obstacles, we analyzed the network using a Boolean logic approach. From literature data, we built logical models of auxin and brassinosteroid signaling, and their interaction. To compare their behavior across conditions, we transformed these discrete dynamic models into qualitative continuous systems, which predict network component states more accurately and can accommodate kinetic data as they become available. Finally, we contrasted the developmental output of our models in the presence or absence of cell type-specific modulators. This combinatorial approach identified a most parsimonious model, which explains a paradoxical mutant phenotype and revealed a physiological feature.

## Positional information through differential endocytosis splits auxin response to drive Arabidopsis root meristem growth

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Transcriptional auxin response across Arabidopsis root meristems peaks around stem cells and decreases as cells divide and differentiate. To investigate why expression of some auxin-responsive genes deviates from this gradient, we created cellular level root meristem models that accurately reproduce distribution of nuclear auxin activity. Dynamic modeling only produced deviating expression profiles after intersection of auxin activity with the observed differential endocytosis pattern and positive regulatory feedback through plasma membrane to nucleus transfer of a transcriptional co-regulator. Our data suggest cell type-specific endocytosis-dependent input into transcriptional auxin perception, which sustains expression of a subset of auxin-responsive genes across the root meristem's division and transition zones and is essential for meristem growth. Thus, the endocytosis pattern provides specific positional information to modulate auxin response.

## The porphyrin pathway and Arabidopsis leaf development

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The vegetative leaves of the Arabidopsis *rugosa1* (*rug1*) mutant display an uneven lamina and are smaller and more irregular in shape than those of the wild type. The most conspicuous trait of *rug1* is the spontaneous development of lesions in its vegetative leaves, which appear as patches of necrotic tissue. This phenotype is reminiscent of the lesion-mimic mutants, which develop lesions in the absence of pathogens. The internal leaf structure is altered in the *rug1* mutant, as shown by light and confocal microscopy. Expression of several cytological and molecular markers associated with disease resistance responses was detected in the damaged areas of *rug1* leaves.

We positionally cloned the *RUG1* gene, which was found to encode phorphobilinogen deaminase (PBGD), also known as hydroxymethyl bilane synthase. PBGD is chloroplast-localized and catalyzes the fifth enzymatic step of the tetrapyrrole biosynthesis pathway, which in higher plants produces chlorophyll, heme, siroheme and phytochromobilin. A microarray analysis showed that nearly 300 nuclear genes are differentially expressed between *rug1* and the wild type, about a quarter of which encode proteins involved in plant defense. Our results suggest that impairment of the porphyrin pathway by defective PBGD activates plant defense mechanisms and alters normal leaf development in Arabidopsis.

## MorphoGraphX: Software for image analysis of surfaces extracted from 3D confocal data

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In plants, the epidermis has special significance for many developmental events, thus it is extremely useful to be able to quantify geometry and fluorescent protein expression in the epidermal layer of cells. Often, however, this layer is not flat, and a simple projection of the data onto a plane may not be sufficient for several reasons. When trying to quantify cell shape change, division angles, or growth, distortions from the projection may be too large. Such errors may also distort fluorescence quantification from genetic constructs intended to track protein expression.

To address this issue we have developed the MorphoGraphX software which specializes in biological image processing on curved surfaces. The software can extract surface geometry from confocal image stacks, and project the fluorescence of the epidermal layer of cells onto the surface. Cell wall markers are used to obtain a curved image of the cell outlines which can be used to segment the surface into cells. Protein fusion constructs collected simultaneousness on a separate channel can be used to quantify protein expression at the cellular, and in some cases sub cellular level. By comparing before and after image stacks from experimental treatments, tissue shape and fluorescence change can be quantified. The technique is much more precise that 2D max projections, and is applicable in many situations where a full 3D segmentation is not possible. MorphoGraphX also allows the direct export of cellular geomtery to files, which can be loaded into simulation tools as the starting point for cellular computer models of plant development.

## Natural genetic variation of gibberellin signaling during Arabidopsis photomorphogenesis

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After germination, seedlings follow two alternative programs depending on the presence or absence of light. The selected program in darkness or skotomorphogenesis is characterized by rapid growth of the hypocotyl, the presence of an apical hook, and small, closed cotyledons folded upon themselves. When light triggers photomorphogenic development, hypocotyl growth slows down, the cotyledons open and expand and the expression of numerous genes is changed, especially those involved in the use of light. Several studies have demonstrated the role of hormones gibberellins (GAs) in the transition between the two types of development (Alabadí et al. 2004; Alabadí et al., 2008).

