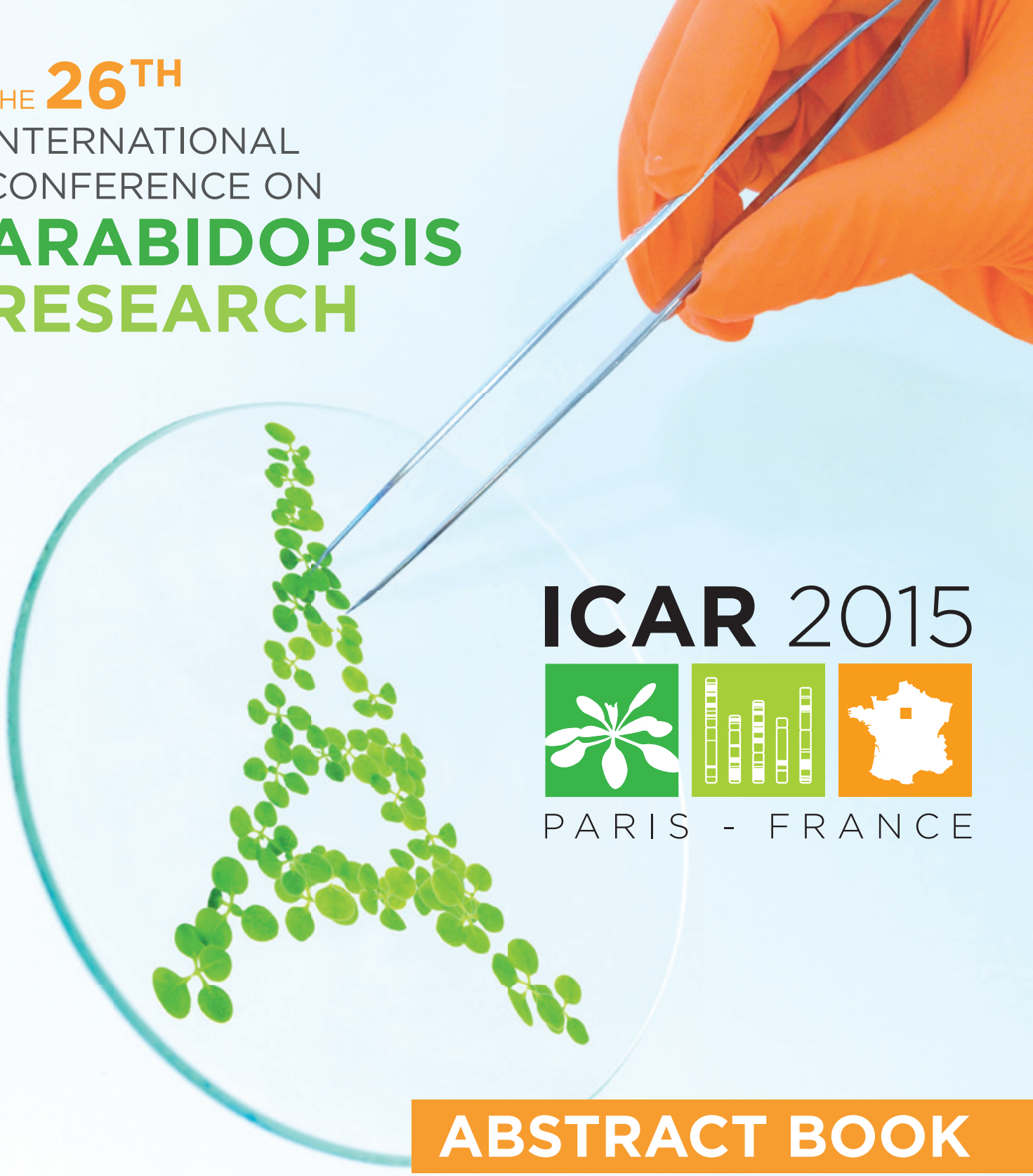
THE 26TH
INTERNATIONAL
CONFERENCE ON
ARABIDOPSIS RESEARCH



ICAR 2015

PARIS - FRANCE

ABSTRACT BOOK

5-9 JULY 2015

PARIS - PALAIS DES CONGRÈS

www.arabidopsisconference2015.org

ADMINISTRATIVE OFFICE

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Product Family for Root Image Analysis and More

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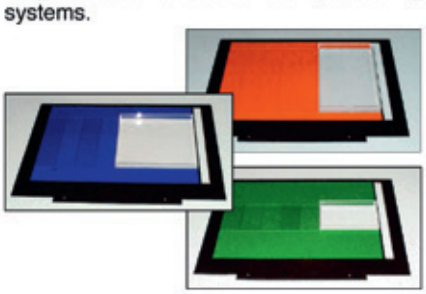
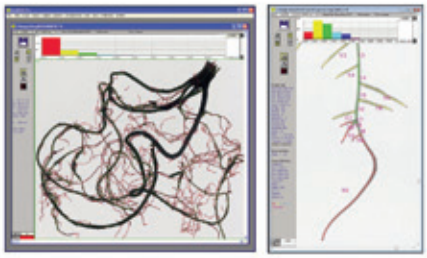
Systems Designed for Automatic Washed Root Analysis

Scan washed roots easily and rapidly with Regent's scanners and root positioning systems.



See analysis results summarized on screen automatically after scanning. Details are autosaved in data files.



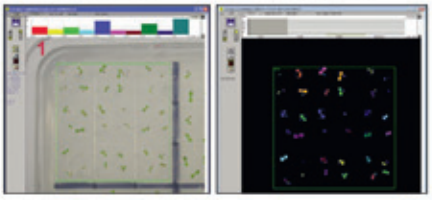
- √ Root morphology in function of root diameter and color: length, area, volume and number of tips
- √ Number of forks and crossings
- √ Root overlap detection for accurate measurement
- √ Topology, link and architecture with fractals
- √ Developmental classification

Arabidopsis

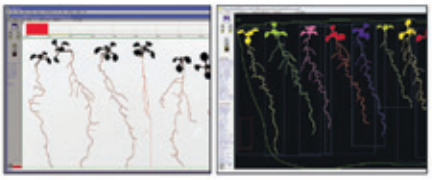
Unique System for Automatic Analysis of Washed Roots and Seedlings in Petri Dish

Analyse seedlings and leaves

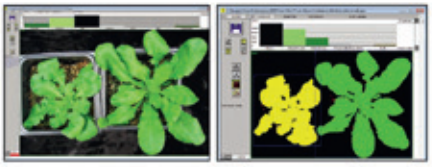
Globally (one analysis per image) or Individually (multiple analyses per image)



- √ Leaf area of seedlings grown in Petri dish
- √ Germination Count



- √ Leaf area - leaf/hypocotyl distinction
- √ Root morphology
- √ Topology and developmental analysis



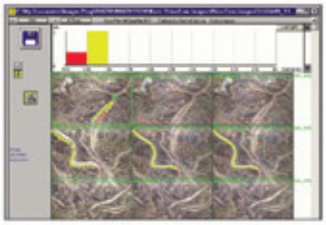
- √ Leaf area, length and width of plants in soil

Tron & Tron MF

Software for Interactive Analysis of Images of Roots in Rhizotron and Soil

Trace roots manually with a mouse or by touching the screen of all-in-one or tablet computers.

Monitor root growth by analysing Multiple Frames (images) of a root system taken at different times.



- √ Root morphology in function of root diameter and color: length, area, volume and number of tips
- √ Topology and developmental analysis
- √ Data retrievable from file names using the ICAP naming scheme
- √ Previous analysis can be retrieved to resume analysis of the same location at a later time simply by adding new or dead roots since the last analysis

Image Analysis Products Available also for the Following Measurements:

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ORGANIZERS

THE FRENCH SOCIETY OF PLANT BIOLOGISTS

The French Society of Plant Biologists (SFBV), formerly the French Society of Plant Physiologists (SFPV) was created in 1955. The SFBV counts approximately 400 members belonging to several universities and national research institutes (INRA and CNRS).

The goals of the SFBV are:

- To promote the engagement of students in plant biology.
- To facilitate the relations between scientists working in several institutions and areas of plant biology in France and abroad.
- To be a representative organ of the French plant biologists in France and abroad.
- To promote and explain, if necessary, to society, via press, media and / or vulgarization conferences, the benefits of research in plant biology and biotechnology.

The SFBV organizes an international congress for plant biologists every other year. It also organizes meetings for young scientists and themed workshops.

For more information on SFBV please consult our web site http://sfbv.snv.jussieu.fr/ or send an email to: sfbv.cordillot@wanadoo.fr.

THE "SACLAY PLANT SCIENCES" LABORATORY NETWORK OF EXCELLENCE

The "Saclay Plant Sciences" Laboratory of Excellence (SPS LabEx, www.saclayplantsciences.fr) is part of the French "Investments for the Future" program and gathers around 50 research teams working on plant sciences in three laboratories from the southern Paris area: the Jean-Pierre Bourgin Institute (IJPB, Versailles), the Institute of Plant Sciences Paris-Saclay (IPS2, Orsay) and the Institute for Integrative Biology of the Cell (I2BC, Gif-sur-Yvette). SPS groups nearly 600 people and is one of the largest European research, teaching, training and innovation consortia centered on plant biology.

The LabEx research activities focus on understanding the genetic, molecular and cellular mechanisms that control the development and physiology of plants, as well as their interactions with the biotic and abiotic environment. These studies range from the gene to the whole plant, and use the concepts and tools of biochemistry, biophysics, imaging, molecular biology, genetics, genomics, cell biology, modeling and bioinformatics. SPS also provides the academic and industrial scientific community with a wide range of high-tech resources.

A set of 20 scientific platforms, with equipment at the cutting-edge of technology, is spread over the four sites. SPS members perform about 7,500 hours of teaching and training and offer more than 100 internships in the LabEx laboratories per year. Finally, the SPS LabEx promotes connections between SPS research teams and private partners (SME / large companies) and provides financial support through a development program for innovative partnership projects (SPS'Innov).

COMMITTEES

LOCAL ORGANIZING COMMITTEE

Chair:
Loïc Lepiniec / Jean-Pierre Bourgin Institute, INRA, Versailles, SPS LabEx Coordinator

Co-chair:
Heribert Hirt / Center for Desert Agriculture, King Abdullah University, Thuwal, SA

Executive coordinators:
Catherine Perrot-Rechenmann / Jean-Pierre Bourgin Institute, CNRS, Versailles – French MASC representative
Marie-Jeanne Sellier / Jean-Pierre Bourgin Institute,INRA, Versailles, SPS LabEx Manager

Members of the organizing sub-committees:
Hélène Barbier-Brygoo / Institute for Integrative Biology of the Cell, CNRS Gif-sur-Yvette)
Michael Hodges / Institute of Plant Sciences Paris-Saclay, CNRS, Orsay
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Rémi Lemoine / Ecology and Biology of Interactions, Poitiers; SFBV Secretary
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Claire Lurin / Institute of Plant Sciences Paris-Saclay, INRA, Orsay
Christian Meyer / Jean-Pierre Bourgin Institute, INRA, Versailles
Martine Miquel / Jean-Pierre Bourgin Institute, INRA, Versailles

With advice from **Joanna Friesner** / NAASC Coordinator and **Luise Brand** / MASC Coordinator

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Jan Traas / RDP, Lyon, France
Klaus Theres / Max Planck Institute for Plant Breeding Research, Köln, Germany
Philip Wigge / Sainsbury Laboratory, Cambridge, UK

ACKNOWLEDGEMENTS

We would like to thank everyone involved in the organization of the Conference; the members of the organizing committees, the session chairpersons, the PhD students as well as the professional conference organizer “Le Public Système” for their important contributions. We deeply acknowledge H. Hirt (co-chair), L. Brand (MASC Coordinator) and J. Friesner (NAASC Coordinator) for friendly advice and valuable information.

We are extremely grateful to the colleagues from the international scientific advisory committee, the local scientific committee and the panel of reviewers, who collectively contributed to the scientific program of ICAR 2015 by proposing and selecting the invited speakers and/or by helping us to select speakers from an impressive number of submitted abstracts. Our thanks are also adressed to all the organizers of the workshops for strengthening so nicely the interest and scientific value of the congress. Finally, we are grateful to the thousand congress participants, from speakers to poster presenters, all discussing their latest findings.

Last but not least, we gratefully acknowledge the generous support of research institutions, research institutes and foundations, the SFBV and all the other partners including sponsors and exhibitors. Without their participation, this congress would not have been possible.

We hope that this Congress will fulfill its main objectives, gathering the scientific community interested in the model plant *Arabidopsis thaliana*, providing the opportunity for colleagues to meet and share cutting-edge results and fostering interactions between young and more established scientists. It will contribute to improve the knowledge of basic biological processes, emphasizing not only the importance and usefulness of *Arabidopsis* as a biological model, but also its impact on other systems and translational research.

Loïc Lepiniec, chair and head of the SPS network of excellence.
Marie-Jeanne Sellier and Catherine Perrot-Rechenmann, executive organizers.

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CLF Plant Climatics

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BAR: BIO-ANALYTIC RESOURCE
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CLF PLANTCLIMATICS GMBH
CNRS: CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
CONVIRON
CSRS: CENTER FOR SUSTAINABLE RESOURCE FOR SCIENCE
ELSEVIER
HETTICH BENELUX
HYBRIGENICS
IBMP: INSTITUT DE BIOLOGIE MOLECULAIRE DES PLANTES DE STRASBOURG
INRA: INSTITUT NATIONAL DE RECHERCHE AGRONOMIQUE
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RIKEN National Research and Development Institute

Since 1917

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http://en.brc.riken.jp/

Under principles of Trust, Sustainability and Leadership:

Founding Biological Resources BRC contributes to research by collecting, preserving and distributing biological resources such as experimental plants and animals, cultured cell lines, genetic materials and associated bioinformatics.

Technology & Development BRC develops novel bioresources to promote research and new technologies to increase the value of bioresources, and implements effective preservation, quality control and usage of bioresources.

Dissemination to and Collaboration with institutions around the world on bioresources, technologies and knowledge.

BRC Experimental Plant Division

http://epd.brc.riken.jp/en/

- We collect, preserve and distribute various mutant Arabidopsis resources that are indispensable for functional genomics and plant science.
- We develop and propagate technologies and databases promoting effective use of biological materials, such as the SABRE database that connects plant cDNA to Arabidopsis genes.
- We maintain and improve plant culture cell lines and genetic materials that can be applied to both basic and applied research on environment, food and useful materials.

RIKEN Center for Sustainable Resource Science (CSRS)

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Enhancing the use of biomaterials and chemicals by understanding the diversity of biological and chemical functions.

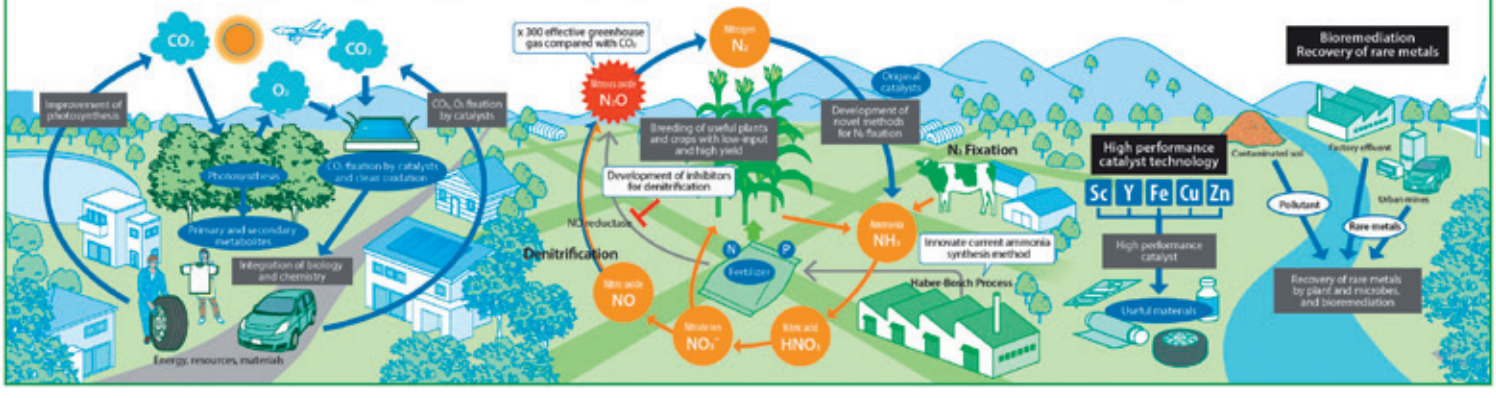
- CSRS comprises three major RIKEN fields: **Plant Science, Chemical Biology,** and **Catalytic Chemistry**.
- Together, Scientists of these three fields research the use of **Carbon, Nitrogen and Metallic elements,** as well as **Research Platforms** to contribute to sustainable production of food, materials and energy:
  - · CO2 Utilization by photosynthesis and catalytic chemistry · Refinement of N2 Utilization
  - · Low-input production of plants · Resilient agriculture · Recovery of metals by plants and microbes
  - · Low cost efficient catalysts · Integration of metabolomics platform and chemical screening platform

CSRS Biomass Engineering Research Division

www.riken.jp/bmep/english/

Biomass Engineering promotes research through collaboration and company cooperation for open innovation by:

- **Discovery of useful genes to improve plant biomass productivity based on genome information of biomass plants.**
- **Establishing innovative cell material production based on synthetic metabolic design.**
- **Establishing and improving practical biopolymer materials to meet the demands of society.**



SCIENTIFIC PROGRAM

SUNDAY, JULY 5, 2015

3:00-7:00 PM **REGISTRATIONS, POSTER SET-UP** — Maillot Hall

4:45-7:30 pm **ICAR 2015 OPENING CEREMONY** — Blue amphi

4:45-5:00 Words of the chair **Loic Lepiniec**
(Jean-Pierre Bourgin Institute, INRA, Versailles, SPS LabEx coordinator)

5:00-6:00 pm **ICAR 2015 KEYNOTE LECTURE** — Blue amphi

Lecture sponsored by RDP
Reproduction and Development of Plants- Lyon

5:00-5:10 Introduction by **Jan Traas** (RDP Lyon), chairman

5:10-6:00 **Elliot Meyerowitz** (California Institute of Technology, Pasadena, CA USA)
Computational morphodynamics of the shoot apical meristem

6:00-7:30 pm **WELCOME RECEPTION** — Maillot Hall

MONDAY, JULY 6, 2015

8:30 am-6:00 pm **REGISTRATIONS, POSTER SET-UP** — Maillot Hall

9:00-10:30 am **PLENARY SESSION: Nutrition and metabolism I** — Blue amphi
Chairman: **Jean-François Briat** (BPMP, Montpellier, FR)

Session sponsored by INRA SCIENCE & IMPACT
BAP Department

9:00-9:25 **Anne Osbourn** (John Innes Center, Norwich, UK)
Unlocking plant metabolic diversity

9:25-9:50 **Wolf Frommer** (Carnegie Institution for Science, Stanford, CA, US)
The role of sugar transporters in carbon allocation

9:50-10:05 **Wolfgang Dröge-Laser** (University of Würzburg, DE)
Low energy Stress response in *Arabidopsis*: the SnRK1-C/S1-bZIP pathway controls metabolic reprogramming to support mitochondrial respiration

10:05-10:15 **Nicole Linka** (Heinrich Heine University Düsseldorf, DE)
In and out - Solute transport across the peroxisomal membrane

10:15-10:30 **Chris Town** (J. Craig Venter Institute, Rockville, MD US)
Araport: your one-stop-shop for *Arabidopsis* data in the 21st century

10:30-11:00 am **COFFEE BREAK** — Maillot Hall

11:00-12:30 am **PLENARY SESSION: Plant Growth and development I** — Blue amphi
Chairman: **Niko Geldner** (DBMV, University of Lausanne, CH)

Session sponsored by ABRC

11:00-11:25 **Dolf Weijers** (Wageningen University, NL)
Control of growth and patterning in the early *Arabidopsis* embryo

11:25-11:50 **Ottoline Leyser** (The Sainsbury Laboratory, Cambridge, UK)
Strigolactone signaling in the regulation of shoot branching

11:50-12:05 **Andrew Millar** (SynthSys, University of Edinburgh, UK)
Daily growth, from clock gene expression to the phospho-proteome and biomass

12:05-12:20 **Sebastian Wolf** (COS Heidelberg, DE)
Receptor-mediated Signalling from the Plant Cell Wall

12:20-12:30 **Hongwei Guo** (Peking University, Beijing, CN)
Suppression of endogenous gene silencing by bidirectional cytoplasmic mRNA decay in *Arabidopsis*

12:30-2:00 pm **LUNCH** — Maillot Hall

2:00-4:00 pm **THEMATIC CONCURRENT SESSIONS**

2:00-4:00 pm **Primary metabolism, photosynthesis, biomass** — Room 242 A-B

2:00-2:20 **Lee Sweetlove** (University of Oxford, UK)
The role of amino acid transporters in nitrogen storage and signalling

Selected talks, 15 min each

Sjef Smeekens (Utrech University, NL)
Sucrose and the control of metabolism and growth
Joshua Heazlewood (University of Melbourne, AU) Poster # 9
Nucleotide sugar transport: Delivering the building blocks of the plant cell wall
Li-Qing Chen (Carnegie Institution for Science, Stanford, US)
SWEET transporters are required for SWEET seeds
Laurent Nussaume (CEA, St Paul lez Durance, FR)
Unravelling the complexity of PHT1 (high affinity phosphate transporters)
Yi-Fang Tsay (Institute of Molecular Biology, Academia Sinica, Taipei, TW)
AtNRT1.13, when mutated, showed altered shoot architecture and late flowering in a nitrogen dependent manner regulations in *Arabidopsis*
Paolo Longoni (University of Geneva, CH) Poster # 16
Dynamics of light-harvesting antenna phosphorylation in *Arabidopsis*

Poster highlight: 2 min talks

Kailash Adhikari (Oxford Brookes University, UK) Poster # 1
A Genome Scale Metabolic Model of *Arabidopsis* helps to understand metabolic role of SBPase and FBPase.
Bianke Loedolff (Stellenbosch University, ZA) Poster # 15
Evidence for a multi-functional α1,6 galactosyltransferase in *Arabidopsis* seeds

2:00-4:00 pm Cell biology — Blue amphi

2:00-2:25 **Olivier Hamant** (RDP, Lyon, FR)
Mechanical signals contribute to the control of cell shape

Selected talks, 15 min each

Oda Yoshihisa (National Institute of Genetics, Mishima, JP)
Secondary cell wall patterning in metaxylem vessels: a spatial interplay between the novel microtubule assembly and disassembly pathways
David Bouchez (INRA IJPB, Versailles, FR) Poster # 31
Arabidopsis trm mutants shed new light on PPB function and plant development
Ying Fu (China Agricultural University, Beijing, CH) Poster # 38
Vesicle Trafficking Driven by Microtubule Growth Regulates Light-Induced Stomatal Opening in *Arabidopsis*
Mathilde Simon (Laboratoire de Reproduction et Développement des Plantes, Lyon, FR)
A PI4P-driven electrostatic field controls plasma membrane identity and plant development
Qiong Zhao (The Chinese University of HongKong, HK) Poster # 72
Fast-suppressor screening identified SOF10 and SOF100 in FREE1-regulated protein trafficking, organelle biogenesis and plant growth in *Arabidopsis*

Poster highlight: 2 min talks

Fatima Awwad (Université de Sherbrooke, CA) Poster # 29
Perturbation of cell wall integrity and the induction of programmed cell death *in Arabidopsis thaliana*
Arthur Molines (I2BC, Gif sur Yvette, FR) Poster # 53
EB1 links microtubule network organization and touch response *in Arabidopsis thaliana*
Fausto Andres Ortiz-Morea (VIB Department of Plant Systems Biology, Ghent, BE) Poster # 58
AtPep1 and its plasma membrane receptors are internalized as a complex via clathrin-mediated endocytosis

2:00-4:00 pm Transcriptional regulations — Maillot room

2:00-2:25 **Lucia Colombo** (University of Milan, IT)
Transcriptional regulatory network controlled by the MADS-domain factor SEEDSTICK

Selected talks, 15 min each

Chloé Zubieta (CNRS, iRTSV/PCV, Grenoble, FR)
Molecular Mechanisms of MADS-domain Transcription Factor Function
Joan Doidy (New York University, US) Poster
Capturing dynamic transcription in gene regulatory networks using affinity-labeled UTP
Julio Saez-Vasquez (LGDP, CNRS Perpignan, FR)
Heat stress induces expression of specific nucleolar proteins and long non-coding rRNA in structurally disorganized nucleoli
Melanie Binkert (University of Geneva, CH) Poster # 78
UV-B-Responsive Association of the *Arabidopsis* bZIP Transcription Factor ELONGATED HYPOCOTYL5 with Target Genes, Including Its Own Promoter
Robert Blanvillain (University Joseph Fournier, iRTSV/PCV, Grenoble, FR) Poster # 79
Nuclear control of chloroplast biogenesis

Poster highlight: 2 min talks

Riccardo Lorrai (Sapienza University of Rome, IT) Poster # 90
DOF AFFECTING GERMINATION 2 is a positive regulator of light-mediated seed germination and is repressed by DOF AFFECTING GERMINATION 1
Mateusz Bajczyk (Adam Mickiewicz University, Poznań, PL) Poster # 76
SERRATE interacts with subunits of the NEXT complex and the polyadenylation machinery in *Arabidopsis thaliana*

4:00-4:30 pm **COFFEE BREAK** — **Maillot Hall**

4:30-6:00 pm **MONDAY WORKSHOPS (W1 to W5)**

4:30-6:00 **Workshop 1 - Abiotic stress responses** — **Maillot room**

Sponsored by the GDRI France-Japan network

Co-chairs: **Toru Fujiwara** (Faculty of Agriculture, University of Tokyo, JP) and **Gwyneth Ingram** (RDP, ENS Lyon, FR)

4:30-5:00 **Toru Fujiwara** (Faculty of Agriculture, University of Tokyo, JP)
A novel mechanism of nutrient sensing mediated by RNA-ribosome complex for regulation of boron transport

5 min - talks selected from abstracts:

Naoyuki Sotta (University of Tokyo, JP) Poster # 525
A mutation in NAC103 suppresses ROS accumulation in root tips caused by excess boron stress
Loren Castaings (LBMP Montpellier, FR) Poster # 464
Regulation of Mn uptake and interplay with Fe transport in *Arabidopsis thaliana*
Yusuke Shikanai (University of Tokyo, JP) Poster # 521
Identification and characterization of low Ca sensitive mutants in *Arabidopsis*
Thibault Dartevelle (CEA Cadarache, FR) Poster # 470
Genetic dissection of the primary root growth response to low phosphate in *Arabidopsis*
Naoto Sano (RIKEN, JP) Poster # 515
Seed Longevity of Arabidopsis Natural Variations after Priming Treatments
Masanori Kaji (Meiji University, JP) Poster # 482
Selection of high temperature resistant germination mutants that have defect in abscisic acid regulation at high temperature
Emeline Sautron (CEA Grenoble, FR) Poster # 517
HMA6 and HMA8 are two chloroplast Cu+-ATPases with different enzymatic properties
Imen Mestiri (IBENS Paris, FR) Poster # 499
Light signaling controls nuclear architecture reorganization during seedling establishment
Akihiro Matsui (RIKEN, JP) Poster # 497
tasiRNA-ARF pathway moderates floral architecture in plants subjected to drought and high-salinity stress
Khurram Bashir (RIKEN, JP) Poster #458
Characterizing the Role of Heat Stress-Inducible Small Open Reading Frames
Loreto Naya Aquilue (IJPB Versailles, FR) Poster # 502
Suppressor screening of autophagy-defective mutants based on early senescence

4:30-6:00 **Workshop 2 - Bioinformatics, Quantitative Techniques and Computational Skills: Current Research and Future Training Needs for 21st Century Plant Biology** — **Room 242A-B**

Sponsored by Arabidopsis.info

Siobhan Brady (NAASC, UC Davis, USA) 10 min
Research in Arabidopsis thaliana: training the next generation of plant scientists.
Blake Meyers and **Mayumi Nakano** (University of Delaware, USA)
Small RNA informatics via Araport, 10 min
Sarah Robinson (University of Bern, Switzerland)
Quantification of the mechanical properties of growing Arabidopsis hypocotyls, 10 min
Nicholas Provart (University of Toronto, Department of Cell & Systems Biology/Centre for the Analysis of Genome Evolution and Function, Toronto, Canada)
ePlant - a new app for exploring Arabidopsis data from the kilometre to nanometre scales, 10 min
Eugenio Azpeitia, (INRIA project-team Virtual Plants, joint with CIRAD and INRA, Montpellier, France)
Intercommunication between wet lab and computational biology, 10 min
Harriet Parsons (University of Cambridge, UK)
An ultra-rapid and quantitative alternative to immunoblotting for determining the organelle composition of tissues homogenates, 10 min
Joanna Friesner (NAASC, University of California, Davis, US)
Moderated interactive panel discussion. *What are the bioinformatics and computational skills needed by plant scientists of the 21st century to deal with more complex datasets (predictive, quantitative and theory-driven)? What are the bottlenecks to providing students with the needed skills? What do employers (of various types) need/want from employees; what are marketable skills in this area?*

4:30-6:00 **Workshop 3 - Cell wall and signaling** — **Blue amphi**

Supported by AuxiWall project ANR-08-BLAN-0219-01,

Stéphanie Robert (Swedish University of Agricultural Sciences (SLU), Umeå, SE)
Distinct mechano-chemical properties participate in the shaping of adjacent pavement cell walls of *Arabidopsis thaliana*, 15 min
Katerina Schwarzerova (Charles University Prague, CZ) Poster # 61
Emerging role of ARP2/3 complex in tissue patterning, 5 min
Petra Stamm (University of Birmingham, UK) Poster # 550
ATHB5 mediates a hypocotyl-specific gene regulatory network driving both expansin gene expression and the final step of *Arabidopsis* seed germination, 5 min
François Jobert (Université de Picardie Jules Verne, Amiens, FR) Poster # 198
Disruption of PME36 activity during seed development alters hormone homeostasis and triggers a shifted compensatory mechanisms in hypocotyl, 5 min
Niko Geldner (University of Lausanne, CH)
The endodermis - a tale of two cell types, 15 min
Fatima Awwad (University of Sherbrooke, CA) Poster # 29
Perturbation of cell wall integrity and the induction of programmed cell death in *Arabidopsis thaliana*, 5 min
Bruce D. Kohorn (Bowdoin College, Brunswick, ME, US)
Cell wall pectin sensing by Wall Associated Kinases (WAKs) 15 min
Julia Richter (University of Natural Resources and Life Sciences, Vienna, AU) Poster # 510
Cross-species-functionality of a *Salix caprea* CELL WALL ASSOCIATED KINASE-LIKE protein in *Arabidopsis*, 5 min
Michael Wrzaczek (University of Helsinki, FIN) Poster # 452
Activity and localization of cysteine-rich receptor-like kinases, 5 min

Conclusions and perspectives by the moderators: **Marie-Theres Hauser** (BOKU, Vienna, AU) and **Grégory Mouille** (IJPB, INRA, Versailles, FR)

4:30-6:00 **Workshop 4 - From Systems Biology to Synthetic biology in plants** — **Room 243**

Supported by frontiers

Rodrigo A. Gutiérrez (P. Universidad Catolica de Chile, Santiago, CL)
Introduction: From Systems to Synthetic Biology in Plants 5 min
Pascal Braun
Systems Analysis of Phytohormone Signal Transduction and Cross-Talk by Interactome Network Mapping 15 min
Gabriel Krouk (CNRS BPMP, Montpellier, FR)
Eukaryotic transcriptional regulatory networks: learning from plants, 15 min
Jim Haseloff (OpenPlant, Department of Plant Sciences, University of Cambridge, UK)
Synthetic Biology and engineering in plants, 30 min

Closing remarks and discussion, round table
Moderators: **Rodrigo Gutierrez, Gabriel Krouk**

4:30-6:00 **Workshop 5 - Ionomics: bringing systems analysis of plant mineral nutrition from Arabidopsis to crops** — **Room 241**

Supported by b.pmp Montpellier

David Salt (University of Aberdeen, Scotland, UK)
Natural diversity in the *Arabidopsis* and rice ionomes 20 min
Nicolaus von WIREN (IPK Gatersleben, DE)
Root plasticity changes in response to the plant nutritional status or shoot ionome 20 min
David Mendoza-Cozatl (University of Missouri, Columbia, US)
Phloem Transport and Seed Loading of Trace Metals in *Arabidopsis* 10 min
Anna Matthiadis (North Carolina State University, Raleigh, US)
Algorithm application to identify novel regulators in the *Arabidopsis thaliana* iron deficiency response 10 min
Marie Barberon (UNIL, Lausanne, CH)
Endodermal development modulates the radial transport of nutrients in roots 10 min

Round table/discussion, moderators: **Jean-François Briat** (BPMP, Montpellier, FR) and **Sébastien Thomine** (I2BC, Gif sur Yvette, FR)

6:00-8:00 pm **POSTER SESSION with drinks** — **Maillot Hall and foyers**

Even # numbers:
Please stand by your poster to present you research during the session

TUESDAY, JULY 7, 2015

8:30 am-6:00 pm **REGISTRATIONS, POSTER SET-UP** — Maillot Hall

9:00-10:30 am **PLENARY SESSION: Responses to the environment I** — Blue amphi

Chairman: **Dominique Roby** (LIMP, Toulouse, FR)

Session sponsored by

9:00-9:25 **Cyril Zipfel** (The Sainsbury Laboratory, Norwich, UK)
Connecting the dots of receptor kinase-mediated immune signaling

9:25-9:50 **Jane Parker** (Max Planck Institute for Plant Breeding Research, Köln, DE)
Intracellular pathogen recognition and immunity

9:50-10:05 **Alain Goossens** (VIB, UGent, BE)
Mapping of protein complexes involved in jasmonate signaling by dynamic tandem affinity purification

10:05-10:20 **Susana Rivas** (LIPM Castanet-Tolosan, FR)
An *Arabidopsis* protease of the subtilase family negatively regulates the transcriptional control of defence responses

10:20-10:30 **Núria S. Coll** (Centre for Research in Agricultural Genomics (CRAG) Cerdanyo, SP)
The role of the metacaspase AtMC1 in aggregate clearance and aging

10:30-11:00 am **COFFEE BREAK** — Maillot Hall

11:00-12:30 **PLENARY SESSION: Reproduction: from flowering to seeds** — Blue amphi

Chairman: **Loïc Lepiniec** (IJPB, Versailles, FR)

Session sponsored by

11:00-11:25 **George Coupland** (MPI, Köln, DE)
Control of floral induction by seasonal cues

11:25-11:50 **Kirsten Bomblies** (Harvard University, Cambridge, MA US)
Meiotic adaptation in *Arabidopsis arenosa*

11:50-12:15 **Ueli Grossniklaus** (University of Zurich, CH)
Molecular control of fertilization and interspecific hybridization

12:15-12:30 **Gwyneth Ingram** (RDP, Lyon, FR)
Seeds feel the pressure

12:30-2:00 pm **LUNCH** — Maillot Hall

2:00-4:00 pm **THEMATIC CONCURRENT SESSIONS**

2:00-4:00 pm **Biotic interactions** — Blue amphi

Session sponsored by

2:00-2:25 **Joy Bergelson** (University of Chicago, IL US)
Diffuse interactions shape the dynamics of a plant pathogen interaction

Selected talks, 15 min each

Panagiotis Sarris (The Sainsbury Laboratory, Norwich, UK)
Insights into intramolecular rearrangements of a TIR-NB-LRR receptor pair upon activation

Souha Berriri (John Innes Center, Norwich, UK) Poster # 100
H2A.Z and SWR1 chromatin remodelling complex components have distinct functions in plant immunity and gene regulation in *Arabidopsis*

Mathilde Fagard (INRA IJPB Versailles, FR)
Impact of nitrogen limitation on the response of *Arabidopsis* to pathogens

Anton Schäffner (Helmholtz Zentrum München, DE) Poster # 144
UGT76B1 and its substrate isoleucic acid in plant defense and development

Agnés Attard (INRA, Sophia Antipolis, FR) Poster # 99
The transcriptome of *Arabidopsis* roots infected with an oomycete identifies genes required for plant defense and susceptibility

Poster highlight: 2 min talks

Safae Hamdoun (University on Maryland Baltimore County, US) Poster # 116
Differential Roles of Two Homologous Cyclin-Dependent Kinase Inhibitor Genes in Regulating Cell Cycle and Innate Immunity in *Arabidopsis*

Tanja Kotur (University of Hamburg/Molecular Plant Physiology, DE) Poster # 124
A Plasma Membrane-Associated Ubiquitin Ligase Regulates Plant Growth and Autoimmunity

2:00-4:00 pm **Development** **Maillot room**

2:00-2:25 **Siobhan Brady** (University of California, Davis, CA US)
How does your garden grow? Transcriptional regulation of *Arabidopsis* cell type development, mineral nutrient acquisition and defense metabolite biosynthesis

Selected talks, 15 min each

Martin Bringmann (Stanford University, US)
Polarization in the *Arabidopsis* leaf epidermis is influenced by mechanical stress.
Hirokazu Tsukaya (University of Tokyo, JP)
Cell-layer-specific coordination between ploidy and cell size in leaves
Yeon Hee Kang (Chonnam National University, Gwangju, KR)
MAKR5 is involved in CLE45 perception in primary root protophloem differentiation
Magdalena Weingartner (University of Hamburg, DE)
MAIL2 is a critical factor for adaption of shoot growth to environmental cues
George Bassel (University of Birmingham, UK)
Graph-based analysis of cellular patterning in plants

Poster highlight: 2 min talks

Daniela Ben-Tov (Hebrew University of Jerusalem Robert H. Smith Faculty of Agriculture, Rehovot, IL) Poster # 164
COBRA-LIKE 2 plays a role in cellulose deposition in *Arabidopsis* seed coat mucilage secretory cells
Margo Smit (Wageningen University , NL) Poster # 242
Linking regulatory networks in vascular development

2:00-4:00 pm **Genome and chromatin dynamics** **Room 242 A-B**

2:00-2:25 **Daniel Zilberman** (University of California, Berkeley, CA USA)
The evolution of genomic imprinting in rice

Selected talks, 15 min each

Danny Wang Ng (Hong Kong Baptist University, HK) Poster # 265
Homoeologous alleles regulations and their contributions in *Arabidopsis* allotetraploids development
Daniel Bouyer (CNRS / IBENS, Paris, FR)
DNA methylation reprogramming during embryogenesis in *Arabidopsis*
Jorge Kageyama (Max Planck Institute for Developmental Biology, Tuebingen, DE) Poster # 262
Methylome variation within and between genetically identical *Arabidopsis* thaliana individuals
Antoine Martin (CNRS, BPMP Montpellier, FR)
Chromatin control of adaptation to nutritional environment in *Arabidopsis*
Alex Mason (University of Washington, Department of Genome Sciences, US)
HSP90 reduction increases penetrance of new mutations

4:00-4:30 pm **COFFEE BREAK** **Maillot Hall**

4:30-6:00 pm **TUESDAY WORKSHOPS (W6 to W10)**

4:30-6:00 **Workshop 6 - Challenges and questions in ambient temperature signaling and acclimation research** **Room 241**

Marcel Quint (Leibniz Institute of Plant Biochemistry, Halle, DE) and
Martijn van Zanten (Utrecht University, NL)
Welcome and introduction, 10 min
Phil Wigge (Sainsbury Laboratory University of Cambridge, UK)
Ambient temperature sensing in plants, 15 min
Carolin Delker (Leibniz Institute of Plant Biochemistry, Halle, DE)
A rendez-vous of signals: regulation of morphogenesis by converging temperature and light stimuli in an intricate signaling network 15 min
Steven Penfield (John Innes Centre, Norwich, UK)
Exploitation of ambient temperature information for control of progeny life history strategy 15 min
Denis Vile (Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, Montpellier, FR)
Analysing GxE to dissect integrated responses of plants to high temperature 15 min

Plenary discussion and collection of audience's viewpoints on the thematic topics, moderators **Marcel Quint** and **Martijn van Zanten**

4:30-6:00

Workshop 7 - Epigenomics

Blue amphi

Doris Wagner (University of Pennsylvania, Department of Biology, Philadelphia, PA 19104-6084, US)
Introduction 10 min
Fred Berger (Gregor Mendel Institute of Molecular Plant Biology GmbH, Dr. Bohr-Gasse 3 1030 Vienna, AU)
Histone variants organise the genome structure, 20 min
Korbinian Schneeberger (Max Planck Institute for Plant Breeding Research Carl-von-Linné-Weg 10 50829 Köln, DE)
Divergent penetrance of DNA methylation among closely related Brassicaceae species, 15 min
Stefan Grob (Institute of Plant Biology, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, CH)
HiC Technology to Characterize Chromosomal Architecture and Identify the KNOT, a Novel Nuclear Structure in Arabidopsis 15 min
Wassim Lakhal (Diagenode, Liege Science Park, Rue Bois Saint-Jean 3, 4102, Seraing, BE) 10 min
Diagenode's activities within the EU EpiTRAITS network 10 min

General discussion, moderators: **Doris Wagner** and **François Roudier** (IBENS, Paris)

4:30-6:00

Workshop 8 - New approaches in plant signaling

Maillot room

Sponsored by the GDRI France-Japan network

Co-chairs: **Yoshihisa Oda** (National Institute of Genetics, Mishima, Shizuoka, JP) and **Annie Marion-Poll** (IJPB, INRA, Versailles, FR)

4:30-5:00 **Yoshihida Oda**
Secondary cell wall patterning in metaxylem vessels

5 min- talks selected from abstracts:

Haniyeh Bidadi (Tsukuba University, JP) Poster # 355
CLE6 expression recovers gibberellin deficiency to promote shoot growth in *Arabidopsis*.
Camille Roux (IJPB, INRA Versailles, FR) Poster # 407
Transcriptome and metabolome analysis of developing seeds of *Arabidopsis nced* mutants deficient in abscisic acid biosynthesis
Taizo Tamura (Nara Institute of Science and Technology, JP) Poster # 95
Detailed analysis on a cis-element sequence of VND7, a master switch of xylem vessel cell differentiation in *Arabidopsis*
Claire Villette (IBMP, CNRS University of Strasbourg, FR) Poster # 250
Isoprenoid homeostasis in *Arabidopsis thaliana*
Satomi Kanno (Kobe University, JP) Poster # 545
Imaging analysis of phosphate absorption and distribution of plants
Mathilde Simon (RDP ENS de Lyon, FR)
A PI4P-driven electrostatic field controls plasma membrane identity and cell signaling in plants
Shogo Takatani (Okayama University, JP) Poster # 62
Arabidopsis NEK6 depolymerizes cortical microtubules by tubulin phosphorylation during directional cell growth
Xiaoyang Zhu (University of Toulouse, FR) Poster # 156
From a phylogenic analysis of Calmodulin-like proteins in green lineage to the role of CML8 in root development and responses to abiotic stress
Hiromi Suzuki (Tokyo Metropolitan University, JP) Poster # 418
Subcellular localization and interaction of Zmphot1 and ZmNPH3-like proteins
Mélanie Ormancey (University of Toulouse, FR) Poster # 135
The calcium-dependent protein kinase CPK3 directly binds to *Arabidopsis* 14-3-3s with various affinities in a calcium- and phospho-dependent manner
Kenta Yamada (Kinki University, JP) Poster # 153
PBL27, a member of RLCKs, directly transduces immune signal from chitin receptor to MAPK cascade in plant immunity
Shugo Maekawa (University of Tokyo, JP) Poster # 130
Proteome analysis using two nuclear proteins with unknown functions provides new insights into the rRNA biosynthesis system in plants

4:30-6:00 **Workshop 9 - Novel Tools and Techniques**
New frontiers in single cell and single molecule biology

Room 243

Sponsored by

Inge Verstraeten (UCR, Riverside, US)
Ligand Fishing with *Arabidopsis* Proteins, 15 min
François-Xavier Ogi (Nanotemper GmbH, München, DE)
Microscale Thermophoresis: A technology for the analysis of biomolecular interactions in free solution, 15 min
Alex Costa (Università degli Studi di Milano, Italy)
Light sheet microscopy: a versatile tool for single cell calcium analysis in *Arabidopsis* root, 15 min
Christopher Grefen (University of Tübingen, DE)
Binary 2in1 vectors allow in planta ratiometric BiFC and improve FRET/FLIM studies, 15 min
Sandra Orthaus-Müller (PicoQuant GmbH, Berlin, DE)
Protein Interaction, Concentration and Mobility Measurements in Plant Cells via FLIM, FRET and FCS
Nicholas Provart (University of Toronto, CA) & **Luise Brand** (University of Tübingen, DE)
The Multinational *Arabidopsis* Steering Committee (MASC) - The role of *Arabidopsis* in single cell, single molecule biology and plant biology, 15 min

General discussion, moderators Luise Brand (MASC) and **Nicholas Provart**

4:30-6:00 **Workshop 10 - The *Arabidopsis* information portal for users and developers**

Room 242 A-B

Agnes Chan (J. Craig Venter Institute)
A Guided Tour of Araport
Matt Vaughn (Texas Advanced Computing Center)
Developing Apps: Exposing your data through Araport
Nick Provart (University of Toronto)
A Community Collaborator Perspective: Case study 1 - BioAnalytic Resource
Blake Meyers (University of Delaware)
A Community Collaborator Perspective: Case study 2 - Small RNA DBs

6:00-8:00 pm **POSTER SESSION with drinks**

Maillot Hall and foyers

Odd # numbers:
Please stand by your poster to present your research during the session

WEDNESDAY, JULY 8, 2015

8:30 am-6:00 pm **REGISTRATIONS, POSTER SET-UP**

Maillot Hall

9:00-10:30 am **PLENARY SESSION: Nutrition and metabolism II**

Blue amphi

Chairman: **Philip Wigge** (Sainsbury Laboratory University of Cambridge, UK)

Session sponsored by

CEPLAS Cluster of Excellence on Plant Sciences

9:00-9:25 **Gloria Coruzzi** (University of New York, NY US)
Nitrogen regulatory networks: From predictive modeling to trait evolution

9:25-9:50 **Andreas Weber** (Heinrich-Heine-University, Düsseldorf, DE)
C4 photosynthesis-understanding the molecular evolution of a complex trait

9:50-10:05 **Mary Wildermuth** (University of California at Berkeley, US)
DNA endoploidy and its impact on metabolism as revealed by the powdery mildew - *Arabidopsis* interaction

10:05-10:20 **Yin Hoon Chew** (University of Edinburgh, UK)
From gene regulatory network to whole-plant biomass via metabolism: Bridging the gap with a multi-scale model

10:20-10:30 **Laurence Lejay** (BPMP, INRA Montpellier, FR)
Regulation of root nitrate uptake in *Arabidopsis* thaliana using NRT2.1 as a target

10:30-11:00 am **COFFEE BREAK**

Maillot Hall

11:00-12:30 **PLENARY SESSION: Plant growth and development II** — **Blue amphi**

Chairman: **Catherine Perrot-Rechenmann** (CNRS IJPB Versailles, FR)

Session sponsored by

11:00-11:15 **Marcos Castellanos** (NASC, Nottingham, UK)
Nottingham *Arabidopsis* Stock Centre (NASC): a quick overview

11:15-11:40 **Eva Benkova** (IST Austria, AU)
Hormonal regulation of root branching

11:40-12:05 **Ykö Helariutta** (University of Helsinky, FI)
The role of symplastic communication on controlling root vascular development

12:05-12:20 **Christian Hardtke** (University of Lausanne, CH)
Phosphoinositide control of root protophloem differentiation and its systemic consequences on hormone activities and root system

12:20-12:30 **Ji-Young Lee** (Seoul National University, KR)
PHABULOSA controls the quiescent center-independent root meristem activities in *Arabidopsis thaliana*

12:30-2:00 pm **LUNCH** — **Maillot Hall**

2:00-4:00 pm **THEMATIC CONCURRENT SESSIONS**

2:00-4:00 pm **Secondary metabolism** — **Room 242A-B**

2:00-2:25 **Tohge Takayuki** (Max Planck Institute, Potsdam-Golm, DE)
Metabolomics-assisted functional genomics on plant phenolic secondary metabolism

Selected talks, 15 min each

Ian Dubery (University of Johannesburg, Auckland Park, ZA)
Metabolomics reveal small molecule dynamics in *Arabidopsis* responding to LPS

Eric Glawischnig (TU München, Freising, DE)
Cytochrome P450 enzymes in the *Arabidopsis* indole-3-acetonitrile metabolic network: Biological functions and protein-protein interactions

Cédric Decourtil (Biopi, Amiens, FR) Poster # 267
Change in secondary metabolism in *Arabidopsis thaliana* pinoresinol reductase mutants.