In order to evaluate the physiological relevance of the regulation of photomorphogenesis by GAs and its importance from the standpoint of adaptation, we have studied the variation in sensitivity to GAs in 150 accessions of Arabidopsis thaliana. As a result of this study we selected 20 accessions with behaviors ranging from hyper- to hyporesistant to paclobutrazol, based on measurements of hypocotyl length and angle between the cotyledons in the dark. The analysis in these 20 accessions of the sequences of 8 genes encoding known components of GA signaling has not established any correlation between allelic variation and the degree of GA sensitivity, with the exception of Bla-1. This accession is hypersensitive to GAs, very likely due to the expression of a truncated version of GAI that behaves as a dominant negative allele even when introduced in a different accession.

Furthermore, a microarray assay between Ler and No-0, two accessions with differential sensitivities to GAs has revealed that there is wide variation at the transcriptomic level and the phenotypic difference found is due to the degree of sensitivity than the intervention of new targets.

In summary, our results suggest that the repression of photomorphogenesis by GAs could be a target with adaptive value in different environmental niches.

Alabadí et al. (2004). *Plant Physiol.* 134: 1050-1057.
Alabadí et al. (2008). *Plant J.* 53: 324-335.

## Temporal and spatial regulation of cell cycle genes by the SHR/SCR network links patterning and growth

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The development of multicellular organisms relies on the coordinated control of cell divisions that lead to proper organ patterning and growth. In some cases these are independent, but in most higher animals and plants they must be coordinated. Disruption of this coordination can lead to the unchecked growth of tumors or misshapen organs. In the Arabidopsis root, the transcription factors SHORTROOT (SHR) and SCARECROW (SCR) regulate the formative division in the immediate progeny of the ground tissue stem cells, known as cortex/endodermis initials (CEI). These are asymmetric cell divisions as the daughter cells adopt distinct developmental fates, one giving rise to the cortex, the other to the endodermis. To understand the specific context and dynamics of the SHR/SCR pathway in regulating these asymmetric divisions, we expressed an inducible version of either SHR or SCR in its respective mutant background, which also contained a ground-tissue specific GFP marker. We used fluorescent activated cell sorting in combination with microarray analysis to examine the transcriptional effects of SHR and SCR induction specifically in the ground tissue over time. This powerful approach allowed us to identify novel downstream genes of SHR and SCR in a dynamic and tissue-specific manner. In addition, to assess which of those genes are directly regulated by SHR, we performed ChIP-chip. Coincident with the onset of formative divisions, a D-type cyclin *CYCD6;1*, was directly activated by SHR and SCR. Remarkably, we observed a highly restricted expression pattern of this D-type cyclin at specific developmental time points and cell-types, as a direct consequence of the tight transcriptional regulation by the developmental patterning genes. Our results indicate that the spatial and temporal activation of specific cell-cycle genes are of special importance for a formative division, thus identifying a direct link between

developmental regulators, specific components of the cell cycle machinery and organ patterning.

# Literature mining for *Arabidopsis* leaf growth and development

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With the quickly expanding primary scientific literature, it is getting increasingly difficult to follow past and recent publications on a given topic. Although text mining efforts provide useful tools for the research community to easily access information, it is far from trivial to transform and simplify complex text-based information into a machine-readable format that can be processed algorithmically to represent knowledge, search for relationships, or build novel hypotheses.

Our concrete goal is to collect information describing processes involved in Arabidopsis leaf growth and development, and to translate it in a format compatible with database mining. As a first step, we used Protégé, a free open-source platform (http://protege.stanford.edu) and its text annotation plug-in Knowtator (Ogren, 2006) to extract information from a training library of approximately 70 primary-research papers. The annotation schema resulting from this initial trial will be presented. Our main focus is to capture knowledge from sentences that describe (i) relationships between genotypes and phenotypes, (ii) genotypes and gene expression, (iii) regulatory mechanisms, and (iv) genetic and molecular interactions. Annotations include existing ontologies whenever possible and is linked to syntax units. Our next challenge is to transpose the collected annotations into a relational database connected to the main AGRON-OMICS information system.

Ogren PV (2006) Proceedings of the 2006 Conference of the North American Chapter of the Association for Computational Linguistics on Human Language Technology 273—275. Knowtator: a protégé plug-in for annotated corpus construction

## High resolution imaging and quantitative genetic analysis of rice root system architecture

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Root System Architecture (RSA) refers to the spatial organization of root systems, which affects their capability to extract resources from the soil. Understanding the genetic and environmental factors that control RSA will be critical for developing sustainable crops for stable world agriculture. However, an understanding of root system growth in crop species has been hampered by severe limitations in the throughput, accuracy, and resolution of available observation methods. We developed a gel-based growth and imaging pipeline that features efficient high-resolution phenotyping of living root systems in three dimensions (3D). We applied this method to capture RSA across five days from ~200 rice recombinant inbred lines that were generated from parents with distinct root morphologies. The resulting images were automatically phenotyped for both standard and novel RSA traits using either the original 2D images or 3D tomographic reconstructions of root systems. These data were subsequently used to identify dozens of QTL at

three developmental time points. In order to prioritize our fine-mapping efforts on the most promising candidates, we performed a multivariate QTL analysis that located several genomic 'hotspots' for RSA traits. In some cases, these hotspots co-localize with previously established meta-QTL, and in some cases they are novel to our study. Efforts toward isolating genes of significance to plant breeding are underway using both traditional fine mapping as well as RNA-Seq to identify candidates. Additional research is focused on modeling individual and collective root growth responses to nutrient availability with high spatiotemporal resolution. Collectively, these efforts represent a powerful approach to pinpointing the genetic basis of root architecture, including plant adaptive responses to environmental stress.

## Identification of novel root vascular patterning mutants

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The plant vascular tissue is necessary for transport of water, nutrients and macromolecules. Vascular plants have a transport system consisting of two tissue types with elongated cell files, phloem and xylem. The network of xylem and phloem is expanded throughout plant life. Until now, little has been known about the genes, important for phloem and xylem development. To identify new genes involved in vascular development, two different genetic screens based on ethyl methane sulfonate mutagenesis has been performed and identified a set of novel mutants with altered AtSUC2-GFP and AHP6-GFP expression. One of the mutants showed expanded AtSUC2-GFP expression and expanded number of phloem cells in the cross sections. Lack of GFP unloading from the phloem suggests that these mutants have defects in phloem development. The mutant shows also severe defects in metaxylem development. Map-based approach was used to identify the gene, causing abnormal phenotype.

The second screen was based on AHP6-GFP missexpression. The identified mutant shows abnormal vascular patterning in the root and the expanded number of phloem cells in root cross sections. This mutant has severely dwarfed, with twisted and malformed organs, develop twisted roots compared to wild type plants. The mutant also exhibit a leaf phenotype having severely affected size and shape, a rumpled surface with asymmetric laminas compared to the wild type plants. Consequently a map based approach of these mutants was used to identify the gene, causing the altered phenotype. Genetic mapping resulted in the localization of the genes on the of bottom part of the chromosome IV of *A.thaliana*. Both mutants display similar root vascular phenotypes that are related to patterning processes and root vascular tissue development. This suggests that these genes are required firstly to maintain the radial pattern of the root and secondly for the specification of cell pattern in the vascular development.

# Natural variation in *Arabidopsis thaliana* shows that metabolic responses to soil water deficit are tightly coordinated and highlights the central role of fumarate, in relation to drought regimes in the native habitats

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In plants, primary metabolism plays a central role to shape growth, development and stress responses. Soluble sugars are a source of energy and exert a tight signaling role. Organic acids, nitrate and potassium are important for osmotic adjustment and thus turgor-driven growth. Amino acids are temporary C and N stores during periods when these resources are low. Metabolites are linked by biochemical reactions and their abundances have often been found to be correlated, at least within biochemical clusters and in response to a variety of stresses. Moreover, evidence has recently emerged that genetic variation of metabolites abundances are differentiated along the climatic gradients of the home-sites.

This study was performed using a genetically diverse set of 90 Arabidopsis accessions aimed at evaluating the degree of connection between metabolites in drought stressed plants, which showed reduced growth and increased metabolic status. We also tested if the reshaping of the metabolic network upon water deficit could be linked to the native climate of the accessions.

Results suggest a moderate degree of connectivity among the 12 metabolites analyzed but clearly show that drought deeply modifies these connections, pointing towards a lower integration of sucrose and a higher integration of nitrate possibly in relation to a role as osmoticum. Moreover, the intensity of most metabolite accumulation was tightly coordinated among accessions with a central position of fumarate and this coordination was further validated using a low fumarate mutant. Finally, the degree of fumarate accumulation upon water deficit was found to be related to the native climate of the accessions, with the highest contrast found for the climatic water balance suggesting that

fumarate accumulation upon water deficit and thus the overall metabolic response genetically depends on the drought regimes encountered by the plants during their recent evolution.