Ella Katz (Tel Aviv University, IL)
The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of *Arabidopsis thaliana*

Ye Xie (Institute of Plant Physiology and Ecology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, CH) Poster # 276
DELLA Proteins Promote Anthocyanin Biosynthesis via Sequestering MYBL2 and JAZ Suppressors of the MYB/bHLH/WD40 Complex in *Arabidopsis* thaliana

2:00-4:00 pm **Reproduction** — **Maillot room**

2:00-2:25 **Raphaël Mercier** (INRA IJPB, Versailles, FR)
Multiple mechanisms limits meiotic crossovers

2:25-2:50 **François Parcy** (CNRS iRTSV, Grenoble, FR)
Structural insights into LEAFY function and evolution

Selected talks, 15 min each

Danny Geelen (Ghent University, BE)
Engineering clonal seed formation in plants through male apomixis

Nicolas Arnaud (INRA IJPB, Versailles, FR) Poster # 279
A conserved role for CUP-SHAPED COTYLEDON transcription factors during ovule development

Ravishankar Palanivelu (University of Arizona, Tucson, US) Poster # 297
Molecular Genetic Analysis of LORELEI Function in Pollen Tube Reception by the *Arabidopsis* Female Gametophyte

Jennifer Doucet (University of Toronto, CA)
Investigating the roles of stigma-expressed RLCKs in compatible pollen signalling

Poster highlight: 2 min talks

Liu Bing (Ghent University, BE) Poster # 282
GA Signaling Playing a Role in *Arabidopsis* Male Meiotic Cytokinesis

Daniel Johnson (University of Toronto, CA) Poster # 289
Autophagy's Link to Self-Incompatibility in the Brassicaceae

2:00-4:00 pm **Post-transcriptional and post-translational regulations** — **Blue amphi**

2:00-2:25 **Pascal Genschik** (CNRS IBPM, Strasbourg, FR)
When protein and RNA degradation pathways meet

Selected talks, 15 min each

Grégory Vert (CNRS I2BC, Gif sur Yvette, FR)
Downregulation of cell-surface proteins by K63 polyubiquitin-mediated endocytosis: how and why?
Roger Hellens (Queensland University of Technology, Brisbane, AU)
Junk RNA? Characterisation of a non-canonical Upstream Open Reading Frame that Regulates Ascorbate Biosynthesis
Martin Crespi (CNRS Institute of Plant Sciences Paris-Saclay, Orsay, FR)
ASCO a long non-coding RNA involved in root development plasticity
Maria Kalyna (University of Natural Resources and Life Sciences - BOKU, Vienna, AU)
Unmasking alternative splicing inside protein-coding exons defines exitrons and their role in proteome plasticity
Emilio Gutierrez Beltran (Swedish University of Agricultural Sciences, Uppsala, SE)
Molecular role of TSN in cytoplasmic messenger ribonucleoprotein complexes during stress

Poster highlight: 2 min talks

Jakub Dolata (Adam Mickiewicz University, Poznan, PL) Poster # 304
Salt stress reveals new role of AGO1 in miRNA biogenesis pathway at both, co-transcriptional and post-transcriptional levels
Trevor Nolan (Iowa State University, Ankeny, US) Poster # 322
Degradation of BES1 mediated by adaptor protein BIP5 controls the balance between plant growth and stress responses in *Arabidopsis*

4:00-4:30 pm **COFFEE BREAK** — **Maillot Hall**

4:30-6:30 pm **THEMATIC CONCURRENT SESSIONS continue**

4:30-6:30 pm **Translational biology and biotechnologies: from *Arabidopsis* to crops** — **Room 242 A-B**

Session sponsored by

4:30-4:55 **Malcolm Bennett** (CPIB, University of Nottingham, UK)
Roots: uncovering the hidden half of crop traits for breeders

Selected talks, 15 min each

Damien Lieberherr (SIB-Swiss Institute of Bioinformatics, Geneva, CH) Poster # 348
Expert curation of *Arabidopsis* proteins in UniProtKB/Swiss-Prot
Sébastien Thomine (CNRS I2BC, Gif sur Yvette, FR)
Duplication of poplar NRAMP3 gene generated two highly homologous transporters with distinct functions
Frederik Faden (Leibniz Institute of Plant Biochemistry, Halle, DE) Poster # 341
Using a low temperature degron cassette exploiting the N-end rule to efficiently control protein abundance and activity in multicellular organisms
Ling Li (Iowa State University, Ames, US) Poster # 347
The *Arabidopsis* QQS orphan gene modulates carbon allocation across species
Pierre Hilson (INRA IJPB Versailles, FR) Poster # 345
Arabidopsis protoplast regeneration sheds light on specific developmental switches

Poster highlight: 2 min talks

Marjorie Guichard (CNRS I2BC, Gif sur Yvette, FR) Poster # 344
From the *Arabidopsis* model plant to the Medicago root hair model system: role of Mechanosensitive channels in legume symbiosis

4:30-6:30 pm **Hormone signaling** — **Blue amphi**

Speaker sponsored by

Plant Physiology

4:30-4:55 **Jennifer Nemhauser** (University of Washington, Seattle, WA US)
From plants to yeast and back again: synthetic biology and plant development

Selected talks, 15 min each

Richard Hickman (Department of biology, Utrech University, NL)
High-resolution RNA-seq time series reveal architecture and regulation of jasmonic acid and salicylic acid modulated transcriptional networks
Claus Schwechheimer (Technische Universität München, DE)
The role of AGC protein kinases in PIN-mediated auxin transport
Aaron Rashotte (Auburn University, US) Poster # 405
Cytokinin Response Factor 6 is a key regulator of cytokinin and oxidative stress
Alexander van der Krol (Plant Physiology Wageningen University, NL)
Strigolactones stimulate plastid stromule formation independent of MAX2 signalling
Reidunn Birgitta Aalen (Department of Biosciences. University of Oslo, NO)
IDA and IDA-LIKE peptide ligands and their receptors: master regulators of cell separation events

Poster highlight: 2 min talks

Isabel Monte (CNB-CSIC, Madrid, SP) POSTER # 393
Rational design of a ligand-based antagonist of jasmonate perception
Tara Enders (Washington University in Saint Louis, US) POSTER # 363
Arabidopsis MAP KINASE1 negatively regulates ROP activity through ROP BINDING PROTEIN KINASE1 in an auxin dependent manner

4:30-6:30 pm **Natural variation and evolution**

Maillot room
Session sponsored by
INRA SCIENCE & IMPACT

4:30-4:55 **Magnus Nordborg** (GMI, Vienna, AU)
Epigenetic variation in A. thaliana

Selected talks, 15 min each

Levi Yant (Harvard University, Cambridge, US)
Allele landscape shifts and convergent evolution associated with serpentine colonization and whole genome duplication in *Arabidopsis arenosa*
Corina M. Fusari (Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, GE) Poster # 436
Identification of regulatory genes involved in central metabolism using Genome Wide Association and Knock-Out analysis
Christos Bazakos (INRA IJPB, Versailles, FR) Poster # 433
Genome-wide expression QTL (eQTL) analysis in interaction with mild drought stress
Françoise Budar (INRA, IJPB, Versailles, FR)
Cytonuclear co-adaptation impacts ecologically relevant phenotypic traits in the annual plant *Arabidopsis thaliana*
Cris Wijnen (Wageningen UR, Plant Sciences Group, NL) Poster # 451
Epistasis Unravelled with a Novel Chromosome Substitution Mapping Set in *Arabidopsis thaliana*

Poster highlight: 2 min talk

Martha Imprialou (University of Oxford, UK) POSTER # 438
Mapping structural variants as quantitative traits in *Arabidopsis* populations

6:30-8:00 pm **POSTER SESSION with drinks**
Even and odd # abstracts: as much as one wants

Maillot Hall and foyers

THURSDAY, JULY 9, 2015

8:30 am-6:00 pm **REGISTRATIONS**

Maillot Hall

9:00-10:30 am **PLENARY SESSION: Responses to the environment II**
Chairman: **Julia Bailey-Serres** (Center for Plant Cell Biology, UC Riverside, CA, US)

Blue amphi
Session sponsored by
cnrs
Institute of Biological Sciences

9:00-9:25 **Caroline Dean** (John Innes Center, Norwich, UK)
Epigenetic switching during vernalization

9:25-9:50 **Christian Fankhauser** (CIG, University of Lausanne, CH)
Insights into the molecular mechanisms enabling plants to grow out of the shade

9:50-10:05 **Amaury de Montaigu** (Max Planck Institute for Plant Breeding Research, Cologne, GE)
Rhythms of gene expression within day/night cycles: natural diversity and phenotypic impact.

10:05-10:20 **Claude Becker** (Max Planck Institute for Developmental Biology, Tübingen, GE)
Exposure to environmental stress induces a transient epigenetic memory response in *Arabidopsis*

10:20-10:30 **Zofia Szweykowska-Kulinska** (Adam Mickiewicz University in Poznan, Poland)
Active 5' splice site regulates the efficiency of biogenesis of *Arabidopsis* microRNAs derived from intron-containing genes

10:30-11:00 am **COFFEE BREAK** — Maillot Hall

11:00-12:30 **PLENARY SESSION:**
Reproduction: from flowering to seeds II — Blue amphi
Session sponsored by SPS
Chairman: **Mathilde Grelon** (IJPB, Versailles, FR)

11:00-11:25 **Claudia Köhler** (Swedish University of Agricultural Sciences, Uppsala, SW)
Epigenetic mechanisms in the endosperm drive plant speciation

11:25-11:50 **Ian Henderson** (University of Cambridge, UK)
Genetic and epigenetic control of meiotic recombination

11:50-12:05 **Doris Wagner** (University of Pennsylvania, Philadelphia)
Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate

12:05-12:20 **Martin Bayer** (Max Planck Institute for Developmental Biology, Tübingen, GR)
YODA signaling in the *Arabidopsis* embryo

12:20-12:30 **Hiroyuki Tsuji** (Kihara Institute for Biological Research, Yokohama City University, JA) Poster # 300
Florigen-induced transposon silencing in the shoot apical meristem in rice

12:30-2:00 pm **LUNCH** — Maillot Hall

2:00-4:00 pm **THEMATIC CONCURRENT SESSIONS**

2:00-4:00 pm **Abiotic stress** — Blue amphi
Session sponsored by

2:00-2:25 **Dirk Inzé** (VIB, Gent, BE)
Environmental control of *Arabidopsis* leaf growth

Selected talks, 15 min each

Jean-Marie Frachisse (CNRS I2BC, Gif sur Yvette FR)
Mechanosensitive MscS-Like10 channel mediate oscillatory perception in *Arabidopsis*
Annemarie Krapp (INRA IJPB, Versailles, FR)
Arabidopsis Responses To Nitrogen Availability Are Regulated By
The Transcription Factors Nin-Like Protein 7 (NLP7) And NLP6.
Thierry Desnos (CEA, St Paul lez Durance, FR)
Genetics dissection of the *Arabidopsis* root growth response to low-phosphate
Louis Grillet (Academia Sinica - Institute of Plant and Microbial Biology, Taipei, TW)
IRON MAN, a novel peptide family activates iron uptake in shoots and roots of angiosperms
Olivier Rodrigues (BPMP, Montpellier, FR) Poster # 512
ABA and flg22 signaling pathways inducing stomatal closure: role of aquaporins in regulation of water and hydrogen peroxide transport

2:00-4:00 pm **Systems biology and new approaches** — Room 242 A-B
Session sponsored by SFBV

2:00-2:25 **Christophe Godin** (INRIA, Montpellier, FR)
Is phyllotaxis deterministic or stochastic?

Selected talks, 15 min each

Patrick Laufs (INRA IJPB, Versailles, FR)
Towards an integrative analysis of *Arabidopsis* leaf margin development
Jamie Waese (University of Toronto, CA)
ePlant: An agile development approach to visualizing multiple levels of biological data.
Yuriko Osakabe (CCAIC, The University of Tokushima, Tokushima-city, JP)
Genetic engineering of abiotic stress response and growth in plants
Marie-Laure Martin-Magniette (Institute of Plant Sciences Paris-Saclay, Orsay, FR)
From gene expression modeling to gene network to investigate *Arabidopsis thaliana* genes involved in stress response
Anna Matthiadis (North Carolina State University, Raleigh, US)
Algorithm application to identify novel regulators in the *Arabidopsis thaliana* iron deficiency response

2:00-4:00 pm **Epigenetics** — Maillot room
Session sponsored by ibmp
Chairman: **Laurence Drouard** (CNRS IBMP, Strasbourg)

2:00-2:25 **Ortrun Mittelsten Scheid** (GMI, Vienna, AU)
Heat stress responses in Arabidopsis nuclei

Selected talks, 15 min each

Franziska Turck (Max Planck Institute for Plant Breeding Research, Köln, DE)
With a little help from my friends: Telobox binding proteins assist Polycomb Group protein complexes in the repression of target genes

François Roudier (CNRS IBENS, Paris, FR)
Role of Polycomb repressive pathways in regulating cell differentiation in *Arabidopsis*

R. Keith Slotkin (The Ohio State University, Columbus, US)
Unraveling How Transposable Elements Mind The Post-Transcriptional To Transcriptional Silencing Gap

Fredy Barneche (CNRS IBENS, Paris, FR)
Light signaling controls nuclear architecture reorganization and massive chromatin state changes during seedling development

Jean-Marc Deragon (Université de Perpignan/CNRS, Laboratoire Génome et Développement des Plantes, FR)
New roles for double-stranded RNA binding proteins in plants

4:00-4:30 pm **COFFEE BREAK** — **Maillot Hall**

POSTER REMOVAL, please remove your posters before the closing keynote lecture

4:30-05:45 pm **ICAR 2015 CLOSING KEYNOTE LECTURE** — **Blue amphi**

Lecture sponsored by

Institute of Biological sciences

4:30-4:40 Introduction by **Jean-Marc Deragon** (LGDP, University of Perpignan), chairman

4:40-5:30 **Robert Martienssen** (Cold Spring Harbor Laboratory, NY US)
Heterochromatin reprogramming with histone variants and small RNA

5:30-5:45 Closing remarks by **Loic Lepiniec** and **Heribert Hirt** and announcement of **ICAR 2016** by **Inhwan Hwang** (KAST, Pohang-si, KR)

7:00-00:30 pm **ICAR 2015 SEINE RIVER CRUISE AND GALA DINNER**

WORKSHOP TOPICS

W1-Abiotic stress responses

organized by the GDRI-IPN network France-Japan
Co-chairs: **Gwyneth Ingram** (RDP, CNRS/ENS Lyon, FR) and **Toru Fujiwara** (Faculty of Agriculture, University of Tokyo, JP)

The workshop will provide a platform for young scientists to showcase their work in the broad research area of plant perception and responses to abiotic stresses, with the aim of stimulating further discussion both during and after the ICAR conference.

W2- Bioinformatics, Quantitative Techniques and Computational Skills: Current Research and Future Training Needs for 21st Century Plant Biology,

supported by NAASC
Co-chairs: **Joanna Friesner** (NAASC, University of California, Davis, CA, US) and **Siobhan Brady** (University of California, Davis, CA, US)
Knowledge gained in Arabidopsis informs our understanding of the genetic basis of plant processes and crop traits, yet the complexity of datasets is only increasing. The challenge for Arabidopsis researchers, and all plant biologists, is to study and understand this complexity, which will require a larger focus on quantitative, systems, and computational approaches. Successful biologists of the 21st century will need to be comfortable with networks containing genes and their products as well as high-throughput, quantitative and dynamic modeling techniques. A key goal for contemporary Arabidopsis researchers is to be skilled with emerging technologies and approaches where using Arabidopsis as a model organism will provide fundamental discoveries and enable translational research in crop species.

1. This workshop will combine two key research threads (wet-lab and computational) by featuring presentations from several early-career scientists that utilize quantitative or computational techniques and approaches in their work.

2. Presenters will be asked to focus a portion of their time discussing what they see as the relevant quantitative, computational, and technological needs for their research program, and for training upcoming graduate students and postdocs to be successful 21st century plant biologists.

3. The workshop will also include brief introduction of several key ongoing or emerging plant biology bioinformatics resources and training initiatives and will conclude with an interactive discussion amongst presenters and participants regarding emerging technologies, research approaches, and training needs for 21st century plant biology.

W3-Cell wall and signaling

Supported by the ANR (French National Research Agency)
Co-chairs: **Marie-Theres Hauser** (BOKU, Vienna, AU) and **Grégory Mouille** (IJPB, INRA, Versailles, FR)

The cell wall contributes to plant architecture, protects cells mechanically and against biotic and abiotic challenges and is the main source of plant produced biomass. Cell walls are constantly reorganized allowing both changes in extension capabilities and integration of novel material. These changes are needed to face periods of growth, organ formation, differentiation, and responses to environmental constraints and thus require sensitive sensing and signaling mechanisms that lead to highly dynamic controls of cell wall synthesis and remodelling. Hormones are known key regulators required for many events during which cell wall remodeling and synthesis occurs. But how hormone signal is translated into cell wall modification are perceived and translated into developmental signals remain a central unresolved question in developmental biology. The purpose of this workshop is to bring together researchers from distinct fields and discuss recent results, their interpretation and perspectives. It will be a rare opportunity to gather a diverse scientific community and stimulate interdisciplinary interactions and future international cooperation.

W4- From Systems Biology to Synthetic Biology in plants

Supported by Frontiers in Plant Science
Co-chairs: **Rodrigo Gutierrez** (P. Universidad Catolica de Chile, Santiago, CL), **Gabriel Krouk** (BPMP,CNRS, Montpellier, FR) and **Gloria Coruzzi** (University of New York, NY, US)

The goal of this workshop is to discuss recent developments in both Systems and Synthetic Biology. To promote the use of these approaches to further plant research and biological engineering of gene networks in plants. This workshop will be the occasion to showcase the different advances in systems/synthetic biology. We plan to bring leading actors around the same table to discuss the latest advances in these research areas.

W5- Ionomics: bringing systems analysis of plant mineral nutrition from Arabidopsis to crops

Supported by BPMP (Montpellier, FR) and ANR project Evometonicks (Gif sur Yvette, FR)
Co-chairs: **Jean-Francois Briat** (BPMP, Montpellier) and **Sébastien Thomine** (CNRS, I2BC, Gif sur Yvette)

Mineral nutrition plays a key role for plant growth and development and can be a major environmental constraint. Sustainable intensification became a major challenge for agriculture. It requires increasing productivity and improving product quality, in a sustainable way to grow plants on marginal lands, poor in mineral nutrients among which some metals (Fe, Zn, Cu, Mn, Mg...), and sometimes contaminated at low levels by harmful trace elements (Cd, As). A major challenge will be to understand how the different sensing and signaling pathways activated in response to changes in nutrient availability are coordinately integrated. Mineral nutrition will have therefore to be considered as a system. It will require developing tools enabling to model integrative gene networks that will take into account the availability of nutrients and their interactions. Undoubtedly Arabidopsis will continue to serve as model to develop concepts and tools in this field of research. Translating this knowledge to crops will contribute to improve their yield, but also importantly the nutritional quality and safety of their derived products.

W6 - Challenges and questions in ambient temperature signaling and acclimation research

Co-chairs: **Marcel Quint** (Leibniz Institute of Plant Biochemistry, Halle, DE) and **Dr. Martijn van Zanten**, (Utrecht University, NL)

The workshop aims at addressing and discussing three main questions:
I) Which are the open questions in ambient temperature signaling?
II) How can these challenges be addressed in a collaborative manner between the leading labs in ambient temperature signaling?
III) How can we translate temperature research in Arabidopsis to relevant crop systems?

W7- Epigenomics

Supported by EPIC and Diagenode
Co-chairs: **Doris Wagner** (University of Pennsylvania, Philadelphia, PA, US) and **François Roudier** (IBENS, CNRS, Paris, FR)

The goal of this workshop is to promote epigenomic and epigenetic research in plants and in particular to highlight novel approaches, tools and resources in this field. This a joint endeavor by EPIC, a research coordination network (RCN) funded by the United States National Sciences Foundation (NSF) and the Multinational Arabidopsis Steering Committee subcommittee on plant epigenetics.

W8- New approaches in plant signaling

organized by the GDRI-IPN network France Japan
Co-chairs: **Annie Marion-Poll** (IJPB, INRA, Versailles, FR) and **Yoshihisa Oda** (National Institute of Genetics, Mishima, Shizuoka, JP)

This workshop will highlight research aimed at the development and/or the application of novel approaches which allow new insights into plant signalling mechanisms at multiple scales.

W9- Novel Tools and Techniques - New frontiers in single cell and single molecule biology

Supported by the DFG (Deutsche Forschungsgemeinschaft) and MASC
Co-chairs: **Nicholas Provart** (University of Toronto, CA) and **Luise Brand** (Masc coordinator, University of Tübingen, DE)

In 2012 the Multinational Arabidopsis Steering Committee (MASC) published a new roadmap with recommendations for Arabidopsis research to the year 2021. One major goal is to foster the understanding of plant development from the single cell to the whole plant and to plant populations. The progress on these frontiers is tremendous due to novel tools and techniques developed in plant biology. This workshop will focus on novel tools and techniques in the field of single cell and single molecule biology. The speakers will therefore present a mix of primary research results including applications of the methods used plus limits/drawbacks of the respective methods, potential future developments, and current methodological frontiers in their research field(s). Finally, an open discussion about the role of Arabidopsis in single cell and single molecule biology and plant biology in general is intented including a brief introduction of the MASC.

W10- The Arabidopsis information portal for users and developers

Chair: **Christopher D. Town** (J. Craig Venter Institute, Rockville, MD, US)

Araport, Arabidopsis information portal (www.araport.org), is a large scale project funded by the US NSF and UK BBSRC that provides a new on-line resource that aims to provide users with a single interface through which to access a wide range of Arabidopsis information - a «one-stop shop» - using state-of-the-art web technologies.

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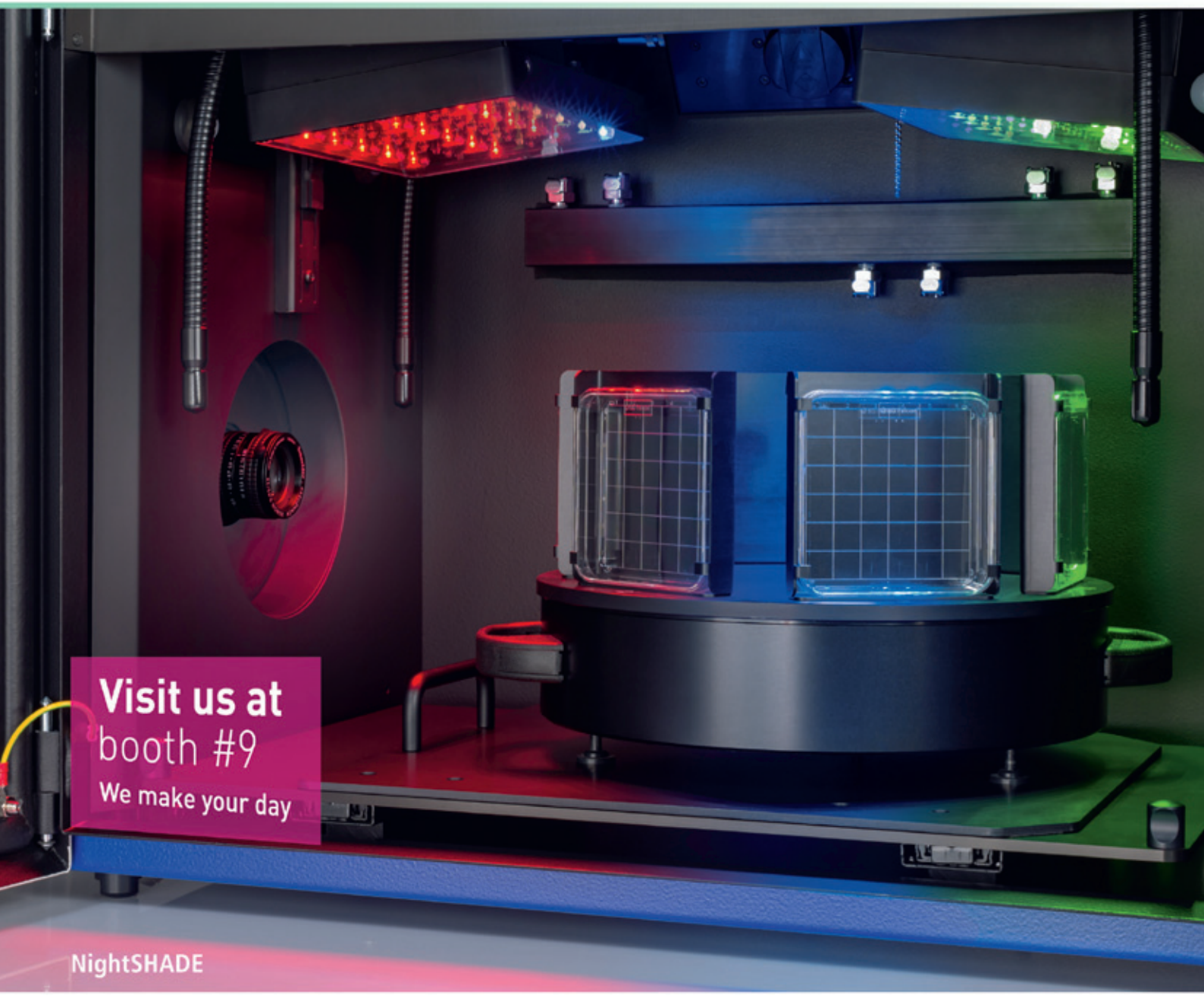

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NUMBERING OF POSTERS

Primary metabolism, photosynthesis, biomass	Posters 001 to 027
Cell biology	Posters 028 to 074
Transcriptional regulations	Posters 075 to 096
Biotic interactions	Posters 097 to 156
Development	Posters 157 to 257
Genome and chromatin dynamics	Posters 258 to 265
Secondary metabolism	Posters 266 to 277
Reproduction	Posters 278 to 301
Post-transcriptional / post-translational regulations	Posters 302 to 338
Translational biology and biotechnologies	Posters 339 to 351
Hormone signaling	Posters 352 to 431
Natural variation and evolution	Posters 432 to 453
Abiotic stress	Posters 454 to 536
Systems biology and new approaches	Posters 537 to 555
Epigenetics	Posters 556 to 573

ORAL ABSTRACTS

Plenary session: Keynote lecture

001 - Computational Morphodynamics of the Shoot Apical Meristem

MEYEROWITZ Elliot[1]

[1] *Howard Hughes Medical Institute and California Institute of Technology, Pasadena, USA*

The shoot apical meristem of *Arabidopsis thaliana* is a complex nanomachine with large numbers of overlapping and distinct patterns of gene expression. Some genes are expressed in a constant relation to the meristem, and others are expressed in relation to the leaf or flower primordia that arise from the meristem. Genes expressed in a constant relation to the meristem include the meristem control genes of the CLAVATA peptide signaling pathway. Recent work in our lab and others shows that one control on the expression of these genes is cytokinin, with positive and negative feedbacks between the genes of the cytokinin pathway, and the CLAVATA genes. Genes expressed in relation to leaf or flower primordia include leaf and flower-expressed genes, and also boundary genes. The regulation of primordial pattern (and therefore, phyllotactic pattern) involves auxin signaling, with auxin concentration controlled by the pattern of auxin flux, as dictated by the PINFORMED1 auxin efflux carrier. One control on PINFORMED is mechanical signaling, which therefore regulates chemical signaling. As auxin changes the mechanical properties of cells, primordium gene expression is under control of a set of feedbacks between mechanical and chemical signals. Auxin and cytokinin signals also interact, making the expression of genes in the meristem a result of many interlocking feedback mechanisms. To explore and test these feedbacks, we iterate experiments with computation, testing explicit mathematical models of meristem feedbacks in silico, and doing experiments *in vivo* to test the validity of the computational predictions. We and our many collaborators call this combined approach Computational Morphodynamics.

Plenary session: Nutrition and metabolism I

002 - Unlocking plant metabolic diversity

OSBOURN Anne[1]

[1] *John Innes Centre, Norwich, UNITED KINGDOM*

Plants produce a tremendous array of natural products, including medicines, flavours, fragrances, pigments and insecticides. The vast majority of this metabolic diversity is as yet untapped, despite its huge potential value for humankind. The recent discovery that genes for the synthesis of different kinds of natural products are organised in clusters in plant genomes is now opening up opportunities for systematic mining for new pathways and chemistries. Improved understanding of the genomic organization and regulation of different types of specialized metabolic pathways will shed light on the mechanisms underpinning pathway and genome evolution. It will also provide grist for the synthetic biology mill.

003 - The role of sugar transporters in carbon allocation

FROMMER Wolf[1], SOSSO Davide[1], QU Xiao Qing[1], LIN I W.[1], EOM Joon-Seob[1], YANG *Jung*-Il (1, SASSE Joelle[1], HOU BiHuei[1], FENG Liang[2]

[1] *Carnegie Institution for Science, Stanford, USA*[2] *Stanford University, Stanford, USA*

Sugar allocation is key to crop yield potential. The phloem as the conduit for sugar translocation connects organs that produce sugars with those that depend on import. Moreover, plants secrete sugars, e.g. in nectaries to attract pollinators, or roots to feed beneficial microorganisms. Pathogens could tap into the system to gain access to the plants resources. Apoplasmic transport requires transport proteins in the plasma membrane. Three types of sugar transporters have been identified in plants: SUTs (sucrose proton cotransporters), SWEETs (hexose and sucrose transporters) and MSTs (monosaccharide transporters). MSTs and SUTs belong to the major facilitator superfamily and contain 12 transmembrane spanning domains (TMs), whereas SWEETs are structurally unique with only seven TMs. The structure of bacterial homologs of the SWEETs has been resolved. The plant genomes analyzed so far contain typically ~20 SWEETs, each fulfilling specific physiological functions. In Arabidopsis, SWEET8 and 13 feed pollen, SWEET11 and 12 appear to efflux sucrose from phloem parenchyma to supply the SUTs in the sieve element companion cell complex for phloem loading; SWEET11, 12 and 15 contribute to seed filling, SWEET16 and 17 are vacuolar hexose transporters, and SWEET9 plays a key role in nectar secretion. The remaining members of the family await characterization; some may play roles in the gametophyte. In rice and cassava, and possibly other pathogen systems, sucrose-transporting SWEETs function as susceptibility loci, potentially hijacked to feed the pathogen. The human genome also contains a glucose transporting SWEET. Further analyses promise new insights into the regulation of assimilate allocation and targets for increasing crop yield.

004 - Low energy Stress response in Arabidopsis: the SnRK1-C/S1-bZIP pathway controls metabolic reprogramming to support mitochondrial respiration

PEDROTTI Lorenzo[1], *WEISTE Christoph*[1], *MAIR Andrea*[2], *TEIGE Markus* [2] , *DRÖGE-LASER Wolfgang*[1]

[1] *University of Würzburg, Julius-von-Sachs-Institute, Würzburg, GERMANY*[2] *University of Vienna , Vienna, AUSTRIA*

Sustaining energy homeostasis is of pivotal importance for all living organisms. In *Arabidopsis*, SnRK1s (Snf1-RELATED KINASES1) are evolutionary conserved kinases related to yeast Snf1 and mammalian AMPK, which control metabolic adaptation during low energy stress. Although not firmly demonstrated, the five group-S1 basic leucine Zipper (bZIP) transcription factors have been implicated in mediating SnRK1 responses. Making use of inducible *SnRK1* and quintuple *bZIPS1* knock-down approaches, transcriptome studies reveal that *Arabidopsis* SnRK1 controls thousands of starvation-related genes whereas a small subset is executed by downstream S1-bZIPs. Combined genetic and pharmacological approaches demonstrate that SnRK1s, S1-bZIPs and their target genes control an alternative pathway supporting mitochondrial respiration and consequently plant survival during starvation. Molecular analyses gained insight into the mechanistic link between SnRK1 activity and transcriptional reprogramming: whereas S1-bZIPs are no SnRK1 targets, SnRK1 mediated phosphorylation of group C-bZIPs leads to phosphorylation-driven heterodimerisation and binding to G-box *cis*-elements. Subsequently, recruitment of the histone acetylation machinery via an N-terminal S1-bZIP recruitment domain facilitates chromatin remodelling and transcription. Taken together, this work reveals a molecular mechanism by which energy deprivation in plants is transduced to reprogram transcription and ultimately drives metabolic adaptation.

005 - In and out - Solute transport across the peroxisomal membrane

LINKA Nicole[1], *BERNHARDT Kristin*[1], *CHARTON Lennart*[1], *HIELSCHER Björn*[1], *KESSEL-VIGELIUS Sarah*[1], *SCHROERS Martin*[1], *WIESE Jan*[1], *WEBER Andreas*[1]

[1] *Heinrich Heine University Duesseldorf, Duesseldorf, GERMANY*

Plant peroxisomes are highly dynamic in metabolism. They are involved in numerous processes of the primary and secondary metabolism, including fatty acid degradation, photorespiration, synthesis of signalling molecules and secondary products. Several metabolic pathways require an interplay with other compartments, such as plastids and mitochondria. Consequently, a high number of substrates, intermediates and cofactors have been transported across the peroxisomal membrane. Considerable progress has been made in peroxisome biology through genomics and proteomics, however, very little is known regarding transport proteins of peroxisomes, mediating the exchange of solutes. Our group has discovered plant peroxisomal carrier proteins mediating the import of the cofactors ATP, NAD and CoA required for a wide range of metabolic reactions inside peroxisomes. We will give a detailed update on the diverse functions of these cofactor transporters in plants. In addition we will present a novel carrier of plant peroxisomes involved in exchange of small organic acids.

006 - Araport: your one-stop-shop for Arabidopsis data in the 21st century

CHAN Agnes[1], KRISHNAKUMAR Vivek[1], FERLANTI Erik[1], CHENG Chia-Yi[1], KIM Maria[1], CONTRINO Sergio[3], MICKLEM Gos[3], MILLER Jason[1], VAUGHN Matt [2] , TOWN Chris[1]

[1] J. Craig Venter Institute, Rockville, MD, UNITED STATES[2] Texas Advanced Computing Center, Austin, TX, UNITED STATES[3] Cambridge University, Cambridge, UNITED KINGDOM

Araport, the Arabidopsis Information Portal, (https://www.araport.org), aims to provide an open-access "one-stop-shop" resource for Arabidopsis research through data federation, integrating data from diverse and geographically dispersed centers via state-of-the-art web technologies. Users can access not only Col-0 gene annotation but also data sourced from both major and community data hubs including UniProt, PubMed , Ensembl, Bio-Analytic Resource, ATTED, EPIC-CoGe and PhytoMine. In addition, Araport inherited from TAIR the responsibility of providing free access to the most up-to-date Col

-0 genome annotation and will release Araport11 later this year that utilizes extensive RNA-Seq data for gene structures and splice isoforms. Araport aims to provide an extensible framework for the incorporation and homogenization of disparate new data sources, to enable integrative cross-data type analysis by both biologists and developers. Future plans include extensions to RNA-Seq expression, pathway and networks, germplasm and phenotypes, and community annotation, and the solicitation and integration of community-generated large-scale datasets. Araport currently uses a dual-model of data warehousing and federation. The warehouse provides a searchable index across all represented data types, while federation delivers in-depth information at run time. Araport incorporates GMOD software including InterMine, JBrowse, Chado, and WebApollo. Supported by the US NSF and the UK BBSRC.

Plenary session: Plant growth and development I

007 - Control of growth and patterning in the early Arabidopsis embryo

WEIJERS Dolf[1]

[1] Wageningen University, Wageningen, NETHERLANDS

Both growth and tissue patterning are processes that occur continuously during plant life. A key question is how these are coordinated in space and time to generate plant shape and function. We use the early Arabidopsis embryo as a simple and highly predictable model in which growth and patterning are intricately coordinated. I will discuss our recent work aimed at understanding the cellular basis for the establishment of multicellular patterns in 3D, as well as its genetic control. I will describe our recent progress in identifying the genetic networks and cellular mechanisms that translate generic hormonal inputs to specific cellular outputs that define identity, growth and pattern formation in the early embryo.

008 - Strigolactone signaling in the regulation of shoot branching

LEYSER Ottoline[1]

[1] Sainsbury Laboratory, University of Cambridge, Cambridge, UNITED KINGDOM

In recent years there has been rapid progress in understanding signal transduction of the plant hormone strigolactone, but there are still many gaps in our understanding. Mutants with reduced in strigolactone synthesis or response are highly branched and strigolactone addition to buds can inhibit their growth. However, under some circumstances, for example in mutants with compromised auxin transport, strigolactones can promote bud growth. A model for shoot branching control that can explain this apparent paradox involves promotion of endocytosis of the PIN1 auxin efflux carriers as a primary target for strigolactone action. There is a substantial body of evidence supporting this mode of action. For example, strigolactone triggers PIN1 depletion from the plasma membrane as a rapid cycloheximide independent response. However, the signal transduction pathway for strigolactone is largely nuclear and it has been proposed that strigolactones act by regulating the degradation of transcription factors, triggering changes in expression of bud regulating genes such as the *BRC1* transcription factor. BRC1 is known to inhibit shoot branching, and in many circumstances its *BRC1* transcription can be induced by strigolactone addition. Our progress in understanding these different modes of action of strigolcatone signalling in shoot branching control will be reported.

009 - Daily growth, from clock gene expression to the phospho-proteome and biomass

MILLAR Andrew[1], CHEW Yin Hoon[1], NOORDALLY Zeenat[1], HINDLE Matthew[1], PRADO Karine[1], SEATON Daniel[1], STITT Mark [2], GRUISSEM Wilhelm[3], SMITH Alison[4], LEBIHAN Thierry[1]

[1] SynthSys and School of Biological Sciences, University of Edinburgh, Edinburgh, UNITED KINGDOM[2] Max Planck Institute for Molecular Plant Physiology, Golm, GERMANY[3] ETH , Zurich, SWITZERLAND[4] John Innes Centre, Norwich, UNITED KINGDOM

For the first time in any multicellular organism, we can understand (explain and predict) how the dynamics of a gene regulatory circuit control biomass at the organismal level. Plant growth in the daily light:dark cycle depends upon molecular, biochemical and physiological responses to light, and on the 24-hour rhythms of the circadian clock. Predictable, seasonal changes in day length demand further adjustment to the plant"s daily programme. The rich data of the Arabidopsis community has allowed us and our partners in the EU FP7 TiMet project to model these processes, from germination to flowering, including the crucial, nightly utilisation of starch carbon stores. We recently combined models from three further biological research areas into the Arabidopsis Framework Model (FM), which predicts biomass quantitatively (Chew et al. PNAS 2014). We have now extended the FM to understand the pleiotropic phenotype of a clock mutant, including altered seedling growth, biomass and flowering time. We are currently testing daily, post-translational regulation by mass spectrometry, revealing rhythmic protein accumulation and phosphorylation, proteome-wide. The challenge remains to understand these organismal processes in the light of the ecological pressures that drove natural selection of the Arabidopsis clock, shaping its dynamic gene regulation. Our data and modelling tools and resources support researchers who share this challenge.

010 - Receptor-mediated Signalling from the Plant Cell Wall

WOLF Sebastian(1), VAN DER DOES Dieuwertje[2] , HOLZWART Eleonore[1], GARNELO-GOMEZ Borja[1], LADWIG Friederike[3] , HARTER Klaus[3] , ZIPFEL Cyril [2], HÖFTE Herman[4]

[1] COS Heidelberg, Heidelberg, GERMANY [2] The Sainsbury Laboratory, Norwich, UNITED KINGDOM[3] ZMBP, Tübingen, GERMANY[4] INRA Versailles, Versailles, FRANCE

Plant growth depends to a large extent on the physico-chemical properties of the cell walls, which dynamically adapt to internal and external cues. This adaptation involves feedback signalling, linking wall sensing with intracellular growthregulating processes. However, very little is known about the nature of these pathways and how signals are transduced to the cytosol. The brassinosteroid (BR) hormone signalling pathway is a central regulator of plant morphogenesis, as indicated by the large amount of BR-responsive cell wall-related genes and the severe growth defects of BR mutants. Recently, we have shown that interference with the major cell wall polysaccharide pectin triggers activation of BR signalling, which in turn orchestrates a compensatory response involving cell wall remodelling. In the absence of BRmediated feedback signalling, altered pectin modification severely compromises cellular integrity, ultimately resulting in cell rupture. Through a forward genetic screen, a receptor-like protein (RLP44) was identified which mediates integration between cell wall and BR signalling through association with the BR receptor complex. Using genetic, biochemical, and transcriptomic approaches, we demonstrate that RLP44 is not itself a component of the BR pathway, but instead conditionally activates hormone signalling when triggered by cell wall-related cues.Thus, feedback information from the cell wall is able to modulate BR signalling to orchestrate growth and development.

011 - Suppression of endogenous gene silencing by bidirectional cytoplasmic mRNA decay in Arabidopsis

ZHANG Xingyan[1], GUO Hongwei[1]

[1] Peking University, Beijing, CHINA

Gene expression and silencing establish the proper transcriptome of eukaryotic cells. Posttranscriptional gene silencing (PTGS), also known as RNA interference, serves as an RNA-based immune system against foreign gene invasion, and is engaged in silencing a subset of endogenous genes, mainly transposons. It remains unclear how plants elaborately avert inappropriate PTGS of endogenous coding genes. Here we demonstrate in *Arabidopsis* that both 5”-3” and 3”-5” cytoplasmic RNA decay pathways act as repressors of transgene and endogenous PTGS. Disruption of bidirectional cytoplasmic RNA decay leads to pleiotropic developmental defects and drastic transcriptomic alterations, which are substantially rescued by PTGS mutants, including *rdr6, sgs3, dcl2/dcl4*, and *ago1*. Upon dysfunction of bidirectional RNA decay, a large number of 21-22 nucleotide endogenous siRNAs (named as coding-transcript-derived siRNA or ct-siRNA) are produced from coding transcripts including multiple miRNA targets, which could interfere with their cognate gene expression and functions. This study highlights the risk of unwanted PTGS and identifies cytoplasmic RNA decay pathways as safeguards of plant transcriptome and development (Zhang et al., Science 2015). We also propose that cytoplasmic RNA decay and siRNA pathways act as crucial components in miRNA regulatory network. After an initial miRNA-directed cleavage, cytoplasmic RNA decay could preclude sustained or exacerbated siRNA-triggered silencing of target gene expression. Notably, miRNA-triggered tasiRNA biogenesis seems not affected by cytoplasmic RNA decay, raising the question as to how aberrant transcripts are sorted and targeted between cytoplasmic RNA decay and PTGS pathways.

Thematic concurrent session: Primary metabolism, photosynthesis, biomass

012 - The role of amino acid transporters in nitrogen storage and signalling

SWEETLOVE Lee[1]

[1] University of Oxford, OXFORD, UNITED KINGDOM

Plants genomes encode large families of amino acid transporters that are involved in transport of amino acids via the phloem and transport within cells between subcellular compartments. An important component of plant nitrogen homeostasis is storage of amino acids in the vacuole. This allows some buffering capacity if the rate of nitrogen assimilation is not precisely matched to the nitrogen demand for protein synthesis and also is important in organs such as fruit where amino acid stores contribute to palatability of the organ to animals that disperse the seed. Proteomic studies of vacuoles isolated from Arabidopsis have so far revealed only a few candidate amino acid transporters and there has been little detailed characterisation of which substrates they may transport. Within the cationic amino acid transporter (CAT) family, for example, several members have been shown to localize to the tonoplast in Arabidopsis (AtCAT2, AtCAT4, AtCAT8, AtCAT9), but the substrate selectivity and transport properties of these proteins remain uncertain. We have shown that CAT9 is an exchanger of GABA, glutamate and aspartate and, in tomato fruit, is pivotal for allowing the decline of GABA and rise of asparate and glutamate during ripening of the fruit. We also have preliminary evidence suggesting that several members of the tonoplast-localised CATs in Arabidopsis are involved in nitrogen status signalling in Arabidopsis roots, making them a potential target for increased nitrogen use efficiency.

013 - Sucrose and the control of metabolism and growth

SMEEKENS Sjef[1], MA Jingkun[1], HUMMEL Maureen[1], LASTDRAGER Jeroen[1], SELVANAYAGAM Jeba[1], HANSON Johannes[2]

[1] Utrecht University, Utrecht, NETHERLANDS [2] Umea Plant Science Centre, Umea, SWEDEN

Sugar availability is a powerful mediator of growth since sugars act both as substrate for intermediary metabolism and as signalling molecules. Plant growth controlling regulatory systems that respond to sugar signals include the centrally important Snf1 Related protein kinase (SnRK1, homolog of animal AMP Kinase), the TOR (Target of Rapamycin) protein kinase, the essential regulatory molecule trehalose 6-phosphate (T6P) and the C/S1 bZIP transcription factor network. In Arabidopsis bZIP transcription factors of the S1 (bZIP1, 2, 11, 44, 53) and C (bZIP9, 10, 25, 63) group from heterodimers and are potent in planta transcriptional regulators that provide the plant with extensive regulatory potential. S1/C bZIP heterodimers affect the level the key growth regulator trehalose 6-phoshate via induction of T6P phosphatase and trehalase genes. Sucrose represses S1 class bZIP activity by arresting translation of S1 bZIP mRNAs via a ribosome stalling mechanism. In the presence of sucrose the “Sucrose Control” (SC) peptide encoded in the 5’-leader of the S1 mRNAs inhibits translation of S1 bZIP main ORFs. This SC peptide is evolutionary conserved in the plant kingdom and found only in homologous plant bZIP genes. Thus, bZIP-mediated reprogramming of metabolism and growth depends on the cellular sucrose level and increasing sucrose levels reduce S1 group mRNA translation. Sucrose as an input signal controls the regulatory module consisting of interacting TOR kinase, SnRK1, T6P and C/S1 bZIP systems. These central regulators are at the heart of physiological and developmental decisions and adaptation to environmental fluctuations.

014 - SWEET transporters are required for SWEET seeds

CHEN Li-Qing[1], LIN I[1], QU Xiao-Qing[1], SOSSO Davide[1], MCFARLANE Heather[2] , SAMUELS A.[2] , FROMMER Wolf[1]

[1] Carnegie Institution for Science, Stanford, USA[2] University of British Columbia, Vancouver, CANADA

A better understanding of the seed filling mechanism is required to increase current crop yield potential. Seeds are heterotrophic organs, requiring sugars supplied from maternal tissues for their development. Sucrose, a major long distance translocation form of sugars for many plants, is translocated from phloem into the maternal seed coat symplasmically. However, how sucrose is transported into the embryo from the seed coat remains poorly understood. Here, we show that sucrose transporters SWEET11, 12 and 15 are expressed in developing seeds, specifically in the different integument layers and the endosperm. A triple *sweet11;12;15* mutant shows retarded embryo development, reduced seed weight and a shrunken seed phenotype. Moreover, the seed coat shows elevated starch accumulation, while the embryo has reduced starch levels. Reciprocal crosses indicate that SWEET11, 12 and 15 are required for the maternal control of seed development. All these findings suggest that SWEET11, 12 and 15 are key for seed filling, which is an important feature for yield potential.

015 - Unravelling the complexity of PHT1 (high affinity phosphate transporters) regulations in Arabidopsis

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Phosphate (Pi) is a crucial and often limiting nutrient for plant growth. It is also a very insoluble ion heterogeneously distributed in soil. The uptake of this element relies on the presence of nine high affinity transporters (PHT1 family) located in plasma membranes[1]. Multiple and complex steps of transcriptional and post-transcriptional regulations (phosphorylation, degradation) of these proteins were identified illustrating the capacity for plants to tightly control the level of these transporters into the cells (2,3,4,5,6). Combining several genetic approaches we reduced 95% of PHT1 family activities offering unique opportunities to investigate the physiological role of these proteins (7). It highlighted their crucial role for Pi absorption and reveals that at least some (if not all) PHT1 members should exhibit dual affinity properties to improve Plant adaptation to the phosphate concentration present in the environment. Innovative techniques, such as radioisotope live micro-imaging system (6) was used to image the spatial distribution of Pi absorption along the root. It revealed unexpected location of the uptake and provides opportunity to dissect the specific role of different root cell layers.

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4 Thibaud et al., (2010). Plant J. / 5 Bayle et al., (2011). Plant Cell / 6 Chen et al., (2015). Plant Cell
7 Ayadi et al., (2015). Plant Physiol. / 6 Kanno et al. (2012) Philos Trans R Soc Lond B Biol Sci.

016 - AtNRT1.13, when mutated, showed altered shoot architecture and late flowering in a nitrogen dependent manner

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Our previous study showed that CHL1/NRT1.1 functions as a nitrate transceptor monitoring nitrate concentration changes in the soil. In addition to external nitrate, internal nitrate also need to be properly monitored to regulate the plasticity of plant growth. To find out the mechanism of internal nitrate sensing, we characterized another transceptor candidate in the NRT1/PTR/NPF family, NRT1.13. *AtNRT1.13* is expressed in parenchyma cells next to xylem, particularly in branching point of inflorescent stem. Subcellular localization study indicated that NRT1.13 is a plasmamembrane protein. In the study of CHL1, an uptake and sensing-decoupled mutant *chl1-9* was used to demonstrate that nitrate transport activity is not required for the sensing function of CHL1, and CHL1 is a nitrate sensor. The *chl1-9* protein, with highly conserved Pro492 mutated to Leucine, exhibits normal sensing activity but loses nitrate transport activity, indicating that Pro492 is important for nitrate uptake but not for sensing. AtNRT1.13 doesn"t carry proline residue at the corresponding position. As expected, when expressed in oocyte, NRT1.13 showed no nitrate transport activity. When Ser 487 at the corresponding position was converted back to proline, NRT1.13 Ser487Pro regained nitrate uptake activity, suggesting that wild type NRT1.13 can"t transport nitrate but could bind nitrate. When plants were grown with normal concentration of nitrate, *nrt1.13* showed no growth phenotype. However, when plants were supplied with low concentration of nitrate, phenotype of *nrt1.13* suggested that NRT1.13 could monitor the nitrate content in the xylem to regulate flowering time, branch outgrowth, and branch initiation, and nitrate allocation.

Thematic concurrent session: Cell biology

017 - Mechanical signals contribute to the control of cell shape and plant development

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Studies in animal single cells have shown that mechanical cues can affect important cell processes such as cell polarity, cell fate or cell division. Here we take advantage of the simpler plant mechanics to investigate this question in a tissue context. Using confocal live imaging, micromechanics and modeling, we found that mechanical signals control the orientation of cortical microtubules, which guide the deposition of cellulose and thus control the mechanical anisotropy of plant cell walls. This in turn supports multicellular morphogenetic events, such as tissue folding, which further consolidates the stress pattern. Interestingly, and depending on stress magnitude, this mechanical feedback loop may also add robustness to individual cell shapes. We also found that this mechanical feedback loop promotes growth heterogeneity in tissues. We propose that the maintenance of a basal level of growth heterogeneity potentiates organogenesis. Conversely, this raises the question of the role of growth heterogeneity in the formation of organs with consistent shapes, and the contribution of mechanical signals in this process. More recently, we started to analyze the contribution of mechanical signals in controlling gene expression patterns and cell fate within the meristem, the plant stem cell niche.