## Identifying and understanding the interactions between genes enhancing leaf size

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As most plant organs, leaves show a determinate growth pattern, resulting in a relatively constant size within a fixed environment, thereby limiting the above-ground biomass.

In the model plant *Arabidopsis thaliana*, leaf primordia emerge as rod-like structures at the flank of the shoot apical meristem. During the early stage of development, leaves grow exclusively through cell division. This phase is followed by cell expansion, which is initiated at the distal tip of the leaf and proceeds gradually in a distal-proximal manner. The regulation of leaf growth is poorly understood but must include a complex spatial and temporal coordination between both processes, which contribute to the final leaf size. Several genes have been described that, when down-regulated or ectopically (over)expressed, increase leaf size in Arabidopsis (1). Although the molecular function of the majority of these so called intrinsic yield genes (IYGs) is known, the downstream molecular mechanisms that result in large leaves are not. In addition, the putative connection between these different growth regulating genes and molecular processes remains elusive. Because many genes are involved in leaf growth and converge to similar processes such as cell proliferation, mechanisms that coordinate these different pathways should exist.

We made crosses of 13 IYG's and analyzed the leaf area of the progeny to identify which gene combinations result in a negative, additive and synergistic effects on leaf growth, the first and last suggesting a potential interaction between both genes. In addition, we started a detailed phenotypic and molecular analysis to reveal the link between *da1* and *eod*, shown to synergistically enhance leaf size when mutated (2).

1. Gonzalez et al. (2010). Increased Leaf Size: Different Means to an End. *Plant Physiol.* 153: 1261-1279.

2. Li et al. (2008). Control of final seed and organ size by the DA1 gene family in *Arabidopsis thaliana*. *Genes Dev.* 22: 1331-1336.

## Brassinosteroids control root-growth dynamics in Arabidopsis

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Brassinosteroids (BRs) are steroid hormones essential for plant development. In a recent work, we have shown how BRs control root development[1]. In addition to their known role in promoting cell elongation[2,3], a quantitative analysis of both gain and loss-of-function BR-mutants and exogenous brassinolide (BL) treated plants revealed that BRs control stem cell homeostasis and root meristem growth by controlling normal progression of the cell cycle[1].

The combined action of BRs, both in cell division and elongation, prompted us to investigate BR-driven cellular dynamics along the different root zones. We have studied how local BRs modulate root growth and stem cell differentiation by driving BRI1 and BES1 expression under cell-type specific promoters. As a complementary approach, we have designed a mathematical model describing the cell population dynamics in each root compartment. By adjusting the model dynamics to quantitative data of wild type and BR mutants we aim at unraveling how BRs control the cell dynamics in each developmental zone.

Herein, we will present our initial results on quantitative compartment-based root dynamics. This multidisciplinary approach allows us to better understand how BRs affect root growth in terms of cellular dynamics.

1. González-García et al. (2011). *Development* 138: 849-859.
2. Müssig et al. (2003). *Plant Physiol.* 133: 1261-1271.
3. Clouse et al. (1996). *Plant Physiol.* 111: 671-678.

## A Unified Model of PIN Allocation in the Shoot Apical Meristem

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We develop a unified model of PIN allocation for the shoot apical meristem of *Arabidopsis Thaliana* based on the flux-based model. It simultaneously reproduces the diffuse PIN distribution of the L1 and the canalization of the L2, with neither hybridization nor alteration of parameters. It is demonstrated on both two and three dimensional tissues, both regular and realistic. Our analysis finds that the different behaviours are due to the L1 and L2 being sink- and source- driven, respectively.

## Reverse genetics of leaf development

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The interest of the study, and eventual manipulation, of leaf development lays on the fact that the leaves are the fundamental photosynthetic organ, of vital importance for life in our planet. Although hundreds of mutants with altered leaf development have been isolated using forward genetics, saturation has not yet been reached for the Arabidopsis genome. The group of Prof. J.R. Ecker, at The Salk Institute, is obtaining a large collection of gene-indexed homozygous T-DNA insertion mutants that will cover the 27,000 genes of the Arabidopsis genome. Aiming to identify novel genes involved in leaf shape and size regulation, we are screening 20,718 of these lines, which correspond to 14,585 genes. So far, we have analyzed the leaf phenotype of 13,367 lines, and identified 382 that show a mutant phenotype with full penetrance and almost constant expressivity. We have genotyped 199 of these genuine leaf mutants, assessing that a T-DNA insertion is homozygous at the annotated locus in 75% of them. A public database will collect the results of our screen and the preliminary characterization of their mutant phenotypes.