018 - Secondary cell wall patterning in metaxylem vessels: a spatial interplay between the novel microtubule assembly and disassembly pathways

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Proper patterning of cell wall deposition is essential for plant cell morphogenesis and cell differentiation. Xylem cells deposit rigid secondary cell walls in distinct patterns such as annular, spiral, reticulate, and pitted patterns to form a water-conducting vessel. In differentiating xylem cells, cortical microtubules are rearranged into specific patterns to direct the secondary cell wall deposition. However, little is known about the mechanism by which the behavior of cortical microtubules is regulated at the subcellular level. Our recent studies have revealed that a ROP11 GTPase is locally activated to promote cortical microtubule disassembly through the MIDD1-Kinesin-13A complex to form secondary cell wall pits in metaxylem vessels. We recently found that a novel xylem-specific microtubule-associated protein, MAP44, globally promoted cortical microtubule assembly, which counteracts the ROP11-MIDD1-Kinesin-13A pathway. Furthermore, a novel ROP effector protein, BDR1, was preferentially localized along the boundary of secondary cell wall pits and maintained the spatial interface between the ROP11-MIDD1-Kinesin-13A pathway and MAP44-dependent microtubule assembly pathway. Our study suggests that the spatial interplay between the microtubule assembly and disassembly pathways is a key step in determining the secondary cell wall patterns.

019 - A PI4P-driven electrostatic field controls plasma membrane identity and plant development.

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Phosphoinositides are minor lipids of the membrane bilayer that provide docking sites on membrane compartments and contribute to their identity. We recently mapped the localization of the different phosphoinositide species in Arabidopsis, and found that they do not accumulate to a specific compartment but rather that each phosphoinositide is distributed to several compartments, albeit at different concentration (Simon et al. 2014 Plant J). These data suggest that the "phosphoinositide code" hypothesis is not sufficient to explain membrane selectivity of lipid binding proteins in plants. Phosphoinositides are anionic lipids and we hypothesized that this physical property might also contribute to membrane identity by regulating surface charges. To investigate membrane electrostatic properties, we designed a set of genetically encoded biosensors able to report membrane surface charges. We found that the plasma membrane (PM) has a specific electrostatic signature that is controlled by the phosphoinositide PI4P. We further show that this PI4P-dependent electrostatic field controls the PM localization and function of several proteins involved in receptor kinase and phytohormone signaling. Our findings uncover that phosphoinositides not only act as biochemical landmarks but that they also regulate organelle identity by establishing their electrostatic fields. Our work shows that this physical lipid property is crucial for membrane homeostasis but also for plant development.

Thematic concurrent session: Transcriptional regulations

020 - Transcriptional regulatory network controlled by the MADS-domain factor SEEDSTICK

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MADS-domain factors have shown to be Master regulators of many developmental processes in plants. Those regulating flower development are among the best studied ones and transcriptional regulatory pathways have been identified for these. For many years we have been studying the MADS-box gene *SEEDSTICK (STK),* which controls ovule and seed development. Using genome wide approaches we have been identified STK’s direct targets, which indicated that this transcription factor has a wide regulatory function, including the regulation of cell wall properties. Our data show that developmental regulators that control tissue identity also accomplish this through the direct regulation of structural genes that control specific properties of the cell.

021 - Molecular Mechanisms of MADS-domain Transcription Factor Function

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[(1)] CNRS/LPCV/iRTSV, Grenoble Cedex 9, FRANCE[(2)] ESRF, Grenoble Cedex 9, FRANCE[(3)] CEA/LPCV/iRTSV, Grenoble Cedex 9, FRANCE

Understanding the molecular mechanisms underlying gene regulation and development is a fundamental challenge in modern biology. Floral organ morphogenesis is a striking example of a series of developmental transitions triggered by the activity of a few transcription factors (TFs) from the MADS-domain TF family. The MADS TFs act as high-level regulators during these important developmental processes. While well studied genetically, the molecular mechanisms of MADS TF function, namely the specificity determinants of protein-protein and protein-DNA complex formation and the dynamics of these interactions, remain less well understood. Biophysical techniques are becoming important tools in unravelling MADS TF function by providing atomic and molecular level information that was lacking in purely genetic approaches. Here we present our results on the structural and in vitro characterisation of two MADS TFs, SEPALLATA3 and SHORT VEGETATIVE PHASE using a combination of protein crystallography, atomic force microscopy and small angle x-ray scattering. We describe the structural determinants of oligomer formation, the overall domain topology of the proteins and discuss the role of complex formation in DNA-binding and downstream gene regulation.

022 - Capturing dynamic transcription in gene regulatory networks using affinity-labeled UTP

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Dynamic transcription events are important in plant responses to environmental signals, but elusive to capture experimentally. Here, we captured such dynamic events by tracking *de novo* synthesis of RNAs made in response to transcription factor (TF) perturbation. To do this, we incorporated 4-thiouracil nucleobase (4tU) to affinity label and capture *only* the newly synthesized transcripts initiated following temporal TF-perturbation. Our proof-of-principle example for the power of this approach is the master TF Basic Leucine Zipper 1 (bZIP1), a central integrator of metabolic signaling by carbon and nitrogen in plants. By capturing only newly synthesized mRNAs, we discovered that *de novo* transcripts initiated by transient TF-target interaction ("Hit"), remain actively transcribed after the TF has left ("Run"). These findings provide experimental support for a "Hit-and-Run" transcription model which posits that a TF can act as a trigger to organize a stable transcriptional complex, after which transcription by RNA polymerase continues without the TF being bound to the gene promoter. In this manner, pioneer TF like bZIP1 mediates rapid and catalytic transcription in response to changes in environment. Therefore, the ability to capture the functional read-out of dynamic TF-target interactions, has uncovered new genome-wide mechanisms for transcriptional control.

023 - Heat stress induces expression of specific nucleolar proteins and long non-coding rRNA in structurally disorganized nucleoli

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The nucleolus is the most prominent nuclear structure and was for a long time essentially considered as a ribosome biogenesis factory. But, now it is clear that this sub-nuclear compartment plays wider roles, notably in cellular responses to intrinsic and environmental changes and in genome stability/organization. Here we present a series of findings that link nucleolus structure and rRNA expression regulation to the heat stress response in *Arabidopsis.* At 37°C, nucleolus organization is dramatically modified and structures associated with rRNA synthesis and ribosome assembly are no longer distinguishable. In this context, 45S rDNA become hypermethylated and silenced while rRNA precursor transcripts over accumulate; two hallmarks of repressed gene expression resulting from TGS and pre-rRNA processing inhibition. Still, a number of rRNA transcripts (whose origin and function are unexplored) are produced/accumulated from intergenic sequences (IgsRNA). These structural and functional changes are reversible upon transfer to favorable temperature. We also demonstrate that "nucleolar" heat stress response involves altered gene expression or nucleolar re-localization of U3snoRNP proteins. The U3snoRNP complex contains nucleolin, fibrillarin and 26S proteasome subunits and is required for coupling RNA transcription and processing. How these and other activities required for processing of non-coding RNA might affect functional and structural nucleolar organization will be reported.

Workshop 1: Abiotic stress responses

024 - A novel mechanism of nutrient sensing mediated by RNA-ribosome complex for regulation of boron transport

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Boron (B) is an essential nutrient but toxic in excess. It is essential for plants to maintain B homeostasis for growth and survival and regulation of transport is essential for homeostasis. Two types of transporters, BORs and NIPs, plays major roles in B transport in plants including *Arabidopsis thaliana* (Takano 2002, Takano 2006 Miwa 2007, Nakagawa 2007, Tanaka 2008, Perez-Castro 2012, Miwa 2013, Tanaka 2013, Hanaoka 2014). Among them, BOR1 and *NIP5;1* are important for adaptation to low B condition in media/soils. BOR1 accumulation is down-regulated in response to high boron condition through endocytosis mediated protein degradation (Takano 2005, Takano 2010, Kasai 2010) and NIP5;1 transcript levels are down-regulated in response to high boron conditions (Takano 2006, Tanaka 2011). These down-regulations of transporters are important for plants not to accumulate too high concentration of B in their tissues. In the present talk, I would like to describe our recent findings of the involvement of RNA-ribosome complex in regulation of *NIP5;1* transcript levels. *NIP5;1* transcript levels are regulated mainly through mRNA degradation and degradation is mediated by B-dependent ribosome stall in the upstream ORF of *NIP5;1* transcript. I will also describe our recent approach to comprehensively understand the overall B transport/distribution in A. thaliana roots through mathematical modeling and analysis using Laser-Ablation ICP-MS (Shimotono and Sotta 2015).

Shimotono and Sotta et al. Plant Cell Physiol 56, 620-630 (2015) /Hanaoka, H et al. Plant J 78, 890-902 (2014)/ Miwa K et al Plant Physiol, 163, 1699–1709 (2013)/ Tanaka N et al. Plant Cell Physiol 54, 2011-2019. (2013)/ Perez-Castro R et al. Plant and Cell Physiol 53,485-494 (2012)/ Tanaka, M et al. Plant Cell 23, 3547-59 (2011)/ Kasai K et al. JBC 286, 6175-6183 (2010)/ Takano, J et al. PNAS 17, 5220-5225 (2010)/ Tanaka M et al. Plant Cell 20,2860-2875 (2008)/ Miwa K et al. Science

318,1417 (2007)/ Nakagawa Y et al. Plant Cell 19, 2624–2635 (2007)/ Takano J et al. Plant Cell 18, 1498

-1509 (2006)/ Takano J et al. PNAS 102, 12276–12281 (2005)/ Takano J et al. Nature 420, 337-340 (2002)

Workshop 2: Bioinformatics, Quantitative techniques and computational skills

025 - Quantification of the mechanical properties of growing Arabidopsis hypocotyls

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Whether is it regulated by changing environmental conditions, pathogen attack or the progression of development; growth is fundamentally a physical process. By developing better investigative tools we can improve our understanding of how this complex network of regulators is integrated with the physical parameters that underpin growth. To this end we developed ACME, an Automated Confocal Micro Extensometer, which enables simultaneous measurement of mechanical properties and visualisation with confocal microscopy. Using ACME we have investigated the material properties of growing tissues in response to hormones as well as in mutants with altered growth rates or anisotropy. In doing so we have been able to make predictive models of the mechanical response of these mutants to hormone application. We are able to make measurements with cellular resolution and, in contrast to other popular indentation methods, we can measure the mechanical properties of the tissue in the direction of principal growth. In addition to measuring the mechanical properties that might underlie growth, we have investigated the possibility of feedback onto gene expression by applying stress or strain while imaging known molecular markers. By combining ACME with finite element modelling and the genetic tools of Arabidopsis we are beginning to better understand the regulation of growth.

026 - An ultra-rapid and quantitative alternative to immunoblotting for determining the organelle composition of tissues homogenates.

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The unequal availability of subcellular antibodies and qualitative nature of immunoblotting are currently limiting factors when estimating organelle composition. A free online tool, Multiple Marker Abundance Profiling, is being developed which permits rapid estimation of organelle composition and sample purity, and requires only a list of proteins as the input, thereby negating the need for immunoblotting in this context. A novel addition to MASCP Gator (http://gator.masc-proteomics.org/) assigns abundance scores to individual proteins based on publically available experimental data. In conjunction with a subcellular localization assigned to each protein using the SUBAcon algorithm (http://suba3.plantenergy.uwa.edu.au/), abundance scores for all major subcellular compartments can be estimated. These scores have been verified using Multiple Reaction Monitoring (MRM) and spectral counting. In MMAP, a list of AGIs generated by e.g. shotgun proteomics is classified and scored using all of the above techniques. Optional weighting of results to account for tissue or growth conditions improves MMAP performance, especially for non-photosynthetic tissues. This enables accurate estimation of organelle enrichment, even in the absence of any experimental data from the original source tissue. MRM of selected marker proteins was also found to be an appropriate stand-alone technique for determining the organelle composition of samples. MMAP will be available at SUBA3 in the near future.

Workshop 3: Cell wall and signaling

027 - Distinct mechano-chemical properties participate in the shaping of adjacent pavement cell walls of Arabidopsis thaliana

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In plants, the epidermis plays an essential role in shaping the entire organism as it is thought to be limiting for growth (Savaldi-Godstein 2008). Another layer of restriction arises from the presence of stiff and cohesive cell walls, which hinder cell-cell movements. Puzzle shape leaf pavement cells displaying interdigitated lobes and indents in the two-dimensional plane of the leaf epidermis, provides a powerful model system to investigate the cellular and subcellular processes underlying cell polarity and shape determination in plant tissues. The formation of these multipolar cells is regulated by the multifunctional phytohormone auxin at the cell plasma membrane via the subcellular compartmentation of Rho GTPases of plants (ROP) signaling pathways (Fu et al. 2002, 2005; Xu at al. 2010), but it remains unclear how such local molecular heterogeneities are translated into the local cell wall properties to generate local shape changes. By probing anticlinal walls on sections using atomic force microscopy (AFM), we determined that cell wall mechanical properties are heterogeneous along the perimeter of pavement cells in Arabidopsis wild-type, but not in the polarity deficient *CA-ROP2* line. Strikingly, we observed the presence of a stiffness gradient across the cell wall, suggesting that two contiguous cell walls can retain distinct mechanical properties. Chemical print of the cell wall by RAMAN spectro-microscopy revealed differential distribution of pectin components along the curvature of the pavement cell walls in the wild type, while straight cell walls of the CA-rop2 were homogenous. Using high-resolution electron microscopy, we showed that although the density of cell wall components was often similar between the lobe and the neck regions of the cells, some of the components were unevenly distributed across the cell wall, thus supporting a scenario in which multipolar pavement cell shape relies on finely tuned modifications of the composition and mechanical properties of cell walls along the perimeter of the cell as well as across the cell wall thickness.

028 - The endodermis - a tale of two cell types

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The endodermis is the main apoplastic diffusion barrier in young, actively absorbing roots of higher plants. Based on cell wall features, classical anatomical literature defines three different stages of endodermal differentiation, of which only two are occuring in Arabidopsis. Establishment of lignified Casparian strips represents the first stage of differentiation, which is followed by a secondary stage, defined by the formation of suberin lamellae all around the endodermal surface. We have found an unexpected plasticity of suberisation in response to nutrients that isregulated by the stress hormones abscisic acid and ethylene. Interestingly, some endodermal cells never form suberin lamellae. These cells were suggestively termed „passage cells“, hinting towards a possible function of those cells in continued uptake of nutrient withinin a mature and otherwise non-permissive endodermis. Passage cells were shown to be always occurring above xylem poles. I will report on the occurence of passage cells in Arabidopsis and on our efforts to understand the mechanisms controlling their numbers and positioning.

029 - Cell wall pectin sensing by Wall Associated Kinases (WAKs)

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The extra cellular matrix or cell walls of angiosperms are composed of a complex arrangement of cellulose, hemicellulose, pectin, and protein.

The pectins can be selectively and locally modified to be cross-linked into a structural network that can have dramatic effects on cell enlargement, but numerous pathogens and mechanical disruptions fragment this pectin network, leading often to a plant stress/defense response. The Wall Associated Kinases (WAKs) are receptor kinases that bind pectin in the cell wall, and span the plasma membrane to place a serine/threonine kinase in the cytoplasm. WAKs are required for cell expansion and have been shown to be involved in the pectin activation of MPK3 and a vacuolar invertase that can increase turgor driven expansion. But WAKs have a preferential binding to pectin fragments or oligogalacturonides (OGs) generated by wounding or pathogens as they invade. We propose that these OGs compete for the native long pectins and cause WAKs to trigger a response to OGs through a distinct signaling pathway and includes a ROS burst, MPK6 activity, and the EDS1 and PAD4 dependent activation of numerous genes. Thus one receptor type monitors the state of pectin in the ECM to drive expansion or a response to pathogen. Phosphoproteonomics, gene expression and genetic analysis has identified signaling components of the OG stimulated pathways.

Workshop 4: From systems biology to synthetic biology in plants

030 - Systems Analysis of Phytohormone Signal Transduction and Cross-Talk by Interactome Network Mapping

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Elucidating how different hormone signals are integrated into an optimal physiological response is critical for understanding the molecular mechanisms regulating agricultural yield and yield stability. Phytohormones play a critical role in integrating environmental cues with internal developmental programs to ensure survival and reproductive success. While principles of plant signal transduction have been revealed over the past decades, and protein-protein interactions were found to play a central role in this process, a systems perspective is still missing and many components mediating signal transduction and crosstalk remain to be discovered. In this project we investigate the Arabidopsis phytohormone signaling network by systematic yeast two-hybrid proteinprotein interaction analysis of 1.200 proteins with a likely or genetically demonstrated role on phytohormone signaling. For these, interactions have been mapping among each other and with all 13.000 proteins in the A.t. ORFeome collection. The bioinformatic analysis of this dataset is currently ongoing. Network dynamics will be analyzed by experimental identification of hormone-dependent protein-protein interactions. In this presentation, we present a first analysis of the phytohormone signaling network map of *Arabidopsis thaliana*.

031 - Eukaryotic transcriptional regulatory networks: learning from plants.

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Deciphering transcriptional gene regulatory networks (GRNs) is a major challenge in the rising field of Systems Biology. Striking advances have recently been made thanks to plant studies. Indeed, experimental and computational evidences converge towards similar conclusions: plant transcriptional networks are very dense and branched in order to i) adapt to the course of evolution or ii) accept many signaling inputs. These observations arise on in particular from a technique that we named TARGET for “Transient Assay Reporting Genome-Wide effect of Transcription Factors” (Bargmann et al., 2013). According to this technique and other observations (Chip-Seq, eYH1, for instance), simple calculations demonstrate that a particular gene in Arabidopsis is likely to be controlled by ~ 7 to 40 transcription factors. We also have recently taken advantage of several studies (Krouk et al., 2013; Para et al., 2014; Medici et al., 2015) to develop and tweak FRANK: a Fast Randomizing Algorithm for Network Knowledge. FRANK generates very large GRNs having the known characteristics of Arabidopsis transcriptional network (and very likely to other eukaryotic genomes) and simulates gene expression (experiments) at a genome-wide scale. In our endeavor to develop stable GRNs (“stable” means: gene expression should be constant or in oscillation) we have defined basic mathematical rules that find echo in network biology. FRANK will help to train machine-learning algorithms in order to build GRNs on real transcriptomic data.

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Workshop 5: Ionomics

033 - Natural diversity in the Arabidopsis thaliana and rice ionomes

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Our focus is on understanding the molecular mechanisms that control and integrate the uptake and accumulation of mineral elements in plants. Over the last 15-years we have used high throughput inductively coupled plasma mass spectrometry (ICP-MS) to analyze the elemental composition of leaves of 187,000 *Arabidopsis thaliana* samples and 32,000 field-grown rice grain samples. We have performed several *A. thaliana* forward and reverse genetic screens, a screen of 1001 natural *A. thaliana* accession and 1,700 accessions of rice. We have successfully used DNA microarray based approaches, QTL and genome-wide association mapping, next generation sequencing and fine mapping to identify genes that control the elemental composition of *A. thaliana* and rice. We have identified genes that encode ion-transporters but also genes that control various developmental and physiological processes in both roots and shoots. Specific genes that are conserved across the plant kingdom are also emerging as ‘hot spots’ controlling natural variation in elements such as Mo, Na and Cd. To maximize the value of this approach we have also developed a publicly searchable online database containing information on the elemental composition of approximately 268,000 samples from over 2,400 different experiments (www.ionomicshub.org) along with various tools to mine these extensive datasets.

034 - Root plasticity changes in response to the plant nutritional status or shoot ionome

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Root growth and development are of outstanding importance for the adaptation of plants to the environment. The shape and extension of the root system affect the plant’s ability to acquire water and nutrients. In order to cope with nutrient limitations, plants have evolved mechanisms to monitor the availability of nutrients and to integrate this information into developmental pathways that regulate the spatial arrangement of roots as described by the root system architecture (RSA). Recent work from several labs has shown that roots can sense the deficiency or heterogeneous availability of some nutrients, thereby leading to modifications in RSA. This systemic and local regulation of root system architecture is nutrient-specific, suggesting that nutrients interfere with the root developmental program at specific steps. This talk highlights some of the molecular players that underlie nutrient sensing processes and may link root trait changes with the plant nutritional status or the shoot ionome.

035 - Phloem Transport and Seed Loading of Trace Metals in Arabidopsis.

MENDOZA-COZATL David[1] , KHAN Mather[1], NGUYEN Nga[1]

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Plants and seeds are the main dietary source of micronutrients (Zn, Fe, Cu, Mn) and also toxic elements (Cd, As)1. We are using cell-specific transcriptomics, functional genomics and ionomics to understand how plants take up, distribute and accumulate trace elements within plant tissues, including seeds. We have identified 70+ Arabidopsis transporters preferentially expressed in phloem-loading cells, most of them with unknown function. We have cloned these transporters in yeast expression vectors and assembled a functional expression library for high throughput screening of substrates using different yeast backgrounds. Initial screens have identified transporters that induce hypersensitivity to cadmium or arsenic. Growth-based assays using yeast deficient in iron uptake suggest some transporters may mobilize Fe into yeast. We are also characterizing plants carrying T-DNA insertions in phloem transporters. Using a recently

described mutant *(opt3-2)* that over accumulates Cd in seeds2, we have identified a transporter that suppresses the Cd sensitivity and over-accumulation of cadmium in *opt3-2*. Radiotracer imaging suggests that this transporter regulates Cd uptake for long-distance transport. Understanding the mechanisms that mediate trace metal accumulation in plants will help developing crops with higher nutritional value and minimal accumulation of non-essential elements such as cadmium and arsenic in edible tissues.

1. Khan et al., (2014). Front Plant Sci 5:51
2. Mendoza-Cozatl et al.,(2014). Mol Plant 7:1455

036 - Endodermal development modulates the radial transport of nutrients in roots

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In higher plants, roots acquire water and nutrients from the soil and transport them upwards to the aerial parts. This function is reflected by their histology: water and nutrients move radially through the concentric layers of epidermis, cortex, and endodermis before entering the central cylinder where they are loaded into the xylem for transport to the aerial parts. The endodermis is a single, epithelium-like cell layer, which surrounds the inner, conductive tissues of roots and forms a barrier that is considered crucial for the controlled uptake of nutrients into the vasculature. The barrier properties of the endodermis are mediated by Casparian Strips (CS) highly localized lignin-based modification of the primary cell wall, surrounding each endodermal cell and by a deposition of suberin lamellae in the secondary cell wall. In the past decades, the endodermis has been largely studied at the histological and physiological level in various plant species. Our recent work in Arabidopsis identified specific mutants, markers and protocols that now provide an unprecedented opportunity to test the roles of the endodermis in nutrient uptake. Our current research indicates that the generally accepted views of endodermal function have been overly simplistic. We could show that compromising the CS integrity does not lead to a massive allocation of nutrients to the aerial parts. Currently, we are investigating in much greater details the significance of the suberin lamellae, its development, function and plasticity. We found that suberin development integrates nutritional and hormonal cues and in turn modulates trans-cellular transport in the endodermis. This will deepen our understanding of the endodermis as a selective barrier for nutrients.

Plenary session: Responses to the environment I

037 - Connecting the dots of receptor kinase-mediated immune signaling

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The first layer of plant innate immunity relies on the recognition of microbes via the perception of pathogen-associated molecular patterns (PAMPs) by surface localized receptors called pattern recognition receptors (PRRs). The mechanisms controlling PRR activation at the plasma membrane and regulating intracellular immune signaling remain however largely unknown. Here, I will present recent work illustrating how activated PRR complexes directly engage with downstream signaling, and how these events are tightly regulated by phosphorylation.

038 - Intracellular pathogen recognition and immunity

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Plants have evolved a multi-layered innate immune system to recognize and respond to pathogenic microbes in the environment. A large, polymorphic family of intracellular nucleotide-binding/leucine rich repeat (NLR) receptors lies at the heart of the resistance signaling network. NLRs act as sensors of microbial effectors which are delivered to host cells to interfere with basal defenses and promote infection. NLR activation by specific effector molecules mobilizes a robust host immune response (called effector triggered immunity, ETI) which involves rapid transcriptional reprogramming of cells for defense. Multiple check points ensure tight control of NLR resistance programs in the absence of pathogen attack because, once activated, they disturb metabolic homeostasis and growth. We"re studying *Arabidopsis* interactions with biotrophic pathogens to understand how NLR receptor signaling networks operate in ETI. We"re interesting in characterizing fundamental cellular mechanisms of NLR-effector activation and NLR connectivities to the transcription machinery. I"ll describe recent genetic, molecular and structural insights to pathogen effector recognition by an *Arabidopsis* nuclear NLR "sensor-signaling" pair, RRS1/RPS4, which triggers transcriptional reprogramming and resistance through the conserved basal immunity regulator, EDS1. Results suggest a short path between NLR pre- and post-activation events at the chromatin and reveal how NLR capture of a broadly acting bacterial effector interference strategy might increase NLR "recognition space" within a single plant genotype.

039 - Mapping of protein complexes involved in jasmonate signaling by dynamic tandem affinity purification

NAGELS DURAND Astrid[1], GOOSSENS Jonas[1], FERNÁNDEZ CALVO Patricia[1], IÑIGO Sabrina[1], RITTER Andres[1], PAUWELS Laurens[1], GOOSSENS Alain[1]

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Jasmonate (JA) regulates many aspects of plant growth, development, and defense in response to endogenous and environmental cues. Within the JA signaling cascade, the JA-Ile conjugate mediates the binding of the JA-ZIM DOMAIN (JAZ) repressor proteins to CORONATINE INSENSITIVE1 (COI1), which forms part of the SCF^{COI1} E3 ubiquitin (Ub) ligase complex. Upon the subsequent destruction of the JAZ proteins by the 26S proteasome, multiple transcription factors (TFs), such as MYC2, are relieved from JAZ-mediated repression, allowing them to activate the first wave of JA-dependent gene expression. Previously, we have applied tandem affinity purification (TAP) to isolate the core JA signaling module from Arabidopsis and discovered that the JAZ proteins recruit the NINJA-TOPLESS co-repressor complex to empower them with the capacity to repress JA-dependent gene expression [Pauwels et al., 2010, Nature 464:788-791]. We have further employed TAP, both in Arabidopsis suspension cells and seedlings, to map the complexes involved in JA signaling. Using NINJA or specific JAZ proteins as baits, novel interacting TFs and E3 Ub ligases, hitherto not linked to JA signaling, were detected. Conversely, using JA-inducible RING E3 Ub ligases with yet unknown activities as baits, novel putative proteasomal degradation targets were discovered. This interactome based screen expands our knowledge on the JA signaling machinery and reveals new facets of JA in the regulation of various cellular processes.

040 - An Arabidospis protease of the subtilase family negatively regulates the transcriptional control of defence responses

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Transcriptional regulation in plant cells plays a crucial role in the establishment of disease resistance. The MYB transcription factor MYB30 positively regulates Arabidopsis defence and modulates hypersensitive cell death/HR-related lipid signaling through the synthesis of sphingolipid-type very long chain fatty acids (VLCFA) after bacterial inoculation. The activity of MYB30 is tightly controlled through different interacting proteins including phylogenetically related MYB96 that works with MYB30 in triggering VLCFA-related HR, or the secreted phospholipase AtsPLA2-a and the E3-ubiquitin ligase MIEL1 that negatively regulate MYB30 through distinct mechanisms. SBT, a serine-type endopeptidase of the subtilase family, was recently identified as an additional MYB30 regulator. The SBT transcript is alternatively spliced giving rise to both a secreted (SBTa) and a nuclear (SBTb) protein. Interestingly, SBTb, but not SBTa, interacts with MYB30 blocking MYB30 DNA binding and transcriptional activation and this appears to be independent of SBT catalytic activity. sbt mutant plants, with no SBTa nor SBTb expression, display enhanced HR and defence and increased MYB30 target gene expression. These phenotypes are reverted by overexpression of SBTb, but not SBTa, in the sbt mutant background, underlining the specific repression of MYB30-mediated defence by SBTb. The coordinated action of these different regulators for the spatiotemporal control of MYB30 activity will be discussed.

041 - The role of the the metacaspase AtMC1 in agregate clearance and aging

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Metacaspases are distant relatives of animal caspases present in fungi, protozoa and plants. Our previous studies established the Arabidopsis metacaspase AtMC1 as a major positive regulator of pathogen-triggered programmed cell death (PCD). We have now unveiled an additional, pro-survival homeostatic function of AtMC1 during aging in plants. This function acts in parallel to a similar pro-survival function of autophagy. Autophagy acts also as a positive regulator of pathogen-triggered PCD in parallel to AtMC1, indicating a common developmental switch for both pathways. The novel pro-survival role of AtMC1 is functionally related to its prodomain-mediated aggregate localization and clearance, in agreement with recent findings using the single budding yeast metacaspase YCA1. The fact that type I metacaspases can have both pro-survival and pro-death roles depending on their spatio-temporal context is of major evolutionary significance. Based on our data we propose a unifying model whereby autophagy and AtMC1 are part of parallel pathways, both positively regulating pathogen-triggered cell death in young plants -when these functions are not masked by the cumulative stresses of aging- and negatively regulating aging in older plants -revealed by the increasing damaged proteins and organelles that require clearing-.

Plenary session: Reproduction: from flowering to seeds I

042 - Control of floral induction by seasonal cues

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The transition from vegetative development to flowering is controlled by seasonal cues such as day length and winter temperatures. We use *Arabidopsis thaliana* to decipher regulatory networks controlling these responses and exploit relative species, particularly perennial Arabis alpina, to determine how these networks change during evolution to confer ecologically significant differences in phenotype. Recent work used comparisons between *A. thaliana* and *A. alpina* to explain how perennial plants delay sensitivity to environmental cues to ensure that they only become competent to flower when they reach a particular age. We are now extending this work to explore how annual plants including *A. thaliana* evolve to overcome this delay and therefore flower at an early age. In addition, we are exploring how the transcriptional cascade that controls response to day length intersects during floral transition with biosynthesis and signaling of the growth regulator gibberellin at the shoot apex. The talk will describe our recent progress in understanding responses of the shoot meristem to day length.

043 - Meiotic adaptation in Arabidopsis arenosa

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Meiosis is essential for the fertility of eukaryotes and its core structures and progression are conserved across kingdoms. In orchestrating recombination, meiosis is also of critical importance in population genetics and breeding. This special cell division neatly halves the genome, resulting in the production of haploid gametes. Failures in this process, however, are not uncommon and can cause aneuploidy or sterility. Genome change and environment are two important factors that can challenge the stability of meiosis. But when these challenges become more than transient, how can evolution alter this tightly constrained process without perturbing its essential functions? My colleagues and I have been investigating how meiosis evolves in response to whole genome duplication, which leads to polyploidy, using a naturally occurring, widespread autotetraploid, *Arabidopsis arenosa*. We used a genome scanning approach to identify genes that were likely important in adaptation to whole genome duplication and found a suite of eight meiosis genes show strong signatures of selection. The proteins encoded by these genes interact to coordinate crossing over and synapsis, This paints a picture of a coordinated multigenic shift likely driven by co-evolution of interacting proteins. We have also found two diploid *A. arenosa* population groups that have shifted the temperature tolerance of meiosis. We are using these to begin exploring the role that environment may play in the adaptive evolution of meiosis, which has important implications for agriculture in the context of climate change. More generally our results provide some insight into how conserved multiprotein processes can evolve in response to diverse challenges from both external and internal contexts.

044 - Molecular Control of Fertilization and Interspecific Hybridization

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Research in our laboratory focuses on the developmental genetics of plant reproduction, with an emphasis on cellular interactions during double fertilization. Fertilization depends on the proper reception of the pollen tube by the synergid cells, where the pollen tube arrests growth and ruptures to release the sperm cells. We have isolated and characterized female gametophytic mutants that disrupt pollen tube reception. Pollen tubes that encounter such mutant female gametophytes are unable to rupture and release the sperm cells (Huck et al., *Development* 130:2149; Kessler et al., *Science* 330: 968). This phenotype suggests that the female gametophyte (embryo sac) controls the behavior of the male gametophyte (pollen) in this process. One of the mutants, *feronia*, was shown to disrupt a receptor-like kinase (Escobar-Restrepo et al., *Science* 317: 656), while another, *nortia*, affects a seven-transmembrane-domain-protein similar to the powdery mildew resistance protein Mlo (Kessler et al., *Science* 330: 968). The identification of additional components in the *FERONIA* signaling cascade suggests the involvement of glycosylation in this recognition process. Furthermore, interspecific crosses between Brassicaceae can result in a similar phenotype, suggesting the cell-cell interactions during pollen tube reception may be involved in interspecific crossing barriers. Using genome-wide association studies, we have been able to identify a factor that plays a specific role in interspecific compatibility while intraspecific crosses are not affected. Thus, pollen tube reception may be involved in establishing crossing barriers essential to maintain species boundaries similar to sperm-egg interactions in animals.

045 - Seeds Feel the Pressure

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Perception of, and response to, changing mechanical tension within developing tissues is thought to play a key role in regulating plant growth and development. Unpicking responses to mechanical signals from responses to other cues can, however, prove challenging in developing systems. Here we present evidence that by studying the dynamic interplay between the zygotic tissues (especially endosperm) and surrounding maternally derived seed coat tissues of the Arabidopsis seed, it is possible to identify novel elements involved in responses to endogenously generated tissue tension during plant development. We have developed genetic and biophysical tools to allow investigation of the mechanics of Arabidopsis seed development. We have identified a Mechanosensitive Cell Layer (MCL) within the seed coat, which reacts to increased tissue tension imposed by the expansion of the endosperm after fertilization, and undergoes cell wall thickening. We propose that this cell layer is a major site of seed growth control. We have identified a mechanosensitive gene, *CYP714A1*, which is expressed in the MCL and encodes a regulator of Gibberellic Acid (GA) homeostasis, providing evidence for a novel link between GA-mediated growth regulation and the perception of mechanical signals in developing seeds. We will also present unpublished work addressing in more detail the specific role of the endosperm in driving the early expansion of the developing seed.

Thematic concurrent session: Biotic interactions

046 - Diffuse interactions shape the dynamics of a plant pathogen interaction

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Theories of host pathogen interactions explain the maintenance of resistance polymorphisms in terms of frequency dependent selection acting between an obligately associated host and pathogen. However, many host-pathogen interactions are not obligate: pathogens often infect multiple host species and hosts harbor complex microbial communities. In this talk, I will tease apart the ecological interactions underlying an ancient balanced polymorphism that we have identified in *Arabidopsis thaliana* in nature. We find that this ancient balanced polymorphism at the *R* gene, *Rps5*, persists amidst a web of complex interactions involving multiple host species, multiple bacterial species and multiple effectors segregating among strains of single pathogen species. These results challenge us to understand how natural selection acts on plant resistance, and how pathogens adapt to their numerous secondary hosts. As a case study, I will assess how *P. syringae* adapts to one of its secondary hosts, *A. thaliana*, through an analysis of whole genome sequences and associated experiments. We find that native strains of P. syringae living in the leaves of *A. thaliana* come to dominate through microbe-microbe interactions rather than enhanced virulence in the host.

047 - Insights into intramolecular rearrangements of a TIR-NB-LRR receptor pair upon activation

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Plant resistance genes (R) usually encode intracellular Nucleotide Binding-Leucine Rich Repeat (NB-LRR) receptor proteins that resemble mammalian NOD-like receptors (NLRs). The NB-LRR proteins enable plants to activate defense upon recognition of specific pathogen effectors. How the NB-LRRs function is still poorly understood, but for some responses, two co-functioning NB-LRR proteins are required. The *Arabidopsis* RPS4/RRS1 is a Toll/Interleukin-1 receptor/Resistance (TIR) NB-LRR pair, which confers resistance to *Pseudomonas syringae* and *Ralstonia solanacearum* carrying the AvrRps4 or PopP2 effectors, respectively, as well as, to certain *Colletotrichum* strains. This dual TIR-NB-LRR receptor appears to be a good system for the study of dual NB-LRRs function. We took cell biology, biochemical approaches as well as functional assays for the investigation of inter- and intramolecular interactions of RPS4/RRS1 protein complex. Our results revealed RRS1 as an essential component for the proper assembling and translocation of the RPS4/RRS1 complex in the plant cell nucleus. Our findings also indicate a heterodimer formation between the N-terminal TIR domains of RPS4/RRS1 upon activation and auto-immunity. Likewise, conformational changes, triggered upon activation, enable the close proximity of the N- and C-terminus of RRS1 protein upon effector perception. Our studies reveal novel insights into the reconfiguration of the RPS4/RRS1 receptor complex upon activation.

048 - Impact of nitrogen limitation on the response of Arabidopsis to pathogens

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Nitrogen (N) is essential for life and is a major limiting factor of plant growth. Because soils frequently lack sufficient N, large quantities of N fertilizers are added for crop production. However, N is a major source of global pollution, because much of the N that is not taken up by plants enters streams, groundwater, and lakes, where it affects algal production and imbalances aquatic food webs. Furthermore, agronomical data indicate that N fertilization has an impact on the incidence of crop diseases. Indeed the higher use of N fertilizers during the green revolution led to an increase in the incidence of several plant diseases. However, there are also examples in which a decrease in N fertilization promotes disease indicating that there is a complex relationship linking N uptake and metabolism to plant disease. Although N availability clearly affects disease, the underlying mechanisms remain unclear. Our goal is to understand the mechanisms that link the plant"s N status to its response to pathogen infection and to identify key regulators that allow plants to adapt their biotic stress response to N availability. We showed that N limitation reduces the non-host resistance of Arabidopsis to E. *amylovora* but increases the resistance of Arabidopsis to *B. cinerea* (Fagard et al, 2014, J. Exp Bot 65: 5643-5656). We will determine which aspects of the interaction are affected by N limitation (defense, transcriptome, metabolome). We are currently analyzing the results of a transcriptome combining N limitation and biotic stress. Preliminary analysis indicates that a number of genes, including defense-associated genes, show a response to the combined stresses that could not be predicted from their profile in response to the each individual stress.

Thematic concurrent session: Development

049 - How does your garden grow? Transcriptional regulation of Arabidopsis cell type development, mineral nutrient acquisition and defense metabolite biosynthesis

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A major component of gene regulation in the model plant, *Arabidopsis thaliana*, is transcriptional regulation. Transcriptional regulation in individual cell types, in response to the environment and of central biological processes is essential for plant growth and function. We have mapped transcriptional regulatory networks spatially and temporally controlling xylem cell development in the root and stem, defense metabolite biosynthesis and central metabolism. Emerging principles from our analyses of these networks include the importance of feedforward loops in ensuring the robust regulation of terminal differentiation, non-linear transcriptional control of metabolite biosynthesis, and combinatorial, treatment-dependent rewiring of mineral nutrient homeostasis.

050 - Polarization in the Arabidopsis leaf epidermis is influenced by mechanical stress.

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The leaf epidermis is a well-suited tissue to study cell fate acquisition in plants. In the stomatal lineage, stem cell-like meristemoids give rise to pavement cells and stomatal guard cells, which together account for over 80% of leaf epidermal cells. Plant cells polarize and divide asymmetrically in order to achieve two daughter cells with different fates. Two proteins have been identified so far that account for cell polarization in the stomatal lineage. Immediately before cell division, these polarized proteins accumulate in membrane subdomains that are inherited by only one of the two daughter cells. Mutations in the corresponding genes lead to loss of asymmetry with regards to both size and fate of the daughter cells. Mechanisms for cell polarization in the stomatal lineage that integrate the plethora of external signals, including hormones, cell-cell communication and mechanical cues are, as of yet, unknown. My research focuses on the interplay of mechanical and chemical signals, apparent in the leaf epidermis. Inducing artificial changes in the mechanical stress environment through cell ablations and tissue stretching, I could show that polarized proteins change their orientation throughout the tissue. This is coherent with changes of the global orientation of the microtubule cytoskeleton. How these processes are coordinated with each other and tie in with peptide signaling among epidermis cells is part of my ongoing investigation.

051 - Cell-layer-specific coordination between ploidy and cell size in leaves

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How organ/cell size is determined? To date, many publications have shown that endoreduplication is tightly linked to the enlargement of cell size in plant tissues. But all of the published data on the correlation between cell size and endoreduplication levels have relied on measurements conducted in epidermal cells. Curiously, the distribution of ploidy levels is thought to be digitized as 2C, 4C, 8C, 16C and 32C also in inner cells, however, the cell size distribution in the palisade layer of a leaf is not digitized, and instead shows a single-peak distribution with a small deviation, as we previously reported. Thus, we hypothesize that digitized, ploidy-dependent cell size control is not as general as previously thought, but may be epidermis-specific. To test this hypothesis, we developed a novel technique for *in situ* imaging of cell volumes and ploidy at the single cell level for mesophyll cells. We found that a correlation between the ploidy level and cell size was much weaker in the palisade cells than in the epidermis. We also found that the correlation between the cell size and ploidy level in a transgenic line in which the epidermis identity gene, *AtML1*, could be ectopically induced. This revealed that the correlation between cell size and ploidy level was under the control of *AtML1*. Therefore, we suggest that that all previous reports on this correlation should be re-examined in light of these new data.

052 - MAKR5 is involved in CLE45 perception in primary root protophloem differentiation

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Peptide ligand/receptor signaling plays a role in various developmental processes in plants. Previous work from our lab has revealed that the CLAVATA3/EMBRYO SURROUNDING REGION 45 (CLE45) ligand/BARELY ANY MERISTEM 3 (BAM3) receptor pair regulates protophloem differentiation. Other signaling components in this pathway are barely known. Second-site null mutation in *MEMBRANE-ASSOCIATED KINASE REGULATOR 5 (MAKR5)*, defined as a homolog of BRI1 KINASE INHIBITOR 1 (BKI1), partially rescues defective primary root protophloem differentiation and associated phenotypes in *brevis radix (brx)* mutants. MAKR5 is a cytosolic protein and localizes in protophloem, metaphloem, companion cells and phloem-pole pericycle cells, which implies that MAKR5 might be involved in protophloem development. The roots of *makr5* mutants resist root meristem growth inhibition caused by exogenous CLE45 application, similar to *bam3* mutants. This result suggests that MAKR5 might have a role in CLE45 perception and/or signaling pathway, and therefore its association with, or dissociation from BAM3 could be a key process of BAM3 function. However, yeast two hybrid results show that MAKR5 does not interact with the BAM3 kinase domain. We expect that ongoing identification of MAKR5-interacting proteins will give us insight into the CLE45/BAM3-MAKR5 relation in protophloem differentiation.

053 - MAIL2 is a critical factor for adaption of shoot growth to environmental cues

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Postembryonic plant development relies on accurate control of gene expression in both a temporal and spatial manner within meristems. This is controlled by a network of transcription factors and chromatin remodeling proteins. We have recently identified in Arabidopsis thaliana three closely related genes, which we named MAINTENANCE OF MERISTEMS (MAIN), MAIN-LIKE-1 (MAIL-1), and MAIN-LIKE-2 (MAIL-2). The encoded proteins share a conserved plant-specific domain that is related to transposases and they are exclusively nuclear localised. Loss of function of MAIN or MAIL1 leads to a drastic short root phenotype associated with loss of cell fate in stem cells and differentiating cells. Here we show that MAIL2 plays an important role in inflorescence and floral meristems. RNAi-mediated down-regulation of MAIL2 expression leads to the development of very short inflorescence stems that produce partially sterile flowers. This phenotype is strictly temperature-dependent and only seen in plants grown at 20C but not at 24C. Moreover RNAi-mail2 plants are impaired in floral commitment and show a floral reversion phenotype when shifted form inductive long day conditions to short day conditions. These phenotypic changes correlate with altered expression of key developmental genes in inflorescence tissues. Our results show that MAIL2 is involved in the control of gene expression in meristems and an important factor for of coordination of developmental programs with environmental conditions.

054 - Graph-based analysis of cellular patterning in plants

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Intercellular communication forms the basis of cellular patterning and self-organization in plant organs. Understanding the organizational properties of these arrangements is key to uncovering multicellular organ function and structure-function relationships. To achieve this, we are looking at plant organs as complex systems of interacting cells and quantifying the topological properties of these systems using graph theory. Image analysis of whole mount 3D segmented organs is used to extract complete cellular connectivity networks, where nodes represent cells and the edges their physical associations. This discretization and abstraction of cellular patterning allows for quantitative topological analyses using a statistical framework developed by the graph theory community. Centrality-based analyses of these structural networks readily identify local irregularities in cellular patterning due to biological noise. Despite these local aberrations, the global topological properties of cellular patterning remain tightly regulated at a whole organ level. A role for path length in the control of communication across plant organs is also emerging. Finally, the topological analysis of mutants enables the contribution of gene activity towards patterning to be quantified on both local and global levels. These analyses draw strength from the complete nature of the networks being analyzed and the robust mathematical framework provided by the graph theory community.

Thematic concurrent session: Genome and chromatin dynamics

055 - The evolution of genomic imprinting in rice

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Arabidopsis thaliana endosperm, a transient tissue that nourishes the embryo, exhibits extensive localized DNA demethylation on maternally-inherited chromosomes, which mediates parent-of-origin-specific (imprinted) gene expression. Endosperm DNA in rice and maize is likewise locally hypomethylated. We have shown that localized hypomethylation in rice endosperm occurs solely on the maternal genome, preferring regions of high DNA accessibility. Maternally expressed imprinted genes are enriched for hypomethylation at putative promoter regions and transcriptional termini, and paternally expressed genes at promoters and gene bodies, mirroring our recent results in A. thaliana. Our data indicate that localized hypomethylation of maternal endosperm DNA is conserved in flowering plants. By examining divergent rice cultivars, we also identified genes the imprinting of which has changed in the course of rice evolution. In some cases, this variation is genetic, with gene expression differences caused by underlying sequence changes, such as transposon insertions. In other cases, methylation differences that control gene expression exist in the absence of significant genetic polymorphism. The results suggest that epigenetic variation contributes to short term plant evolution and may influence traits selected by crop breeders.

056 - DNA methylation reprogramming during embryogenesis in Arabidopsis

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We have discovered extensive DNA methylation reprogramming during embryo development and germination, which affects both genes and transposable element (TE) sequences, but very differently in each case. Specifically, approximately 10% of genes gain or lose body methylation (which is almost exclusively in the CG context) during the transition from embryonic to seedling growth. In contrast, a large number of TEs are subject to a major gain of DNA methylation specifically during late embryogenesis and predominantly in the CHH context, which is a hallmark of RNA-directed, de *novo* DNA methylation (RdDM). Furthermore, this gain of CHH methylation is rapidly erased following germination. Embryonic-like development continues if chromatin reprogramming by the Polycomb Repressive Complex 2 (PRC2) fails, which leads to a global loss of the repressive H3K27me3 mark. Mutants in the central component of PRC2, *FIE*, maintain high CHH methylation levels that originate from mature embryos and coincide with particularly enriched sRNA abundance. We will discuss these findings and their implications for our understanding of how DNA methylation patterns are established and transmitted within and across generations in plants.

057 - CHROMATIN CONTROL OF ADAPTATION TO NUTRITIONAL ENVIRONMENT IN ARABIDOPSIS

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Plants are constantly challenged by variations in their nutritional environment, and adapt their growth and development accordingly. To this end, the expression of root nutrient uptake and assimilation systems are the subject of fine-tuned regulations by nutritional conditions, but also by developmental cues. We have recently shown that nitrogen-dependent expression of the major root nitrate transporter in Arabidopsis, *NRT2.1*, relies on IWS1/HNI9, an essential chromatin factor of the Pol II large complex, and is associated with chromatin changes. It has suggested for the first time in plants that chromatin dynamics is an important component of the adaptation to the nutritional environment. To further address this issue, we now investigate how chromatin states determine nitrogen-dependent genome reprogramming, what is the importance of cell-type specificity, and what is the link between environmental and developmental regulations. Using these approaches, we show for instance that Polycomb members have very specific roles in the spatiotemporal regulation of the nitrogen response, and that chromatin state of nitrogen-responsive genes is cell-type specific. We also reveal a role for specific histone variants in response to nitrate signalling, and suggest new hypothesis on the role of HNI9 in the regulation of nitrogen-responsive genes. This work will allow a better understanding of the contribution of chromatin regulations to the adaptation to nutritional environment in plants.

058 - HSP90 reduction increases penetrance of new mutations

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Phenotypic robustness is the ability of organisms to develop into "wild-type" adults despite genetic and environmental perturbations. Due to this observation and similar observations in diverse model organisms, we hypothesize that differences in robustness among humans may account for the variability in penetrance of disease alleles and the "missing heritability" of complex traits. The best characterized regulator of robustness is the molecular chaperone HSP90, which allows for accumulation of phenotypically silent genetic variation in its select group of client proteins.

In *Arabidopsis thaliana*, we have previously shown that by inhibiting HSP90, the penetrance and heritability of natural genetic variation increases. We therefore hypothesize that *A. thaliana* seedlings with low-levels of HSP90, either by genetic or pharmacological insult, will show a higher frequency of aberrant phenotypes after introduction of genome-wide new mutations via chemical mutagenesis. By creating a mutant phenotype index to measure severity of phenotypes, we observe that HSP90-reduced seedlings show a higher frequency of detrimental phenotypes after mutagenesis compared to controls. Additionally, we have screened our mutagenized populations for new HSP90 buffered phenotypes. We have identified a line that has phenotypes which increase in penetrance under HSP90-reduced conditions and current work is focused on characterizing the variants that underlie these phenotypes.

Workshop 6: Challenges and questions in ambient temperature signaling and acclimation research

060 - A rendezvous of signals: temperature and light stimuli converge at multiple knots in an intricate signaling network to trigger morphological changes

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The predicted rise in global ambient temperature poses a significant threat to natural plant populations and industrial crops alike. Identification of molecular mechanism involved in temperature compensation and acclimation become paramount to meet future challenges e.g., to securing food sustenance. While responses to many environmental factors have been extensively studied in the past, the effects and signal transduction of moderate temperature increases have only recently gained a surge in attention. Initiated by forward genetic approaches we present data on extensive interconnection of light and temperature signaling. Well known regulators of photomorphogenesis such as the DET1/COP1-SPA/HY5 cascade as well as components of the circadian clock are involved in the regulation of PIF4-mediated temperature responses and form a highly interconnected network of regulators that can integrate multiple stimuli into architectural or developmental adaptations.

062 - Analysing GxE to dissect integrated responses of plants to high temperature

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Phenotypic plasticity to high temperature (HT) is inevitably multidimensional and multifactorial. Multidimensionality is inherent to the concept of phenotype where any trait contributes to shaping the phenotypic space. Plasticity to HT is multifactorial because in natural and even in supposedly controlled environments HT is associated with other site-(or experiment-)specific environmental factors that often include stresses such as water deficit. We used combinations of environmental (E) factors (water, light, CO2) to dissect the genetic bases of Arabidopsis responses to HT. Our results show that plant growth under HT strongly depends on carbon metabolism and that allometric constraints play a significant role in plant tolerance to HT. Specifically, a size-dependent response of water use efficiency (WUE) to HT but not to soil water deficit, indicated that most of the plasticity of carbon acquisition through photosynthesis and of WUE to HT was controlled by pleiotropic loci that control variation of development, growth, and transpiration. Based on this framework, we currently develop a multi-traits-multi-environments comparative approach that will allow identifying the adaptive strategies of different crop species and the possible constraints that may operate on plant breeding for ideotypes targeted to specific environments. This approach that has its roots into evolutionary ecology will continue to challenge the development of high throughput phenotyping platforms.

Workshop 7: Epigenomics

063 - Histone variants organise the genome structure

BERGER Fred[(1)]

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Histone variants play crucial roles in gene expression, genome integrity and chromosome segregation. However, to which extent histone variants control chromatin organization remains largely unknown. Here, we show that the previously uncharacterized histone variant H2A.W plays a crucial role in condensation of heterochromatin. Genome wide profiling of all four types of H2A variants in *Arabidopsis* shows that H2A.W specifically associates with heterochromatin. H2A.W recruitment is independent of H3K9me2 and DNA methylation that mark heterochromatin. Genetic interactions show that H2A.W acts in synergy with H3K9me2 to maintain genome integrity. In vitro, H2A.W enhances chromatin condensation by higher propensity to make fiber-to-fiber interactions via its conserved C-terminal motif. In vivo, elimination of H2A.W causes decondensation of heterochromatin and conversely, ectopic expression of H2A.W promotes heterochromatin condensation. H2A variants containing the H2A.W C-terminal motif exist in mammals and our study will shed light on understanding heterochromatin condensation disorders in human health.

064 - Divergent penetrance of DNA methylation among closely related Brassicaceae species

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Cytosine methylation is a heritable DNA modification and is involved in transcriptional silencing of genes and repetitive elements, whereas its role in gene body methylation is not fully understood. Despite the broad distribution of cytosine methylation across the entire eukaryotic phylogeny and the high degree of conservation of DNA methylation between closely related species, methylation can vary greatly between distantly related species. However deciphering the evolutionary history of changes in DNA methylation remains fuzzy when analyzing distantly related species only. Recently we found striking abnormalities within the DNA methylation patterns of *Arabis alpina* a close relative of *Arabidopsis thaliana*. Even though the typical patterns of DNA methylation including repeat and gene body methylation were conserved the degree of methylation as assessed on individual residues was highly different. In particular methylation in CG context was found not to be complete and symmetrical between the two strands of DNA. As the presence of complete and symmetrical CG methylation is a footprint of methylation maintenance during replication, this indicates divergent evolution of DNA methylation maintenance between these species. Here I will present a survey of DNA methylation and its regulatory pathways across additional genomes of the Brassicaceae family guided by their evolutionary relationship to explore additional DNA methylation phenotypes along with their phylogenetic distribution.

065 - HiC Technology to Characterize Chromosomal Architecture and Identify the KNOT, a Novel Nuclear Structure in Arabidopsis

GROB Stefan[(1)] *SCHMID Marc*[(1)], *GROSSNIKLAUS Ueli*[(1)]

[(1)] *Institute of Plant Biology, University of Zurich, Zurich, SWITZERLAND*

Efficient storage and readout of genetic information is not only dependent on tight epigenetic regulation, but also on the spatial organization and folding of chromosomes. The epigenome of Arabidopsis thaliana has been extensively studied; however, until recently, little has been known about the interplay with chromosomal architecture. Employing HiC technology, we and others (Feng et al., 2014; Wang et al., 2014; Grob et al., 2014) demonstrated that chromosomal architecture is tightly linked to the epigenetic state. Furthermore, we reported how physical constraints, such as nuclear size, can influence the folding principles of chromatin. In addition to global principles of chromatin organization, we described a novel nuclear structure, termed *KNOT*, in which genomic regions of all five *Arabidopsis* chromosomes highly interact. These *KNOT-ENGAGED-ELEMENT (KEE)* regions represent heterochromatic-like islands within euchromatin. *KEEs* show similarities to piRNA clusters in *Drosophila*: They are enriched in associated small RNAs and are preferred landing sites for transposable elements (TEs). These findings suggest that *KEEs* are involved in TE defense.

066 - Diagenode's activities within the EU EpiTRAITS network

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Epigenetic gene regulation confers stability of gene expression patterns through cell divisions while allowing changes in expression in response to environmental or developmental cues. Changes in epigenetic gene regulation are a major cause for trait variation in crops. EpiTRAITS European project focuses on one of the key plant traits, flowering, which is controlled by various epigenetic mechanisms. The scientific program aims to bridge the gap between fundamental and applied research by translating results from epigenetic research in model organisms to improved technologies for crop breeding and molecular diagnostic tools. Diagenode, as a leading company specialised in Epigenetics and Human molecular diagnostics, is the only company providing a complete solution for epigenetics research, including state-of-the-art products and technologies for DNA sonication, best-in-class antibodies, and high-quality kits for chromatin immunoprecipitation followed by High throughput sequencing (ChIP-seq) and methylation studies. Diagenode activities within EpiTRAITS consist on the development of robust marketable ChIP-seq protocols compatible with most important model plants. In addition, Diagenode will provide Next Generation Sequencing (NGS) data to EpiTRAITS partners in order to enrich the scientific program of EpiTRAITS (*).

Keywords: Diagenode, EpiTRAITS, Epigenetics, ChIP-seq, model plants

(*): Some parts of the abstract were taken from EpiTRAITS website

Workshop 9: Novel Tools and Techniques - New frontiers in single cell and single molecule biology

067 - Ligand Fishing with Arabidopsis Proteins

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To identify and characterize protein-ligand interactions, we developed a workflow that involves LC-TOF MS analyses of compounds that co-purify with affinity-tagged proteins. Proteins are produced in tobacco, and using the HA-Precision protease-Biotin (HPB) tag, purified with streptavidin beads. The bound compounds are interrogated by LC-TOF MS. To optimize this workflow, we characterized the interaction of the herbicide mesotrione with its target 4-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD was produced in μg quantities in *N. benthamiana* and ~30% of the purified HPPD contained ligand. Secondly, in vivo interaction of ABA with the receptor PYL5 (IC50 ~20 nM) was detected when HAB1 was co-expressed. We next tested if ligands for proteins of unknown function could be identified using this method and focused on proteins containing the polyketide cyclase/START-domain. These proteins were individually purified from tobacco and bound material was analysed by LC-TOF. The XCMS software platform was used to identify compounds enriched in experimental pulldowns relative to controls. Using this approach, we successfully determined a family of endogenous lipid ligands. In addition, we generated transgenic Arabidopsis lines expressing biotin tagged versions of ~80 START-proteins and we will present ligand fishing data from these lines. In summary, we have successfully assigned a ligand to a protein of unknown function using a relatively simple untargeted co-purification approach.To identify and characterize protein-ligand interactions, we developed a workflow that involves LC-TOF MS analyses of compounds that co-purify with affinity-tagged proteins. Proteins are produced in tobacco, and using the HA-Precision protease-Biotin (HPB) tag, purified with streptavidin beads. The bound compounds are interrogated by LC-TOF MS. To optimize this workflow, we characterized the interaction of the herbicide mesotrione with its target 4-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD was produced in μg quantities in *N. benthamiana* and ~30% of the purified HPPD contained ligand. Secondly, in vivo interaction of ABA with the receptor PYL5 (IC50 ~20 nM) was detected when HAB1 was co-expressed. We next tested if ligands for proteins of unknown function could be identified using this method and focused on proteins containing the polyketide cyclase/START-domain. These proteins were individually purified from tobacco and bound material was analysed by LC-TOF. The XCMS software platform was used to identify compounds enriched in experimental pulldowns relative to controls. Using this approach, we successfully determined a family of endogenous lipid ligands. In addition, we generated transgenic Arabidopsis lines expressing biotin tagged versions of ~80 START-proteins and we will present ligand fishing data from these lines. In summary, we have successfully assigned a ligand to a protein of unknown function using a relatively simple untargeted co-purification approach.

068 - Microscale Thermophoresis: A technology for the analysis of biomolecular interactions in free solution

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Microscale Thermophoresis: A technology for the analysis of biomolecular interactions in free solution

The analysis of biomolecular interactions, such as protein-protein, protein-nucleic acid or protein-small molecule, not only helps to develop therapeutics or diagnostics techniques, but it also provides important insights into cellular processes. Here we present an establish technology to analyze the affinity of biomolecular interactions in free solution, which is based on the method Microscale Thermophoresis (MST). MST analyzes the directed movement of molecules in optically generated microscopic temperature gradients. This thermophoretic movement is determined by the entropy of the hydration shell around the molecules. Almost all interactions and also any biochemical process relating to a change in size, charge and conformation of molecules alters this hydration shell and is thus detectable by MST. A case study of a screening done in collaboration with Sanofi, Paris, will be presented. In addition, examples are shown how MST can measure interactions with high selectivity in complex bioliquids like cell lysate or blood serum using a non-intrinsic source of fluorescence.

069 - LIGHT SHEET MICROSCOPY: A VERSATILE TOOL FOR SINGLE CELL CALCIUM ANAYSIS IN ARABIDOPSIS ROOT

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Translucent plants, including *Arabidopsis thaliana*, can be imaged at high spatial resolution by light sheet microscopy techniques, such as Selective Plane Illumination Microscopy (SPIM). Plant growth can be observed over various time scales, from seconds to days, using proper mounting protocols that keep the sample in an upright position, in close-to physiological conditions [1]. SPIM combined with Förster Resonance Energy Transfer is well suited to observe Ca2+ dynamics in single plant root tip cells, under external stimuli (ATP, Glutamate, Auxin, etc.)[2]. In particular, ATP treatment induces a Ca2+ wave propagating shootward, from the tip to the mature zone of the root. The fast acqusitions of SPIM enables to monitor such Ca2+ wave in 4D (three dimension plus time), revealing a prominent role of endodermal cells in the Ca2+ signal propagation. Moreover, thanks to the large field of view of this technique, it is feasible to image large tissue volumes maintaining single cell resolution. Such property allows for example, in a single experiment in time lapse, monitoring of multiple root hairs (RH) growth (from minutes to hours), with the associated tip Ca2+ oscillations. In order to accurately quantify Ca2+ oscillations we have developed an automatic routine able to register single RH during their growth, producing statistically relevant data regarding Ca2+ tip oscillations. These features pave the way for the comparison of RH growth in different genotypes.

070 - Binary 2in1 vectors allow in planta ratiometric BiFC and improve FRET/FLIM studies

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Protein-protein interaction analyses shape our understanding of cellular functions on a mechanistic level. Fluorescence-based techniques in particular are invaluable, enabling measurements of interactions in real-time context and *in-vivo* environment. An inherent flaw, however, with most current protein-protein interaction techniques is the variability in expression levels for fusion proteins when using several individual plasmids. We have generated a number of vectors that incorporate the benefit of the recombination-based 2in1 cloning system with the latest state-of-the-art fluorophors for optimal FRET/FLIM output [1] or the possibility to ratio Bimolecular Fluorescence Complementation (rBiFC, [2]). In rBiFC both candidate genes are simultaneously cloned into a single vector backbone containing an internal fluorescent marker for expression control and ratiometric analysis. rBiFC significantly increases the credibility of protein-protein interaction results allowing ratiometric comparison between different protein pairs. Under conditions of high efficient (co-) transformation and accumulation, techniques such as FRET can realize their potential for providing highly accurate and quantitative interaction data. FRET/FLIM allows measuring transient interactions as well as the detection of mere proximity of two proteins.

[1] Hecker A, Wallmeroth N, Peter S, Harter K, Blatt MR and Grefen C (2015) Plant Physiology. in revision

[2] Grefen C and Blatt MR (2012) Biotechniques. 53: 311-14

071 - Protein Interaction, Concentration and Mobility Measurements in Plant Cells via FLIM, FRET and FCS

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The turn-key upgrade of Confocal Laser Scanning Microscopes towards time-resolved methods alllows for new measurement modes like Fluorescence Lifetime Imaging (FLIM), lifetime based Förster Resonance Energy Transfer (FRET) and Fluorescence Correlation Spectroscopy (FCS). The fluorescence lifetime can change, e.g. depending on the fluorophore environment (polarity, pH, ion concentration) and thus enables for sensing of the local environment inside cells. Furthermore, FLIM is applied to discriminate multiple labels and to eliminate signal artefacts (e.g. sample background) thereby allowing a higher detection efficiency and accurate marker localization. Also, the autofluorescence is characteristic for a certain cell type and is therefore used, e.g., for tissue characterization. FRET enables to determine intra- and intermolecular interactions in vivo and in vitro. Furthermore, FRET sensors allow to monitor environmental conditions in cells like pH and ion concentration. Such FRET measurements are significantly improved and even quantified using FLIM. Changes in the donor fluorescence lifetime are monitored which are in a broad range concentration independent. Other than intensity-based approaches, FLIM-FRET can reveal sub-populations and allows to determine the fraction of free compared to associated donor molecules within a complex. FCS allows to measure dynamics, interaction and concentration of molecules in vivo providing insights into their function and temporal regulation.

Plenary session: Nutrition and metabolism II

072 - Nitrogen regulatory networks: From predictive modeling to trait evolution

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A goal in systems biology is to predict how networks will respond to perturbation [1]. Our first step connected nitrogen (N) response genes using metabolic, protein, and regulatory interactions using the "VirtualPlant" platform (www. virtualplant.org) [2]. The subnetworks uncovered new hypotheses including N-control of the clock [2] and miRNA regulation of lateral roots [4]. Next, using time-series data, we inferred N-regulatory networks able to predict gene responses at future time-points, the ultimate goal of systems biology [5]. To validate the networks, we developed *TARGET*, a cell-based system for TF perturbation [6]. We discovered a new mechanism of "Hit-and-Run" transcription underlying rapid N-signaling [7], and a TF that integrates N and phosphate signaling [8]. To explore the N-response as an integrated system, we uncovered systemic N-supply & demand signaling using a "split-root" set-up [9], and exploiting natural variants [10]. Lastly, to uncover genes underlying trait evolution, we constructed a phylogenomic tree of 150 species available as a web-resource (http://nypg.bio.nyu.edu/bp/) [11].

Refs: [1] Krouk 2013 *Genome Biol.*14:123;[2] Katari 2010 *Plant Physiol.*152:500;[3] Gutiérrez 2008 *PNAS* 105:4939; [4] Gifford 2008 *PNAS* 105:803;[5] Krouk 2010 *Genome Biol* 11: R123; [6] Bargmann 2013 *Mol Plant* 6:978;[7] Para 2014 *PNAS* 111;10371;[8] Medici 2015 Nature Comm. 6:6274 ; [9] Ruffel 2011 PNAS 108:18524; [10] Rosas 2013 *PNAS* 110:15133; [11] Lee 2011 *PLoS* Gen 12:e1002411.

073 - C4 photosynthesis - understanding the molecular evolution of a complex trait

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C4 photosynthesis is a remarkable example of convergent evolution of a complex trait. It has independently evolved more than 70-times in monocotyledonous and dicotyledonous flowering plants, including at least two independent origins in the Brassicales. With few exceptions, all C4 plant species display a distinct leaf anatomy called Kranz-anatomy as well as similar metabolic and biochemical features. Using computational modeling, we have shown that C4 photosynthesis evolves from C3 photosynthesis on a smooth upward trajectory via C3-C4 intermediate states (Heckmann et al., 2013). C3-C4 intermediacy evolves via simple loss of function mutations and causes a nitrogen disbalance between leaf mesophyll and bundle sheath cells. Overcoming this nitrogen disbalance requires the shuttling of organic acids between mesophyll and bundle sheath cells, which under permissive conditions sets the system on a slippery slope towards C4 photosynthesis (Mallmann et al., 2014).

Heckmann D, Schulze S, Denton A, Gowik U, Westhoff P, Weber APM, Lercher MJ (2013) Predicting C4 photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. Cell 153: 1579-1588.

Mallmann J, Heckmann D, Bräutigam A, Lercher MJ, Weber APM, Westhoff P, Gowik U (2014) The role of photorespiration during the evolution of C4 photosynthesis in the genus Flaveria. eLife, 3. doi: 10.7554/eLife.02478.

074 - DNA Endoploidy and Its Impact on Metabolism as Revealed by the Powdery Mildew - Arabidopsis interaction

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Powdery mildew fungi are obligate biotrophs that alter plant cellular architecture and metabolism to acquire their nutrients from the plant, while limiting plant defense. Using site-specific analyses, we found that the powdery mildew *Golovinomyces orontii* induces endoreduplication in plant mesophyll cells underlying the fungal feeding structure concomitant with fungal proliferation (*PNAS* 107:460-5). Multiple rounds of DNA replication without mitosis result in enhanced cell ploidy with ~16-fold elevated DNA content. Increased endoploidy has long been associated with enhanced metabolism, but the detailed mechanisms underlying this relationship are not well understood. We identified a conserved eukaryotic transcription factor and plant-specific factors that control the extent of induced ploidy and found the extent of fungal proliferation correlates with DNA ploidy levels (*MPMI* 26:537-45). This suggests ploidy-dependent enhanced host metabolism is needed to support the metabolically demanding proliferation phase of the powdery mildew life cycle. Using RNAseq, genetic, and biochemical analyses we have now identified specific ploidy-dependent alterations in *Arabidopsis* primary metabolism that are utilized by the powdery mildew to fuel its proliferation, providing a mechanistic underpinning to link enhanced ploidy to elevated metabolic capacity.

075 - From gene regulatory network to whole-plant biomass via metabolism: Bridging the gap with a multi-scale model

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Mechanistic mathematical models linking across multiple biological levels, from explicit representation of gene functions, could bridge the gap from genotype to phenotype and guide both breeding and synthetic biology to improve crop yield. Arabidopsis provides the ideal system for testing the feasibility of such models since it has abundant data, which motivated our Arabidopsis Framework Model (Chew et al, PNAS 2014). We have now extended our multi-scale model to include known circadian-clock gene regulation of starch turnover (Seaton et al, Interface 2014). We found that our new model could predict the reduced plant biomass of the starch-excess lsf1 mutant, but overestimated the biomass of the *prr7prr9* clock mutant that also accumulates excess starch. Comparison of measured metabolite levels between genotypes revealed high accumulation of a second specific carbon store in *prr7prr9*. The growth phenotype of *prr7prr9* was fully accounted for when the model considered this accumulation that sequesters carbon from growth. In conclusion, our multi-scale model links the dynamics of a gene regulatory network to biomass phenotype at a level as high as the whole plant, for a multi-cellular model organism for the first time. This work was funded by the EU FP7 TiMet grant.

076 - Regulation of root nitrate uptake in Arabidopsis thaliana using NRT2.1 as a target

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In *Arabidopsis* the *NRT2.1* gene encodes a main component of the root high-affinity nitrate uptake system (HATS). Its regulation has been thoroughly studied at the mRNA level, showing a strong correlation between *NRT2.1* expression and HATS activity in response to carbon (C) and nitrogen (N) treatments. However, despite its central role in plant nutrition, little is known concerning the molecular mechanisms involved in its regulation. Over the past few years, we combined different approach to study both the control of *NRT2.1* expression by C signaling and the regulation of *NRT2.1* at the protein level. We showed that NRT2.1 is induced by C through an original mechanism linked to the Oxidative Pentose Phosphate Pathway (OPPP) and that posttranslational regulatory mechanisms occur at the protein level. More recently, to decipher and model the gene regulatory networks involved in the control of root nitrate uptake by C and N we started a systems biology approach. For this work, we used *NRT2.1* as a target gene along with several treatments combining different amount of light, sucrose and nitrate. This approach allowed us to find a previously uncharacterized transcription factor involved in the regulation of both *NRT2.1* and *NRT2.4* by light depending on the level of nitrate nutrition. To our knowledge, this is the first time that a gene involved in the regulation of root ion transporters by light has been identified. Unpublished data will be presented.

Plenary session: Plant growth and development II

077 - Nottingham Arabidopsis Stock Centre (NASC) : a quick overview

CASTELLANOS Marcos[1], GILLETT Linda[1], KHAN Iqbal[1], ALJAFER Naofel[1], LODGE Stuart[1], EDGE Dan[1], JAVED Kashif[1], AMOS Beatrice[1], MAY Sean T.[1]

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The Nottingham Arabidopsis Stock Centre (NASC), based at the University of Nottingham, collects, preserves, reproduces and distributes diverse seed and other stocks of the model plant Arabidopsis thaliana and related species for research and education. Established in April 1991 with British public funding, NASC"s seed collection approaches one million stocks including insertion lines covering 28,937 genes and over 1,400 distinct natural accessions. NASC"s activities are coordinated with those of the Arabidopsis Biological Resource Center, (ABRC) based at Ohio State University, USA. Both stock centres offer several advantages over alternative providers: they provide security and stability as seed stocks are preserved under the best possible conditions; they have no interest in or claims to intellectual property, and they maintain and curate large numbers of stocks according to common standards. NASC has traditionally been funded by the Biotechnology and Biological Sciences Research Council (BBSRC) with a small proportion of operational costs also covered through user fees. This combination of government funding and cost recovery represents a reasonable balance between community services provided and financial support required. The usage of NASC resources has increased continuously since its inception. The number of seed and DNA stocks sent annually is >100,000, a rate that substantially exceeds anything imagined in the beginning. As part of the AraPort community, our data are freely available through web services and we are currently expanding our integration with other community resources.

078 - Hormonal regulation of root branching

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Plant hormones are important signaling molecules that control many developmental processes, including cell division, cell differentiation, organogenesis, and morphogenesis. They can regulate multitude of apparently unrelated physiological processes, with often overlapping roles. This implies that synergistic and antagonistic interactions between plant hormones play an important role in the regulation of plant development. Physiological and genetic studies have dissected the molecular components of signal perception and transduction of the individual hormonal pathways. However, over recent years it has become evident that hormones do not act only in a linear pathway. Hormonal pathways are interconnected by a complex network of interactions and feedback circuits that determines the final outcome of the individual hormone actions. This raises questions about the molecular mechanisms underlying hormonal cross talk. To identify novel components of the auxin-cytokinin interaction, we applied two main methodologies, transcriptome profiling and forward genetic screening. Genes recovered from both the transcriptome profiling and the forward genetic screen represent strong candidates for novel cross-talk components to reveal mechanisms that integrate auxin and cytokinin signaling pathways. Progress in our understanding of auxin-cytokinin crosstalk in regulation of root system architecture will be discussed.

079 - The role of symplastic communication on controlling root vascular development

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Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. Phloem is adapted for transporting organic molecules from the source to sink tissues. It consists of the transporting sieve elements

(SE) and companion cells (CC) that support these highly specialized enucleate cells with various characteristic subcellular adaptation including some distinctive cell wall properties. During root primary development, phloem identity is established through a set of asymmetric cell divisions that will also give rise to part of the pluripotent procambial tissue that will develop as vascular cambium later during secondary development. We have analyzed phloem development based on genetic and molecular approaches and identified a set of key regulators, such as transcription factors that are controlling phloem identity (APL, Bonke et al 2003 Nature) and phloem differentiation (NAC 45/81 Furuta et al 2014 Science). Finally, through identification of dominant mutations affecting callose biosynthesis, we have engineered a temporally and spatially controlled system to control plasmodesmatal trafficking during phloem development (Vaten et al. 2011 Developmental Cell; Bishopp et al. 2011 Curr Biol).

080 - Phosphoinositide Control of Root Protophloem Differentiation and its Systemic Consequences on Hormone Activities and Root System Architecture

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In Arabidopsis mutants with impaired primary root protophloem differentiation, *brevis radix (brx)* and *octopus (ops)*, meristematic activity and overall root growth are strongly reduced. 2nd site mutation in the protophloem-specific 5-phosphatase COTYLEDON VASCULAR PATTERN 2 (CVP2) partially rescues *brx* defects. Consistently, both CVP2 hyperactivity and mutation of protophloem-specific 5-kinases result in brx/ops root phenotypes. Paradoxically however double mutants of *cvp2* and its homolog *cvl1* with strongly reduced 5-phosphatase activity also display

brx/ops root defects. Thus, tightly balanced phosphatidylinositol-4,5-biphosphate (PIP2) levels are essential for protophloem differentiation. Genetically, OPS is an effector of PIP2 balance, since *cvp2* mutation cannot rescue ops defects, whereas increased *OPS* dosage rescues *cvp2 cvl1* defects. Finally, all protophloem mutants display systemic shifts in auxin response, where auxin activity is reduced in the meristematic zone, but increased in the differentiation zone. This phenotype is associated with a higher frequency of lateral root initiation and can also be created through peptides that specifically suppress protophloem differentiation. Discontinuous protophloem strands thus create an "auxin traffic jam" as a consequence of suboptimal auxin delivery into the meristem, thereby systemically shaping root system architecture through a range of secondary effects that encompass formative cell divisions as well as hormone activities.

081 - PHABULOSA Controls the Quiescent Center-Independent Root Meristem Activities in Arabidopsis thaliana

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Plant growth depends on stem cell niches in meristems. In the root apical meristem, the quiescent center (QC) cells form a niche together with the surrounding stem cells. Stem cells produce daughter cells that are displaced into a transit-amplifying (TA) domain of the root meristem. TA cells divide several times to provide cells for growth. SHORTROOT (SHR) and SCARECROW (SCR) are key regulators of the stem cell niche. Cytokinin controls TA cell activities in a dose-dependent manner. Although the regulatory programs in each compartment of the root meristem have been identified, it is still unclear how they coordinate one another. Here, we investigate how PHABULOSA (PHB), under the posttranscriptional control of SHR and SCR, regulates TA cell activities. The root meristem and growth defects in *shr or scr* mutants were significantly recovered in the *shr phb* or *scr phb* double mutant, respectively. This rescue in root growth occurs in the absence of a QC. Conversely, when the modified *PHB*, which is highly resistant to microRNA, was expressed throughout the stele of the wild-type root meristem, root growth became very similar to that observed in the *shr;* however, the identity of the QC was unaffected. Interestingly, a moderate increase in *PHB* resulted in a root meristem phenotype similar to that observed following the application of high levels of cytokinin. Our protoplast assay and transgenic approach using *ARR10* suggest that the depletion of TA cells by high PHB in the stele occurs via the repression of B-ARR activities. This regulatory mechanism seems to help to maintain the cytokinin homeostasis in the meristem. Taken together, our study suggests that PHB can dynamically regulate TA cell activities in a QC-independent manner, and that the SHR-PHB pathway enables a robust root growth system by coordinating the stem cell niche and TA domain.

Thematic concurrent session: Secondary metabolism

082 - Metabolomics-assisted functional genomics on plant phenolic secondary metabolism

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Plant secondary metabolites are widely diversified in their chemical structures, since during the long evolutionary period wherein plants have adapted to the environmental niches, several strategies such as gene duplication and convergent evolution of some key enzymatic genes have contributed to the evolution of the secondary metabolism. For the reason that phenolic secondary metabolites play important roles in both biotic and abiotic defences in seed plants as well as being potentially important bioactive compounds with both nutritional and medicinal benefits for animals and humans, investigation of this metabolism has been highlighted for long years, especially focusing on crop species such as maize, bean and tomato. Recent technical developments allowing affordable whole genome sequencing as well as omics studies and available of several resources such as knockout mutant library, QTL lines and wild accessions, have resulted in dramatic increase in the number of approaches for functional genomics, namely metabolic phenotype screening, network analysis, mQTL and GWAS studies. We integrated these approaches with metabolomic analysis to refine biosynthetic structure of phenolic secondary metabolism including natural variance, tissue and species specificity. I will present our metabolomics-assisted functional genomics approach which resulted in discovery of key genes involved in chemical diversity in Arabidopsis and crop species.

083 - Metabolomics reveal small molecule dynamics in Arabidopsis responding to LPS

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A metabolomic approach was used to elucidate changes in the metabolome of *Arabidopsis thaliana* following lipopolysaccharide (LPS) treatment where cells and leaves were elicited with LPS (80 µg/ml) and incubated for 8 h, 12 h, and 24 h (cells) and 24 h (leaves). Metabolites were extracted from both the cell (intracellular and extracellular) and leaf samples using boiling methanol. UHPLC-MS profiled the extracts and supplied detailed retention, mass and intensity values. Multivariate data analyses extracted meaningful information from the complex LC-MS data for interpretation of metabolic changes. PCA scores plots revealed time-dependent (cell study) and treatment-related (leaf study) variations. LPS-induced metabolic changes were translated into structurally elucidated metabolites through the use of chemometric tools and various databases. Camalexin and indolic glucosinolates (glucobrassicin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin and sulfoglucobrassicin) were identified from cell and leaf extracts and positively correlated with the LPS treatment. Other annotations included compounds from the shikimate-phenylpropanoid-flavonoid pathways. The metabolomic data reveal new insights into the LPS induced differential changes / metabolic reprogramming in the metabolome of *A. thaliana*, leading to variation in the synthesis of defence-related secondary metabolites from various metabolic pathways in support of innate immunity and defence.

084 - Cytochrome P450 enzymes in the Arabidopsis indole-3-acetonitrile metabolic network: Biological functions and protein-protein interactions

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In the Brassicaceae, pathogen infection induces the biosynthesis of a range of tryptophan-derived phytoalexins. Characteristic for Arabidopsis is the formation of indole-3-caboxylic acid (ICOOH) derivatives and of the anti-fungal metabolite camalexin. The camalexin biosynthetic pathway branches off indole glucosinolate biosynthesis by the conversion of indole-3-acetaldoxime to indole-3-acetonitrile catalysed by the closely related cytochrome P450 monooxygenases CYP71A12 and CYP71A13. CYP71A13 physically interacted with CYP71B15 (PAD3), the essential bifunctional enzyme of the pathway indicating channeling of IAN into camalexin biosynthesis. As *CYP71A12* and *CYP71A13* are localized in tandem, stable double knockout lines were created via a TALE-nuclease mediated approach, following the establishment of very efficient somatic mutagenesis. Double mutants synthesized only traces of camalexin, demonstrating that CYP71A12 contributes to camalexin biosynthesis in leaves, and a major role of CYP71A12 was identified for inducible biosynthesis of ICOOH. Based on these results we present a model for the complex network of IAN-metabolism in Arabidopsis.

085 - The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of Arabidopsis thaliana

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The glucosinolate breakdown product indole-3-carbinol functions in cruciferous vegetables as a protective agent against foraging insects. While the toxic and deterrent effects of glucosinolate breakdown on herbivores and pathogens have been studied extensively, the secondary responses that are induced in the plant by indole-3-carbinol remain relatively uninvestigated. Here we examined the hypothesis that indole-3-carbinol has a role in influencing plant growth and development through manipulating auxin signaling. We show that indole-3-carbinol rapidly and reversibly inhibits root elongation in a dose-dependent manner, and that this inhibition is accompanied by a loss of auxin activity in the root meristem. One hour following I3C treatment of seedling root tips, about 300 genes are differently regulated, including genes that are known to be activated by auxin. A direct interaction between indole-3-carbinol and the auxin perception machinery was suggested, as application of indole-3-carbinol rescues auxin-induced root phenotypes. *In vitro* and yeast-based protein interaction studies show that indole-3-carbinol perturbs the auxin-dependent interaction of TIR1 with Aux/IAA proteins, further supporting the notion that indole-3-carbinol acts as an auxin antagonist. The results indicate that chemicals induced by herbivory, such as indole-3-carbinol, function not only to repel herbivores, but also as signaling molecules that directly compete with auxin to fine tune plant growth and development.

Thematic concurrent session: Reproduction

086 - Multiple mechanisms limit meiotic crossovers

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Meiotic crossovers (COs) have two important roles, shuffling genetic information and ensuring proper chromosome segregation. Despite their importance and a large excess of precursors (i.e DNA double strand-breaks, DSBs), the number of meiotic COs is tightly regulated, typically one to three per chromosome pair. Nevertheless, the mechanisms that ensure DSBs repair mostly as non-crossovers, and the evolutionary forces that impose this constraint, are poorly understood. Following a specific genetic screen, we identified several proteins that antagonize crossover formation in Arabidopsis Thaliana. This includes the helicase FANCM (Crismani et al, Science 2012) and its two co-factors MHF1 and MHF2 (Girard et al, NAR 2014), the BLM-like helicase RECQ4, the TOPOISOMERASE3a (TOP3A) (Seguela-Arnaud et al, PNAS in press) and the AAA-ATPase FIDGETIN-LIKE1. Strikingly, the concomitant disruption of several of these activities led to a nine fold increase in CO frequency, without affecting chromosome segregation and fertility. This shows that several parallel pathways actively limit CO formation and supports the idea that crossover number is restricted not because of mechanical constraints but likely because of long-term costs of recombination. Furthermore, this demonstrates how manipulating a few genes holds great promise for increasing recombination frequency in plant breeding programs.

087 - Structural insights into LEAFY function and evolution

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The LEAFY(LFY) transcription factor is a key regulator of flower development in angiosperms. We use an integrated structural approach to explore LFY function and evolution. By solving the structure of its two conserved domains in various species, we better understand the role and the mode of action of this unique plant transcription factor. Manipulating the structure of the LFY DNA binding domain revealed a new facet of LFY function in meristem emergence. We have also unraveled the evolutionary pathway of LFY DNA binding specificity. Finally, we discovered that LFY is able to form oligomers, a property that shapes its genomic binding landscape.

088 - Engineering clonal seed formation in plants through male apomixis

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Apomixis refers to a collection of different mechanisms by which the sexual reproduction system generates clonal seed. In naturally occurring apomixis it is usually the female organ that omits meiosis and develops an embryo without fertilization (parthenogenesis). Here, we present an alternative method whereby the male reproductive organ generates clonal pollen that are used to fertilize eggs that selectively eliminate their own genome input upon fertilization. As a result clonal seeds are formed without the need for apomeiotic eggs. Male apomeiosis can be obtained by combining loss of meiotic recombination and reductional cell division. This was achieved by combined loss of AtSPO11-1 and JAS, e.g. two proteins essential for meiotic recombination and reductional cell division respectively, converting the meiotic cell division into a mitotic one and hence yields clonal 2n pollen. The fertilization of an Arabidopsis genome elimination line yields diploid progeny plants that are genotypically identical to the pollen donor plant. We conclude that the combined loss of JAS and AtSPO11-1 confers male apomeiosis and hence provides a molecular basis for the synthetic engineering of “male apomixis” in plants. Progress to increase the frequency of clonal 2n pollen and methods to engineer a fully male apomictic *Arabidopsis* line will be presented.

089 - Investigating the roles of stigma-expressed RLCKs in compatible pollen signalling

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Species in the Brassicaceae have dry stigmas, meaning pollen must first be recognized by the stigma for successful germination. Upon compatible pollen recognition, water and enzymes are transported through vesicle trafficking from the stigmatic papillae to the pollen, allowing pollen hydration, germination and pollen tube penetration for subsequent pollen tube growth and fertilization. Despite known physiological responses following compatible pollen recognition, the signalling events that trigger these responses remain unknown. We used a reverse genetics approach to identify candidate genes involved in compatible pollen acceptance signalling by searching for stigma-expressed genes in publicly-available microarray datasets. We identified two stigma-expressed Receptor-Like Cytoplasmic Kinases (RLCKs) and confirmed their expression profiles using RT-PCR. These RLCKs are conserved within the Brassicaceae, supporting their possible involvement in a family-wide compatible pollen recognition pathway. These two RLCKs were knocked-down in A. thaliana using RNA silencing though an artificial microRNA construct; CRISPR-Cas9 knockouts for both genes have also been generated. These transgenic lines are currently being assessed for altered stigmatic papillar responses to compatible pollen, and preliminary results show reduced pollen adhesion, pollen tube penetration, and seed set. This project represents an exciting first step towards understanding compatible pollen acceptance signalling.

Thematic concurrent session: Post-transcriptional / post-translational regulations

090 - When protein and RNA degradation pathways meet

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Post-transcriptional gene silencing (PTGS) mediated by siRNAs is an evolutionary conserved antiviral defense mechanism in higher plants and invertebrates. In this mechanism, viral-derived siRNAs are incorporated into the RNA-induced silencing complex (RISC) to guide degradation of

the corresponding viral RNA. In Arabidopsis, a key component of RISC is ARGONAUTE1 (AGO1), which not only binds to siRNA but also carries the RNA slicer activity. At present little is known about post-translational mechanisms regulating AGO1 turnover. Recently, we found that the P0 protein, which is the viral suppressor of RNA silencing from Polerovirus, acts by addressing AGO1 protein for degradation through the autophagy pathway and that P0 most likely highjacks an endogenous pathway that control AGO1 accumulation in plant cells. The role of autophagy in the control of ARGONAUTE proteins accumulation is conserved in human and, in worm, autophagy seems also to have an impact on RNA silencing activity. Using a P0-inducible transgenic line, we have generated a P0 suppressor screen that allowed us to identify mutants affected in the P0-dependent degradation of AGO1. Here we present the first characterization of one of these mutants.

091 - Downregulation of cell-surface proteins by K63 polyubiquitin-mediated endocytosis : how and why?

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Brassinosteroids (BRs) are plant hormones that control many aspects of plant growth and development, and are perceived at the cell surface by the receptor kinase BRI1. Here we show that BRI1 is post-translationally modified by K63 polyubiquitin (Ub) chains *in vivo*, largely independently from BR receptor activation. Using both BRI1 artificial ubiquitination and generation of an ubiquitination-defective BRI1, we demonstrate by high-resolution TIRF imaging that Ub promotes BRI1 internalization from the cell-surface, and is absolutely essential for its recognition at the *trans*-Golgi network/early endosomes for vacuolar targeting. We also demonstrate that, although not regulated by BRs, BRI1 Ubmediated endocytosis is an important mechanism dictating the levels of receptor in the cell. Interestingly, high temperature growth conditions trigger Ub-mediated endocytosis of BRI1 and lead to decreased BRI1 protein levels specifically in roots, unraveling the existence of a crosstalk between BR signaling and temperature. Consistently, plants grown at higher temperature are resistant to the inhibitory effect of BRs on root growth. Finally, we will present some results about the mechanisms of temperature-dependent root growth control. Altogether, our results identify K63-linked polyUb chain formation as a dual targeting signal for BRI1 internalization and sorting along the endocytic pathway, and highlight its role in hormonally and environmentally controlled plant development.

092 - Junk RNA? Characterisation of a non-canonical Upstream Open Reading Frame that Regulates Ascorbate Biosynthesis

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Much of the RNA that is transcribed could mistakenly be thought of as "junk" that is just spliced away or that flanks the peptide coding sequence. We have discovered an unusual open reading frame in the 5"UTR and demonstrated its role in feedback regulation of ascorbate bisoynthesis [1]. Ascorbate (vitamin C) is an essential antioxidant and enzyme cofactor in both plants and animals. Ascorbate concentration is tightly regulated in plants, partly to respond to stress. Here, we demonstrate that ascorbate concentrations are determined via the posttranscriptional repression of GDP-l-galactose phosphorylase (GGP), a major control enzyme in the ascorbate biosynthesis pathway. This regulation requires a cis-acting upstream open reading frame (uORF) that represses the translation of the downstream GGP open reading frame under high ascorbate concentration. Disruption of this uORF stops the ascorbate feedback regulation of translation and results in increased ascorbate concentrations in leaves. The uORF is predicted to initiate at a non-canonical codon (ACG rather than AUG) and encode a 60- to 65-residue peptide. Analysis of ribosome protection data from Arabidopsis thaliana showed colocation of high levels of ribosomes with both the uORF and the main coding sequence of GGP. Together, our data indicate that the non-canonical uORF is translated and encodes a peptide that functions in the ascorbate inhibition of translation. Small peptides such as these raises an inconvenient question: are we missing a vast library of biologically important peptide signals because our bioinformatic analyses are not yet well enough designed to detect them [2]?

1 Laing, W.A., *et al.* (2015) An Upstream Open Reading Frame Is Essential for Feedback Regulation of Ascorbate Biosynthesis in Arabidopsis. *The Plant cell*

2 Waterhouse, P.M. and Hellens, R.P. (2015) Plant biology: Coding in non-coding RNAs. *Nature*

093 - ASCO a long non-coding RNA involved in root development plasticity

NATALI Romero-Barrios[1], FEDERICO Ariel[1], FLORIAN Bardou[1], VAN DU Tran [2] , OUSSAMA Souiai [2] , DANIEL Gautheret [2] , CRESPI Martin[1]

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Long non-coding RNAs (lncRNAs) are emerging as key players in the regulation of varied important developmental processes. They can act directly in a long form by lncRNA-protein interactions or be processed into shorter small si/miRNAs, leading to mRNA cleavage, translational repression or epigenetic DNA/chromatin modification of their targets. Hence, lncRNAs are highly regulated presenting specific temporal and spatial expression patterns. In our Lab, a bioinformatic approach identified 76 *Arabidopsis thaliana* lncRNAs. The *ASCO* lncRNA (for Alternative Splicing COmpetitor) interacts with NSRs (Nuclear Speckles RNA-binding proteins) and affects the splicing patterns of several NSR-regulated mRNA targets. The *nsra/b* double mutant and *ASCO* overexpressing lines treated with auxin show altered ability to form lateral roots. A new bioinformatical approach "RNAprof" allowed the genome-wide detection of 1885 differential RNA processing events in auxin-treated *nsra/b* compared to wt including genes involved in lateral root development. Auxin treatment induces *NSRb* in plantlets and decreases *ASCO* expression in roots. In *ASCO* overexpressing lines, *NSRb* is over-accumulated whereas, in the RNAi *ASCO* lines, *NSRb* induction is lost. A cooperative action of *ASCO* and NSRs in alternative splicing regulation during lateral root development is proposed.

094 - Unmasking alternative splicing inside protein-coding exons defines exitrons and their role in proteome plasticity

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Alternative splicing (AS) diversifies transcriptomes and proteomes and is widely recognized as a key mechanism for regulating gene expression. Previously, in an analysis of intron retention events in Arabidopsis, we found unusual AS events inside annotated protein-coding exons (Marquez et al. 2012). As these events involve introns with features of both introns and protein-coding exons, we name them exitrons (exonic introns). Though exitrons were detected as a subset of retained introns, they are clearly distinguishable, and their splicing results in transcripts with different fates. Exitron splicing occurs in about 3.3% and 3.7% of Arabidopsis and human protein-coding genes, respectively. Intriguingly, intronless genes can be also alternatively spliced via exitron usage. Splicing of exitrons affects protein domains, disordered regions and various post-translational modification sites, thus broadly impacting protein function. Exitron splicing is regulated across tissues, in response to stress and in carcinogenesis. At least some exitrons originate from ancestral coding exons and their evolution involves intron loss. We propose a "splicing memory" hypothesis whereby intron loss and imprints of former exon borders defined by vestigial splicing regulatory elements could drive the evolution of exitron splicing. Our studies show that exitron splicing is a conserved strategy for increasing proteome plasticity in plants and animals complementing the repertoire of AS events.

095 - Molecular role of TSN in cytoplasmic messenger ribonucleoprotein complexes during stress

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Tudor staphylococcal nuclease (TSN) is an evolutionarily conserved protein involved in transcriptional and posttranscriptional regulation of gene expression in animals [1, 2]. Although TSN was found to be indispensable for normal plant development and stress tolerance, the molecular mechanisms underlying these functions remain elusive. We have found that *Arabidopsis thaliana* TSN is essential for the integrity and function of cytoplasmic messenger ribonucleoprotein (mRNP) complexes called stress granules (SG) and processing bodies (PB), sites of post-transcriptional gene regulation during stress [3]. We have further revealed that TSN as a key enzymatic component of catabolic machinery responsible for the processing of mRNAs in the mRNP complexes. Notably, TSN is stably associated with both SG and PB, suggesting that it may serve scaffolding role to recruit other proteins to the mRNP complexes. As the first step to address this point and to advance our understanding of molecular role of TSN in SG and PB, we have characterized TSN interactome using tandem affinity purification (TAP) combined with bimolecular fluorescence complementation (BiFC).

1. Paukku, K., J. Yang, and O. Silvennoinen. Mol Endocrinol, 2003. 17(9): p. 1805-14.

2. Scadden, A.D. Nat Struct Mol Biol, 2005. 12(6): p. 489-96.

3. Gutierrez-Beltran, E., et al. Plant Cell, 2015.

Thematic concurrent session: Translational biology and biotechnologies

096 - Roots: uncovering the hidden half of crop traits for breeders

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Crop production has to double by 2050 to keep pace with global population growth. This target is even more challenging given the impact of climate change on water availability. Root architecture critically influences water and nutrient uptake efficiency. Despite this knowledge, very few genes that regulate root architectural traits such as angle, depth and density have been identified in crops. A key impediment has been the ability to image roots grown in soil non-invasively. I will describe the Hounsfield Facility, a new fully roboticised X-ray CT based imaging platform, designed to phenotype crop root architecture and uncover novel water and nutrient foraging traits. I will also describe examples of how genes originally identified in model plants like Arabidopsis are being used to manipulate root architecture in cereal crops.

Thematic concurrent session: Hormones and signaling

097 - From plants to yeast and back again: synthetic biology and plant development

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The fundamental building blocks of plant development - cell-cell communication, cellular differentiation, cell growth and proliferation - are characterized by complex gene regulatory networks, many of which include plant hormones. By recapitulating the *Arabidopsis thaliana* auxin signal transduction pathway in *Saccharomyces cerevisiae*, we were able to identify and analyze the parameters of auxin response without interference from other networks. This work revealed that members of the large Aux/IAA family exhibit a range of degradation rates and that Aux/IAA degradation rates drive transcriptional dynamics. These synthetic experiments and subsequent experiments in transgenic plants demonstrate that Aux/IAA degradation rate can set the pace for critical developmental events. Our findings lead us to conclude that, by acting as auxin-initiated timers, Aux/IAAs facilitate coordinated cell behaviors during developmental transitions. Recent extensions of these findings will be discussed.

098 - High-resolution RNA-seq time series reveal architecture and regulation of jasmonic acid and salicylic acid modulated transcriptional networks.

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The plant hormones jasmonic acid (JA) and salicylic acid (SA) are core regulators of plant immunity, whose signalling pathways cross-communicate to provide highly tailored and effective immune responses. To investigate the architecture, dynamics and potential regulatory mechanisms underlying the JA- and SA-modulated networks, we have used RNA-seq to profile these hormone induced transcriptional responses at high temporal resolution. Computational data-analysis reveals large-scale, rapid, and dynamic reprogramming of gene expression, with distinct and overlapping sets of genes responding to both hormones. The responses to JA and SA can be separated into distinct transcriptional phases, allowing for inferences regarding gene regulatory network structure to be made. Clustering of the highly informative expression profiles revealed regulatory modules that are enriched for specific functions and biological processes. Promoter analysis using these modules reveal specific TF-binding motifs that are overrepresented in the promoters of coexpressed genes which, combined with an analysis of TF-families that are active in each time-series, predict *cis*- and *trans*-acting factors that may discriminate between and coordinate the distinct transcriptional responses modulated by JA and SA. In addition, novel transcriptional regulators of early hormonal responses were inferred from the predicted gene regulatory network and are being experimentally validated using mutant gene lines.

099 - The role of AGC protein kinases in PIN-mediated auxin transport

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The plant hormone auxin controls virtually all aspects of plant development. Auxin is directionally distributed within the plant through a system of auxin import and export transporters. PIN auxin exporters are polarly distributed in the plasma membranes of many cells and their polar distribution gives directionality to auxin transport streams. Through our investigations of the basally localized D6 PROTEIN KINASES (D6PKs), we could show that PIN protein-mediated auxin efflux strictly requires activation by PIN phosphorylation, e.g. by D6PKs or other AGC kinases. The plasma membrane-associated D6PKs rapidly traffic to and form the plasma membrane and the presence and absence of D6PKs at the plasma membrane strictly controls PIN-mediated auxin efflux. Thus, D6PKs are essential switches for auxin transport in the plant. Interestingly, auxin also controls D6PK-dependent PIN phosphorylation, D6PK plasma membrane association and D6PK polarity, pointing at a role for D6PKs as integrators of auxin response and auxin transport. We mapped the phosphorylation sites of D6PK on PINs and detected all four phosphorylation events of PIN1 at the basal plasma membrane. At the same time, we find PIN1 unphosphorylated during trafficking and or at the apical membrane. While our results clearly demonstrate a role for D6PK and related AGC kinases in activating PIN-mediated auxin transport, they put into question existing models on the polarity control of PIN proteins by the AGC kinase PINOID.

100 - Strigolactones stimulate plastid stromule formation independent of MAX2 signalling

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Stromules are tubular structures extending from plastids and visualized by GFP-tagged plastid stromal proteins. The Stromule Frequency (SF) in hypocotyl epidermal cells of Arabidopsis WT, Strigolactone (SL) signaling mutant max2 and SL-biosynthesis mutant max3 was used to study the effect of SLs on stromules. In max2 the SF is significantly higher, while in max3 SF is significantly lower than in WT. SL biosynthesis is under MAX2-signalling dependent negative feedback regulation, resulting in higher SL levels in max2 compared to WT. The SF in WT and max2 and max3 therefore seems to reflect endogenous SL levels. Also, addition of synthetic strigolactone GR24 increases SF. SL synthesis increases in plants exposed to low phosphate (Pi) (but not in max3). Indeed SF is dramatically increased on low Pi in WT and max2, but not in max3. Moreover, the high SF in max2 is reduced by the SL specific inhibitor D2. Combined the results indicate a SL-dependent stimulation of Stromule formation, independent of MAX2 signaling. Stromules function in exchange of molecules between plastid and other cell compartments. By stimulating SF SL may therefore have an important role in controlling flux of compounds to and from plastids, e.g. SLs may stimulate their own flux from plastid to ER under low Pi. We are testing the role of SLs in low Pi induced exchange of plasma-membrane phospholipids by plastidal glucolipids and a role in flux of the plastidal monoterpene biosynthesis pathway.