# PIN mediated auxin accumulation during apical hook development

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Auxin is an essential plant regulator whose asymmetric distribution controls differential growth during plant development. One example of differential growth is the apical hook formation. Recent experimental data show that it depends on the asymmetric distribution of auxin, which is regulated by auxin efflux carriers of the PIN family. Particularly important are PIN3 and PIN4 proteins, whose expression is enhanced on the convex side and severely reduced on the concave side of the hook in the cortex tissues (Žádníková et al, 2010). This asymmetry presumably explains the transport-dependent auxin accumulation on the concave side of the hook.

A computer model operating on a digitized transversal template of the hook (z-stack confocal images of the hook were processed in MorphoGraphX image processing software) was used to examine conditions under which auxin fluxes generate an auxin maximum in the hook. When differential *PIN3/4* expression, correlated with the gravity vector, was limited to the cortex walls, auxin accumulated in the cortex tissue on the concave side of the hook. However, if the differential *PIN3/4* expression was extended to epidermal cells, the auxin maximum was predicted in the epidermis. We analyzed DR5 auxin reporter expression on the transversal sections of the apical hook to dissect which of these predictions corresponded to the actual auxin distribution in the hook. Enhanced response of the *DR5* reporter was found in the epidermal cells on the concave side of the hook, thus supporting our second *in silico* prediction. Furthermore, quantification of PIN3 and PIN4 on cell membranes in the hook epidermis revealed a significantly stronger PIN4

expression in the cells on the convex side. Correspondingly, quantification of the DR5 response revealed that auxin maximum is broader and extends towards epidermal cells on the convex side of the hook in *pin4* but not in *pin3* mutant. Identical auxin distribution pattern was predicted by computer model. In summary, our results reveal that during apical hook formation auxin accumulates in epidermis and PIN3 and PIN4 auxin efflux carriers are involved in the fine tuning of this auxin distribution.

## Roles of cytokinins in cambium activity during secondary development in *Arabidopsis* root

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Our previous discoveries have suggested the role of cytokinin in regulating cambial functions in *Arabidopsis*. We and the others have been able to show that cytokinin signalling induces cambial growth and cytokinins are major hormonal regulators required for cambial development. To identify genes involved in cambium development and to identify the components downstream of cytokinin signalling, a genome-wide gene expression profiling in combination with Fluorescence Activated Cell Sorting (FACS) was performed. Sorting of cambial cells was based on the procambium/cambium specific marker gene ARR15::GFP and RNAs from the cambial cells representing three different developmental zones from either cytokinin treated or non-treated *Arabidopsis* roots were used for whole genome chip hybridization. Gene expression data obtained were compared against databases representing gene expressions in other *Arabidopsis* root cell types and about 500 genes were defined as “cambium enriched”, from which a group of genes seemed to be cytokinin responsive. These genes are the potential targets of cytokinin signaling in cambium development.

By promoter GUS fusion, we are able to validate the secondary development related expression patterns for some of our candidate genes. In addition, since the role of most of them in vascular development is uncharacterized, we are currently analyzing the identified genes functionally. We have been characterizing corresponding mutant lines and analyzing over expression lines of the genes of interests.

## Cell wall DUF642 proteins role in root development

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Cell wall proteomic data provide insights into proteins of unknown function that may be important in cell wall structure or cell-to-cell communication processes. DUF642 family members have been detected in the cell wall proteomes from different tissues and plants. In particular, *At4g32460* and *At2g41800* have been described as auxin up-regulated genes. Accordingly, we conducted an expression study using GFP under the control of the putative promoter region of *At4g32460* and *At2g41800* during plant development and callus induction. In general, *At4g32460* was highly expressed in the vascular system of sink tissues, such as the root meristem, lateral root primordial, petals and stamens and *At2g41800* was expressed mainly in the epidermal cells of the root meristem and during lateral root emergence. In particular, *At4g32460* expression was observed in the emerging primordia of lateral roots, specifically in pericycle-derived cells whereas *At2g41800* was detected in the epidermal/cortical cells overlaying the lateral root primordial. These expression patterns were also detected during callus induction. The cell wall localization of protein encoded *At2g41800* was confirmed using the quimeric 41800-GFP protein under the control of the *At2g41800* promoter. GFP was detected in the cell wall of the root meristematic region epidermic whereas in some damaged cells located in the root transition zone GFP was only detected in the tangential cell wall. These results suggest that the At2g41800 protein could have polarity shifts localization in response to environmental signals. We concluded that the At4g32460 and At2g41800 cell wall proteins are factors of the lateral root initiation program.

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