101 - IDA and IDA-LIKE peptide ligands and their receptors: master regulators of cell separation events

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Plant architecture and reproduction are dependent on generation of new organs, but also on cell separation events. In *Arabidopsis* the peptide ligand INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) and its receptors, the leucine-rich repeat receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2), control the shedding of floral organs (Butenko et al., Plant Cell 2014). *IDA* and *IDA-LIKE (IDL)* genes encode proteins with a conserved C-terminal motif encompassing 12 amino acids shown for IDA to constitute a highly efficient hydroxyprolinated peptide that can activate and bind these receptors. Intriguingly, IDA/IDL-HAE/HSL signalling is also at work in cell separation processes the root. IDA and its receptors control separation of the endodermal, cortical and epidermal cells overlaying lateral root primordia and facilitate their emergence (Kumpf et al., PNAS, 2013; Aalen et al., JXB, 2013). The columella root cap protects the root apical meristem during the root"s penetration of the soil. We have genetic and biochemical evidence for a novel IDL-HSL peptide-ligand receptor pair involved in sloughing and renewal of columella root cap tiers. Phylogenetic analyses demonstrate the presence of the *IDL* and *HSL* gene families in all angiosperms. We hypothesize based on expression data that IDL/HSL signalling in general function as master regulator of cell separation events, and thus may also control seed, fruit and leaf abscission in other species than Arabidopsis, including important crops.

Thematic concurrent session: Natural variation and evolution

102 - Epigenetic variation in A. thaliana

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Epigenome modulation potentially provides a mechanism for organisms to adapt, within and between generations. However, neither the extent to which this occurs, nor the mechanisms involved are known. Here we investigate DNA methylation variation in Swedish *Arabidopsis thaliana* accessions grown at two different temperatures. Environmental effects were limited to transposons, where CHH methylation was found to increase with temperature. Genome-wide association studies (GWAS) revealed that the extensive CHH methylation variation was strongly associated with genetic variants in both *cis* and *trans*, including a major *trans*-association close to the DNA methyltransferase CMT2. Unlike CHH methylation, CpG gene body methylation (GBM) was not affected by growth temperature, but was instead correlated with the latitude of origin. Accessions from colder regions had higher levels of GBM for a significant fraction of the genome, and this was associated with increased transcription for the genes affected. GWAS revealed that this effect was largely due to *trans*-acting loci, many of which showed evidence of local adaptation.

103 - Allele landscape shifts and convergent evolution associated with serpentine colonization and whole genome duplication in Arabidopsis arenosa

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Evolutionary genomics allows us to identify alleles that may be important in adaptation and to understand how selection shapes genomes. Comparative studies allow us to ask how repeatable genomic responses to selection are in independent adaptations, as well as how previous selection events might influence subsequent ones. Here, we use evolutionary genomics coupled with elemental profiling to assess how autotetraploid *Arabidopsis arenosa* adapted to a multi-challenge serpentine habitat. We find that serpentine-endemic plants exhibit highly altered elemental accumulation levels and discover evidence for multiple selective sweeps, indicating a polygenic, multitrait basis for serpentine adaptation. Comparing to a serpentine colonization in *A. lyrata*, we find that selection acted on 15 of the same genes in these two species, a striking example of convergent evolution. Finally, in *A. arenosa*, earlier adaptation to whole genome duplication (WGD) and more recent colonization of serpentine reveal that selection acted on similar ion homeostasis processes in each, and identical polymorphisms show progressive increases in frequency through these serial events. Thus, increases in relevant alleles due to adaptation to WGD may have facilitated colonization of challenging habitats by bringing relevant alleles to higher frequency. We propose that the adaptability of some polyploid lineages may be rooted in their prior genetic adaptation to physiological challenges resulting from WGD.

104 - Cytonuclear co-adaptation impacts ecologically relevant phenotypic traits in the annual plant Arabidopsis thaliana

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Co-adaptation of organelle genomes (mitochondria and plastids) with the nucleus results from the fine-tuning of the cooperation between genomes for the optimal functioning of the crucial functions operating in mitochondria and chloroplasts. While often evidenced by the alterations of fitness related traits after interspecific crosses, cytonuclear coadaptation has much less been explored within species so far. In many organisms, including plants, cytoplasmic variation contributes to local adaptation, which suggests a relevant source for non-neutral cytoplasmic natural variation within species. To explore the effects of a disruption of cytonuclear coadaptation at the within-species level in plants, we produced dedicated genetic resources in *A. thaliana*. These

resources, named cytolines, combine the cytoplasmic genomes from one natural accession with the nucleus of another one. Fifty-two cytolines and eight parents were grown in semi-natural experimental conditions. Traits related to germination, phenology, resource acquisition, plant architecture and seed dispersal, seed production, survival, and response to aggressors were scored during the whole life cycle. Our results show that cytonuclear interactions effects impact more traits than solely cytoplasmic effects. We will present a comprehensive testing of pairwise cyto-nuclear epistasis designed to reveal traits impacted by a disruption of cytonuclear coadaptation.

Plenary session: Responses to the environment II

105 - Epigenetic switching during vernalization

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Plants monitor seasons in order to align the transition to reproduction with favourable environmental conditions. We have focused on how long-term temperature cues influences flowering through the process of vernalization. In Arabidopsis, the pathways that determine the need and ability to respond to prolonged cold quantitatively modulate expression of the flowering repressor locus *FLC*. Molecular analysis of these pathways has led us into the dissection of conserved chromatin mechanisms underlying epigenetic switches. Prolonged cold epigenetically silences *FLC* expression through a Polycomb-mediated cell-autonomous switch promoted by cold. *FLC* is then switched back on in developing embryos to ensure each generation of plants requires vernalization. Yet another chromatin switching mechanism determines *FLC* expression levels in the warm. The talk will describe our latest understanding of these conserved mechanisms, how they intersect to give robust and quantitative regulation of this developmental regulator and how these mechanisms have been modulated during adaptation.

106 - Insights into the molecular mechanisms enabling plants to grow out of the shade

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Arabidopsis is a typical shade-avoiding plant that displays a suite of growth and developmental adaptations in response to foliar shade, known as the Shade Avoidance Syndrome (SAS). Phytochrome B (phyB) is the primary photosensory receptor detecting competition for light from neighboring plants. The SAS involves phyB directly controlling protein abundance of Phytochrome Interacting Factors 4 and 5 (PIF4 and PIF5). PIF4 and PIF5 rapidly promote elongation growth via auxin-mediated processes and simultaneously turn on HFR1, a negative regulator of the SAS [1-3]. We are using a combination of computational modeling and experimental validation to study the SAS regulatory network. This lead to the identification of a novel role of HFR1 and further insight into the mechanism enabling PIFs to control auxin biosynthesis and sensitivity [4]. We are comparing mechanism controling hypocotyl elongation in response to shade with the molecular events underlying the shade response in leaves: petiole elongation, reduced blade growth and leaf hyponasty. To this end we developed a novel phenotyping platform allowing us to analyze leaf growth and positioning with great spatial and temporal resolution [5].

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107 - Rhythms of gene expression within day/night cycles: natural diversity and phenotypic impact.

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Most organisms have adapted to the daily rotations of the earth by evolving temporal regulation mechanisms that synchronize biological activities with cycling environmental conditions. The diversity of temporally regulated processes in plants ranges from stress resistance to metabolism, and includes developmental traits of high agricultural value. Temporal patterns of regulation are often conferred by genes whose expression oscillates on a daily basis, with peaks of expression usually occurring at the time when a gene is required to fulfil its function. Although variation in the timing of gene expression is predicted to provide adaptive benefits in natural environments, the range of this variation in natural genotypes remains unexplored, and its impact on downstream phenotypes is poorly understood. By using *GIGANTEA* (GI) as a model diurnally regulated gene and by studying its expression at high resolution in 77 Arabidopsis accessions, we found that the timing of *GI* expression does not vary to the same extent in all day length conditions. Within long days where the variation was broadest, natural alleles precisely modified the temporal waveform of *GI* expression predominantly by altering light signalling pathways rather than circadian rhythms. These changes in the *GI* expression waveform were then shown to have detectable effects on growth, thereby providing a paradigm for how changes in daily rhythms of gene expression contribute to phenotypic variation in natural genotypes.

108 - Exposure to environmental stress induces a transient epigenetic memory response in Arabidopsis

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Environmental fluctuations occur constantly to varying extents, yet rarely cause detrimental effects to the survival of organisms. Most organisms adapt slowly to novel environments by selecting genetic variants; it has been suggested, however, that induced epigenetic changes could accelerate this process especially in non-motile organisms such as plants. Here we report the identification of epigenetically labile regions of the Arabidopsis genome that are targeted by transient de novo methylation and demethylation in response to hyperosmotic stress. These epigenetic changes are associated with transient adaptive phenotypic stress responses in the direct progeny; they correlate with gene expression changes that are partially mediated by methylation-associated long-noncoding RNAs. The effects, however, are not transmitted equally through male and female sexual lineages due to an active epigenetic reprogramming operating in male gametes. Furthermore, contrary to current views, this “stress memory” is rapidly lost in subsequent generations once normal conditions resume, i.e. in the absence of stress. Our data thus collectively indicate that plants have developed a highly dynamic “short-term memory” stress response, to perceive and respond to environmental stimuli via directing transient epigenetic changes to discrete loci, enabling direct offspring with mechanisms to cope with fluctuations in the environment.

109 - Active 5' splice site regulates the efficiency of biogenesis of Arabidopsis microRNAs derived from introncontaining genes

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We showed previously that intron and active 5" splice site stimulate the accumulation of microRNAs encoded within the first exon of an intron-containing *MIR* gene. Our model assumed interactions between the plant microprocessor and U1 snRNP components. Here, we show that the SERRATE protein (the key component of plant microprocessor) interacts with selected U1snRNP proteins. Is there also a crosstalk between the spliceosome and miRNA microprocessor during plant intronic microRNA biogenesis? We selected miR402 located within the first intron of a protein coding host gene and found upregulation of the mature miR402 level in heat-stress conditions. Its accumulation was correlated with the inhibition of splicing of miR402-carrying intron and intronic proximal polyA site selection. We generated constructs containing the miR402 host-gene with mutated splice sites of the first intron and transiently expressed them in *N. benthamiana* leaves. The strong accumulation of the mature miRNA was observed only in the case of constructs carrying mutated constitutive 5"ss. The results of our experiments confirmed the strong competition between U1snRNP and plant microprocessor in the case of miR402 processing: efficient splicing of miRNA-carrying intron results in lower accumulation of mature miR402. Our results show new ways of plant *MIR* genes expression regulation and consequently regulation of their target genes.

Plenary session: Reproduction: from flowering to seeds II

110 - EPIGENETIC MECHANISMS IN THE ENDOSPERM DRIVE PLANT SPECIATION

KÖHLER Claudia[1], KRADOLFER David[1], WOLFF Philip[1], MORENO-ROMERO Jordi[1], SCHATLOWSKI Nicole[1], JIANG Hua[1], REBERNIG Carolin (1, LAFON-PLACETTE Clement[1]

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Polyploidization is a widespread phenomenon among plants and is considered a major speciation mechanism. Polyploid plants have a high degree of immediate post-zygotic reproductive isolation from their progenitors, as backcrossing to either parent will produce mainly nonviable progeny. This reproductive barrier is called triploid block and it is caused by malfunction of the endosperm. Our work has revealed that deregulated parent-of-origin specific genes (imprinted genes) are causal for the response to interploidy hybridizations, revealing an epigenetic basis of this phenomenon. I will discuss epigenetic changes in response to interploidy hybridizations and their consequences for endosperm development. I will furthermore discuss an epigenetic method for the generation of viable triploids, providing an impressive example for the potential of epigenome manipulations for plant breeding. Lastly, I will discuss a recently evolved interspecies hybridization barrier in the genus Capsella that reveals striking similarities to interploidy hybridization barriers, suggesting a common mechanistic basis.

111 - Genetic and Epigenetic Control of Meiotic Recombination

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During meiosis homologous chromosomes pair and undergo reciprocal genetic exchange, termed crossover. This process of genetic recombination has a profound effect on patterns of genetic diversity within sexually reproducing species, in addition to being a vital tool during crop breeding. The frequency of crossover is highly variable along the length of chromosomes and is typically focused in narrow hotspots. We are using a combination of genetic approaches to investigate the control of meiotic recombination distributions along Arabidopsis chromosomes. I will present data showing the influence of natural genetic variation on recombination patterns, including evidence for both cis and trans modifiers. Additionally, epigenetic modification of chromatin plays a major role in shaping crossover distributions. At the fine-scale crossovers are focused at hotspots associated with gene promoters and terminators. In contast, heterochromatic repeats such as transposons are suppressed for crossover. I will show data demonstrating that distinctive epigenetic modifications associated with genes versus repeats are critical for determining these opposite recombination profiles. Together an understanding of the genetic and epigenetic factors that influence recombination will allow this process to be manipulated in ways that are beneficial to crop breeding and genome engineering.

112 - Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate

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Reprogramming of cell identities during development frequently requires changes in the chromatin state that need to be restricted to the correct cell populations. Here we identify an auxin hormone-regulated chromatin state switch that directs reprogramming from transit amplifying to primordium founder cell fate in *Arabidopsis* inflorescences. Upon auxin sensing, the MONOPTEROS transcription factor recruits SWI/SNF chromatin remodeling ATPases to increase accessibility of the DNA for induction of key regulators of flower primordium initiation. In the absence of the hormonal cue, auxin sensitive Aux/IAA proteins bind to MONOPTEROS to block recruitment of the SWI/SNF chromatin remodeling ATPases and to recruit a co-repressor/histone deacetylase complex. This creates a repressive chromatin state. This simple and elegant hormone-mediated chromatin state switch is ideally suited for iterative flower primordium initiation and likely orchestrates additional important auxin-regulated cell fate transitions.

113 - YODA Signaling in the Arabidopsis Embryo

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In *Arabidopsis thaliana*, the fertilized egg cell or zygote elongates approximately three-fold before it divides asymmetrically. This first zygotic division marks a crucial cell-fate decision as the two daughter cells not just differ in size but also follow different developmental routes. While the smaller apical cell forms the spherical pro-embryo, the cells of the basal lineage continue to divide horizontally to form the stalk-like suspensor. Zygote elongation and suspensor formation are regulated by a MAP kinase signaling pathway including the MAPKK kinase YODA (YDA). Little is known, how YODA is differentially activated in the two daughter cells of the zygote but the membrane-associated receptor-like cytoplasmic kinase SHORT SUSPENSOR (SSP) seems to play a central role in this process. *SSP* expression is tightly regulated by an intriguing mechanism: *SSP* transcripts accumulate specifically in sperm cells, while the SSP protein can only be detected transiently after fertilization. This suggests that *SSP* transcripts are inherited during the fertilization events and are then translated into protein in the zygote. Here we present our latest data, how SSP expression is post-transcriptionally regulated in sperm cells. Furthermore, we will present novel pathway members that form a second, possibly SSP-independent signaling input of the embryonic YODA pathway including a putative receptor complex. This receptor kinase pathway most likely resembles the evolutionary older state with *SSP* as a relatively new, *Brassicaceae*-specific addition.

Thematic concurrent session: Abiotic stresses

114 - Environmental control of Arabidopsis leaf growth

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Plant growth is a complex process, continuously fine-tuned by the environment and actively inhibited under various abiotic stresses such

as drought. We aim to get insight in how plants reduce leaf growth in response to stress by identifying the regulators of this process. We developed an *in vitro* stress assay based on mannitol-supplemented growth medium, reducing leaf growth similarly as mild drought. This *in vitro* proxy enabled us to unravel the successive steps of the pathway underlying leaf growth inhibition. The response consists of a complex regulatory network involving crosstalk between the phytohormones ethylene and gibberellins. In parallel, we unravel the drought response in soil using the WIWAM automated machine to precisely control drought regimes. Combining both approaches showed that, although early regulators might not be the same *in vitro* and in soil, more general growth mechanisms uncovered *in vitro* can successfully be used to improve growth under mild drought.

115 - Mechanosensitive MscS-Like10 channel mediate oscillatory perception in Arabidopsis

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For plants, wind represents the major stimulation responsible for recurring mechanical load from the environment. Mechanical stimulation induces short-term cellular responses, such as signaling components variations, activation of mechanoresponsive genes followed by long-term responses consisting in structural reinforcement. At whole-plant level the effect of repetitive mechanical stimulation produces plant shortening, increasing of stem and trunk diameter. However at cellular level, perception and transduction of oscillatory mechanical stimulation is still elusive. Here, we show that Mechanosensitve channel Small conductance-Like 10 (MSL10) contributes to frequency perception in plant. We show that MSL10 channel is rapidly activated and deactivated upon pulse membrane stretching. The channel activity is modulated by sinusoidal pressure stimulation at different frequency (0.3 Hz to 30 Hz) with a higher open probability upon oscillatory than during sustained stimulation. At plant level, performing oscillatory mechanical stimulation of Arabidopsis stem, we evidenced the responsiveness of mechanoresponsive genes (ZAT7, ZAT12 and TCH4). Using knock-out mutant and overexpressing lines, we identify MSL10 to be necessary for ZAT7 gene activation by stem oscillation. Our results demonstrate that MSL 10 behave as a frequency transmitter. Therefore MSL10 is, to our knowledge, the first example of mechanosensor *per se* involved in early frequency perception in plant so far.

116 - Arabidopsis Responses To Nitrogen Availability Are Regulated By The Transcription Factors Nin-Like Protein 7 (NLP7) And NLP6.

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Nitrogen (N) is an essential element for plant growth. Nitrate, beside its role as nutrient, acts as a signal molecule triggering many adaptive responses to changes in N availability. In particular, nitrate triggers important modifications of gene expression, the so called primary nitrate response. How such nitrate specific mechanisms are regulated at the molecular level is poorly understood. We identified the Arabidopsis NIN-like protein 7 (NLP7), a member of the RWP-RK family of putative transcription factors, as an important element involved in the adaptation to N availability and as the major player of the primary nitrate response (Castaings et al. Plant J, 2009; Marchive et al. Nature Comms, 2013). The Arabidopsis *NLP* gene family contains 9 members, including *NLP6* which is very closely related to *NLP7* and recent data showed that all NLPs can bind to a nitrate-responsive cis-element (NRE, Konishi & Yanagisawa, Nature Comms, 2013). We show that indeed the double mutant *nlp6nlp7* displays an important growth delay compared to the single mutants which is partly dependent on the presence of nitrate whereas *nlp6* single mutants display only slight modifications of the various responses to nitrate. Overexpression of NLP6 in the *nlp7* mutant background can partly compensate for the loss of NLP7. Altogether, we propose NLP7 as the master regulator of early nitrate signaling, with NLP6 involved in the responses to N availability with slightly redundant functions to NLP7.

117 - Genetics dissection of the Arabidopsis root growth response to low-phosphate

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The Arabidopsis primary root growth is rapidly inhibited when grown on a low-phosphate (Pi) synthetic medium. We used a classical genetics approach to identify genes involved in this growth response. By QTL analysis we identified the *LPR1* gene which inhibits the primary root growth in low-Pi. LPR1 is a multicopper oxidase that genetically interacts with PDR2 (a P-type-5 ATPase), and both proteins are located in the endoplasmic reticulum. We observed that expression of *LPR1* in only few specific cells of the primary root tip is sufficient to fully inhibit root growth in low-Pi conditions. Starting from a genetic screen for EMS mutants with altered root growth in low-Pi, we identified STOP1 and ALMT1, two proteins already known for their role in root response to low-pH and toxic Al3+. STOP1 is a transcription factor regulating the expression of *ALMT1,* which encodes for a malate efflux transporter. We showed that STOP1 goes to the nucleus, binds to the promoter of *ALMT1* and that a dominant negative Stop1 mutant is fully complemented by constitutively expressing *ALMT1.* We will show more details about the characterization of these different mutants and genes.

118 - IRON MAN, a novel peptide family activates iron uptake in shoots and roots of angiosperms

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Root iron (Fe) uptake is adjusted to the plant demand through complex signaling pathways. Several transcription factors directly activate Fe uptake genes, but the cascade leading to their transcriptional activation is yet to be deciphered. We identified genes encoding peptides of unknown function that are up-regulated in several plant species when subjected to Fe deficiency. Constitutive expression of these genes caused over-accumulation of Fe, zinc and manganese in roots and leaves, facilitated Fe loading into seeds, and induction of Fe acquisition genes in roots. We named these genes *IRON MAN (IRON AND MANGANESE ACCUMULATOR*). Heterologous expression of *AtIMA1* in tomato resulted in a phenotype similar to that of Arabidopsis lines. IMAs do not possess any known functional domain but proteins with similar size and a highly conserved amino acids motif are present in all angiosperms. Proteins harboring partial deletion of the conserved motif were non-functional whereas deletions of other parts of the sequence did not affect its function. AtIMA3 shares little identity with AtIMA1 except for the conserved motif, and also triggered a Fe deficiency response in Fe-replete *Arabidopsis* plants. Thus, the conserved motif is critical for IRON MAN function. While the molecular mechanism underlying IRON MAN function remains elusive, we uncovered a novel peptide family that promotes Fe deficiency response in both shoots and roots through a mechanism that is common among angiosperms.

Thematic concurrent session: Systems biology and new approaches

119 - Is phyllotaxis deterministic or stochastic ?

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Over the last two centuries, multidisciplinary research on phyllotaxis has led to a common deterministic explanation of the striking symmetries displayed by the arrangement of organs along stems. In this view, recently created primodia at the tip of plant axes inhibit the initiation of new primordia in their vicinity by depleting auxin. Due to growth, these already existing primordia get progressively away from the initiation zone, leaving periodically space for new initiations at the tip. This deterministic scheme is now widely accepted as the "standard model" of phyllotaxis. In recent years, however, several studies have reported perturbations and abnormalities in the phyllotaxis patterns of different plants and mutants. In several cases, these perturbations have been identified as permutations of varying complexities of organs along the stem. Here, I will present how the standard model can be revisited to integrate stochasticity and locality as central components of the system to account faithfully for these observations. I will also discuss the predictions and consequences of such a new stochastic view of phyllotaxis.

120 - Towards an integrative analysis of Arabidopsis leaf margin development

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Leaves show a tremendous diversity in their sizes and shapes. Though, they all originate as small, finger-shaped primordia at the flanks of stem cells-containing groups of undifferentiated cells, the meristems. Leaf shape is established later during development, mainly as a result of differential growth of their margins. *CUP-SHAPED COTYLEDON (CUC)* genes have emerged during the last years as essential determinants of leaf margin patterning and growth. Nevertheless, how these genes control leaf morphogenesis remains mostly unclear. Here, we will present an integrative approach aiming at providing a better understanding of leaf development that we will exemplify by analysing the role of the *CUC* genes during leaf development. We have developed a software that allows reconstructing the developmental trajectories of developing leaves and defining a framework on which cellular or molecular data can be mapped. We have developed an imaging analysis method that allows extracting cellular parameters in developing leaves. To investigate gene activity, we have developed a set of reporter lines and quantification tools, which we have used to precisely determine the contribution of the microRNA miR164 to the regulation of *CUC2* expression during leaf development. Finally, we will present progress towards the identification of the CUC-centred gene regulatory network controlling leaf development.

121 - ePlant: An agile development approach to visualizing multiple levels of biological data.

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The Arabidopsis community has generated numerous large data sets for exploring multiple facets of plant biology. Tools for visualizing this data have flourished over the past several years. The vast majority of these tools are designed to visualize a single level of data such as gene expression patterns, protein-protein interactions, molecular models, and genomic sequences. Since most visualization tools have their own user interface and methods for selecting and loading data, integrated multi-level research requires a significant learning curve. An integrated tool that visualizes multiple levels of data from macro to micro, all on the same screen with a unified user interface, can lead to better hypothesis generation by helping researchers make connections between biological levels of organization that are normally considered separately. We present ePlant 2.0 , an effort to leverage current thinking about data visualization and user experience design to help Arabidopis researchers explore multiple levels of data at the same time. We have adopted an agile development process, making iterative changes based on community feedback and suggestions. User testing at ICAR 2014 led to several new features: RSVP (rapid serial visual presentation) display, loading genes by describing a desired expression pattern, multiple tab views, heat map overviews, and others (to be presented). One more round of user testing is planned for ICAR 2015 before ePlant will be launched officially on the Bio-Analytic Resource at bar.utoronto.ca and with Araport.org.

122 - GENETIC ENGINEERING OF ABIOTIC STRESS RESPONSE AND GROWTH IN PLANTS

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Genome editing is utilized in genome modification by using CRISPR/Cas9 and becomes more widely in various organisms. We designed several sequences as gRNAs with low risk for off-targets for the target genes that function in signal transduction pathways and stress responses in Arabidopsis and tomato. We generated the CRISPR/Cas9 cassettes using these sequences and the plant codon-optimized Cas9 driven by the constitutive promoter. The Cas9 and gRNA expression levels were monitored in the each transgenic line, and the selected plants or calli were used for the detection of newly generated mutations using Cel-1 assay and sequence analysis. The results showed that the mutations were generated in the target genes with the 12.5-60% efficiency. The reduction of the number of nuclear localization signals decreased the mutation efficiency. The mutations were also detected in the next generation in Arabidopsis and in the newly developed tomato shoots. Our data suggested that the CRISPR/Cas9-mediated mutation was successfully obtained in both Arabidopsis and tomato. Using our genome editing systems, we will focus on the further applications of molecular breeding to improve plant growth and stress responses.

123 - From gene expression modeling to gene network to investigate Arabidopsis thaliana genes involved in stress response

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The gap between the structural annotation of a genome and the functional one still remains wide. Recent studies have estimated that 20% to 40% of the predicted genes have no assigned function in eukaryotic organisms whose genome is completely sequenced. Transcriptome data allow investigating the gene behaviors and co-expression studies have rapidly been considered as a way to identify sets of candidate gene modules. Generally co-expression is established by analyzing correlations between all gene pairs in multiple microarray experiments collected from public repositories. Such approaches may suffer from both heterogeneity of data and the choice of the clustering method, usually based on gene pairs. Tackling these limitations, we propose an analysis based on a large and homogeneous set of transcriptome data extracted from CATdb: 387 stress conditions organized into 9 biotic and 9 abiotic stress categories. Instead of correlation analysis, a model-based clustering was applied to identify clusters of co-expressed genes per stress category. Various resources were then analyzed and integrated to characterize functions associated with genes in these clusters. Protein-protein interactions and transcription factors-targets interactions were exploited to display gene networks. All the results are stored and managed in GEM2Net, a new module of CATdb (Zaag et al., 2015). We are currently demonstrating that this resource provides a valuable starting point to study stress responses and to propose a high-throughput functional annotation of *Arabidopsis thaliana* genome.

124 - Algorithm application to identify novel regulators in the Arabidopsis thaliana iron deficiency response

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Iron deficiency is a significant issue for agriculture and human sustainability. Plants have evolved to respond to iron deficiency through strategies that make iron more available in the soil and maintain required but not toxic levels of iron within a plant. This critical balance requires precise and complex regulation via gene regulatory networks. We are using a collaborative systems biology approach to unravel the regulatory responses following exposure to iron deficiency. The basis of this project is a time-course microarray dataset of global transcriptional response in the *Arabidopsis thaliana* root following the shift of seedlings from iron sufficiency to deficiency. Computational approaches that have been used to extract causal relationships from such datasets are limited by sparse time points and low signal levels. We address these limitations by developing the Cluster and Differential Alignment Algorithm (CDAA). This algorithm was utilized to identify novel transcription factors, previously unlinked to iron homeostasis, that influence the activity of known regulators of the iron deficiency response. A network of candidate regulator-target relationships was validated using qRT-PCR and Y1H analysis. The CDAA, therefore, represents a useful tool to highlight missing regulatory nodes as well as to make specific regulatory predictions. The results of this project extend beyond the immediate field of iron nutrition and can inform similar systems biology approaches.

Thematic concurrent session: Epigenetics

125 - Heat stress responses in Arabidopsis nuclei

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Acute heat stress in plants induces fast signalling cascades and production of heat-protective factors. In *Arabidopsis thaliana*, adapted to habitats with rather moderate temperatures, enduring heat stress conditions result also in activation of additional sets of genes, including repetitive elements, which are otherwise transcriptionally silent. The classical epigenetic features like DNA methylation and histone modification are affected only marginally, while nucleosome occupancy and chromatin organization within the nucleus undergo substantial changes, possibly modifying the accessibility of DNA for transcription. We study the effect of heat stress on nuclear organization, applying high resolution imaging. The results show that nuclear architecture in *Arabidopsis thaliana* is dynamic and responsive to environmental stimuli. Although most of these changes are transient, they have the potential to result in lasting effects in some cells, but so far we could not find evidence for effects on growth or phenotype in subsequent generations.

126 - With a little help from my friends: Telobox binding proteins assist Polycomb Group protein complexes in the repression of target genes

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The mechanism underlying Polycomb-mediated repression has been understood as linear process in which the writer complex PRC2 sets the first modification, H3K27me3, while recognition of this mark by the reader complex PRC1 leads to chromatin compaction. Recent data have challenged this rather simple view. In Arabidopsis, in particular the function of the unique PRC1 chromodomain component, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) remains a conundrum: although LHP1 is encoded by a single copy gene; phenotypic changes in lhp1 mutants are mild compared to those observed in other PRC1 and PRC2 component mutants. We carried out a genetic enhancer screen in the lhp1 background to identify components acting either redundantly or independently of LHP1. Two causal mutations were mapped to Myb-domain transcription factors previously shown to associate with telomeric repeats. By ChIP-seq analysis, we showed that these factors bind to thousands non-telomeric sites and that teloboxes or related cis-elements are strongly overrepresented in the peak regions. These were also found overrepresented when we compared global seedling distribution of H3K27me3 in a panel of mutants. While lhp1 mutants, as previously reported, showed very little variation in H3K27me3, this was different for curly leaf (clf) mutants which were affected at almost thousand genes. Enrichment of telobox-like elements was strongest for genes showing reduced H3K27me3 levels. We present data supporting a model by which telobox-binding factors act redundantly with LHP1 to repress target genes. Since presence of LHP1 prevents access to many target sites, the telobox-binding factors are likely to act as repressive back-up. In contrast, the correlation of telobox-like elements with fragile H2K27me3 in the clf mutant points toward an antagonistic role of these elements with gene repression. Our current work aims to combine these observation to present an unified model of the interaction of telobox-like elements with the PcG-pathway.

127 - Role of Polycomb repressive pathways in regulating cell differentiation in Arabidopsis

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Studies in the model plant A*rabidopsis thaliana* have provided important insights into chromatin components and regulatory pathways as well as epigenome organization. The emergent picture is one of conserved mechanisms between plants and animals with plant-specific innovations related to their developmental and life strategies. Our current efforts aim at understanding the role of chromatin-based mechanisms in the regulation of cell differentiation using the Arabidopsis root as a model, with an emphasis on the transcriptional switches mediated by Polycomb repressive pathways. To this end, cell-type specific approaches are combined with genetics and comparative epigenomics in order to determine the dynamics of PRC2 activity and its relation to transcriptional regulation at successive stages of the differentiation process, from stem cells to terminally differentiated cells. Our goal is to characterize the regulatory principles associated with Polycomb-mediated repression and its interplay with the gene regulatory networks underpinning cell fate specification and maintenance of cell identity during root development.

128 - Unraveling How Transposable Elements Mind The Post-Transcriptional To Transcriptional Silencing Gap

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In plant genomes the vast majority of transposable elements (TEs) are found in a transcriptionally silenced state that is epigenetically propagated from generation to generation. Although the mechanism

of this maintenance of silencing has been well studied, it is now clear that the pathways responsible maintaining TEs in a silenced state differ from the pathways responsible for initially targeting the TE for silencing. Recently, attention in this field has focused on investigating the molecular mechanisms that initiate and establish TE silencing. My laboratory has focused our attention on 21-22nt siRNAs produced from Pol II transcripts via endogenous RNAi, and their role in the establishment of DNA methylation and chromatin modification. Using deep sequencing of small RNAs and DNA methylation patterns, for the first time we have generated a comprehensive view of the various pathways responsible for degrading TE transcripts into siRNAs and how these different pathways target DNA methylation. Our results demonstrate how siRNAs generated from post-transcriptional silencing inform the cell which regions of the genome are TEs and should undergo chromatin modification and transcriptional silencing.

129 - Light signaling controls nuclear architecture reorganization and massive chromatin state changes during seedling development

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The impact and extent of chromatin dynamics associated with light-driven developmental transitions are poorly understood. We investigated chromatin organization as well as functional aspects of histone modifications for transcriptional regulation during de-etiolation. First, we observed that global engagement in transcription of RNA Pol II increased, suggesting that cotyledon photomorphogenesis involves a transition from globally quiescent to more active transcriptional states. Accordingly, chromatin states of hundreds of genes were subjected to extensive changes within the first hours of light exposure, a process that frequently contributed to their optimal induction. We also found that light triggers a switch between two different nuclear architectural schemes during cotyledon development: nucleus expansion and higher-order heterochromatin organization dynamics are initially similar under light and dark conditions during germination, but later steps leading to "mature" nuclear morphology are light-dependent and involve DET1 and COP1 integrators as well as cryptochrome-mediated photoperception. Heterochromatin rearrangements appear to be independent of nuclear size variations and of DNA methylation-based processes. This multilevel study allows us to propose that light-triggered changes in chromatin states and in nuclear architecture underlie interplays between chromatin reorganization and transcriptional reprogramming associated with the establishment of photosynthesis.

130 - New roles for double-stranded RNA binding proteins in plants

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Animal double-stranded RNA binding proteins (DRBs) are involved in numerous cellular mechanisms ranging from localization and transport of messenger RNAs, through maturation and degradation of RNAs, viral response, signal transduction and RNA interference processes. In comparison, little is known on plant DRBs. Arabidopsis DRB1 and DRB4 are the most studied, and both proteins are cofactors of DICERs involved in small RNA biogenesis. To learn more on plant DRBs, we retrace their evolutionary history and identify two new families (DRB6 and DRB7), one of which turned out to be a DCL4 cofactor involved, with DRB4, in epigenetically activated siRNAs (easiRNA) biogenesis. We have previously shown that the loss of DRB2 results in a significant increase in the population of RNA polymerase IV (p4) dependent siRNAs, which are involved in the RNA-directed DNA methylation (RdDM) process. We therefore decided to explore the putative role of DRB2 in RdDM. Surprisingly, we observed that DRB2 is part of a high molecular weight complex that does not involve RdDM actors but several chromatin regulator proteins, such as MSI4, PRMT4B and HDA19. We show that DRB2 can bind transposable element (TE) transcripts in vivo but that *drb2* mutants do not have a significant variation in TE DNA methylation. We propose that DRB2 is part of a repressive epigenetic regulator complex involved in a negative feedback loop, adjusting epigenetic state to transcription level at TE loci, in parallel of the RdDM pathway. Loss of DRB2 would result in an increased production of TE transcripts, readily converted in p4-siRNAs by the RdDM machinery. This represents the first indication that a DRB protein could help the targeting of an epigenetic complex by binding to nascent transcripts.

Plenary session: Keynote lecture

131 - Heterochromatin reprogramming with histone variants and small RNA

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Epigenetic inheritance is more widespread in plants than in mammals, in part because mammals erase epigenetic information each generation by germline reprogramming. To assess the extent of germline reprogramming in plants, we sequenced the methylome from sperm cells (SC), the vegetative nucleus (VN), and the precursor microspore from developing haploid pollen. We found that asymmetric CHH methylation is lost in microspores and sperm cells, but restored in the VN and in fertilized seed. In the VN symmetric CG methylation is lost from targets of the DNA glycosylases DEMETER (DME) and REPRESSOR OF SILENCING 1 (ROS1) including transposons near imprinted genes, which contributes to imprinting via RNA directed DNA methylation and 24nt siRNA. In contrast, most active transposons give rise to 21nt "epigenetically activated" small RNA in DECREASE IN DNA METHYLATION 1 (DDM1) mutants, in tissue culture and in the VN, which loses heterochromatin. Biogenesis of 21nt epigenetically activated siRNA requires miRNA and RNA DEPENDENT RNA POLYMERASE 6. Loss of heterochromatin in the VN is not the direct result of loss of DNA methylation but instead may be due to histone replacement with variants resistant to modification. Thus genome reprogramming in pollen contributes to epigenetic inheritance, transposon silencing, and imprinting, guided by small RNA.

POSTER ABSTRACTS

Primary metabolism, photosynthesis, biomass

Posters 001 to 027

P001 (Talk) - A Genome Scale Metabolic Model of Arabidopsis helps to understand metabolic role of SBPase and FBPase.

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The level of sedoheptulose-bisphosphatase (SBPase) and fructose-bisphosphatase (FBPase) activity in chloroplast affects the photosynthetic performance along with altered starch accumulation in the plants, but there are no clear explanations to this relation. We use Flux Balance Analysis (FBA) on a GSM of Arabidopsis to investigate the metabolic significance of these enzymes. We removed reactions catalysed by these enzymes, individually and in combination, from the model, to identify the potential physiological impact. The results suggest that individual knock out of SBPase or FBPase will not be lethal to the plant as each of them in conjunction with transaldolase can maintain the flux through the regenerative limb of the Calvin cycle. In fact dual knockout of SBPase and FBPase will also be possible; in this case the flux through the regenerative limb in the chloroplast is maintained by transaldolase in conjunction with cytosolic isomer of FBPase. Change in flux values to all the reactions responsible for starch production was noted in all mutants and compared to wild type solution. We also performed the gene knockout experiments to study the growth of mutant plants. Though with compromised growth, homozygous SBPase and FBPase knockout mutants were found to be viable. Experimental validation for the dual knockout is currently in progress.

Funding: The AccliPhot consortium (http://accliphot.eu/), EU's 7th Framework Programme through the grant agreement PITN-GA -2012-316427.

P002 - Identification of a triacylglycerol-lipase by functional proteomics

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Hydrolysis of storage oil is the first committed step to carbon reserve mobilization in oil-seeds and is catalyzed by a triacylglycerol (TAG) lipase. In Arabidopsis, the SDP1 locus codes for a TAG lipase which is bound to oil bodies. This lipase has been shown to control fat storage breakdown in seedlings as the vast majority of the oil is not hydrolyzed during post-germinative growth of the *sdp1* mutant[1]. However, SDP1 lipase represents less than 20% of total TAG lipase activity. We have used a functional proteomic approach to identify the TAG lipase responsible for the main activity recorded in *Brassica* napus seedlings as previously described [2] . TAG lipase activity (measured on triolein as a substrate) was enriched about 100-fold from a 4-day-old seedling extract. It was labeled with radioactive tetrahydrolipstatin, an inhibitor that binds covalently to lipase active serine residue. A single, 42 kDa band was labeled and the protein identified by mass spectrometry. Analysis of a mutant for the orthologous gene of Arabidopsis showed a > 90% reduction in soluble lipase activity from seedling extract. Further characterization of this enzyme will be presented.

References: Eastmond PJ (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell 18: 665-675

Dhouib R, Laroche-Traineau J, Shaha R, Lapaillerie D, Solier E, Ruales J, Pina M, Villeneuve P, Carriere F, Bonneu M, Arondel V (2011) Identification of a putative triacylglycerol lipase from papaya latex by functional proteomics. Febs J 278: 97-110

P003 - The influence of the Target of Rapamycin (TOR) on starch metabolism in Arabidopsis thaliana

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The target of rapamycin (TOR) kinase is a central coordinator of nutrient, energy, and stress signaling networks. Attempts to decrease the activity of the components of TOR complex in plants revealed broad changes in primary metabolism, including carbon partitioning and a starch excess phenotype. The main goal of this work is to elucidate the influence of TOR on the diurnal metabolism of starch in leaves. Therefore, *Arabidopsis thaliana* col-0 seedlings were grown hydroponically for about 12 days prior to treatment with the specific ATP-competitive inhibitor AZD-8055 (TOR repressor) or DMSO (control), applied at dawn and dusk in parallel experiments. Samples were collected every 6 hours along the day-night cycle to identify if the starch excess is due to increased starch synthesis or impaired starch degradation. Preliminary results indicated that AZD treated plants have reduced starch degradation rates independently from the time of drug administration (end of the day or end of the night, 12 or 24h of treatment, respectively). Furthermore, metabolite profiling analysis is ongoing to look for changes in primary metabolism. These preliminary results will be useful as starting point to characterize the mode of action of TOR on starch metabolism and to dissect its function in plant metabolism.

P004 - Identification of New Components Involved in Chlorophyll Biosynthesis and Turnover

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Chlorophylls are predicted to be dephytylated during several important biological processes within the chloroplasts. However, the identities of the dephytylating enzymes involving in most of these processes remain elusive. Here, we show that an Arabidopsis locus encodes an enzyme with chlorophyll dephytylating activity, hence named *CHLOROPHYLL DEPHYTYLASE1 (CLD1).* A missense mutation of *CLD1*, causing the replacement of the conserved Gly193 by Asp, was mapped in a semi-dominant heat-sensitive mutant (*cld1-1*). Introducing this mutant allele into the wild-type background recapitulated the heat-sensitive phenotype. CLD1 contains putative transit peptide and shares homology with pheophytinase that dephytylates pheophytin but not chlorophyll during leaf senescence. CLD1-GFP fusion proteins were found in chloroplasts when transiently expressed in protoplast; subcellular fractionation showed that CLD1 was associated with the thylakoid. Recombinant CLD1 without the transit peptide dephylated chlorophyll a/b but not pheophytin in vitro; the G193D mutant enzyme showed substantially higher activity; mutation in the putative hydrolase motif abolished the enzyme activity. In seedlings harboring the *cld1-1* allele, chlorophyllide a/b were highly accumulated after heat treatment, suggesting CLD1 dephytylates chlorophylls in vivo. Manipulation of CLD1 expression perturbed the homeostasis of chlorophyll *a* and *b* under normal and heat stress conditions, suggesting a role of CLD1 in chlorophyll biosynthesis and turnover.

P005 - Investigation of a novel peroxisomal transport protein in Arabidopsis thaliana

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We identified a novel transport protein in Arabidopsis thaliana termed PERTL6 that is located in peroxisomes. Here we report a detailed phenotypic analysis of two Arabidopsis T-DNA insertion lines lacking PERTL6. Since microarray analysis revealed that PERTL6 gene expression is highly up regulated upon heat stress, we demonstrated that both independent *pertl6* mutant plants were unable to recover after heat treatment. In addition, we observed reduced root and hypocotyl growth in the absence of sucrose, indicating a defect in fatty acid oxidation. However, the conversion of the synthetic auxin precursor was unaffected

in these mutant lines, suggesting that Y-oxidation itself is not impaired by loss of PERTL6. Currently, we are investigating the biochemical transport function of PERTL6 using recombinant protein reconstituted into liposomes for uptake studies. Thus, our work will expand the small group of characterized peroxisomal transport proteins of this crucial, but long time neglected organelle.

P006 - The long-term adaptive response of air-grown Arabidopsis ggt1 photorespiratory mutants involves a drastic reduction in leaf RuBisCO content

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Photorespiration results from the oxygenase activity of the ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (RuBisCO). It was believed to be wasteful since this carbon (C)-recycling pathway requires ATP and releases CO_2 and NH3. However, in air, mutants deficient for photorespiratory enzymes are affected in growth and development, thus showing the importance of this pathway. In high CO_2 conditions (inhibiting photorespiration), no growth phenotypes are observed. Here, we have analyzed T-DNA insertion mutants encoding glutamate:glyoxylate aminotransferase (GGT1) that exhibit delayed growth in air, and low net CO_2 assimilation rates (A_n) due to reduced leaf RuBisCO content. Interestingly, leaf C/N (nitrogen) balance was not altered. *ggt1* mutants transferred from high CO_2 to air exhibited a rapid decrease of A_n and ATP/ADP ratio in the light, with an increase in non-photochemical chlorophyll fluorescence quenching (NPQ) without changes in either RuBisCO content or activation state. Such data suggest a limitation of RuBP regeneration due to reduced photorespiratory C-recycling in *ggt1* leaves. It is proposed that the low A_n will limit N-assimilation, which in turn will decrease leaf RuBisCO content until plants attain a new homeostatic state that maintains a constant C/N balance. The need to balance plant N demand with available assimilated C produces smaller, slower growing plants and probably involves an adaptive reprogramming of gene expression.

P007 - The role of trehalose-6-phosphate in plant metabolism and development

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In plants trehalose is synthesized via a phosphorylated intermediate - trehalose 6-phosphate (Tre6P). Tre6P has a large influence on plant metabolism, growth, and development. It has been demonstrated that Tre6P acts as a specific signal of sucrose availability. Tre6P is also proposed to act as a negative feedback regulator of sucrose levels, helping to keep these close to the optimum level for a given cell or tissue type. Here, we describe the generation of a cell- and tissue-specific system for inducible expression of heterologous Tre6P synthases (TPS) and Tre6P phosphatases (TPP). We are using this inducible system to investigate cell- and tissue-specific mechanisms of Tre6P signaling in Arabidopsis. It has been shown that sucrose plays a critical role in triggering bud outgrowth of decapitated pea plants. By measuring metabolites in axillary buds of decapitated pea (*Pisum sativum* cv. Torsdag) plants, we observed that Tre6P starts to rise in the second axillary bud within 3 hours of decapitation, indicating a potential role for Tre6P in the sucrose driven initiation of bud outgrowth. Further evidence for a role of Tre6P in the regulation of shoot branching is provided by Arabidopsis mutants with constitutive or tissue-specific changes in Tre6P content. Plants with high Tre6P, from over-expression of TPS, have increased shoot branching and reduced apical dominance, whereas lowering Tre6P by over-expression of TPP has the opposite effects.

P008 - Investigating a key metabolic step of NAD biosynthesis involved in stress resistance in Arabidopsis thaliana

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Nicotinamide adenine dinucleotide (NAD) and its derivative nicotinamide adenine dinucleotide phosphate (NADP) are essential co-factors in various metabolic events for numerous redox reactions in living organisms. Recent advances in the characterization of the *Arabidopsis thaliana* show that NAD biosynthesis genes have shed light on the significance of NAD biosynthesis in response to environmental stresses and various developmental processes. We investigate the roles of NAMNAT (nicotinate mononucleotide adenyltransferase), an enzyme which is involved in both the *de novo* biosynthesis and recycling of NAD, and whose gene is strongly induced under stress conditions. In Arabidopsis, *in silico* analyses reveal that three putative mRNAs/proteins are encoded by the single gene *At5g55810*. Work is under way to determine the subcellular targeting of the corresponding proteins and their biochemical properties, since it seems that part of the protein could carry a second enzyme activity (NMNAT; nicotinamide mononucleotide adenyltransferase), more efficient in NAD recycling, and that could play a crucial role in the metabolic adjustment that occurs under stress conditions.

P009 (Talk) - Nucleotide sugar transport: Delivering the building blocks of the plant cell wall

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Glycosylation reactions require activated glycosyl donors in the form of nucleotide sugars to drive processes such as post-translational modifications and polysaccharide biosynthesis. Most plant cell wall polysaccharides are synthesized in the Golgi apparatus from cytosolic-derived nucleotide sugars, which are actively transferred into the Golgi lumen by nucleotide sugar transporters (NSTs). We recently perfected a yeast proteoliposome transport assay coupled to LC-MS, specifically designed to screen substrates of plant encoded NSTs. We have now screened the > 40 members of the plant NST family and identified candidate transporters for 13 distinct substrates. To further support these *in vitro* assays, we have been conducting in vivo analysis of NST function in Arabidopsis using reverse genetics. These approaches have been used to recently confirm a clade of six bi-functional UDP-Rha / UDP-Gal transporters, a family of 3 UDP-Xyl transporters and a GDP-Fuc transporter. We are currently in the process of validating the in vivo functions for the other nucleotide sugar substrates. Interestingly, it appears that specific family members within a clade have distinct functional roles with regard to the biosynthesis of cell wall polymers. These observations support the notion that some NSTs likely form specific biosynthetic complexes and are involved in substrate channeling.

P010 - A plant-specific carrier involved in mitochondrial sulfate transport

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Our group has discovered a carrier localized in the mitochondrial membrane: The inorganic sulfate transporter (SIT1) catalyzing the import of sulfate into mitochondria. Sulfate is presumably required for the counter exchange with dicarboxylic acids via the mitochondrial dicarboxylate carriers (DIC). This mechanism fuels the tricarboxylic acid (TCA) cycle of mitochondria and ultimately the respiratory energy production in form of ATP. Most prominent features of Arabidopsis sit1 *knockout* lines included reduced plant and root growth, delayed flowering and an increased number of non-germinating seeds. These emerging phenotypes are thought to be a result of a reduced ATP synthesis rate. Phosphates provided for ATP synthesis were shown to act as substrates for the DIC proteins instead of sulfate, therefore the phosphate pool is shared between energy synthesis and dicarboxylic acids import leading to a highly limited ATP production. We were able to show that SIT1 plays a role in mitochondrial energy metabolism significantly contributing to the TCA cycle and the overall energy supply.

P011 - Biotechnological Improvement of Woody Biomass through Developing Xylem Preferential Gibberellin Production

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Woody biomass is gaining popularity as environmentally friendly, renewable and sustainable sources for liquid fuel production. Here we demonstrate biotechnological improvement of the quantity and quality of woody biomass by employing developing xylem (DX)-specific production of gibberellin (GA), a phytohormone positively regulating stem growth. Firstly, we produced transgenic *Arabidopsis* plants expressing GA20-oxidase from *Pinus densiflora* (PdGA20ox1) under the control of either 35S promoter (i.e., 35S::PdGA20ox1) or DX-specific promoter (i.e., DX15::PdGA20ox1), respectively. As we hypothesized, both transgenic Arabidopsis plants exhibit an accelerated stem growth and resulted in a massive increase of biomass up to 300% compared to wild-type control plants, together with increased secondary wall thickening and elongation of fiber cells. Next, we applied our proved concept into the production of transgenic poplar trees. As expected, both transgenic poplars showed dramatic increase of biomass up to 250% with accelerated stem growth and xylem differentiation. However, undesirable phenotypes of 35S::PdGA20ox1 poplar, including poor root growth and smaller leaf development, were found. Interestingly, DX15::PdGA20ox1 poplar resulted in much reduced undesirable phenotypes. Our results indicate that the controlled production of GAs through a utility promoter can be utilized as an efficient biotechnological tool for producing enhanced plant biomass minimizing undesirable effects

P012 - Phosphoregulation of photorespiratory enzymes in response to light and CO2 content in Arabidopsis thaliana.

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Photorespiration is an essential process for all oxygenic photosynthetic organisms. It allows the removal of toxic metabolites and the recycling of the carbon atoms of 2-phosphoglycolate produced by the oxygenase activity of ribulose

-1,5-bisphosphate carboxylase/oxygenase. Although photorespiration has been extensively described, nothing is known about the regulation of this cycle. A global quantitative phosphoproteomic analysis was undertaken using leaf extracts of Arabidopsis thaliana plants subjected to 4 h of light either at 100ppm, 380ppm or 1000ppm of CO_2 in 21% O_2 to modify the photorespiration rate. Light-dark changes in protein phosphorylation were also analyzed at 380ppm of CO_2. Amongst the 264 phosphopeptides showing significant changes in their phosphorylation status between different conditions, phosphopeptides corresponding to glycolate oxidase and serine hydroxymethyltransferase were detected. Hydroxypyruvate reductase and glycine decarboxylase were also found as phosphorylated enzymes but without any significant modification in their phosphorylation status. To explore the function of these phosphorylation events, phosphorylated residues were changed to either an alanine/valine or an aspartate (phosphorylation-mimic) and recombinant enzyme activities were analyzed. The expression of mutated enzymes in corresponding knock-out mutants has been undertaken. The results suggest a potential role of phosphorylation in photorespiratory cycle regulation.

P013 - Sulfur assimilation in NAD kinase overexpressing Arabidopsis plants

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Pyridine nucleotides are essential for numerous redox reactions and serve as co-factors in multiple metabolic processes in all organisms. It is known that NAD kinase (NADK) is an enzyme involved in the synthesis of NADP+ from NAD+ and ATP. Arabidopsis has three types of NADK (AtNADK1-3). AtNADK2 is a chloroplast localizing enzyme, and provides recipients of reducing power in photosynthetic electron transfer process. Our previous studies have indicated that overexpression of AtNADK2 stimulates carbon and nitrogen assimilation in Arabidopsis. In this study, we analyzed the sulfur assimilation in AtNADK2 overexpressing Arabidopsis (OX). Microarray analysis of OX plants demonstrated up-regulation of several genes whose expressions were known to be stimulated under the sulfate deficient condition. When the OX plants were grown on the MS medium supplemented with 5 mM sulfate, growth promotion and glutathione accumulation were appeared. On the other hand, OX did not show resistance to hydrogen peroxide. These data suggest that the AtNADK2-overexpression has an effect on promoting primary metabolism such as carbon, nitrogen and sulfur assimilation.

P014 - Effect of 3 CO2 concentrations on Arabidopsis growth and sugar transport.

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The predicted rising in atmospheric CO_2 raises a number of questions about the effects on plants, and controversial results on short and long-term adaptation to high CO_2 were obtained. As part of our studies on understanding phloem sugar transport and source-sink interactions, *Arabidopsis thaliana* Col0 plants were grown at 3 different CO_2 concentrations: ambient (400 ppm), 1000 and 1500 ppm. Preliminary experiments were run to determine optimal culture conditions (pot size, nutrient supply and light intensity). Plants were sown in ambient CO_2 and transfered after 7days in different CO_2 conditions and harvested after 25 and 32 days. Whereas leaf projected area slitghly decreased, the rosette biomass increased with elevated CO_2. The Speficic Leaf Area (SLA in cm2.g-1) decreased at high CO_2. Several parameters linked to photosynthesis were also studied. Stomatal conductance was higher at 1000 ppm whereas stomatal density decreased with increasing CO_2. Nevertheless, the chlorophyll content increased with CO_2 suggesting an increase in photosynthetic capacity. This was confirmed on ultra thin sections of minor veins observed in electrom microscopy : chloroplasts in sheath cells were bigger with large starch granules. Moreover the structure of the veins seemed affected as veins of plants grown at 1500 ppm had more phloem parenchyma cells than plants grown at 400 ppm. Further analyses are underway to relate these data with sugar transport.

P015 (Talk)- Evidence for a multi-functional α-1,6 galactosyltransferase in Arabidopsis seeds

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Raffinose family oligosaccharides (RFOs) are sucrosyl oligosaccharides that accumulate exclusively in plants. In Arabidopsis raffinose (Raf, Suc-Gal1) is the only RFO oligomer accumulating in leaves and roots. However, in seeds both raffinose and stachyose (Sta, Suc-Gal2) occur. While Raf biosynthesis in vegetative tissues is well described, little is known of the biosynthetic genes responsible for RFO accumulation in seeds. Despite Sta being the predominant RFO in the seeds, there are no reports concerning the molecular identity of an Arabidopsis stachyose

synthase (*SS*) or its role in RFO biosynthesis. Using both classical reverse and forward genetic-approaches we have characterised Arabidopsis *AtSS* (*At4g*01970), providing evidence that it is a functional *SS*. Mutant (*atss*) seeds accumulate no Sta but hyper-accumulate Raf 3-fold more than controls (Col-0). When we created a double mutant using the raffinose synthase mutant *atrs5*, reported to contain 50% less Raf in the seeds but normal amounts of Sta we surprisingly observed complete RFO deficiency in the seeds. This provided the first hint at multi-functionality. We subsequently constitutively over-expressed *AtSS* in the Col-0 background and were able to demonstrate that leaves unusually accumulate both Raf and Sta to concentrations of about 29 and 2 mg. gDW-1, respectively. This confirmed that *AtSS* is a multi-functional A-1,6 galactosyltransferase responsible for the biosynthesis of both Raf and Sta in Arabidopsis seeds.

P016 (Talk) - Dynamics of light-harvesting antenna phosphorylation in Arabidopsis

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The major antenna (LHCII) is a crucial component of the photosynthetic machinery, playing a central role in light capture and acclimatory responses to changing light conditions. A large part of this antenna is present in the form of homo- and hetero-trimers composed of three isoforms: Lhcb1, Lhcb2, and Lhcb3. They have different relative abundance, with Lhcb1 estimated to represent two thirds of the total, Lhcb2 one fourth and Lhcb3 the remaining part, approximately one tenth. The two phosphorylatable subunits, Lhcb1 and Lhcb2, have different roles in the formation of supercomplexes with the photosystems (PSI and PSII) and in their phosphorylation pattern (Galka et al., 2012; Leoni et al., 2013; Pietrzykowska et al., 2014). In the present work we evaluate LHCII phosphorylation in *Arabidopsis thaliana* using a quantitative approach. We observe that Lhcb1 and Lhcb2 differ in their total phosphorylation: only 30% of Lhcb1 is phosphorylated in light conditions favoring PSII excitation, while around 50% of Lhcb2 is phosphorylated. We also elucidate their phosphorylation status in different supercomplexes of the thylakoid membrane. Our results showed that Lhcb2 is completely phosphorylated in the state transition complex (PSI-LHCI-LHCII) while Lhcb1 is almost completely unphosphorylated. This reveals the central role of phospho-Lhcb2 in binding the mobile LHCII antenna to PSI.

P017 - pH, N-source and dose interact to regulate GS and GDH enzymes

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Ammonium (NH_{4+}) and nitrate (NO_{3-}) are the main nitrogen (N) forms available in the soil for plants nutrition. NO_{3-} is reduced to NH_{4+} which is then assimilated into nitrogenous molecules. However, when NO_{3-} reduction step is skipped by the use of NH_{4+} as sole N source, plants display toxicity symptoms. Several factors have been associated to NH_{4+} toxicity, among others 1) decreased uptake of essential cations 2) disproportionate use of carbon skeletons 3) excessive + consumption of energy for ammonium efflux or 4) disorder in pH regulation. Concerning pH, when plants absorb NH4 equal amounts of H^+ are released acidifying the apoplast and/or rhizosphere and alkalinizing the cytosol. In this sense, pH buffering has been shown to alleviate NH_{4+} stress. Indeed, better adapted plants to acidic conditions are usually ammonium tolerant. Similarly, NH_{4+} assimilation is essential to avoid toxicity and is mainly achieved through GS/GOGAT cycle. Although controversial, glutamate dehydrogenase (GDH), that catalyzes the reversible deamination of glutamate to 2-oxoglutarate, might also be collaborating in NH_{4+} assimilation. In the context of NH4+ assimilation role on its tolerance, we have evaluated Arabidopsis roots and shoots GS and GDH enzyme activity and gene expression as metabolic mechanisms to favor NH_{4+} tolerance under two pH conditions (5.7 and 6.7) with NO_{3-} or NH_{4+} as N-source (2 and 10 mM).

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P018 - A novel protein regulates cyclic electron transport around photosystem I mediated by NDH in Arabidopsis

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Cyclic electron transport around photosystem I (CET) is indispensable for efficient photosynthesis. There are two main CET pathways in higher plants. One depends on PROTON GRADIENT REGULATION 5 (PGR5) or PGR5-Like1 (PGRL1) (PGR5/PGRL1 pathway) and the other is mediated by the chloroplast NAD(P)H dehydrogenase-like (NDH) complex (NDH pathway). Although significant achievements have been made in identification of the subunit compositions and regulators of the complexes, the regulator involved in CET is still not fully understood. Here we isolated an *Arabidopsis thaliana* nuclear gene *13-C1* through the secondary EMS mutagenesis on *pgr5* background. We identified *13-C1* as a heat shock protein 40 (Hsp40) and found it is located in the chloroplast. Mutation of 13-C1 caused the inactivation of NDH pathway. Coimmuoprecipitation and yeast hybrid results show that 13-C1 interacts with NdhI and a chloroplast stromal protein, CRR6, which is involved in the process of NdhI and other subunits assembly. Based on these results, we suggest that 13-C1 participates in the NDH complex assembly through interacting with CRR6, thereby regulates NDH pathway.

P019 - The Putative Transcription Factor STKR1: A Novel Component Of SnRK1 Mediated Signaling?

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In plants, the Sucrose non-fermenting (SNF1)–related protein kinase 1 (SnRK1) represents a central integrator of low energy signaling and acclimation towards environmental stress responses. However, many components of SnRK1-regulated signaling pathways remain to be elusive. Recently, we have demonstrated that proteins containing a domain of unknown function (DUF) 581 interact with the catalytic alpha subunits of SnRK1 (AKIN10/11) from *Arabidopsis thaliana*. Arabidopsis possesses 19 DUF581 proteins, which consist of a variable N-terminal domain and a plant-specific highly conserved C-terminal DUF581 domain containing a novel type of C4-zinc finger that mediates the interaction with AKIN10/11. Bimolecular fluorescence complementation (BiFC) analysis confirmed interaction inside the plant nucleus. Yeast-two hybrid analysis revealed several putative interaction partners of DUF581 proteins, including Storekeeperrelated 1 (STKR1), a putative transcription factor that also interacts with AKIN10/11 in yeast and inside the nucleus of plant cells. Thus, we hypothesize that DUF581 proteins may modulate the interaction of SnRK1 and its potential targets such as STKR1. Interestingly, Arabidopsis plants overexpressing STKR1 show growth retardation and display a phenotype being reminiscent of plants overexpressing SnRK1, including anthocyanin accumulation and delayed senescence. These data suggest a possible involvement of STKR1 in SnRK1 mediated signaling.

P020 - Variation of source-sink relationship in Arabidopsis thaliana plants during circadian clock, full development cycle and under an osmotic stress.

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Source-sink transport of sucrose is one of the major determinants of plant growth. In plants, sucrose produced in leaves is exported to sink organs through the phloem. Carbohydrates partitioning require the specific activity of membrane transporters. In *Arabidopsis thaliana* plants, two types of transporters are involved in the phloem loading of sucrose in source organs: AtSUCs and AtSWEETs transporters but, little is known about sucrose transporters and their activity during phloem unloading in sink organs especially in roots. This study is focused on the identification of sucrose transporters in leaves and sink organs (e.g. roots) and their role

in source-sink carbohydrates partitioning during a circadian-clock period, a full development cycle and in response to an osmotic stress (hydroponic culture with poly-ethylene-glycol 6000 addition). Among the 9 AtSUCs present in *A. thaliana*, we identified 4 *AtSUC* 1-4 genes expressed in leaves and roots. For the 7 AtSWEETs, *AtSWEET* 11-13 and 15 are expressed in leaves and roots, but *AtSWEET* 14 is root specific. Under osmotic stress, we noticed a decrease of *AtSUC1* and *AtSUC2* transcripts in roots. In roots *AtSWEET* 11-13 and 15 transcripts level is reduced but in leaves, these transcripts are increased. Source-sink C partitioning was estimated by the measurement of soluble sugars and starch contents in source and sink organs in the three conditions studied and by following [U-^{14}C] sucrose transport during A.thaliana development.

P021 - A novel protein-targeting mechanism is important for starch biosynthesis in Arabidopsis.

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The domestication of starch crops underpinned the development of human civilisation, yet we still do not fully understand how plants make starch. Starch is composed of glucose polymers that are branched (amylopectin) or linear (amylose). The amount of amylose strongly influences the physico-chemical behaviour of starchy foods during cooking and of starch mixtures in non-food manufacturing processes. The GRANULE BOUND STARCH SYNTHASE (GBSS) is the glucosyltransferase that specifically elongates amylose polymers, and was previously thought to be the only protein required for amylose biosynthesis. We now present evidence that another protein, PROTEIN TARGETING TO STARCH (PTST), is also required for amylose synthesis in Arabidopsis. PTST fulfills a previously unknown function in targeting GBSS to starch granules. It is a plastidial protein possessing an N-terminal coiled coil domain and a C-terminal carbohydrate binding module (CBM). Arabidopsis *ptst* mutants synthesise amylose-free starch and are phenotypically similar to mutants lacking GBSS. Analysis of starch granule-bound proteins showed a dramatic reduction of GBSS protein in *ptst* mutant starch granules. Pull-down assays with recombinant proteins *in vitro*, as well as immunoprecipitation assays in planta, revealed that GBSS physically interacts with PTST via a coiled coil. Furthermore, the CBM domain of PTST, which mediates its interaction with starch granules, is required for correct GBSS localisation. Fluorescently-tagged Arabidopsis GBSS, expressed either in tobacco or Arabidopsis leaves, required the presence of Arabidopsis PTST to localise to starch granules. These findings shed new light on the importance of targeting biosynthetic enzymes to sub-cellular sites where their action is required. Importantly, PTST represents a promising new gene target for the biotechnological modification of starch composition, as it is exclusively involved in amylose synthesis. *Reference: Seung D et al. (2014). Protein Targeting to Starch is required for localising Granule Bound Starch Synthase to starch granules and for normal amylose synthesis in Arabidopsis. PLoS Biol 13[(2)] : e1002080.*

P022 - Characterization of the impact of pgr5-deficient mutation on ATP synthesis in the chloroplast

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Cyclic electron transfer around PSI (CET) is required for optimizing photosynthesis, because CET induces to dissipate excessive light energy as heat (qE), supplies ATP to CO_2 assimilation processes via formation of proton motive force (pmf) and alleviates over-reduction of the stroma. Arabidopsis *pgr5* mutant showed largely decreased CET activity (Munekagae et al., 2002), thus the decreased amount of synthesized ATP could be expected. However H^+ efflux via ATP synthase, evaluated as gH^+ (a parameter of the Electro-Chromic Shift measurement), was higher in *pgr5* in the leaf (Avenson et al., 2005; Wang et al., 2014). To reveal how the *pgr5* mutation impacts on ATP synthesis in the chloroplast, we characterized *Arabidopsis* ruptured chloroplasts by ECS measurement and quantification of ATP synthesized during electron transfer. The size of *pmf* and the amount of synthesized ATP in *pgr5* was the same as WT under the condition, where linear electron transfer (LET) is dominant. In contrast to the LET-condition, the amount of ATP and the size of *pmf* were smaller in *pgr5* under the CET-condition, suggesting that deficient CET decreased ATP synthesis due to less proton translocation from stroma to lumen. Our results also suggested that ATP synthesized through ET was correlated to the size of pmf, but gH^+ was not the case, at least in *vitro* experiments. We would also discuss the correlation between the rate of CET and the amount of ATP synthesized during electron transfer.

P023 - New prospects on the regulation of AtFer1 expression

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Iron (Fe) is an essential element for life as it is involved in many fundamental reactions such as photosynthesis, respiration or DNA synthesis. However, an excess of Fe results in the production of ROS (reactive oxygen species) that are deleterious to the cells. Ferritins are proteins involved in the transient storage of Fe in a nontoxic form preventing oxidative damages that would be deleterious to the plant growth and biomass production. *AtFer1* encodes the most abundant ferritin present in *Arabidopsis thaliana*, and its transcript accumulation is tightly connected to Fe availability. In addition, *AtFer1* expression is also regulated at the transcriptional level in response to various abiotic signals such as oxidative stress, phosphate nutrition, light and the circadian clock. In order to gain new insights into the regulatory network that controls *AtFER1* expression, and by extension Fe homeostasis, we are currently following different strategies: (i) in depth study of *AtFER1* promoter structure, (ii) study of the putative role of a long non-coding RNAs that fully overlap *AtFER1* sequence, and (iii) analysis of the dynamic response to an excess of Fe at the whole transcriptome level. Recent advances issued from these three axes will be discussed.

P024 - The PsbO1 protein of photosystem II protects the oxygen-evolving complex from the reducing power of luminal ascorbate

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Ascorbate (Asc) plays essential roles in cellular development, cell wall synthesis, modulation of gene expression and signaling and it also scavenges reactive oxygen species produced during photosynthesis. The oxygen-evolving complex (OEC) is a vulnerable component of the photosynthetic electron transport chain. Upon heat stress, the extrinsic proteins of the OEC are released (primarily PsbO) and this is followed by the release of Mn, Cl and Ca ions into the thylakoid lumen, a process functionally inactivating the OEC. In this work, we aimed at understanding the possible role of Asc as a reductant during the inactivation of the Mn-cluster. To this end, we crossed *psbO1* and Asc-deficient *vtc2-1 Arabidopsis thaliana* mutants. The lack of the PsbO1 protein leads to retarded growth and a strong decrease in OEC activity relative to wild-type plants; in our *psbO1xvtc2-1* lines the phenotype and photosynthetic efficiency were partially restored. Also, we observed that in isolated photosystem II (PSII) membrane particles lacking the PsbO1 protein Asc inactivated the Mn-cluster. On the other hand, we demonstrated earlier that in heat-treated leaves Asc is an alternative electron donor and it provides electron to TyrZ+ in PSII. Therefore, our results suggest that upon heat stress the PsbO1 protein is released from PSII and in its absence, Asc over-reduces and thereby inactivates the Mn-cluster. Once this occurs, Asc provides electrons to PSII at a relatively slow, but continuous rate.

P025 - Transcriptional regulation of oil accumulation in the endosperm of oilseeds

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In oilseeds of Arabidopsis, 90% of oil is stored in the embryo and 10% within the endosperm. If recent advances have contributed to the elucidation of the transcriptional regulation of oil storage within

embryos, our understanding of the regulatory processes controlling oil accumulation within the endosperm remains very limited. MYB118, a transcriptional factor specifically induced in the endosperm at the onset of seed maturation, functions as a negative regulator of maturation-related genes limiting the amount of oil stored in the endosperm (Barthole et al., 2014). The characterization of MYB115, its closet homolog, which is also expressed in the endosperm of maturing seeds, may contribute to a better understanding of the control of lipid synthesis in this tissue. To test the putative functional redundancy existing between MYB118 and MYB115, expression of maturation-related genes was studied in different genetic backgrounds (Col-0, *myb115*, *myb118*, and *myb115 myb118*). They allowed identifying common target genes for the two transcription factors. These were further validated by transient activation assays in *Nicotiana benthamiana*. The functional redundancy between the two transcription factors will be further evaluated through a complementation assay of the *myb118* phenotype with a *ProMYB118:MYB115* construct. Finally, a fine characterization of the oil content in the embryo and within the endosperm of the single and double mutants will be realized.

P026 - Cysteine biosynthesis in Arabidopsis: functions of the serine acetyltransferase (SERAT) and O-acetylserine (thiol) lyase (OASTL) gene families

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Sulfur is an essential nutrient for plant growth. Sulfate, the major form of inorganic sulfur, is taken up from soil by roots and reduced to sulfide by the assimilatory sulfate reduction pathway in plants. Sulfide is incorporated into organic compounds *via* cysteine (Cys) biosynthesis. Cys synthesis is carried out by two sequential reactions by serine acetyltransferase (SERAT) and O-acetylserine (thiol)lyase (OASTL). SERAT catalyzes the formation of *O*-acetylserine (OAS) from serine and acetyl coenzyme A. OASTL incorporates sulfide into OAS to produce Cys. Both the SERAT and OASTL enzymes are encoded by members of large gene families and are localized in the cytosol, plastids and mitochondria. SERAT plays a regulatory role on sulfur assimilation through the production of OAS, which is not only a limiting factor for Cys synthesis but has also been suggested to be a positive regulator of the sulfur assimilation pathway and a specific gene set, the OAS cluster genes. Two regulation mechanisms for SERAT activity allow maintaining intracellular OAS and Cys levels: Cys feedback-inhibition of SERAT activity and formation of the SERAT-OASTL complex which modulates each enzyme"s activity. In this study, Arabidopsis T-DNA mutants of *SERATs* and *OASTLs*, including single and multiple knockout mutants, have been analyzed. This study demonstrates the functions of individual SERAT and OASTL isoforms and the subcellular- and tissue-specific regulation of OAS and Cys synthesis in Arabidopsis.

P027 - Energy starvation signaling for Arabidopsis organ growth regulation

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Plants with a sessile lifestyle control organ growth under the regulation of developmental programs and environmental signaling cues. For example, a phytohormone ethylene modulates organ growth in an environmental signaling context. Ethylene inhibits *Arabidopsis* hypocotyl growth in the dark, but promotes its growth in the light. To understand a dual action of ethylene in organ growth regulation, molecular mechanisms underlying the integration of the phytohormonal and environmental signaling need to be elucidated.

Recently we have developed a fluorescence lifetime image microscope-adopted assay to monitor photochemical efficiency at a single cell resolution and demonstrated that ethylene sensitivity modulates photochemical efficiency affecting cellular energy status. Energy starvation signaling-mediated by an evolutionarily conserved energy sensor AKIN10 then controls *Arabidopsis* hypocotyl growth. In this study we have unraveled the longstanding puzzle of dual function of ethylene for *Arabidopsis* hypocotyl growth regulation with respect to lights.

Cell biology

Posters 028 to 074

P028 - Prefoldin Subunits Identified As Interactors Of REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) And RUP2

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Survival of plants in sunlight requires UV-protective responses. Plants respond to low levels of UV-B radiation with a coordinated photomorphogenic response that allows acclimation to this environmental stress factor. The positive regulators in this UV-B response are UVR8 (UV-B photoreceptor), COP1 (an E3 ubiquitin ligase), and HY5 (a bZIP transcription factor). UVR8 homodimers sense UV-B, resulting in their monomerization (Rizzini *et al.*, 2011). Elevated UV-B–specific response in UVR8-overexpression lines is associated with dwarf growth, indicating the importance of balancing UV-B–specific signalling (Favory *et al.*, 2009). Recently, we described two highly related WD40-repeat proteins, RUP1 (REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1) and RUP2, that facilitate UVR8 redimerization and thus function as repressors of UV-B signalling (Gruber *et al.*, 2010; Heijde and Ulm, 2013). In order to find new interactions partners for RUPs, tandem affinity purification combined with MS-based protein identification was performed. We identified the prefoldin subunit PFD3 as putative interactor of GS-TAG-tagged RUP2 expressed in Arabidopsis suspension cultured cells. PFD subunits compose the heterohexameric prefoldin complex that is involved in the correct folding of cytoskeletal components such as tubulin and actin. However, there is growing evidence that interaction partners of PFD subunits are not restricted to cytoskeletal proteins. Moreover, interactions can occur with the complex or individual subunits (Locascio *et al.* 2013; Millán-Zambrano *et al.* 2013). We will present our attempts to identify a potential role of PFDs in UV-B signalling.

P029 (Talk) - Perturbation of cell wall integrity and the induction of programmed cell death in Arabidopsis thaliana

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Thaxtomin A is a phytotoxin produced by the causative agent of common scab disease in potato, *Streptomyces scabies*. Thaxtomin A is essential for the development of disease symptoms such as the formation of lesions on tubers. This toxin inhibits cellulose synthesis, leading to cell swelling and growth inhibition. We have shown that thaxtomin A and other cellulose biosynthesis inhibitors (CBI) like isoxaben activate a specific programmed cell death that can be distinguished from a typical hypersensitive response. In *Arabidopsis thaliana* wild type cell suspensions as well as seedlings, CBI-induced cell death can be inhibited by the addition of auxin. We present here experimental evidence that polar transport of auxin might be implicated in the process. Our results also suggest that CBI effects are mediated by calcium efflux but do not involve MAPK signaling pathway. This study brings a deeper understanding of cellular responses induced by cell wall perturbations and may provide new insights for improving plant protection.

Keywords : Thaxtomin, Cellulose Biosynthesis, Cell Wall, Programmed Cell Death, Auxin.

P030 - BIRD proteins confine SHORTROOT to single layer through spatial protein complex distribution and differential transcriptional regulation.

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In Arabidopsis roots, movement of the cell fate regulator SHORT-ROOT (SHR) from the stele to the ground tissue has been associated with transferring positional information across tissue layers. Here we show that the zinc finger BIRD proteins JACKDAW (JKD) and BALDIBIS (BIB) constrain SHR to a single layer in a mechanism that involves multimeric protein complex formation and nuclear retention. This action is critical for the establishment and maintenance of the boundary between stele and ground tissue. Using FRET/FLIM technology, we visualized protein complexes between SCARECROW (SCR), SHR and BIRDs in living Arabidopsis roots at the cellular resolution. With endogenous promoters, we show that these complexes are abundant only in subsets of cells and not throughout the domains where they co-localize; SHR-SCR complex was more prominent in the cortex/endodermis (CEI/CEID) stem cells while interactions of BIRDS with SCR occurred predominantly in the endodermis while with SHR, protein heterodimers were only detected in the QC and the stem cells. Our transcription assays indicate that JKD and BIB have different actions on promoter activity; they restrict CYCLIND6 (CYCD6) expression to the CEI/CEID; and promote SCR expression, we also show that SCR and SHR are not sufficient to activate key downstream target genes, but require tethering by BIRD proteins. Our findings provide evidence on how in a multicellular organism, a given protein complex restricts target gene expression to a specific cell type to confer proper cell fate.

P031 (Talk) - Arabidopsis TRM mutants shed new light on PPB function

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The TTP complex (TON1, TRM, PP2A) controls the organization of cortical microtubule arrays in plant cells, both during interphase and at the G2/M transition for preprophase band (PPB) setup. Recent characterization of G2/M specific TRMs contributing to a mitotic isoform of the TTP complex allowed us to produce triple mutant *Arabidopsis* plants specifically devoid of PPB, with no cumulative defects carried over from misfunction of the interphase cortical array of microtubules (ICMT) prior to G2/M. Mutant *Arabidopsis* plants specifically devoid of PPB display suprinsingly mild defects in term of morphology and fertility. Analysis of the geometry of cell division in the root meristem of such PPB-less plants revealed that the mean orientation of division planes was not altered in the mutant compared to the wild type, remaining perpendicular to the cell file"s axis. However, the variance of division planes was significantly higher in mutant tissues, in terms of both angle and axial position. We thus conclude that the PPB, contrarily to a widely accepted view, is not a primary determinant of division plane positioning, since plants specifically lacking PPBs are able to position their division planes almost properly. However, the PPB is necessary to reduce variations in division site positioning, and as such should be viewed more as part of a noisereducing or reinforcement process rather than as a "decision-making" one.

P032 - Establishment of the glycerolipidome of Arabidopsis plastoglobules suggests presence of microdomains at the thylakoid membrane.

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Plastoglobules are lipid droplets localized inside plastids. Studies from the last decade demonstrated that plastoglobules are active compartments at the crossroads of different metabolic pathways. Yet, these particles are also believed to store carotenoids during chromoplastogenesis and to participate in the turnover of lipids during senescence, in particular by storing lipids coming from the dismantlement of thylakoids. Plastoglobules are surrounded by a leaflet contiguous with the thylakoid outer leaflet and therefore share a physical link with thylakoids. This connection probably allows the free exchange of molecules between these two compartments. While the protein composition of plastoglobules is well known, a precise description of the lipid content is still missing. We thus decided to establish the glycerolipidome of Arabidopsis plastoglobules. The results we obtained demonstrate that the plastoglobule surrounding leaflet has a specific lipid composition different from the thylakoid leaflet from which it derives, suggesting that microdomains might exist in thylakoids that would favor plastoglobule formation.

P033 - Differential regulation of type-I and type-II ROPs by RhoGDI

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Rho of Plants (ROPs) function as polarity determinants that orchestrate cytoskeleton organization, endocytosis and were implicated in responses to several hormones, biotic and abiotic stress. Based on their amino acid sequences ROPs have been divided into two subgroups designated type-I and type-II. It has been shown that type-I ROPs undergo posttranslational prenylation whereas type-II ROPs undergo stable C-terminal S-acylation. The distribution of Rho proteins between the plasma membrane and the cytosol is regulated by RhoGDIs (Rho Guanine nucleotide Dissociation Inhibitors), which are thought to interact with prenylated ROPs. Given that the interaction of ROPs with RhoGDI depends on prenylation it was expected that type-I but not type-II ROPs would interact with RhoGDI. In yeast two hybrid assays, only type-I but not type-II ROPs interacted with RhoGDIs. Next, I tested the ability of recombinant *Arabidopsis* RhoGDI1 to extract type-I and type-II ROPs from the plasma membrane. This analysis showed that RhoGDI1 could extract transiently expressed type-I ROPs: ROP2, ROP4 and ROP6 from the plasma membrane but not the type-II ROPs: ROP9, ROP10 and ROP11. It has previously been shown that the RhoGDI1 mutant scn1 (supercentipide 1) develops abnormal root hairs with multiple tips. My results indicate that this phenotype is associated with compromised regulation of type-I ROPs.

P034 - Complexity of LEFKOTHEA protein targeting in nucleus and chloroplast

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A crucial parameter establishing the canonical cellular homeostasis is the dynamic communication between different cellular compartments. To decipher the origin of the perplexed mechanisms underlying compartmental communications leading to proper plant growth, we have focused on embryonic development of *A. thaliana*. We have identified and characterized a mutant named *lefkothea (lefko)* with improper embryo development, more specifically embryo arrest at the heart-stage. The LEFKOTHEA protein contains a plant specific domain and *in silico* analysis has shown that LEFKOTHEA protein has a putative transit peptide for chloroplast targeting and signals for nuclear localization (NLS) and export (NES). Microscopic analysis of *LEFKO* gene constructs fused to *YFP* confirmed the dual targeting properties of LEFKO to both chloroplasts nucleoids and nucleus foci. The *lefkothea* phenotype results from disturbed equilibrium of the protein in the nucleus due to enhanced protein export. Therefore, the truncated version of LEFKOTHEA protein missing the transit peptide at the N-terminus complements the

lefkothea phenotype, highlighting the crucial role of LEFKOTHEA in the nucleus. Multi sub-cellular localization provide an elegant network for plant signaling response to developmental cues and our aim is to identify the mechanisms responsible for the LEFKOTHEA protein translocation to both nucleus and chloroplast for canonical embryo development, a key parameter in plant productivity.

P035 - intracellular anion dynamics

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Every important physiological process in living cells involves ion transport across a membrane, like nutrition, signalling, stomatal movements, generation of membrane potentials… Ion transport is controlled by specialized membrane proteins called transporters. Some of these proteins work as active transporters while others as passive transporters (channels). Whereas anion fluxes across cellular membranes play a major role in plant physiology (e. g. nutrition and stomata movement) little is known about anion dynamic in intracellular compartments (e. g. concentration, connection between a physiological response and anion dynamic…). This work focuses on two proteins belonging to the ChLoride Channel family (CLC) expressed in the tonoplast. In *Arabidopsis thaliana* there are seven members of the CLC family being all intracellular proteins. Only two *Arabidopsis thaliana* CLCs have been functionally characterized so far (CLCa, CLCb). The aims of this project are: 1) the molecular characterization of the transport processes catalyzed by two CLCs from *Arabidopsis thaliana* (CLCc and CLCg); and 2) exploring the dynamic changes of intracellular anion fluxes in living plant cells using biosensors and the role of different anion transporters. The combination of electrophysiology (molecular level) and microscopy (cellular level) will allow us to have new perspectives of the function of intracellular anion fluxes.

P036 - Chitin-induced and CERK1-dependent endocytosis of the LysM-RLKs LYK5 and LYK4

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Plants recognize potentially harmful fungi by detection of chitin, a component of the fungal cell wall. In *Arabidopsis*, the chitin-binding LysM-RLK CERK1 is essential for chitin perception. *Arabidopsis* contains two more LysM-RLKs with chitin binding activity, LYK4 and LYK5, which have recently been proposed to form a receptor complex with CERK1. In this work, we analysed the subcellular behavior of fluorescence protein-tagged CERK1, LYK4 and LYK5 using confocal microscopy. Our analyses confirm that all three LysM-RLK are localized to the plasma membrane (PM). The subcellular localization of CERK1-GFP is not altered upon chitin treatment but experiments with Brefeldin A revealed constitutive cycling in early endosomes (EE). Unlike CERK1-GFP, LYK4-mCitrine and LYK5-mCitrine undergo chitin induced endocytosis. Inhibitor studies suggest that LYK5-mCitrine is shuttled from the PM to EE and then to late endosomes (LE) / multivesicular bodies (MVBs). Application of kinase inhibitors completely blocked chitin-induced endocytosis of LYK5-mCitrine. Interestingly, chitin-induced endocytosis of LYK5-mCitrine does not occur in a *cerk1*-2 knock-out background, suggesting that functional CERK1 is required for LYK5 internalization. Moreover, we could show that LYK4-mCitrine and LYK5-mCitrine are phosphorylated *in planta* after chitin treatment in a CERK1-dependent manner. Our data favor a model in which ligandbinding induces CERK1-dependent phosphorylation and endocytosis of LYK4 and LYK5.

P037 - The ROP effector ICR2 is a microtubule-associated protein that affects abiotic stress responses

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Plants have a single family of RHO GTPases, which are called ROPs (Rho of Plants). Members of a plant specific family of ROP effectors designated ICR (Interactor of Constitutively active ROP) have been identified in our laboratory. The ICR family consists of five members, designated ICR1-ICR5. The ICRs are coiled coil proteins that associate with microtubules and function as scaffolds that interact with specific group of proteins. My research focuses on the function Arabidopsis ICR2. ICR2 is expressed in vegetative and reproductive meristems, in vascular tissues, stomata, stamens, and developing seeds. Co-expression analysis shows that ICR2 is co-expressed with several microtubules (MTs) associated proteins (MAPs) and actin associated proteins, that have been implicated in regulation of cell division, possibly linking ICR2 to regulation of MTs during the cell division. BiFC and subcellular localization assays showed that ICR2 interacts with ROPs and associates with MTs. Interestingly, a loss of function null mutant of ICR2 displayed decreased sensitivity to abiotic stress, ABA and the GA synthesis inhibitor paclobutrazol. To conclude, my observations suggest that ICR2 is a MAP that regulates ABA and abiotic stress responses and possibly also cell division and growth.

P038 (Talk) - Vesicle Trafficking Driven by Microtubule Growth Regulates Light-Induced Stomatal Opening in Arabidopsis

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Spatial positioning and motility of vesicles and organelles are fundamental to cellular functions in all eukaryotic cells, and are primarily governed by the cytoskeleton. In plant cells, it has long been accepted that the vesicle trafficking is predominantly dependent on actin-based myosin. Recent findings suggested that microtubules (MTs) and their motors might anchor or slow down organelles/vesicles for their targeting to appropriate destinations. However, few report described MT growth-driven vesicle trafficking. In response to various hormonal and environmental stimuli, stomatal opening and closing are precisely regulated by guard cells to control gas exchange and water transpiration, and restrict pathogen invasion. During stomatal movement, vesicle trafficking of ion channels in guard cells plays an important role. In our study, we demonstrated that Arabidopsis AUGMIN subunit 8 (AUG8), a MT plus-end binding protein, directly interacted with a SNARE (N-ethylmaleimidesensitive factor adaptor protein receptor) protein that mediates the light induced-trafficking of a K^+ channel to the plasma membrane (PM) in guard cells. Live cell imaging revealed that SNARE protein-associated vesicles co-localized with AUG8 at the growing ends of MTs, and was propelled by the growth of MTs to the PM for the exocytosis. Our study revealed a novel mechanism of vesicle trafficking driven by MT growth, which is required for efficient exocytosis to modulate light-induced stomatal opening.

P039 - Investigating the role of potassium transporters in cesium accumulation by Arabidopsis thaliana.

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Radioactive isotope of cesium (Cs), have been released during the Fukushima nuclear accident. Among solutions investigated for the rehabilitation of agricultural land contaminated with radioCs, is the use of adapted plants for safefood or phytoremediation. In addition to the Cs availability in soil, plant properties have a major influence on the intensity of Cs uptake which is highly heterogenic between species. Identify and characterize transport proteins involved in Cs accumulation could participate to the understanding of this heterogeneity. Due to analogy with potassium (K), Cs is supposed to pass through K carriers. Influx experiments on wild type Columbia 0 seedlings show that, as for K, at least two systems are involved in Cs uptake. Testing 3 different amounts of K, we find that Cs accumulation is significantly higher in starved plant. Lower competition between K and Cs cannot explain entirely this result. We supposed that the HAK5 transporter, which is induced at low K concentration and involved in Cs uptake, participate also to this increase of Cs uptake. Testing Cs toxicity between different mutant lines, we find that a mutant disrupted in a transporter belonging to the same family than HAK5 was more sensitive compare to the wild type. Ongoing influx experiments tend to demonstrate that Cs roots uptake is not affected. Thus we are now investigating the expression pattern and the localization of the protein disrupted as well as its long-term accumulation pattern.

P040 - Characterization of TyrosylProtein SulfoTransferase in Casparian strip formation

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The Casparian Strip (CS) is a specific lignin-like cell wall impregnation that surrounds each endodermal cell as a belt providing a seal of the extracellular root space. We identified a novel CS mutant, *schengen2 (sgn2*), that presents a delay in the formation of a functional CS barrier. This mutant presents the CS extracellular material deposited in a non-continued fashion in younger root parts. *SGN2* encodes for TyrosylProtein SulfoTransferase (*TPST*) that mediates sulfation of Tyrosine (Tyr) as post-translational modification. Tyr-sulfated peptides have been found to be of crucial importance for normal growth and development in plant roots. Phytosulfokine (PSK) and plant peptide containing sulfated tyrosine 1 (PSY1) are involved in cell elongation activity, and root meristem growth factor (RGF) is essential for meristematic activity. Analysis of *sgn2/tpst* mutant growth in medium containing the chemically synthesized sulfated form of PSK, PSY1 and RGF1 indicated that these peptides are not the responsible for the CS defects. When TPST is express specifically in the endodermis in *sgn2/tpst*, the CS interruptions cannot be abolished. This result suggests that the corresponding Tyr-sulfated peptide required for the CS initial formation is produced in other tissue that is not the endodermis, being a mobile ligand. Overall, the identification of this small peptide will allow us to better understand how plants develop their extracellular diffusion barrier in roots.

P041 - Retinoblastoma resets the cell cycle after S phase through a system of interconnected feedback loops

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Cyclin-dependent kinases (CDKs) change the activity of their substrates by phosphorylation and by that promote progression into the next cell cycle phase. CDKA;1, a homolog of Cdc2/CDC28, is one of the major cell cycle kinases in *Arabidopsis*. Although the onset of the S phase has been intensively studied, how the S-phase state is repressed, especially after DNA replication is completed, remains largely unclear. In our work, we focus on *Arabidopsis* retinoblastoma-related protein (RBR1), a repressor of S-phase genes. It has been proposed that in late G1 phase, CDKA;1 phosphorylates RBR1, by that phosphorylated RBR1 dissociate from E2F-DP heterodimeric complexes which are responsible for the transcription of S-phase genes. Here we present the identification of two negative feedback loops that control the level of RBR1. We show that the expression of *RBR1* is directly regulated by RBR1 itself. Importantly, we reveal that RBR1 is a poor CDK substrate compared to other targets of S-phase CDK activity and thus, inactivation of RBR1 results in the expression of effective competitors in RBR1 phosphorylation. Molecular modeling shows that the combination of these regulatory elements can effectively reset the S-phase state.

P042 - RUBY Encodes a Glyoxal Oxidase Needed For Cell Wall Strengthening

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In Arabidopsis thaliana, seed coat epidermal cells extrude mucilage when exposed to an aqueous solution. Like primary cell walls, the mucilage is composed of pectin, cellulose and hemicellulose, and it envelops the seed to produce a structured, highly-adherent capsule. Since seed mucilage is abundant and not essential to seed viability, we are using it as a model to study cell wall structure and function. The *MUM2* gene encodes a B-galactosidase secreted by the seed coat to modify mucilage, loosening it to allow expansion during hydration. Mutations in *MUM2*result in seed mucilage that cannot extrude. We have used genetic suppressor analysis to identify a new gene, *RUBY*. Mutations in *RUBY* completely suppress the *mum2* mucilage extrusion phenotype and also result in the separation of the seed coat epidermal cells from the seed. Extractable *ruby* mutant mucilage contains 20 times more galactose and 30 times more arabinose than wild type, with linkages typical of arabinogalactan protein (AGP) side chains. These phenotypes suggest that RUBY is needed to strengthen connections within the mucilage and middle lamella that involve the attachment of AGPs or its side chains. RUBY encodes a glyoxal oxidase-related protein (GLOX) that it is expressed late in seed coat differentiation. GLOX proteins produce hydrogen peroxide that might catalyze cross-links between cell wall proteins and/or their side chains and these genes have been shown to be up-regulated in response to pathogen attack.

P043 - The SLOW GROWTH 3 pentatricopeptide repeat protein is required for the splicing of mitochondrial nad7 intron 2 in Arabidopsis

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Mitochondria play an important role in maintaining metabolic and energy homeostasis in the cell. In plants, impairment in mitochondrial functions usually has detrimental effects on growth and development. To study genes that are important for plant growth, we have isolated a collection of *slow growth* (slo) mutants in Arabidopsis. One of the slo mutants, *slo3*, has a significant reduction in mitochondrial complex I activity. We used map-based cloning to identify that the slo3 mutant had a four-nucleotide deletion in *At3g61360* encoding a pentatricopeptide repeat (PPR) protein. The SLO3 protein contains 9 classic PPR domains belonging to the P subfamily. The small deletion in the *slo3* mutant changes the reading frame and creates a premature stop codon in the first PPR domain. We demonstrated that the SLO3-green fluorescent protein (GFP) is localized to the mitochondrion. Further analysis of mitochondrial RNA metabolism revealed that the slo3 mutant was defective in splicing of *nad7* intron 2. This specific splicing defect led to a dramatic reduction in complex I activity in the mutant as revealed by blue native gel analysis. Complementation of *slo3* by *35S:SLO3* or *35S:SLO3-GFP* restored the splicing of *nad7* intron 2, the complex I activity, and the growth defects of the mutant. Together, these results indicate that the SLO3 PPR protein is a splicing factor of *nad7* intron 2 in Arabidopsis mitochondria.

P044 - Arabidopsis Mitochondrion Protein EDR1 is Essential for Embryo Development

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Several lines of evidences have shown that the mitochondrion is deeply involved in many cellular processes in *Arabidopsis*, including gametophyte development, the defense response to oxidative stress, calcium signaling, the regulation and execution of programmed cell death. Based on a previous study on proteome of plant mitochondrial nucleoids, we describe a novel *Arabidopsis* mitochondrion protein named embryo development retardation1 (EDR1) that affects nutrition accumulation during embryogenesis, with less protein and lipid body production in the embryo. Whole-mount preparation of samples shows that *edr1* embryo development arrest occurs by approximately early globular stage, but finally formed a fully differentiated bent-cotyledon embryo. Transmission electron microscopy suggests mitochondria in edr1 embryo often have electron-lucent interiors and chloroplasts are less developed with no starch. Therefore, with the exception of the decrease of the nutrition, mitochondrial and chloroplast alterations are the first alterations observed at the ultrastructural level. EDR1 is mainly expressed in the embryo sac and pollen, localized to mitochondria. In conclusion, EDR1 functions in the mitochondria, possibly by targeting components of the metabolic regulatory pathway, thus affecting the production of energy sources.

P045 - Functional analysis of DMP proteins in Arabidopsis thaliana

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DMPs (DUF679 Membrane Protein) are small integral proteins containing 4 transmembrane domains. The ubiquitous occurrence of DMP proteins in green plants and their absence from other kingdoms indicate a role in plant-specific processes. Ten *DMP* genes are found in *Arabidopsis thaliana* with marked tissue- and development-specific expression patterns. DMPs have been shown to be mostly retained in the ER membrane or targeted to the tonoplast using fusion proteins with the fluorescence tag eGFP. However, the biological function of these genes remains unknown to date. The current study provides the first insights into the molecular function of DMP proteins using metabolite profiling of DMP overexpressor lines in *Arabidopsis thaliana* and the heterologous organism *Saccharomyces cerevisiae*. Further analyses in yeast including complementation and toxicity assays reinforce the functional characterization of DMP proteins.

P046 - Cytosolic targeting factor AKR2A captures chloroplast outer membrane-localized client proteins at the ribosome during translation

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In eukaryotic cells, organellar proteome biogenesis is pivotal for cellular function. Chloroplasts contain a complex proteome, the biogenesis of which includes post-translational import of nuclear-encoded proteins. However, the mechanisms determining when and how nascent chloroplast-targeted proteins are sorted in the cytosol are unknown. Here, we establish the timing and mode of interaction between ankyrin repeat-containing protein 2 (AKR2A), the cytosolic targeting factor of chloroplast outer membrane (COM) proteins, and its interacting partners during translation at the single-molecule level. The targeting signal of a nascent AKR2A client protein residing in the ribosomal exit tunnel induces AKR2A binding to ribosomal RPL23A. Subsequently, RPL23A-bound AKR2A binds to the targeting signal when it becomes exposed from ribosomes. Failure of AKR2A binding to RPL23A *in planta* severely disrupts protein targeting to the COM; thus AKR2A-mediated targeting of COM proteins is coupled to their translation, which in turn is crucial for biogenesis of the entire chloroplast proteome.

P047 - Identification of Arabidopsis messenger RNAs transported between tissues

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The concept that proteins and small RNAs can move to and function in distant body parts is well established. However, the non-cell-autonomy of small RNA molecules such as micro RNA or siRNA raises the question to which extent other RNA molecules such as protein-encoding messenger RNAs (mRNAs), ribosomal RNAs (rRNAs), and transfer RNAs (tRNAs) are also transported to distant tissues in plants. By using hetero-grafted *A. thaliana* plants and by DNA- and RNAseq - based SNP analysis algorithms we identified 2006 endogenous Arabidopsis genes producing mobile long RNAs including mRNAs. Most of these mobile transcripts seem to follow the phloem-dependent allocation pathway transporting sugars from photosynthetic tissues to roots via the vasculature. Notably, a surprisingly high number of transcripts also move in the opposite, root-to-shoot direction, and some of these seem to move specifically from roots into specific above ground tissues such as rosette leaves or flowers (Thieme et al. 2015, Nature Plants). Currently, we are addressing the potential function of mobile mRNAs as signals, their transport pace and destination specificity. Notably, we found evidence that some mobile mRNAs harbour a special transport motif mediating their transfer into flower tissues or root apices. We could show that artificial mRNAs harbouring a transport motif are translated and functional at the destination side. The extensive mobility of mRNAs suggests that a tissue-specific gene expression profile might not be a reliable indicator for a particular cell type in which a transcript exerts its function.

P048 - Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9

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[1] Stanford University, Stanford, CA, UNITED STATES [2] Carnegie Institution for Science, Stanford, CA, UNITED STATES[3] Max Planck Institute for Chemical Ecology, Jena, GERMANY[4] University of Minnesota Duluth, Duluth/, MN, UNITED STATES Angiosperms developed floral nectaries that reward pollinating insects. Although nectar function and composition have been characterized, the mechanism of nectar secretion has remained unclear. Here we identify SWEET9 as a nectary-specific sugar transporter in three eudicot species: *Arabidopsis thaliana, Brassica rapa* (extrastaminal nectaries) and *Nicotiana attenuata* (gynoecial nectaries). We show that SWEET9 is essential for nectar production and can function as an efflux transporter. We also show that sucrose phosphate synthase genes, encoding key enzymes for sucrose biosynthesis, are highly expressed in nectaries and that their expression is also essential for nectar secretion. Together these data are consistent with a model in which sucrose is synthesized in the nectary parenchyma and subsequently secreted into the extracellular space viaSWEET9, where sucrose is hydrolysed by an apoplasmic invertase to produce a mixture of sucrose, glucose and fructose. The recruitment of SWEET9 for sucrose export may have been a key innovation, and could have coincided with the evolution of core eudicots and contributed to the evolution of nectar secretion to reward pollinators.

P049 - Targeting of the V-ATPase complex in Arabidopsis thaliana

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The vacuolar H+-ATPases (V-ATPases) are multisubunit complexes that are responsible for the acidification of various cellular compartments in all eukaryotic cells.The localization of this multifaceted enzyme is determined by the isoforms of the membrane integral subunit, VHA-a. The incorporation of VHA-a1 targets the V-ATPase to the trans-Golgi network/early endosome (TGN/EE) whilst the incorporation of VHA-a2 and VHA-a3 targets the V-ATPase to the tonoplast. The molecular mechanisms responsible for differential V-ATPase trafficking have not yet been determined. In order to discern the elusive targeting signal in VHA-a1, we made use of chimeric constructs which consisted of increasing lengths of the VHA-a1 N-terminus fused to decreasing lengths of the C-terminal domain of VHA-a3. By this approach we have narrowed the responsible region down to 12 base pairs. In our quest to uncover the targeting signal in VHA-a3, we have initially focused on two conserved cysteine residues in the N-terminus of all VHA-a3-related sequences. We could show that these residues are the target of the S-acylation of VHA-a3, however we found that S-acylation is not responsible for tonoplast targeting. We are thus now investigating the alluring role of S-acylation in the regulation of the tonoplast V-ATPase activity and membrane subdomain partioning.

P050 - Purification and characterization of M20, a novel endonuclease from pollen mitochondria

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In plant cells, mitochondria contain their own genomes that cooperate with nuclear genome to harmonize the plant development. In most angiosperm species, the quantities of mitochondrial DNA (mtDNA) decrease during pollen maturation. This phenomenon leads to a maternal mitochondrial inheritance and raises a question of how the mtDNA is regulated during pollen development. Here, we gathered abundant maize (*zea mays*) pollen, isolated highly purified mitochondria and finally identified a novel 20 kD nuclease that may function in diminishing circular mtDNA in the pollen through consecutive steps. We obtained its sequence by LC-MS/MS and term this protein as M20. Bioinformatic annotation revealed that M20 was a putative endonuclease and our experiments proved it a thermo-stable endonuclease which can degrade the linear and circular DNA with the present of Mg2+ or Mn2+. The significant sequence feature of M20 is the H-N-H/N domain and its homologous protein AtM20, in Arabidopsis (*Arabidopsis thaliana*), was explored as well. AtM20 is expressed mainly in immature pollen, located in mitochondria and have the same nuclease activity as M20 *in vitro*. The obscurity of gaining efficient AtM20 knock-down or knock-out plants implies this protein unusual and maybe crucial to plants.

P051 - Autophagic turnover of the 26S proteasome is mediated by the dual ubiquitin/ ATG8 receptor RPN10

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Selective protein turnover by the ubiquitin-proteasome system is critical in eukaryotes, hence 26S proteasome levels are tightly controlled by multiple co-ordinated mechanisms. Here we demonstrate that proteasome levels are regulated by ATG8-mediated autophagic turnover, a process we have termed proteophagy. Using *Arabidopsis* proteasomes tagged with GFP, we observed their delivery into vacuoles via a route that requires components of the autophagy machinery. This transport can be separately induced by nitrogen starvation through the ATG1 kinase sensor and by chemical or genetic inhibition of the proteasome, implying that multiple induction mechanisms exist. Proteasome inhibition stimulates comprehensive ubiquitylation of the complex, along with increased association of the ubiquitin receptor RPN10 via these added ubiquitin moieties. The ensuing proteophagy then uses this bound RPN10, which can independently bind ATG8 to form a tripartite ubiquitin-RPN10-ATG8 bridge, to tether proteasomes to expanding autophagic vesicles. Collectively we propose that *Arabidopsis* RPN10 act as a selective autophagy receptor that targets damaged or inactive 26S proteasomes for breakdown by simultaneous interaction with ubiquitylated proteasome subunits/targets and lipidated ATG8 that lines the enveloping autophagic membranes.

P052 - New roles for oil body oleosins during seed development

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Oil bodies (OBs) are lipid storage organelles that allow accumulation of neutral lipids in seed, and sustain plantlet development after the onset of germination. OBs are covered with specific proteins embedded in a single layer of phospholipids. Using fluorescent dyes and confocal microscopy, we monitored the dynamics of OBs in living Arabidopsis embryos at different stages of development. Analyses were carried out with different genotypes i.e. the wild type and 3 mutants affected in the accumulation of various oleosins (OLE1, OLE2 and OLE4), three major OB proteins. Image acquisition was followed by a detailed statistical analysis of OB size and distribution during seed development in the 4 dimensions (X, Y, Z, and time). Our results indicate that OB size increases sharply during seed maturation, in part by OB fusion, and then decreases until the end of the maturation process. In single, double and triple mutant backgrounds, the size and spatial distribution of OBs are modified, affecting in turn the total lipid content, which suggests that the oleosins studied have specific functions in the dynamics of lipid accumulation. Various chimeric oleosins are being expressed *in planta*, in order to get more information on those functions, especially with regard to the importance of the N- and C-termini of oleosins. We aim at modeling the dynamics of OB during seed development and shed light on the role of each oleosin in the oil accumulation process.

P053 (Talk) - EB1 links microtubule network organization and touch response in Arabidopsis thaliana

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In plants cells, the fine-tuning of the microtubule (MT) network organization is crucial for multiple processes such as division and growth. However, the precise molecular mechanisms sustaining the overall MT array deployment are not well understood. We question here the involvement of AtEB1a and AtEB1b, two of the three *A. thaliana* orthologs of the +TIPs (plus-End-Tracking Proteins) EB1 (End-Binding 1), in the cortical MT network architecture. Unlike animals, precise functions and regulations of EB1 plant counterparts are still to be elucidated. We tackled the study of EB1 functions in elongating epidermal cells using chimeric lines of plants expressing GFP-fused tubulin in wild type or in double mutant background. Using confocal and TIRF microscopy, combined with anisotropy calculation of fibers distribution, we observed a significant disorganization of the MT network in the double mutant. Super-resolution microcopy, combined with an original image analysis process, revealed a marked decrease of MT bundling. Moreover, double mutated plants display significant defects in growth tropism, underlying the functional relationships between MTs and development. Altogether, our data suggest that EB1a and b contribute both to the bundling and to the 3D organization of plant MTs, the two events being possibly linked. We are currently investigating both cell wall architecture and cell mechanical properties in order to correlate the plant growth defect to the sub-cellular phenotype.

P054 - Function of the deubiquitinating enzyme AMSH3 and an SH3 domain containing protein in intracellular trafficking in Arabidopsis

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Endocytosis is important to regulate protein abundance at the plasma membrane, by which transmembrane proteins can be transported to and degraded in the vacuole. In many cases, ubiquitination initiates the endocytosis of a protein, upon which the cargo protein is transported to the vacuole for degradation by the endosomal sorting complexes required for transport (ESCRT) I, II, and III. Ubiquitination, and hence substrates stability, can be regulated by ubiquitinating- as well as deubiquitinating enzymes (DUBs). We are interested in a class of metalloprotease DUBs, ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM (AMSH). The Arabidopsis genome encodes three *AMSH* homologs. We have previously

shown that AMSH1 and AMSH3 interact with subunits of ESCRT-III and that they are involved in the endocytic- and autophagic protein degradation pathway. To understand the molecular mechanism by which AMSH3 is regulating cellular trafficking events, we wanted to identify further interacting proteins of AMSH3 and found an Arabidopsis SH3 domain containing protein as a yeast two-hybrid interactor of AMSH3. We are currently conducting further analyses with the aim to understand the molecular framework supporting AMSH3- and SH3 domain containing protein function in intracellular trafficking.

P055 - Auxin and Rho-of-plant direct actin-mediated polar nuclear migration in Arabidopsis root epidermal hair cells

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Polar nuclear movement at the subcellular level is crucial during multiple events in eukaryotic development. In most cases, components and regulators of the cytoskeleton as well as nuclear envelope proteins are major components involved, as demonstrated by severe developmental disorders in respective mutants. In the Arabidopsis root epidermis, the nucleus initially assumes a position at the inner lateral membrane during cell elongation and moves towards the root hair after hair bulging. Except for its actin-dependence demonstrated by pharmacological studies, little is known about dynamic hallmarks and regulatory mechanisms underlying this polar nuclear migration. Here, we report that nuclear auxin signaling and Rho-of-Plant (ROP) signaling direct actin-dependent polar nuclear migration towards the root hair bulge. Time-lapse imaging reveals that the nucleus located at the inner lateral membrane starts its actin-associated movement towards the outgrowing root hair in an ACTIN7 (ACT7)-dependent manner. Loss of *ACT7* function as well as reduced or enhanced activation of ROP signaling alter polar nuclear migration. High auxin concentration or reduced *CTR1* kinase function induce nuclear mis-positioning that is suppressed by *ARF7 ARF19* loss of function, revealing that nuclear auxin signaling regulates nuclear migration. Our findings establish a mechanistic framework of auxin- and ROP-signaling-directed, ACT7-dependent polar nuclear migration in the Arabidopsis root epidermis.

P056 - CO2 activation of the guard cell S-type anion channel is impaired in an Arabidopsis mutant with non-chlorophyllous stomata

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Guard cells in most plant species have photosynthetically active chloroplasts. Guard cell chloroplasts have been proposed to play an important role in the osmoregulatory mechanisms mediating stomatal movements, although their function has been a subject of debate and remains to be confirmed. In this study, we isolated an *Arabidopsis gles1 (green less stomata*

1) mutant that had non-chlorophyllous stomata. In this mutant, most of the guard cells had either chloroplasts with a faint amount of chlorophyll auto-fluorescence or chlorophyll less chloroplasts, whereas normal chlorophyllous mesophyll cells were present. To elucidate the role of guard cell chloroplasts in stomatal movement, we measured the stomatal response to CO_2, light and abscisic acid (ABA). CO_2 and light dependent stomatal responses were severely disturbed in the nonchlorophyllous stomata of the *gles1* mutant. Whole-cell patch-clamp experiments indicated that in the mutant guard cells, CO_2 activation of the S-type anion channels was impaired. On the other hand, this mutant showed a functional response to ABA. These results provide direct evidence to support the idea that guard cell chloroplasts are essential for lightinduced stomatal opening and CO_2-induced stomatal closure.

P057 - Arabidopsis Lost Its Sister: Why Brachypodium Is a Better Model to Study PIN-Mediated Auxin Transport in the Shoot

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In the *Arabidopsis* shoot the auxin-transport protein PIN-FORMED1 (AtPIN1) positions incipient organ primordia by concentrating auxin into local maxima. From these maxima, AtPIN1 transports auxin internally, along paths that pattern vasculature. We identified a PIN clade sister to AtPIN1, here termed Sister-of-PIN1 (SoPIN1), which is present in all sampled angiosperms but lost from Brassicaceae, including *Arabidopsis*. In the grass *Brachypodium*, SoPIN1 is highly expressed in the epidermis and polarized toward maxima of the DR5 auxin-signaling reporter, which suggests SoPIN1 functions to localize new primordia. In contrast, the duplicate *Brachypodium* PIN1 proteins, PIN1a and PIN1b, are expressed in internal tissues, and polarize away from epidermal DR5 maxima, suggesting a role in vascular patterning. SoPIN1 and PIN1b co-localization shows that each protein can polarize differently within a single cell, suggesting their disparate polarization modes are protein sequence and not expression context dependent. Remarkably, when SoPIN1 and PIN1b are hetrologously expressed in *Arabidopsis*, only SoPIN1 is able to create the convergent polarization patterns thought necessary to form auxin maxima, and accordingly, only SoPIN1 is able to rescue the naked inflorescence phenotype characteristic of the *Arabidopsis pin1* mutant. Thus in most flowering plants multiple PIN family members with different functional properties may mediate organ initiation and vein patterning in the shoot.

P058 (Talk) - AtPep1 and its plasma membrane receptors are internalized as a complex via clathrin-mediated endocytosis

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Recently, a growing number of small signaling peptides have been discovered to play an active role in a variety of biological and physiological responses in plants. Amongst them is the plant elicitor peptide1 (Pep1), which has been shown to induce innate immune responses and to be expressed upon biotic stresses. In Arabidopsis, the AtPep1 binds the plasma membrane receptors PEPR1 and PEPR2 with high affinity. Although a large amount of information about the components and responses triggered by AtPep1 is available, its subcellular dynamics remains largely unknown. In the present study, we developed a bioactive fluorescently labeled AtPep1 to study its behavior in living cells. We found that the labeled AtPep1 was able to bind the plasma membrane very quickly in a receptor-dependent manner. Subsequently the receptor-ligand complex was internalized via clathrin-mediated endocytosis and trafficked to the lytic vacuole, passing through early and late endosomal compartments. Impairment of AtPep1/PEPRs complex internalization compromised the innate immune responses. Our findings provide the first in vivo visualization of a signaling peptide in plant cells, thus giving new insights on its intracellular fate and dynamics, and also serve as an excellent model to study the implications of endocytosis in plant immunity.

P059 - UUAT1: an UDP-uronic acid transporter involved in cell wall composition

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The cell wall is a complex extracellular matrix mainly composed of polysaccharides synthetized in the Golgi apparatus by glycosiltransferases (GTs). UDP-glucuronic acid (UDP-GlcA) is a key molecule involved in this process since it serves as precursor for the synthesis of polysaccharides. UDP-GlcA is synthesized in the cytosol and needs to be transported into the Golgi apparatus lumen, where the catalytic site of the epimerases and GTs are located. In this process Nucleotide Sugars Transporters (NSTs) are key elements. To date, no UDP-GlcA transporters have been identified and their role in polysaccharides biosynthesis remains unclear. Here we present the identification and characterization of UUAT1, the first UDP- GlcA transporter located in the Golgi apparatus. *UUAT1* is highly expressed in seed coat, trichomes, roots, stems and flowers. Sugar composition analyses on these organs showed that *uuat1-2* mutant have deficiencies in galacturonic acid and/or arabinose, two sugars derived from the UDP-glcA metabolism, depending on the tissue investigated. Additional analyses of the seed coat showed that arabinan is reduced in the distal cell walls, which is concomitant with an altered mucilage release and an increase in the mucilage homogalacturonan methylation. Our results provide evidences for the existence of a UDP-GlcA transporter, which provides the substrate for the biosynthesis of polysaccharides, in a very specific and highly regulated manner. Thanks: Fondecyt 3140415.

P060 - Isolation and characterization of the suppressor mutants of ht1-2 in Arabidopsis

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CO_2 is not only a carbon donor for photosynthesis but also an environmental signal that regulates stomatal aperture. With a high-throughput leaf thermal imaging screen, several mutants have been identified that are impaired in stomatal response to CO_2 concentration changes. *Arabidopsis ht1 (high leaf temperature 1)* is one of the identified mutants that are insensitive to CO_2. The stomatal aperture of the *ht1* is reduced under both low- and high-CO_2 conditions. The *HT1* gene encodes a protein kinase that is expressed predominantly in the guard cells and functions as a major negative regulator of CO_2-induced stomatal closing. However, the molecular mechanisms that underlie stomatal CO_2 signaling have remained obscure, so we attempted to isolate components of stomatal CO_2 signaling pathway. We performed preliminary screening using infrared thermography from a population of nearly 8,000 mutagenized *Arabidopsis ht1-2* lines, and selected 6 mutant lines. While the *ht1-2* mutant exhibits reduction of stomatal aperture, the stomata of the selected 6 mutant lines opened under both low- and high-CO_2 conditions. These mutants showed functional response to abscisic acid, the hormone that triggers closing of the stomata, suggesting that they are specifically deficient in the CO_2-signaling pathway. Further analysis of these mutants may identify unknown components specific to the CO_2-signaling pathway.

P061 (Talk) - Emerging role of ARP2/3 complex in tissue patterning

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The ARP2/3 complex consists of seven protein subunits and is well conserved in eukaryotes. The main role of ARP2/3 complex is well established in yeast and animals, where it nucleates actin cytoskeleton into branched network and the loss of the ARP2/3 complex is often detrimental. The loss of ARP2/3 complex in plants results in rather mild phenotypes like distorted shapes of trichomes or changes in pavement cell morphology, as studied in *Arabidopsis*. However, in the present study, we identified new phenotypes in mutants of *Arabidopsis* plants lacking ARP2, ARPC4 and ARPC5 subunits, which include decreased number of cell layers in leaves and stems of mutants, thinner organs, and overall changes in the organization of cells within tissues. Thinner cell walls in cells of interfascicular tissues and generally reduced cellulose content in inflorescence stems was accompanied by changes in lignin deposition. Moreover, auxin response reporter DR5::GUS as well as auxin influx carrier AUX1-YFP were reduced in the vascular bundles suggesting that auxin distribution and transport was also altered in the mutants. Our results indicate that either ARP2/3-mediated actin nucleation is of primary importance for the processes of cell wall synthesis and localization of auxin carriers, or that the malfunction of ARP2/3-driven nucleation triggers a set of compensatory mechanisms characterized by complex changes in tissue and organ morphogenesis.

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P062 (Talk) - Arabidopsis NEK6 depolymerizes cortical microtubules by tubulin phosphorylation during directional cell growth.

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Microtubules control cell expansion and division through its dynamic behavior in response to the internal and external signals. Here, we analyzed the function of Arabidopsis NIMA-related kinase 6 (NEK6) in phosphorylation-dependent microtubule regulation during cell growth. The *nek6* mutants exhibited decreased elongation and ectopic outgrowth of hypocotyl epidermal cells in association with microtubule disorganization. The suppression of cell elongation and the ectopic outgrowth were observed in the different regions along the hypocotyl. The estradiol-induced expression of *NEK6* also suppressed cell elongation and disorganized cortical microtubules, suggesting that NEK6 activity is tightly regulated to direct cell expansion. Live cell imaging of NEK6-GFP and mCherry-TUB6 demonstrated that NEK6 localized to the shrinking ends of microtubules. Furthermore, we identified five phosphorylation sites of beta-tubulin for NEK6 and analyzed their functional significance. The substitution of phosphorylation sites by alanine promoted microtubule localization of GFP-tubulin, whereas the phosphorylation-mimic substitutions enhanced the cytoplasmic localization of GFP-tubulin. These results elucidate that NEK6 depolymerize cortical microtubules by tubulin phosphorylation during directional cell expansion.

P063 - Pleckstrin Homology domain protein 1 (AtPH1) controls the subcellular localization of Natural Resistance Associated Macrophage Protein 1 (AtNRAMP1)

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Pleckstrin Homology domain containing proteins bind phosphorylated phosphatidylinositols. However, their functions in plant cells are still poorly understood. We found that loss of function mutations in Arabidopsis Pleckstrin Homology protein 1 (*AtPH1*) rescued the defects of *nramp3nramp4* mutant impaired in metal efflux from the vacuole. AtPH1 specifically binds phosphatidylinositol 3 phosphate (PI3P). AtPH1 was localized in late endosomes and this association was disrupted by wortmanin, an inhibitor of PI3P biosynthesis, as well by a point mutation in the PH domain. We tested the hypothesis that loss of AtPH1 function could result in the localization to the vacuolar membrane of a metal transporter which resides on a different compartment in wild type cells. In agreement with this hypothesis, we found that AtNRAMP1, which is targeted to the plasma membrane to take up Mn under Mn deficiency, accumulated at the vacuolar membrane in *ph1* cells. In *nramp3nramp4ph1*, the presence of AtNRAMP1 in the vacuolar membrane compensates for the absence of AtNRAMP3 and AtNRAMP4 on this compartment. This work identified AtPH1 as a regulator of AtNRAMP1 and possibly other membrane proteins sorting to the vacuole.

P064 - The dynamics of trans-Golgi network (TGN) in plants

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In all eukaryotic cells, the post-Golgi organelles, such as the *trans*-Golgi network (TGN), endosomes, vacuoles, and the plasma membrane, are connected by a network of membrane traffic. The TGN is required for protein transport at the post-Golgi network and functions as a sorting station that directs cargo proteins to a variety of post-Golgi compartments. Initially, the TGN was defined as a specialized compartment on the most *trans*-side of the Golgi apparatus that is responsible for delivering proteins to the post-Golgi network. However, the knowledge about the TGN in plants is still limited. To elucidate the dynamics of plant TGN, we established transgenic plants expressing GFP-SYP43, the ortholog of Tlg2/syntaxin16, which is localized to the TGN in yeast and mammalian cells, under the control of the native promoter as a TGN marker. Observation by confocal laser scanning microscopy and super-resolution confocal live imaging microscopy revealed two types of TGN in Arabidopsis root: the GA-TGN (Golgi-associated TGN), located on the trans-side of the Golgi apparatus, and the GI-TGN (Golgi-released independent TGN), located away from the Golgi apparatus and behaving independently. The GI-TGN is derived from a population of GA-TGN by segregation, although the core of the GA-TGN remains even after the generation of GI-TGN. We further found that abundance of the GI-TGN differs between observed tissues. Our results indicate that the dynamic features of the TGN in plant cells differ from those of animal and yeast cells. In addition, we have already reported that the SYP4 group regulates the secretory and vacuolar transport pathways in the post-Golgi network and maintains the morphology of the Golgi apparatus and TGN. Moreover, SYP4 proteins are required for biotic and abiotic responses. In this conference, we will report and discuss our recent results about the TGN dynamics under the biotic stress conditions.

P065 - Calcium is required for auxin-regulated endocytosis

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Auxin is a major decisive signal for plant growth and development. Its distribution within tissues and organs regulates cell division, expansion and cellular reprogramming throughout the plants life. Critically important for such auxin distribution patterns is active polar auxin transport mediated by PIN proteins which are asymmetrically localised in the plasma membranes. Via inhibition of endocytosis, auxin is believed to enforce PIN polarisation and thus coordinated auxin transport across tissues. Recently, the apoplastically localised ABP1 has been connected to auxin-inhibited endocytosis via ROP and RIC signalling. Auxin is known to induce fast Ca^{2+} signals through an unknown mechanism. Here, we explored the origin of auxin-induced Ca^{2+} signals and tested the impact of manipulating Ca^{2+} on endocytosis. We found that Ca^{2+} is required for the inhibitory effect of auxin endocytosis. Conversely, increasing cytosolic Ca^{2+} by inhibiting Ca^{2+} ATPases was sufficient to inhibit endocytosis. These findings are consistent with a model in which auxin inhibits endocytosis via activation of Ca^{2+} signalling. However, thusfar, none of the tested Ca^{2+} signalling or transport mutants showed obvious defects in auxin-regulated endocytosis.

P066 - Ethylene induces microtubule reorientation and fast inhibition of Arabidopsis root elongation in a TIR1/AFBs-Aux/IAAs dependent manner

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Microtubules are essential regulator of plant cell shape and morphogenesis. Ethylene-induced microtubule reorientation has been discovered for decades, and is believed to mediate the effect of ethylene on anisotropic cell expansion. However, signaling mechanism underling this long-existing observation has rarely been touched. Here, we revealed by live confocal imaging and root growth kinetic assay that the time courses of ethylene-induced microtubule reorientation and root elongation inhibition well resemble each other, suggesting a close correlation between these two processes. Our genetic analysis demonstrated that the effect of ethylene during these two processes is mainly transduced through the canonical linear ethylene signaling pathway. Functional ETR1, EIN2 and EIN3/EIL1 are all required for full ethylene responsiveness, although an EIN3/EIL1-independent modest response is observed in both microtubule reorientation and root elongation inhibition. We found through pharmacological and genetic analysis that disruption of endogenous auxin biosynthesis and transport abolishes ethylene-induced microtubule reorientation and root elongation inhibition, and the TIR1/AFBs-Aux/IAAs-ARFs auxin signaling pathway, but not the ABP1-ROP6-RIC1 signaling branch, is essential during these processes. Unexpectedly, dynamic imaging and 3D quantification of the DII-Venus auxin sensor indicated that increasing of local auxin level is not involved in fast ethylene-induced responses in *Arabidopsis* root, suggesting ethylene signaling may directly interact with signaling components of TIR1/AFBs-Aux/IAAs-ARFs pathway during fast root ethylene responses. Together, we present a genetic mechanism for ethylene-induced microtubule reorientation and fast root elongation inhibition in *Arabidopsis*, which involves an essential role of TIR1/AFBs-Aux/IAAs-ARFs auxin signaling pathway.

Keywords: microtubule reorientation, root elongation, ethylene, auxin

P067 - Plant Nitric Oxide Sensors - Tip of the Iceberg?

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Nitric oxide (NO) is a key signaling molecule in many plant processes including fertilization. In pollen, NO has been identified as a signal for pollen-stigma interaction and in guiding the growing tube to the ovule. NO generation in pollen has been demonstrated and intracellular regulatory roles for NO include actin organization, vesicle trafficking and cell wall deposition. However, sensors for NO have remained elusive. Here, we show that the pollen-specific *Arabidopsis thaliana* Diacylglycerol Kinase 4 (AtDGK4) contains a domain with key residues of the heme-binding centers of annotated gassensing hemoproteins and demonstrate that AtDGK4 senses NO. This binding is significantly reduced in mutants with altered residues in the NO-binding site. Importantly, both reducing agent

and NO caused rapid dose-dependent Soret peak changes diagnostic for sensors. AtDGK4 has NO-dependent diacylglycerol kinase activity *in vitro* catalyzing the conversion of diacylglycerol to phosphatidic acid. In atdgk4 knock-down mutants, pollen germination, growth rate and pollen tube orientation show less NO-dependent inhibition consistent with a role as an NO signal transducer. Preliminary results of two other Arabidopsis candidate NO sensors harboring similar heme center motif are also shown. Since this motif is highly conserved across bacterial, plant and animal proteins many of which are not currently annotated as NObinding, we anticipate that they also bind to and are directly regulated by NO.

P068 - Transmembrane region of the Arabidopsis guard cell SLAC1 anion channel involved in stomatal CO2 response

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The *Arabidopsis* SLAC1 channel is a key component in the control of ABA- and CO_2-induced stomatal closure. Regarding the regulation of the SLAC1 channel, phosphorylation of Serine 59 and Serine 120 in its N-terminal region by CPK6 and OST1 has been reported to be crucial for ABA-induced SLAC1 activation. In contrast, the mechanism of CO_2-induced SLAC1 activation remains unclear. To investigate regions of SLAC1 protein involved in stomatal response to CO_2 *in vivo*, we analyzed *slac1* mutants transformed with N-, C- or both terminals of the SLAC1 truncated constructs (ΔN, ΔC and ΔNC). Measurement of gas exchange of the transgenic plants showed that all the lines restored CO2 responses. However, these transgenic lines did not restore the ABA regulation of stomatal movement. Furthermore, whole-cell patch clamp experiments revealed that in the ΔNC transgenic line, bicarbonate activation of the S-type anion currents was restored in guard cells, whereas ABA activation of the anion currents is completely disrupted. These results indicate that sites involved in stomatal CO_2 response exist in the transmembrane region of SLAC1 and could be different from the sites of ABA activation.

P069 - Temporal and Spatial Regulation of Cell Wall Regeneration on Arabidopsis Mesophyll Protoplasts

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Plant cell wall is a complex and dynamic structure that is a highly organized composite of many polysaccharides and proteins. Although various types of structural and functional factors are supposed to contribute to the assembly and remodeling of the cell wall, the mechanism of dynamic regulation of the cell wall is almost unknown. To clarify the mechanisms of cell wall construction, we developed an improved experimental procedure for cell wall regeneration in isolated Arabidopsis mesophyll protoplast. The application of imaging technique for visualizing and quantifying the fine structure of the cell wall greatly facilitated the value of this experimental system. For example, the imaging and quantification of cellulose orientation during regeneration from the protoplast showed that ordered cellulose deposition is strictly regulated. We also identified the proteins secreted into cell wall spaces in regenerating protoplast by an integrated omics approach. Combined omics-based identification of the cell wall proteins and the new experimental system for investigating the cell wall structure will assign specific roles to these proteins in the assembly and remodeling of the cell wall in regenerating protoplast.

P070 - The Distinct Functions of Individual Carbonic Anhydrases in CO2 Control of Stomatal Movements

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Elevated carbon dioxide (CO_2) reduces stomatal apertures. However, early mechanisms by which the CO_2 signal is transduced remain enigmatic. Research has shown key functions of the *γ*-carbonic anhydrases CA1 and CA4 (*γ*CAs) in rapid CO_2-induced stomatal movements by catalytic transmission of the CO_2 signal in guard cells. However, the mechanisms remain unclear, as initial studies indicate *γ*CAs are targeted to distinct compartments in tobacco cells and which cellular location of these enzymes plays a key role in guard cells in CO_2-regulated stomatal movements remains unknown. Here we show that the specific locations of *γ*CA4 at the plasma membrane and *γ*CA1 in chloroplasts each function in rapid CO_2 control of stomatal movement. Combined with localization and complementation analyses using a mammalian ACAII-YFP protein in guard cells and the mathematical CO_2 flux modeling, the presented data suggest that intracellular HCO3- concentration dynamics in guard cells is a mechanism mediating CO_2-regulated stomatal movements.

P071 - Phytochrome A signalling - by taxi to the nucleus

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Phytochromes are plant red/far red photoreceptors which control growth and development according to environmental conditions. Arabidopsis thaliana has five phytochromes, from which phytochrome A (phyA) is important for early seedling development. Light-activated phyA translocates from the cytoplasm into the nucleus. Nuclear localization of phyA leads to a change in transcription of numerous genes. However not all physiological responses can be explained through that mechanism suggesting the existence of cytoplasmic phytochrome functions. Translocation of phyA requires the presence of two signaling components: FHY1 (far-red elongated hypocotyl) and FHL (FHY1-like), with FHY1 playing the dominant role. Nuclear import of phyA in the *fhl/fhy1* double mutant is undetectable. FHY1 contains a Nuclear Localization Signal (NLS) recognized by importins, which are involved in the classical nuclear import of proteins. Importins can use actin filaments to move other molecules into the nucleus and it could be shown that the cytoskeleton is also involved in phyAs nuclear import. Protein-protein binding assays suggest that the interaction between FHY1 and importin alpha 1 (IMPa1) is not depending on phyAs presence. Therefore the regulation of phyA import by FHY1 has to rely on changing the affinity between phyA and the FHY1-IMPa1 complex, possibly by light-dependent secondary modifications of either partner. Moreover FHY1/FHL appears to shuttle between nucleus and cytoplasm since FHY1 contains also a Nuclear Export Signal (NES) recognized by exportins. The mechanism of phyA transport and FHY1 shuttling will be discussed.

P072 (Talk) - Fast-suppressor screening identified SOF10 and SOF100 in FREE1-regulated protein trafficking, organelle biogenesis and plant growth in Arabidopsis

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Membrane trafficking is essential for plant growth and responses to external signals. We have recently demonstrated the multiple functional roles of FREE1, a unique FYVE domain containing protein, in regulating protein trafficking and organelle biogenesis in plants, including formation of MVB, PM protein degradation in the vacuole, vacuolar transport, central vacuole biogenesis, autophagic degradation and autophagosome-vacuole

fusion [1-3]. To further understand why and how FREE1 depleted cells contain fragmented small vacuoles and how FREE1 loss-of-function leads to seedling death, we performed a forward genetic screen for mutants that suppressed the seedling lethal phenotype of FREE1 RNAi transgenic plants. The obtained mutants are termed as suppressors *of free1 (sof)* [4]. Two candidates SOF genes (SOF10 & SOF100) have been identified as null mutants harboring premature stop codon mutations. Subcellular endosomal localization of SOF10 and SOF100 showed that these two genes participated in membrane trafficking. Co-localization studies showed that both SOF10 and SOF100 positive endosomes correlated with autophagasomes and FREE1-labeled endosomes. Here we will present an update about our study on SOF10 and SOF100 identification and function in Arabidopsis. Supported by grants from the Research Grants Council of Hong Kong (AoE, CRF and GRF) and the Shenzhen Peacock Project (KQTD201101).

Keywords

FREE1, SOF, Autopahgosome, Endomembrane trafficking, Arabidopsis,

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P073 - Roles of SH3P2 in Plant Autophagosome Biogenesis

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Macroutophagy (hereafter as autophagy) plays important physiological roles in eukaryotic cells, but the underlying mechanisms of autophagosome formation remain largely unknown in plant. Here we show that the *Arabidopsis* SH3P2, which contains a N-terminus BAR (Bin-Amphiphysin-Rvs) domain and C-terminus SH3 (Src homology 3) domain, associates with the PI3K complex and interacts with ATG8 to regulate autophagy. Dynamic and ultra structural analysis reveals that SH3P2 localizes on the preautophagosome structure throughout its expansion process and highlights the endoplasmic reticulum (ER) as a common membrane source for autophagosome formation in plant. RNAi knock-down of SH3P2 is developmental lethal and suppresses the autophagosome formation and autophagic flux. Recently, we have also demonstrated that SH3P2 interacts with a unique plant ESCRT component FREE1, which is required for multivesicular body (MVB) protein sorting and vacuole biogenesis in plant, hence linking the possible crosstalk between endosome and autophagosome machinery. Taken together, our findings provide a conclusive model for autophagosome formation involved with ER membrane in plant and identify a novel regulator that mediates autophagosome biogenesis in *Arabidopsis thaliana*, which may facilitate the membrane deformation that is necessary for autopahgosome membrane expansion/maturation steps. Supported by grants from the Research Grants Council of Hong Kong (AoE, CRF and GRF) and the Shenzhen Peacock Project (KQTD201101).

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P074 - Carotenoid derived signals alter leaf development

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The functionality of chloroplasts depends on a differentiation process in response to specific signals and in coordination to the tissue differentiation. The nucleus encodes the majority of the structural and regulatory proteins that modulate chloroplast development. However, the developing plastids also generate signals that modulate the expression of nuclear-encoded genes diverse signaling pathways. Experimental evidences have shown that the differentiation of the organelle also impacts the overall leaf development, but the signals responsible for this regulation are largely unknown. The characterization of an *Arabidopsis clb5* mutant, impaired in early chloroplast development, provided genetic and molecular evidences of a novel signal that profoundly affects leaf development. We found that this signal is generated from the accumulation of linear carotenoids as consequence to a defect in the zeta carotene desaturase (ZDS) activity. The observed phenotypes are specific for this mutation as are not present in other carotenoid deficient plants. Our data demonstrate that the yet unidentified signal responsible for the *clb5* phenotypes is produced through specific cleavage of phytofluene or z-carotenoids by the carotenoid digoxigenase 4 enzyme. Using different strategies, including wide genome analysis, we have shown that this signal affects the expression of various nuclear-encoded genes, important for proper leaf development. We have demonstrated that the regulation of expression depend on cis-acting sequences located upstream promoter region. To advance in the molecular events associated with the leaf morphological alterations the expression pattern of the ZDS gene has been analyzed during leaf development.

Transcriptional regulations

Posters 075 to 096

P075 - Global identification of transcription factors targeted by the histone acetyltransferase GCN5 in Arabidopsis thaliana

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GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) appears to be an important histone acetyltransferase required for gene expression involved in many development pathways in plants and animals. Mutations in Arabidopsis thaliana GCN5 (AtGCN5) show various pleiotropic defects as a consequence of affecting the activity meristem activity. Although AtGCN5 plays an essential role in chromatin modification and transcriptional regulation, its mode of action is still not understood. Proteins involved in chromatin remodelling control the development of plants and animals through directly regulating the expression of specific developmental transcription factors. In this work, we have identified a set of potential direct target genes of AtGCN5 through a chromatin immunoprecipitation/ DNA sequencing (ChIP-Seq) approach. A global analysis revealed that AtGCN5 targets are enriched in specific biological processes including metabolisms, nutrient transport and transcription. Among these targets, we identified transcription factors belonging to different families. Using a genetic and chemical approach, these transcription factors have been validated as direct targets of AtGCN5. Functional analysis will reveal the role of these transcriptions factors in plant development and their genetic interaction with AtGCN5 in arabidopsis thaliana.

P076 (Talk) - SERRATE interacts with subunits of the NEXT complex and the polyadenylation machinery in Arabidopsis thaliana

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The *Arabidopsis thaliana* SERRATE protein (SE) is involved in two important pathways of RNA metabolism: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA biogenesis, where together with DCL1 (Dicer like 1) and HYL1 (HYPONASTIC LEAVES 1) form a core of the plant microprocessor. In this complex SE influences the accuracy of pri-miRNA cleavages catalyzed by DCL1. The Arabidopsis se null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the cap binding complex (CBC), have been also ascribed to splicing of pre-mRNA. In order to understand this dual role of SE in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the se-1 mutant genetic background. The SE-bound proteins were identified by mass spectrometry, and the putative interactions were confirmed by the yeast two hybrid system and pull-down experiments. Our results have clearly demonstrated that SE contacts directly both the NEXT complex and the polyadenylation machinery. We suggest that these interactions are important for the quality control of miRNA precursors and/or degradation of pri-miRNA fragments after miRNA excision.

P077 - Role of H3K27me3 and PRC2 members in the regulation of NRT2.1 in response to nitrogen variations

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Nitrate is an essential source of nitrogen for plants and also controls many physiological responses as a signaling molecule. Root nitrate transporters, such as *NRT2.1*, are therefore subjected to complex transcriptional regulations by nutritional and developmental signals. In eukaryote, chromatin controls genome expression by arranging different chromatin states allowed by chromatin marks deposition such as H3K27me3 deposited by the Polycomb Repressive Complex 2 (PRC2). We have demonstrated previously that repression of *NRT2.1* by high nitrogen supply (HN) is correlated with an increase in H3K27me3, a mark of transcriptionally silent chromatin. However, the role of H3K27me3 enrichment in this response is still unclear. Here, we show first that *NRT2.1* transcriptional repression precedes by far H3K27me3 deposition at *NRT2.1* locus, suggesting that H3K27me3 enrichment is used to reinforce the repression by HN. We also use PRC2 mutants to analyze *NRT2.1* spatiotemporal regulation, and demonstrate that H3K27me3 is mainly involved in the developmental, rather than in environmental, coordination of *NRT2.1* expression. Finally, we develop a cell-type specific approach to identify chromatin state variations in response to nutritional changes. This work will highlight the importance of chromatin dynamics in the adaptation of plants to their nutritional environment.

P078 (Talk) - UV-B-Responsive Association of the Arabidopsis bZIP Transcription Factor ELONGATED HYPOCOTYL5 with Target Genes, Including Its Own Promoter

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Exposure to UV-B radiation triggers responses in plants that minimize damage caused by UV-B. The bZIP transcription factor ELONGATED HYPOCOTYL5 (HY5) acts downstream of the UV-B photoreceptor UV RESISTANCE LOCUS8 (UVR8) and promotes UV-B induced photomorphogenesis and acclimation. Expression of HY5 is induced rapidly by UV-B and HY5 protein is required for the majority of UV-B responsive genes. Up to now the transcriptional framework mediating UV-B induced gene expression is not well established. Our recent work focuses on the regulation of HY5 transcription in response to UV-B and the impact of UV-B on the association of HY5 with its target promoters. Using chromatin immunoprecipitation studies we show that UV-B signaling regulates the dynamics of HY5 binding to the promoters of UV-B-responsive genes. HY5 enrichment at target promoters is enhanced by UV-B in a UVR8-dependent manner and overexpression of REPRESSOR OF UV-B PHOTOMORPHOGENESIS2 (RUP2), a negative regulator of UVR8 function, blocks the UV-B effect on HY5 binding. Moreover, we have identified a T/G-box in the HY5 promoter that is required for its UV-B-responsiveness. We show that HY5 and its homolog HYH bind to the T/GHY5-box cis-acting element and that they act redundantly in the induction of HY5 expression upon UV-B exposure. We conclude that HY5 enrichment at target promoters is an integral part of the UV-B response in a UVR8 photoreceptor dependent manner and that HY5 and HYH interact directly with a T/G-box cis-acting element of the HY5 promoter, mediating the transcriptional activation of HY5 in response to UV-B.

P079 (Talk) - Nuclear control of chloroplast biogenesis

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The plant life cycle depends on the swift acquisition of photoautotrophy after seed germination and light perception by the seedling. The early steps of light responses, known as photomorphogenesis, involve photoreceptors transducing light signals into a genetic response that trigger plastid differentiation into functional chloroplasts. Plastids are genetically semi-autonomous organelles supporting photosynthesis, essential biosynthetic pathways (amino-acids, fatty acids, phytohormones...), and other diverse functions (immunity, pigmentation...) depending on their differentiation state. They possess their own genome and a machinery to express it, but they highly depend on the import of nuclear-encoded proteins from the cytosol. Recent data including some from our team indicate that the major functional plastid-encoded RNA polymerase complex (PEP complex) contains four plastid-encoded subunits and a minimum of 10 nuclear-encoded proteins (called PEP-associated proteins or PAPs) that are essential for its transcriptional activity. Individual genetic excisions of the *PAPs* lead to severe albino phenotypes indicating that the PEP complex is a keystone of chloroplast biogenesis whereas very little is known about its formation and dynamics. Our working model is that alteration of any PAP leads to complex instability, masking the biochemical function of each individual component but emphasizing the importance of the whole. Co-expression- and promoter analysis suggest the existence of a master regulator of the PAPs. To identify this putative regulator in *Arabidopsis*, we are setting specific marker lines of the PAP-expression pattern in order to develop targeted mutant screens. In-depth analyses of PAP promoters in transgenic plants revealed common patterns of GUS expression but also diverging patterns among the different PAPs. Out of 10 potential marker lines PAP 2,

5 and 8 were pre-selected as starter lines for mutagenesis. PAP2 promoter is active as early as during embryogenesis preceding the photosynthetic phase of the embryo, indicating light and also development-dependent regulations.

P080 - Role of LEC2 during seed development: identification of partners and biochemical characterization of the complex.

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The ability to make seeds is a fundamental and specific trait of higher plants. Seeds are the main resource for animal and human nutrition and the means for improving agricultural practices and managing genetic resources. Genetic studies mostly carried out in the model species *Arabidopsis thaliana* have delineated a complex network of transcriptional regulators controlling gene expression programs essential to accomplish the maturation phase (such as accumulation of seed storage compounds lipids and proteins, inhibition of differentiation, acquisition of desiccation tolerance…). Key transcriptional activators of the maturation phase include founding members of the B3 domain family of DNA-binding proteins, namely ABSCISIC ACID INSENSITIVE3 (ABI3), FUSCA3 (FUS3), and LEAFY COTYLEDON2 (LEC2). This AFL (ABI3/FUS3/LEC2) network acts in concert with protein homologous to the HAP3 subunit of the CAAT

box-binding protein called LEC1 and LEC1-LIKE. We have investigated the relationships between LEC2 and Oleo1(S3) promoter activity, alone and in combination with other AFLs. Our results indicate a synergetic effect of AFLs on S3 transcription level and suggest the formation of a putative complex between LEC2, ABI3 and LEC1. The objective of this study is to validate members of the complex and will be undertaken by *in vitro* and *in vivo* analysis using protein-tagging approaches. Moreover, precise localisation and accumulation pattern of LEC2 and LEC1 during seed development is being analysed using specific antibodies.

P081 - Analysis of cell cycle regulation by the SnRK1 kinase using a protoplast transient expression system.

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Plant carbon and energy status integrate very diverse developmental and stress signals, enabling optimal growth and survival in a continuously changing environment. The exact molecular mechanisms involved are still largely unknown. The key regulators HXK1 and TOR respond to sugar supply, while SnRK1 plays an important role in reprogramming metabolism and limiting growth for energy homeostasis and survival in metabolic stress conditions. Plant growth results from coordinated cell division, expansion and differentiation and several studies point to a direct and possibly conserved metabolic control of the eukaryotic cell cycle in organisms from yeast to man. As studies of cell division and cell cycle progression are hampered by their limitation to meristematic tissues in multicellular organisms and/or the complicated genetics of vital processes, we are setting up a convenient experimental system reconstructing the cell cycle regulatory machinery by transient co-expression in Arabidopsis leaf mesophyll protoplasts. In these differentiated cells, G1/S and G2/M phase transition-specific promoter-luciferase reporters (based on S phase-specific *MCM5* and *ORC2* and M phase-specific *KNOLLE* and *CYCB1.1* gene expression) are responsive to direct upstream regulator co-expression. We are now expressing the entire CYCD/CDKA-RBR-E2F-DP (G1/S) and CYCB/CDKA-MYB3R (G2/M) pathways to identify the exact points and mechanisms of regulation of the plant cell cycle by the energy sensing SnRK1 kinase.

P082 - Bacterial LPS perception leads to dynamic changes in the miRNA profiles of Arabidopsis thaliana cells and leaf tissues

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MicroRNAs (miRNAs) are non-coding RNA molecules which have recently emerged as important gene regulators in plants and their gene expression analysis is becoming increasingly important. miRNAs regulate gene expression at the post-transcriptional level by translational repression or target degradation of specific mRNAs and gene silencing. In order to profile the microtranscriptome of *Arabidopsis thaliana* leaf and callus tissues in response to bacterial lipopolysaccharide (LPS), small RNA libraries were constructed at 0 and 3 h post induction with LPS and sequenced by Illumina sequencing technology. Differential regulation of subset of miRNAs in response to LPS treament was observed. Small RNA reads were mapped to the miRNA database and 358 miRNAs belonging to 49 miRNA families in the callus tissues and 272 miRNAs belonging to 40 miRNA families in the leaf tissues were identified. Moreover, target genes for all the identified miRNAs families in the leaf tissues and 44 of the 49 miRNAs families in the callus tissues were predicted. The sequencing analysis showed that in both callus and leaf tissues, various stress regulated-miRNAs were differentially expressed and real time PCR validated the expression profile of miR156, miR158, miR159, miR169, miR393, miR398, miR399 and miR408 along with their target genes. *A. thaliana* callus and leaf callus tissues respond to LPS as a microbe-associated molecular pattern molecule through dynamic changes to the microtranscriptome associated with differential transcriptional regulation in support of immunity and basal resistance.

P083 - Identification of Regulators of Polypyrimidine Tract Binding Protein Splicing Factors in Arabidopsis

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Alternative splicing (AS) is widespread in plants, affecting more than 60 % of all intron-containing genes in *Arabidopsis thaliana*, and has been linked to fundamental aspects of plant development. The AS outcome depends on the action of splicing factors, only few of which have been examined in plants so far. Among those, the Polypyrimidine Tract Binding Proteins (PTBs) AtPTB1 and AtPTB2, members of the heterogeneous nuclear ribonucleoproteins (hnRNPs), have been demonstrated to regulate several hundred AS events in *A. thaliana*. Given that AS decisions are typically the result of an interplay of diverse factors, we are interested in the identification of modulators of PTB activity. In an unbiased approach, we identified Transportin1 as an interaction partner of AtPTB2. The interaction was independently validated and its impact on PTB function was addressed. Furthermore, as the Serine/Arginine-rich proteins (SRs) have been described as antagonists of hnRNPs, we are determining their effect on the PTB-mediated AS of a reporter construct. Current research aims at the identification of common splicing targets of PTB2 and antagonistically acting SRs. The results of these studies are expected to contribute to a better understanding of the complex mechanisms controlling AS, and thereby to provide novel insight into the regulation of plant development and adaptation to stress.

P084 - Transcription factor target analysis for the Arabidopsis root stem cell niche

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Genes of the *PLETHORA (PLT)* clade of double AP2-domain transcription factors are master regulators controlling multiple aspects of root development in both a dose-dependent and specific manner. Acting in parallel with the PLT pathway in specification and maintenance of the root stem cell niche are the *SHORTROOT (SHR)* and *SCRARECROW (SCR)* genes that belong to the GRAS family of transcription factors. Transcriptomics of niche cells was performed to assess where and how PLT and SHR/SCR pathways converge in stem cell maintenance. In addition, PLT proteins display a gradient expression with maxima in the root stem cell niche and have been implicated to promote stem cells and meristematic activity whilst inhibiting exit from the meristem and differentiation. Phenotypic analyses confirms transcriptomics and ChIP-seq data that reveal PLT redundant regulation of proliferation and differentiation related genes. This is reflected by upregulated target expression patterns in the niche and meristem, whereas downregulated genes predominantly express beyond the gradient of PLT accumulation. For PLT2, both SELEX/EMSA and bioinformatics analysis of ChIP-seq peaks appoint the same binding site consensus. Modification of such predicted binding sites in target promoters revealed their importance in PLT directed transcriptional regulation in planta. Together, these results provide us with a handle to investigate how the PLT gradient is translated into the discrete expression patterns of its targets.

P085 - Functional analysis of bromodomain-containing BRD1, BRD2 and BRD13 proteins in Arabidopsis

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The SWI/SNF chromatin remodeling complexes perform essential role in control of transcription in eukaryotic cells. SWI/SNF complexes in mammals (PBAF), yeast (RSC) and Drosophila (pBAP) include proteins with multiple-bromodomains (Polybromo), which are assumed to target complexes to specific chromatin sites through interaction of bromodomains with acetylated lysines of the core histones [1]. Arabidopsis genome does not contain genes encoding proteins with multiple bromodomains. It is however possible that the functions of Polybromo are fulfilled in plants by several proteins with single bromodomain [2]. Arabidopsis genome encodes 29 bromodomain-containing proteins [3]. Studies in our laboratory as well as the published data [4] suggested that of these proteins BRD1, BRD2 and BRD13 could be involved in chromatin remodeling. Here, we present analysis of direct protein interactions between BRD and the subunits of SWI/SNF complex. Moreover, our phenotypic analysis of *brd* mutants suggests that BRD proteins are involved in leaf development and flowering regulation. Our results provide insights into the role of these newly identified SWI/SNF subunits in plant development.

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P086 - Identification of plant genes involved in resistance of various plant viruses based on transcriptome analysis

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Chrysanthemum plants have been widely cultivated for floriculture and are hosts for many viral pathogens causing reduction of quality and quantity of chrysanthemum production. In order to generate genetically modified (GM) chrysanthemums resistant to viral pathogen, we recently performed gene expression profiles of the chrysanthemum in response to three plant viruses such as *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV) and *Potato virus X* (PVX) using a chrysanthemum135K microarray. We identified several differentially expressed genes and 33 genes were commonly regulated by three viruses. Of identified 33 genes, we validated expression of six genes including ethylene-responsive transcription factor 4, protein phosphatase 2c, ribonucleoside-diphosphate reductase, basic 7c globulin, GDSL esterase lipase, and an unknown function gene by real time RT-PCR. To characterize functions of those genes in detail, we are now producing transgenic *Arabidopsis* plants overexpressing individual chrysanthemum gene. The generated transgenic *Arabidopsis* plants will be tested for the virus resistance. A chrysanthemum gene showing strong virus resistance will be selected for the generation of GM chrysanthemum resistant to various viral pathogens. Taken together, our study is the first comprehensive chrysanthemum transcriptome analysis to identify genes involving in virus infection. In addition, the functional study using *Arabidopsis* plants is an effective approach to select a chrysanthemum gene for the GM chrysanthemum resistant to viral pathogens.

P087 - The 5'UTR-intron of the Gladiolus polyubiquitin promoter GUBQ1 enhances translation efficiency in Gladiolus and Arabidopsis

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Introns have been known to affect both the level and tissue-specific expression of genes in many plants. This study characterizes the levels of GUS expression and levels of uidA mRNA that code for Y-glucuronidase (GUS) expression in leaves of Gladiolus and Arabidopsis using GUBQ1, a polyubiquitin promoter with a 1.234 kb intron, isolated from the non-cereal monocot Gladiolus, and an intronless version of this promoter. Gladiolus and Arabidopsis were transformed with the uidA gene that was under control of either the GUBQ1 promoter (1.9 kb), a 5′ GUBQ1 promoter missing its

1.234 kb intron (0.68 kb), or the CaMV 35 S promoter. Histochemical staining showed that GUS was expressed throughout leaves and roots of Gladiolus and Arabidopsis with the 1.9 kb GUBQ1 promoter. GUS expression was significantly decreased in Gladiolus and abolished in Arabidopsis when the 5′UTR-intron was absent. In Arabidopsis and Gladiolus, the presence of uidA mRNA was independent of the presence of the 5′UTR-intron. The 5′-UTR intron enhanced translation efficiency for both Gladiolus and Arabidopsis.

P088 - Temporal dynamics of NAC regulatory networks underlying leaf aging

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Aging and senescence are induced by an extensive range of developmental and environmental signals and controlled by multiple, cross-linking pathways. Elucidation of this complex process requires the systems-level understanding of molecular networks and network modules, overcoming the current, individual component-based view. Furthermore, aging and senescence are dynamic process, which are regulated by temporal changes of molecular networks for the functional and regulatory transition of cells, organs, and organisms. We previously identified the trifurcate feed forward pathway for age-dependent cell death involving EIN2, ORE1, and miR164 in *Arabidopsis*, which revealed fundamental characteristics of network-level control of plant aging and senescence. ORE1 is one of 109 NAC transcription factors that form heterodimers and transcriptionally regulate themselves. We extended this network structure for a better understanding of the aging network, through analyzing gene regulatory networks for leaf-expressed *NAC* genes along aging. In-depth analyses revealed that the genetic regulatory networks are composed of highly linked regulatory modules and these key modules involving the positive and negative regulation may have an important role in maintaining the balance between cell maintenance and cell death. We also discovered temporal changes of the key regulatory interactions. We, eventually, aim to unveil operating system principles for temporal design of functional network modules underlying leaf aging.

P089 - New mechanistic links between Topoisomerase VI and the transcriptional response to photooxidative stress

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Environmental stresses disrupt the metabolic balance of cells, resulting in the increased photoproduction of reactive oxygen species (ROS) in chloroplasts. The generated ROS may then act as signalling molecules that are involved in plastid-to-nucleus retrograde signalling and eventually activate nuclear genes and the plant defense responses. A genetic screen aimed to identify new factors involved in ROS retrograde signalling in Arabidopsis has allowed us to disclose the central role of the topoisomerase VI (Topo VI) as a key regulator in the plant response to environmental stress conditions, by controling the expression of ROS-responsive genes. Using a yeast-two-hybrid screen, we have identified an interaction between Topo VI and a RNA polymerase II (Pol II) general transcription factor (TFII). This interaction has been confirmed *in planta* and gives the first hints of possible mechanisms by which Topo VI may regulate gene expression, namely by directly interacting with the transcription machinery. Mutant lines defective for each interactor are hypersensitive to photooxidative stress. A RNA-seq comparison of the gene expression profiles shows that more than 85% of the photooxidative stress-induced genes whose expression is compromised in the Topo VI mutant are similarly affected in the TFII mutant. This high similarity in gene expression between the two mutants is consistent with Topo VI acting

with Pol II complexes during the response of plants to photooxidative stress.

P090 (Talk) - DOF AFFECTING GERMINATION 2 is a positive regulator of light-mediated seed germination and is repressed by DOF AFFECTING GERMINATION 1

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The Arabidopsis protein DOF AFFECTING GERMINATION (DAG2) shares a high degree of aminoacidic identity with DAG1, a negative regulator of the phytochromeB-mediated seed germination process. We have previously shown that inactivation of the Dof proteins DAG1 and DAG2 affects in opposite ways seed germination: *dag2* mutant seeds required more light and GA than wild-type seeds to germinate, whereas germination of *dag1* seeds was less dependent on these factors. We also pointed out that DAG1 acts downstream of PHYTOCROME INTERACTING FACTOR3-LIKE 5 (PIL5), and it negatively regulates GA biosynthesis by directly repressing the GA biosynthetic gene *AtGA3ox1*. More recently, we proved that DAG2, opposite to DAG1, functions as a positive regulator in the molecular pathway controlling seed germination, downstream of PIL5 and DAG1, and that it is directly repressed by DAG1.

P091 - Characterising the Regulation Network of Transcription Factor MYB26 during Anther Dehiscence and Pollen Release in Arabidopsis thaliana

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Release of pollen and anther development affects the success of fertilisation and thus indirectly crop yields. During the dehydration of the anther secondary thickening, which is regulated by the MYB26 transcription factor, produces mechanical force in the anther endothecium layer to facilitate anther dehiscence; mutation of MYB26 results in a failure of anther dehiscence and functional male sterility. However the regulatory network of *MYB26* remains to be fully identified. Two NAC domain transcription factors, that act redundantly, have been linked to the regulation of anther secondary thickening and dehiscence, these are *NAC SECONDARY WALL-PROMOTING FACTOR1 (NST1)* and *NST2* (Mitsuda et al., 2005). These show activation by increased expression of MYB26, and down-regulation in myb26 mutant. Chromatin Immunoprecipitation(ChIP)-PCR results provide evidence of MYB26 binding to promoter region of *NST1* and *NST2*. Additional targets are being identified for ChIP screening by analysis of microarray data from the myb26 mutant. Preliminary results suggest that MYB26 binds to promoter regions of *NST1* and *NST2*. However electrophoretic mobility shift assay (EMSA) didn"t show consistent results, possibly due to the requirement of additional proteins to facilitate MYB26 binding, thus protein pull-down experiment coupled with mass spectrometry and fluorescence resonance energy transfer (FRET) will be adopted to study the MYB26 protein complex.

P092 - Transcriptional control of four pollen specific receptor like cytoplasmic kinases during late pollen development and pollen tube growth in Arabidopsis

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In land plants, the correct growth of the pollen tube is essential for a successful reproduction. This process requires the mature pollen grain to sense the receptive stigma and then to grow and reach the ovule. Although many of these processes have been well characterised at physiological level, the underlaying molecular mechanisms has not been well established yet. Several genes have been related to transcriptional control of pollen specific genes, however there is no universal mechanism that regulates this process. Here we analysed the transcriptional control of four pollen specific receptor like cytoplasmic kinases *(PS-RLCKs)* by the trihelix transcription factor *GT-4* and the RNP variant *STEP1* as a putative ortholog to the *Lilium* germline restrictive silencing factor in *Arabidopsis thaliana*. Quantitative real-time PCR shows a decrease of *PS-RLCKs* transcripts in *GT-4* mutants and a slightly increase in *STEP1* mutant lines. *In vitro* pollen germination of mutant plants showed abnormal pollen tubes suggesting that the decrease of pollen specific genes in the *GT-4* mutant lines is correlated with the abnormal phenotypes and reduced seed set in these plants.

P093 - Transcriptome of Oriental Melon

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Oriental melon (*Cucumis melo L. var. makuwa*) is one of six subspecies and cultivated widely in China, Japan, and Korea. Comparing to its economic importance in Asia market and the distinctive genetic features of oriental melon to other subspecies, there are few reports of genome scale researches about oriental melon. We performed RNA-sequencing and generated 30.5 Gbp and 36.8 Gbp raw data from female flower, male flower, leaf, root and fruit of two oriental melon varieties, KM (Korea landrace) and NW (Breeding line of NongWoo Bio Co.), respectively. From the raw reads, 64,998 transcripts from KM and 100,234 transcripts from NW were de novo assembled. Assembled transcripts were used for detection of single nucleotide polymorphisms (SNPs) and simple sequence repeat (SSR) markers, screening of tissue-specific genes, and construction of linkage map using the markers. Between KM and NW, 234 SNP markers of the total 7,871 candidates and 25 SSR markers of the total 8,502 candidates showed polymorphism in the two cultivars. Finally, 234 SNP and 25 SSR markers were screened in 94 F2 population and showed clear co-dominant type Mendelian segregations. 259 markers were used to construct linkage map and 248 markers were distributed in twelve linkage groups and 11 markers were unlinked. It is promise that genomic sequence data and molecular markers of oriental melon in this study may provide useful information to improve the molecular breeding of oriental melon and other related species.

P094 - Molecular Basis for Protein Interaction and the Control of Auxin Response Repression

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Auxin signaling guides plants through nearly every aspect of growth and development. PB1 domain interactions among AUXIN RESPONSE FACTOR (ARF) and AUXIN/INDOLE 3-ACETIC ACID (Aux/IAA) proteins regulate auxin-responsive gene transcription. Understanding the driving forces behind these protein-protein interactions that facilitate auxin signal transduction is vital. The crystal structure of the C-terminal interaction domain of Arabidopsis ARF7 reveals a Phox and Bem1p (PB1) domain that provides both positive and negative electrostatic interfaces for directional protein interaction. In this work, we investigate the attractive forces that influence ARF-ARF self-interaction. We found using isothermal titration calorimetry and NMR chemical shift experiments that key residues on both the basic and acidic faces of the domain contribute to and organize coordinately to stabilize PB1 domain binding. Our data suggest the positively charged residues on the basic face of the PB1 domain drive the interaction, whereas the less structured acidic domain face coordinates around these residues to anchor the binding. These thermodynamic and structural analyses uncover the driving forces and key residues involved in PB1 domain interactions that universally organize cellular signaling scaffolds. Our data suggest a revised model of auxin response in which ARF and Aux/IAA multimerization are necessary for regulation of auxin response.

P095 (Talk) - Detailed analysis on a cis-element sequence of VND7, a master switch of xylem vessel cell differentiation in Arabidopsis

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Xylem tissues of vascular plants contain vessel cells and fiber cells, which function in water transport and supporting, respectively. Their cell differentiation processes are regulated by a certain group of NAC transcription factors including VASCULAR-RELATED NAC-DOMAIN7 (VND7) and SECONDARY WALL–ASSOCIATED NAC DOMAIN PROTEIN1 (SND1). VND7 and SND1 are considered as transcriptional switches for differentiation of xylem vessel cells and fiber cells, respectively; however, molecular functional analysis revealed that they can recognize similar DNA sequences as target cis-elements, suggesting that our knowledge is still not enough to understand how VND7 and SND1 induce the differentiation of different cell types. To obtain the detailed information on the binding affinity of VND7 or SND1 to DNA sequences, we established the Fluorescence Correlation Spectroscopy (FCS)-based system, in which the binding affinities between recombinant proteins and fluorescence-labeled DNA are measured and a cis-element sequence can be determined at a single nucleotide resolution. By using the promoter sequence of *XCP1* gene, a well-known direct target of VND7, we successfully refined the cis-element sequence for VND7. The binding affinity of SND1 to this sequence was clearly lower than that of VND7. Based on the results, we will discuss if the difference of DNA sequence preference between VND7 and SND1 can simply explain their distinct activity of cell differentiation induction.

P096 - Arabidopsis MYC transcription factors control the jasmonate-responsive expression of the ORA47 gene encoding a regulator of jasmonate biosynthesis

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Jasmonic acid (JA) and related oxylipins, collectively known as jasmonates (JAs), are key regulators of plant development and plant responses to abiotic and biotic challenges. Upon herbivore or pathogen attack plants produce JAs. The regulation of their biosynthesis is not well understood at the molecular level. We found that overexpression of the AP2/ERF-domain transcription factor ORA47 leads to elevated expression of all JAs biosynthesis genes and to elevated levels of JAs, indicating that ORA47 controls the positive feedback loop. ORA47 is itself encoded by a JAs-responsive gene. We explored the hypothesis that the *ORA47* gene is regulated by the functionally redundant JAs-responsive transcription factors MYC2, MYC3 and MYC4. Our results show that the MYC proteins can trans-activate the *ORA47* promoter via interaction with a single G-box. Triple knockout of *MYC* genes or overexpression of a stable JAZ1 derivative abolished JAs-responsive *ORA47* expression, demonstrating the crucial role of the MYC-JAZ module in regulation of *ORA47* expression.

Biotic interactions

Posters 097 to 156

P097 - The role of Argonaute 2 protein in the defense against viral and bacterial pathogens

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RNA silencing is a major mechanism of constitutive antiviral defense in plants. *Arabidopsis* encodes four Dicer-like (DCL) proteins and ten different Argonaute (AGO) proteins that are specialized to function in different RNA silencing-related mechanisms. To date, AGO1, AGO2 and AGO5 are known to possess antiviral activity against different viruses. We have previously shown that AGO2 plays an important role in protecting plants against viruses, including Potato virus X (PVX) in Arabidopsis. To investigate which Argonautes are required for resistance against PVX in Arabidopsis, we have used transient expression of AGO proteins in the susceptible host Nicotiana benthamiana. Using this system we found that AtAGO2, but not NbAGO2, possesses intrinsic antiviral activity against PVX. Consistent with this, we find that activity of NbAGO2, but not AtAGO2, is supressed by the PVX suppressor of RNA silencing, P25. In addition, we have observed natural variation in AtAGO2 in Arabidopsis ecotypes and found that certain polymorphisms correlate with the degree of susceptibility to PVX. To investigate whether these polymorphisms specifically affect virus resistance, we challenged Arabidopsis introgression lines carrying different AtAGO2 alleles with Pseudomonas syringae. Our results indicate that natural variation in RNA silencing components can have important effects on plant-pathogen interactions

P098 - MLP124478 : Subcellular localization and alteration of transcriptional status

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Pathogen secrete effectors into the host cells to suppress host immunity or facilitate invasion. In plant cells, host proteins associated with effectors can be classified as facilitators or targets. However, few effectors have known function in host cells and the host molecules they target are largely unknown. To address this question, the genome of Melampsora *larici-populina* (MLP) (poplar leaf rust) was mined and revealed over 1200 candidate effectors. Confocal microscopy revealed fluorescent signal of MLP124478-GFP (a candidate rust effector) in nucleus and nucleolus. Moreover, truncation and site-directed mutagenesis was performed to assess the amino acids contributing to the nucleolar localization. Truncated proteins revealed similar localization. However, mutagenized protein (K67N and K73N) showed diminished fluorescence from the nucleolus, indicating that these two lysine could be responsible for nucleolar localization. Ion torrent based transcriptome assay was performed for genes whose expression is explicitly altered in response to the constitutive expression of 124478-GFP. TFBSs database (AthaMap, Pscan and PlantPan) revealed four different TFs (ABF1, TGA1a, ABH5 & TCP16) over-represented in 73 genes with altered expression in the transgenic. The latter result suggests that MLP124478 affects transcription to suppress the normal transcriptional response to pathogen.</p>

P099 (Talk) - The transcriptome of Arabidopsis roots infected with an oomycete identifies genes required for plant defense and susceptibility

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Little is known about the responses of plant roots to filamentous pathogens, particularly for oomycetes. We investigated the overall changes in gene expression in *A. thaliana* roots challenged with *Phytophthora parasitica*. We analyzed various infection stages, from penetration by the pathogen and establishment of the interaction to the switch from biotrophy to necrotrophy. We then carried out functional analyses, to identify the functions involved. The A. thaliana transcriptome displays a dynamic response to *P. parasitica* infection, from penetration onwards. Some genes were specifically coregulated during penetration and biotrophic growth of the pathogen. In addition, many genes encoding DC1 domain-and VQ motif-containing proteins were found to be upregulated in plant roots, early in infection. Inactivation of two *DC1*-and one *VQ*-genes significantly increased susceptibility to *P. parasitica* infection. These proteins thus contribute to root defense responses, restricting penetration and the biotrophic growth of the oomycete pathogen. By contrast, inactivation of another DC1 domain-containing proteins and a transcription factor showed these proteins promote *P.* parasitica development in roots. Our data suggest that the particular genetic program specifically activated during penetration may determine the outcome of pathogen invasion. These results and the functional analysis of genes will be presented. Their potential use for plant protection purposes will be discussed.

P100 (Talk) - H2A.Z and SWR1 chromatin remodelling complex components have distinct functions in plant immunity and gene regulation in Arabidopsis

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In order to adapt to the environmental conditions, plants need to efficiently modulate their gene expression. Transcriptional reprogramming is important in growth and developmental processes as well as in response to stresses. Gene expression regulation is controlled by multiple molecular mechanisms, especially by the chromatin structure dynamics. Chromatin remodeling complexes control gene expression by modulating chromatin architecture or by incorporating histone variants. The SWR1 complex (SWR1c), a Swi2/Snf2-related ATPase-containing chromatin remodeling complex, catalyzes the incorporation of the histone variant H2A.Z into nucleosomes. In Arabidopsis H2A.Z and homologs of SWR1c components have been shown to be important for the transcriptional regulation underlying development, thermosensory responses and defense. We are interested in deciphering the role of SWR1c/H2A.Z in Arabidopsis immunity. Through a systematic analysis, we have characterized the function of the putative Arabidopsis SWR1c subunits PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 (PIE1), ACTIN-RELATED PROTEIN6 (ARP6), and SWR1 COMPLEX 6 (SWC6), as well as H2A.Z, in different layer of immunity and in gene regulation. Our data indicates that although they belong to the same complex, Arabidopsis SWR1c components are functionally specialized and have non-redundant functions in plant immunity.

P101 - A tandem affinity purification (TAP)-based strategy to study the Arabidopsis MEKK1-MKK2-MPK4 module

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Mitogen-activated protein kinases (MAPKs) are present in all eukaryotes. They function in signal transduction modules where a MAPK kinase kinase (MAPKKK) activates by phosphorylation a MAPK kinase (MAPKK) which in turn activates by phosphorylation a MAPK. The MEKK1-MKK1/MKK2-MPK4 module, involved notably in Arabidopsis immunity, has been well studied at the genetic level and some substrates of MPK4 have been identified, but relatively little is known about the protein regulation of this cascade. Are there MAPK scaffold proteins like in yeast and mammals? Are there proteins implicated in the spatial distribution of this cascade? To try to answer such questions, a tandem affinity purification (TAP)-based strategy was employed. Stably transformed plants were first obtained where a TAP-tagged MAPK was expressed in the corresponding *mpk* knock-out genetic background. TAP experiments with tagged MEKK1, MKK2 and MPK4 plant lines were then realized in several replicates and purified proteins were identified via liquid chromatography coupled to tandem mass spectrometry analysis. Several putative interactors of the MAPK module were cloned and are currently tested by yeast two-hybrid and bimolecular fluorescence complementation assays. In parallel, knock-out and tagged lines of selected components were isolated and are undergoing molecular phenotypic analysis in the context of Arabidopsis immunity.

P102 - Large-scale screening reveals a genetic framework of stomatal immunity.

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Stomata are microscopic pores at the surface of the leaf that regulate gas exchanges but they also provide direct entry points for pathogens to enter plant tissues. To prevent invasion, stomata can close in response to so-called pathogenassociated molecular patterns (PAMPs) such as chitin and flagellin, through the action of the cognate pattern recognition receptors. Even if a number of components required for stomata closure upon biotic stress have been previously identified, we still lack a complete understanding of the pre-invasive immunity mechanism. To address this question, we developed a high throughput method of stomata imaging, allowing us to measure the stomata pores for a large set of selected mutants (200 genotypes) in various conditions. The analysis of the responses to PAMPs, ABA and second messengers allow us to build biotic and abiotic signalling networks. The method robustly identifies stomata phenotypes of known mutants in development, abiotic stresses and immunity, but more interestingly it highlights members of the vesicle trafficking pathway (CHC, VPS37, SNAREs) as being key regulators of stomatal movement after PAMP perception. Further analysis of *CHC* mutants reveal that clathrin-mediated endocytosis contributes to signalling cues important for the full deployment of plant defences against bacterial pathogens. This complete approach allow us to compare signalling networks leading to stomatal closure as well as identifying new genes involved in immunity.

P103 - Microbial sphingosylphosphorylcholine (lysosphingomyelin) is a potential activator of the innate immune system of Arabidopsis thaliana

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Plants live in constant interaction with microbes of various life styles. In pathogenic interactions the plant´s response to microbial infection is triggered by receptor-mediated recognition of microbe-associated molecular patterns (MAMPs), i.e. bacterial flagellin, lipopolysaccharides, peptidoglycans or fungal or oomycete proteins, peptides or glucan and chitin oligomers. In this study we used diverse methods to identify a novel putative MAMP of the class of sphingolipids, i.e. sphingosylphosphorylcholine (SPC). SPC is a ubiquitous molecule of plant-associated bacteria of the genus Sphingomonas. We show that SPC activates plant defense responses on transcriptional and physiological levels. Upon exposure of Arabidopsis leaves to SPC, elevated Ca2+ levels correlated with membrane depolarizations, genes of the innate immune system were activated, and levels of the plant defense hormone salicylic acid and the phytoalexin camalexin were increased in wild type but not in mutants which are defective in SA and camalexin synthesis (*sid2* and *pad3*). Moreover, SPC enhanced resistance against the biotrophic pathogen *Hyaloperonospora arabidopsidis*. As little is known about plant immunity-related responses involving sphingolipids, the study of SPC can provide deeper insight into the diversity of MAMPs and the molecular mechanisms underlying chemical communication in plant-pathogen interactions and cellular outputs.

P104 - Identification of Resistance Genes against Albugo candida Using CRISPR-Cas9 in Arabidopsis thaliana

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Albugo candida is an obligate biotrophic oomycete that causes white rust disease on many Brassicaceae including the crop species *B. oleraceae* and *B. juncea*. Some *A. candida* races can also infect the model plant *Arabidopsis thaliana*. Therefore, *A. thaliana/A. candida* pathosystem can be utilized to clone *White Rust Resistance (WRR)* genes that can be transformed directly into various Brassicaceae crops.

All the *A. thaliana* lines tested so far are resistant to the *Brassica oleraceae*-infecting race AcBoT. This therefore hinders genetic studies in order to identify non-host resistance genes against this *A. candida* race in *A. thaliana*. Since *eds1* mutants of *A. thaliana* are fully susceptible to this race, AcBoT resistance in *A. thaliana* is most likely due to TIR-NB-LRR encoding resistance genes. We therefore developed a strategy to generate null alleles of TIR-NB-LRR genes using CRISPRCas9 genome editing, in order to create susceptible A. thaliana lines to AcBoT. We designed 68 gRNAs targeting 89 TIRNB-LRR encoding genes annotated from Col-0 TAIR10. Based on identity in nucleotides, some gRNAs target more than one gene. We are currently cloning these gRNAs together with

Cas9 in two different T-DNAs by Golden Gate cloning and transforming *A. thaliana* lines. Any AcBoT susceptible mutants derived from those lines will be used to identify AcBoT resistance genes. Progress in identification of CRISPR-induced mutations will be presented.

P105 - Roles of tomato ERF38 and ERF35 in stress responses

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Ethylene-response factors (ERFs) are a large family of plant-specific transcription factors involved in various stress responses; however, our knowledge about their functions and the involved regulatory mechanisms is still rudimentary. In this study, five uncharacterized members of tomato ERF group VIII (*SlERF35/36/37/38/39*) were studied for their roles in plant response to bacterial wilt (BW) caused by Ralstonia solanacearum and water deficit (WD). The studied ERFs localize in nucleus and confer transcriptional repression activity. These genes display differential transcriptional expression patterns in various tissues and under stress and hormone treatments. Transient gene silencing assays suggest their positive role in tomato defense against BW and negative role in the tolerance to WD. Furthermore, by characterizing *SlERF38*-overexpressor transgenic plants, we propose that *SlERF38* may de-repress ethylene (ET) signaling and repress jasmonic acid (JA) signaling, leading to its effect on reproduction and responses to diseases and WD. Moreover, by characterizing *SlERF35*-overexpressor transgenic plants, it is suggested that *SlERF35* is involved in the chlorophyll degradation induced by stress hormones and the tolerance to BW. These results reveal overlapping as well as differential roles of these regulators in plant development and stress responses.

P106 - Negative regulation of the central immune regulator BIK1 by the Arabidopsis protein phosphatase type-2C PIB

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The *Arabidopsis* leucine-rich repeat receptor kinases (LRR-RKs) FLS2 and EFR are the immune receptors for the bacterial pathogen-associated molecular patterns (PAMPs) flagellin and EF-Tu, respectively. Upon elicitation, EFR and FLS2 recruit the co-receptor BAK1 to initiate PAMP-triggered immunity (PTI). The cytoplasmic kinase BIK1 is a direct substrate of the receptor complex and plays a crucial role in the activation of PTI signaling. BIK1 phosphorylates and activates the NADPH oxidase RBOHD leading to a PAMP-induced burst of reactive oxygen species (ROS). RBOHD-mediated ROS production is required for PAMP-induced stomatal closure, which is thought to restrict bacterial entry into the apoplast. Appropriate immune signaling initiation, timing and amplitude must be carefully regulated to avoid excessive activation of immune responses, which can lead to autoimmune diseases and severe growth defects. However, the mechanisms that negatively regulate PTI in plants are not fully understood. Here, we identify a protein phosphatase type-2C, tentatively named PIB, as a negative regulator of PTI. We show that *PIB* associates with and dephosphorylates BIK1. Accordingly, PIB overexpression compromises PAMP-triggered ROS burst, stomatal closure and anti-bacterial immunity. Notably, *PAMP* perception leads to PIB phosphorylation and release from BIK1, which most likely enables full BIK1 activation. Our results uncover a novel component involved in the tight regulation of plant immune signaling.

P107 - Identification and characterization of desert plant bacterial endophytes inducing abiotic stress tolerance in Arabidopsis thaliana

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Food security is of major importance globally and harvest losses due to abiotic stresses amount to more than 60% of total productivity, making abiotic stress tolerance the main goal of crop improvement worldwide. The ability of a variety of plants to cope with stress conditions depends on their association with rhizosphere microbes and can potentially help increase food production in a sustainable way. However, so far neither the microbial diversity nor the mechanisms of their beneficial interaction with plants are sufficiently understood. Our project is to isolate and characterize endophyte microbial strains that can help plants to survive and develop in harsh conditions. From an endophyte bacterial library isolated from desert plant roots of the Jizan region in Saudi Arabia, we have established a screening protocol to select strains that can enhance plant tolerance to salt stress in *Arabidopsis thaliana*. Using a number of anatomical and physiological parameters, we identified 37 strains, classified as STPRs (Salt Tolerance Promoting Rhizobacteria). We currently characterize a dozen of these STPRs at the molecular level by determining their genome sequences and changing transcriptome patterns during the infection process. Genetic analysis and genome-wide association studies of Arabidopsis with selected STPRs identified a number of candidate genes that could be key regulators in the plant-microbe interaction. Field trials on wheat, barley and pearl millet seem to confirm our strategy for identifying STPRs. Overall, our approach promises to not only reveal some of the basic physiological processes involved in the enhancement of stress tolerance mechanisms but also be of direct practical use for agriculture.

P108 - Natural variation of nitrogen nutrition effect on Arabidopsis thaliana tolerance to Dickeya dadantii

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Nitrogen fertilizers are commonly used in agriculture as they allow an important increase in crop yields. However, plants cannot absorb all the nitrogen present in the soil. The unabsorbed amounts of nitrogen compounds are found in groundwater and constitute a danger to human health. Pathogens cause important losses in agriculture each year and plant resistance can be changed by the use of nitrogen fertilizers. Indeed nitrogen intake may promote or decrease infection depending on the plant species and the pathogen. Identifying cultivars efficient for nitrogen use and resistant to a wide range of pathogens is essential. To do this it is important to understand the role of nitrogen nutrition on plant’s susceptibility to pathogens. In this study, we investigated the effect of nitrogen nutrition on the susceptibility level of a panel of *Arabidopsis thaliana* natural accessions. For this study we used the pathosystem *A. thaliana*/bacterium *D. dadantii* because several reports indicated that infections by this bacterium are affected by nitrogen supply on chicory. We used the following accessions: Col-0, Cvi-0, Bur-0, Shadara, Edi-0, Oy-0, Can-0, Ge-0. Plants were grown under limiting or non limiting nitrogen nutrition then infected with the pathogenic bacterium D. dadantii. We scored symptoms, defense markers (ROS, *PR* gene expression) and metabolites. Our data indicate that these accessions have contrasting behaviors depending on nitrogen. In some accessions nitrogen nutrition has a strong effect on susceptibility to *D. dadantii*, and on others, nitrogen supply does not affect the level of susceptibility. These data suggest that it is possible to find genotypes harboring stable resistance to pathogens whatever the nitrogen supply.Nitrogen fertilizers are commonly used in agriculture as they allow an important increase in crop yields. However, plants cannot absorb all the nitrogen present in the soil. The unabsorbed amounts of nitrogen compounds are found in groundwater and constitute a danger to human health. Pathogens cause important losses in agriculture each year and plant resistance can be changed by the use of nitrogen fertilizers. Indeed nitrogen intake may promote or decrease infection depending on the plant species and the pathogen. Identifying cultivars efficient for nitrogen use and resistant to a wide range of

pathogens is essential. To do this it is important to understand the role of nitrogen nutrition on plant’s susceptibility to pathogens. In this study, we investigated the effect of nitrogen nutrition on the susceptibility level of a panel of *Arabidopsis thaliana* natural accessions. For this study we used the pathosystem *A. thaliana*/bacterium D. dadantii because several reports indicated that infections by this bacterium are affected by nitrogen supply on chicory. We used the following accessions: Col-0, Cvi-0, Bur-0, Shadara, Edi-0, Oy-0, Can-0, Ge-0. Plants were grown under limiting or non limiting nitrogen nutrition then infected with the pathogenic bacterium *D. dadantii*. We scored symptoms, defense markers (ROS, *PR* gene expression) and metabolites. Our data indicate that these accessions have contrasting behaviors depending on nitrogen. In some accessions nitrogen nutrition has a strong effect on susceptibility to D. dadantii, and on others, nitrogen supply does not affect the level of susceptibility. These data suggest that it is possible to find genotypes harboring stable resistance to pathogens whatever the nitrogen supply.

P109 - Impact of nitrogen supply on the resistance of A. thaliana to bacterial phytopathogens

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Plants are confronted to a combination of different biotic and abiotic stresses that limit crop yields. Nitrogen (N) is a key mineral nutrient playing a crucial role in plant growth and development that influences strongly both induced and constitutive resistance. Our work focuses on the effect of N on the resistance of A.thaliana to bacterial pathogens Erwinia amylovora and Pseudomonas syringae. We showed a decrease in non-host resistance of A.thaliana to E. amylovora in low N (Fagard et al 2014). In order to understand this effect, different aspects of the defense response such as defense-related gene expression, phytohormones, reactive oxygen species and plant metabolite accumulation will be tested. We are interested in the genetic basis of the impact of N on the responses of plants to biotic stress. Rasmussen et al, 2013 revealed that 61% of the transcriptome changes in response to double stresses were not predictable from the responses to single stresses. We therefore studied the transcriptome modification of A.thaliana grown in high and low N, inoculated by E.amylovora. Our data revealed difference in gene expression under single and double stresses suggesting important interactions between the two stress treatments. Groups of genes coexpressed under combined stresses have been identified and will be further studied.

References: Fagard et al. 2014. Journal of Experimental Botany 65, 5643-5656. Rasmussen et al. 2013. Plant Physiology 161, 1783-1794.

P110 - Arabidopsis MPK4 regulates immunity through inducing degradation of its substrate MKS3

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Mitogen-activated protein kinase (MAPK) cascades are highly conserved signaling pathway that transduces extracellular stimuli into the nucleus in all eukaryotes. MAPK cascades play central roles in plant immunity. In response to pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and pathogen effectors, activation of MAPK cascade leads to multiple defense responses through phosphorylation and activation of varying effector proteins. In *Arabidopsis thaliana*, the MEKK1-MKK1/MKK2-MPK4 cascade is one of the best-characterized MAPK signaling pathways. MPK4 was shown to be a negative regulator of the immune responses. However, how MPK4 regulates the defense responses is still largely unknown, since only a few MPK4 substrates has been identified. To this end, we employed a modified version of yeast two-hybrid system to screen for MPK4 putative substrates. Here, we show that MKS3 (MAP Kinase Substrate 3) interacts with MPK4 through its N terminal domain and acts as a substrate of MPK4. Phosphorylation of MKS3 by MPK4 upon PAMPs perception leads to its degradation, thereby down-regulates plant immunity. Our data provide new insights into the regulatory mechanism of *Arabidopsis* MPK4 cascade.

P111 - Dissecting Temperature-Induced Defence Breakdown in Arabidopsis

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As sessile organisms, plants need to fine-tune growth and development to suit their environment and ensure reproductive success. Environmental factors such as temperature greatly modulate the growth and development of plants. Moderate increases in temperature accelerate growth in plants but can compromise their immune responses, thus increasing their susceptibility to pathogens. This is one of the key determinants for plant disease epidemics as a result of climate change. Increased severity and extended ranges of crop diseases are predicted to follow local increases in temperature according to recent predictions. The molecular mechanisms underpinning this temperature induced disease susceptibility are currently poorly understood. In this study, a forward genetic approach in *Arabidopsis* was used to isolate *resilient (res)* mutants that are able to maintain their defences at higher temperatures. The *res* mutants display altered defence and thermosensory responses. They offer a novel tool to dissect the growth/ defence interface and most importantly the molecular basis for temperature induced defence breakdown. Knowledge generated from this study will have many potential implications, including devising strategies for climate resilient disease resistance in crops.

P112 - Cellulose-binding containing proteins from oomycetes triggered immunity and resistance to the root pathogen Phytophthora parasitica in Arabidopsis

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Oomycetes are fungal-like organisms which produce cell wall cellulose-binding containing proteins, recognize as PAMP during plant infection. Using isothermal titration and depletion assays conjugated with SAXs experiments, we characterized the affinity for cellulosic substrate of the *Phytophthora parasitica* PAMP Cellulose Binding Elicitor Lectin (CBEL). We then used CBEL to identify *Arabidopsis thaliana* accessions and mutants impaired in CBEL responses. Upon screening, as compared to the Col-0 responsive line, three insensitive-CBEL lines were unable to mount a necrotic response after CBEL treatment (Ws-4, Oy-0, Bla-1). Ws-4 and Oy-0 were also impaired in the production of an NADPH oxidase-dependent oxidative burst and expression of defense genes, while Bla-1 was only partially affected in these responses. Although necrotic responses were observed, CBEL-responses were also impaired in *Atrboh D/F* mutants and bak1-4. While all the lines were well colonized by Pp310 strain (compatible), *AtrbohD/F* and *bak1-4*, but not *Ws-4* and *Oy-0*, displayed a significant increase in Pp0 (incompatible) susceptibility. Thus CBEL-triggered immunity required the *BAK1* and *AtRBOH* genes which also control resistance to the non-adapted *P. parasitica* strain (Pp0). However, natural variability in CBEL responses is not correlated to the outcome of the interaction suggesting that other mechanisms are involved in *Phytophthora* resistance.

P113 - Reprogramming Of The Two Major Phloem Resident SWEET Transporters Does Not Support Biotrophy Of Three Adapted Microbial Arabidopsis Pathogens

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In compatible interactions, biotrophic microbial phytopathogens rely on the supply of host assimilates and therefore need to reprogram host metabolism. In rice and cassava, SWEET (Sugars Will Eventually be Exported Transporter) sucrose transporters are induced by bacterial effectors to establish compatibility. The two main Arabidopsis phloem

loading transporters *At*SWEET12 and/or *At*SWEET11 are induced in the interactions with the (hemi)biotrophic bacterial and fungal pathogens *Pseudomonas syringae* pv. *tomato (Pst), Erysiphe cruciferarum (Ec)* and *Colletotrichum higginsianum (Ch)* and represent potential susceptibility determinants. *At*SWEET12-YFP reporter gene activity driven by the endogenous promotor increased in the vasculature upon challenge with all three pathogens and also accumulated in close proximity of *Ch* and *Pst* interaction sites, while *At*SWEET11-YFP was only increased in vasculature of *Ec* infected leaves. A substantial reporter protein induction was absent from the plant-pathogen interface of all three studied pathosystems, which indicates that *At*SWEET12 is not induced by the action of pathogen effectors. A loss of either or both transporters had no influence on susceptibility to *Ec* and *Pst*. In contrast, *sweet11/sweet12* double mutants exhibited increased resistance to *Ch*. A stronger salicylic acid (SA) accumulation and an enhanced SA response in the absence of Ch indicate that constantly elevated levels of soluble sugars trigger defence priming in the double mutant.

P114 - Constitutively active MAPKs as a tool to understand MAPK signal transduction in plants

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In eukaryotes, Mitogen-Activated Protein Kinases (MAPKs) define key regulatory modules of signal transduction. In *Arabidopsis*, the most studied MAPKs are MPK3, MPK4 and MPK6 which are activated by biotic and abiotic stresses. In our laboratory, we previously identified mutations which render MAPKs constitutively active (CA). To demonstrate that they define a useful tool to investigate MAPK functions, we generated lines in which the endogenous MPK4 is replaced by a CA form and we confirmed the role of MPK4 as a negative regulator of defense responses and SA (Salicylic Acid) accumulation (Berriri *et al.*, 2012 Plant Cell). We are now focusing our work on another stress-activated MAPK. Plants expressing this CA-MAPK show spontaneous cell death, accumulation of reactive oxygen species and total SA but not free SA. Interestingly, these results are reminiscent of *mpk4* phenotype. We also observed a very strong resistance to the necrotrophic fungus B*otrytis cinerea* and constitutive accumulation of camalexin. Larger transcriptomic and metabolomic analysis, as well genetic studies, will help us to understand which pathways are regulated by this MAPK. In conclusion, CA mutation allowed us to reveal new specific roles for this stress related MAPK. This new tool provides answers that go beyond classical genetic analysis using loss-of-function mutants and may become an interesting way to engineer crops able to better cope with environmental constraints.

P115 - Molecular mechanisms for anchoring remorin to plasma membrane nanodomains

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Remorins belong to a plant-specific multigenic family, classified in 6 phylogenetic groups with 16 isoforms in Arabidopsis (Raffaele *et al.*, 2007) involved in plant-microbe interactions during nodule formation, Phytophthora infection and virus intercellular trafficking (Lefebvre *et al.*, 2010, Raffaele *et al.*, 2009, Bozkurt *et al.*, 2015). The remorin protein family accumulates in a range of co-existing plasma membrane (PM) inner leaflet nanodomains, but the PM anchoring and nanoclustering mechanisms are still unknown. Our group has defined the 28 aa Remorin C-terminal Anchor domain (RemCA,) as a novel membrane binding domain shaped by convergent evolution (Raffaele *et al.*, 2013) necessary for remorin localization and subsequent function, and sufficient to target a soluble protein (GFP) to the PM (Perraki *et al.*, 2012). To Challenge the capability of Rem-CA to anchor the PM, we reinvestigated Rem-CA structure and behavior by coupling *in silico* Molecular Dynamic approaches (Deleu *et al.*, 2014) and *in vitro* biophysical experiments. This work revealed that Rem-CA had a preference for domains enriched in sterols and phosphoinositides (PIP_2), 2 components found in inner leaflet rafts. Moreover, we established that Rem-CA was composed of 2 distinct domains: the C-terminal extended sheet domain that acts as the driver for PM targeting and PM inner leaflet penetration, whereas the N-terminal A-helical domain stabilizes Rem-CA at the PM surface via interaction of K residues with PIP_2.

P116 (Talk) - Differential Roles of Two Homologous Cyclin-Dependent Kinase Inhibitor Genes in Regulating Cell Cycle and Innate Immunity in Arabidopsis

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Precise cell cycle control is critical for plant development and responses to pathogen invasion. Two cyclin-dependent kinase inhibitor genes, SIAMESE (*SIM*) and its close homolog SIM-RELATED 1 (*SMR1*) were recently shown to be involved in Arabidopsis defense regulation based on the phenotypes conferred by a *sim-1smr1-1* mutant. However whether these two genes play differential roles in cell cycle and defense control is unknown. In this report, we examined plants impaired in *SIM* and/or *SMR1* genes for cell cycle and defense phenotypes. Our data show that while *SIM* plays a major role in promoting trichome development, a process tightly associated with endoreduplication, *SMR1* has stronger effect on non-trichome leaf cell ploidy. In addition, we found that a *smr1-1* but not a *sim-1* mutant was more susceptible to *P. syringae* and this susceptibility could be rescued by activating salicylic acid (SA)-mediated defense. Consistent with these results, the *smr1-1* mutant but not the *sim-1* mutant partially suppressed dwarfism, high accumulation of SA, and cell death phenotypes exhibited by the acd6-1 mutant, a convenient genetic tool that has been used to gauge the change of defense levels. Thus *SMR1* plays a stronger role at least partly through the SA pathway in defense control. Furthermore cell ploidy analysis by flow cytometry with an SA activator or with SA mutants revealed that SA signaling is necessary and sufficient to disrupt cell cycle progression. Interestingly, a mutant with disrupted *CYCD3* genes and increased endoreduplication also led to compromised disease resistance to *P. syringae*. Together, this study further support the importance of cell cycle control during host-pathogen interactions and also reveals differential roles of two homologous CKIs in regulating cell cycle progression and innate immunity in Arabidopsis.

P117 - The activity of ammonium transporter is regulated by signaling regulator CBL-CIPK network

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Nitrogen is quantitatively the most important mineral nutrient. Ammonium is one of the three major forms of nitrogen acquired by plants. Assimilation of ammonium into amino acids is less energy consuming relative to nitrate. However, ammonium, when given alone, leads to toxicity in bacteria, fungi, animals and plants. Therefore, ammonium transport (AMT) is highly regulated. Our lab identified the first ammonium transporters from any organism, and uncovered an allosteric regulatory mechanism that can be used to rapidly shut down cellular import of ammonium. Besides of it"s transport activity, AMT have While we could show that phosphorylation of the C-terminus is important for allosteric inactivation of AMT complexes, the protein kinases required for the phosphorylation of the C-terminus AMTs and the components participated in the signaling pathway are still unknown. In this study, CBLs-CIPKs (Calcineurin B-like and CBL-interacting protein kinase, respectively), primary signaling regulators involved in all major abiotic and biotic pathways, were co-expressed with AMT1;1 in the heterologous *Xenopus laevis*oocyte system using Two Electrode Voltage Clamp. Results shown that multi CBLs and CIPKs significantly affect the transport activity of AMT1;1 while co-expressing in oocytes. These results demonstrate CBL-CIPK network novelly participates in the regulation of AMTs" activity and candidates that may control allosteric inhibition of AMT complexes through interaction and phosphorylation. Other members of AMT family will be further tested with same CIPKs in

oocytes to determine whether other members of the AMT family using the same phosphorylation mechanism. The in vivo phosphorylation status of AMT1;1 and ammonium signal responses in CIPKs mutants and the ammonium toxicity growth assays will also be further determined *in planta* to understand the mechanism of regulation as well as the biology of ammonium toxicity. Our research here promises to make important contributions towards the development of more nutrient efficient plats for sustainable agriculture.

P118 - RRS1 is required for RPS4 nuclear localization and effector-independent EDS1 association

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Plant resistance (R) genes encode Nucleotide Binding-Leucine Rich Repeat (NB-LRR) proteins, which structurally and functionally resemble mammalian Nod-like receptors (NLRs). R proteins enable plants to activate defense upon recognition of specific pathogen effectors. However, the mechanisms of plant NB-LRR activation upon effector recognition remain unclear. The *Arabidopsis* RPS4/RRS1 is a Toll/Interleukin-1 receptor (TIR) NB-LRR pair and confers resistance to *Pseudomonas syringae* strains carrying the AvrRps4 effector or to *Ralstonia solanacearum* carrying the acetyltransferase effector PopP2. Both AvrRps4 and PopP2 are detected through interaction with the WRKY DNA-binding domain of RRS1. The RPS4/RRS1 complex is a good model system for in depth studies on dual NB-LRRs function. RPS4-dependent cell death in tobacco is compromised in the presence of RRS1. Interestingly, RRS1 protein forms homodimer in the absence of RPS4, but in contrast, RPS4 does not form homodimers in the absence of RRS1. Furthermore, RRS1 stabilizes RPS4 and increased the abundance of an RPS4-EDS1 complex in the nucleus. The effector protein, AvrRps4, does not affect RPS4-EDS1 association in presence of RRS1. In the absence of RRS1, AvrRps4 interacts with EDS1, the formation of which is disturbed by the co-expression of PAD4 but not by SAG101. Our biochemical approaches suggest that the large, nuclear pre-activation RPS4/RRS1 immune complex might undergo conformational changes after activation by effector recognition.

P119 - Tracking Down Evolutionarily Conserved Immune Signalling Mechanisms of Coiled-Coil Type NLRs

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The nucleotide-binding domain leucine-rich repeat containing (NLR) family mainly functions as innate immune sensors. These modular receptors perceive non-self or modified-self inside the cell and activate innate immunity, often associated with a local cell death. The NLR MLA confers isolate-specific disease resistance against the phytopathogenic grass powdery mildew fungus in barley. MLA is functional in *Arabidopsis thaliana*; hence monocots and dicots share the underlying resistance signalling machinery. MLA-mediated responses are mainly initiated via its N-terminal coiled-coil (CC) domain. We have generated stable *Arabidopsis* transgenic lines expressing the MLA CC domain (MLA_{CC}) under chemically-inducible promoters. MLA_{CC} expression triggers responses resembling the immune responses upon pathogen attack. These transgenic lines have been used to define evolutionarily conserved MLA signalling components: i) we performed biochemical purification of MLA_{CC} complexes; ii) EMS mutagenesis yielded three candidate mutants impaired in MLAcc-mediated responses. Subsequent genome-wide SNP mapping identified candidate mutant loci distinct from known loci required for NLR function (e.g. *NDR1, SGT1, RAR1*, and *SID2*). We also conducted a yeast two-hybrid assay using MLA_{CC} as bait against transcriptional regulators. Functional characterization of the candidate interactors/mutants is in progress with the aim of identifying signalling components for MLA and, possibly, other CC-type NLRs.

P120 - Pairs of paired plant immune receptors, RPS4/RRS1 and RPS4B/RRS1B; it takes two to tango

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Diverse microbes cause plant disease, and plants have evolved a robust innate immune system that recognizes pathogen molecules and then activates defense. Immunity involves cell surface receptors and also intracellular nucleotide binding, leucine rich repeat immune receptors, encoded by *Resistance (R)* genes, that resemble mammalian NOD-like receptors (NLRs). For some responses, two co-functioning NLR proteins are required. The adjacent, divergently transcribed, Arabidopsis RPS4 and RRS1 genes, encoding TIR-NLR proteins, are both required for resistance to bacteria that deliver AvrRps4 or PopP2 effectors, and for resistance to certain *Colletotrichum* fungal strains. An additional gene pair, RPS4B and RRS1B, is closely linked to RPS4/RRS1 and confers recognition of AvrRps4 but not PopP2. Both RRS1 and RRS1B carry a C- terminal WRKY domain that targeted by AvrRps4 and PopP2, suggesting these effectors target WRKY domains. Using R gene enrichment sequencing (Renseq), we explored the diversity of such "integrated decoy" and other NLRs in Brassicaceae and Solanaceae. We have used genetics, biochemistry and cell biology to investigate the functions and mechanisms of the RPS4/RRS1 complex, and I will suggest how these and other paired R proteins might convert pathogen effector recognition into defense activation.

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P121 - Association mapping reveals novel regions affecting response to clubroot in Arabidopsis

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Clubroot is a devasting *Brassica* crop disease caused by the obligate biotrophic protist *Plasmodiophora brassicae* and the development of resistant cultivars is currently the most efficient way to control it. Quantitative resistance constitutes an additional layer of resistance in the absence of R-mediated resistance and is displayed to be more durable due to the combination of small-effect distinct genetic mechanisms. To characterize the natural diversity of Arabidopsis clubroot response, the effect of *P. brassicae* infection was evaluated in 137 accessions through gall size and location on the root system as well as foliar development and pathogen proliferation. The identification of causal components of quantitative resistance was then carried out using a genome-wide association analysis. Arabidopsis accessions showed a wide range of phenotypic variation for each assessed trait from full susceptibility to high level of resistance. Association mapping identifies 19 genomic regions associated with phenotypic variation for at least one of the traits. Five regions colocalize with the 4 genomic regions previously identified in the partially resistant accession Bur-0 and the major resistance gene *RPB1*. In addition to the identification of 14 new regions controlling clubroot resistance, this study delimits a short list of candidates for further genetic

and functional studies and reveals that various combinations of small-effects loci confer clubroot quantitative resistance.

P122 - Piriformospora indica colonization leads to early flowering in Arabidopsis thaliana

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The transition from vegetative growth to generative stage is regulated by a combination of environmental and internal signals, which triggers the transcription of a number of integrator genes responsible for initiating the floral induction program. *Piriformospora indica*, is a widespread plant root colonizing endophytic fungus that positively affects different aspects of plant performance, such as growth, nutrient uptake, and biotic and abiotic stress tolerance. To check whether *P. indica* colonization impacts *Arabidopsis* flowering, we established an inoculation protocol to colonize *Arabidopsis* seedlings in soil. In average, inoculated plants flowered four days earlier than control plants. At the time of flowering, the number of rosette leaves was significantly lower in inoculated plants (8 leaves) than control (10 leaves). These results indicate that *P. indica* promotes early flowering in *Arabidopsis* under long day condition. To get insight into the molecular mechanism of this *P. indica* effect, we quantified the expression of different flowering genes; FT, LFY, AP1 and FLC. Our data showed that FT mRNA level strikingly induced 10 days after inoculation, while that of LFY and AP1 was slightly increased. These expression changes can explain the observed early flowering phenotype.

P123 - Antagonistic regulation of MAP kinase cascades in PRRs-mediated immunity

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Mitogen-activated protein kinase (MAPK) cascades are highly conserved signaling modules downstream of plasma membrane receptors that transduce extracellular stimuli (e.g. secreted peptide hormone and pathogen associated molecular patterns (PAMPs)) into intracellular responses. We identified Arabidopsis receptor-like cytoplasmic kinase PBL27, as an ortholog of OsRLCK185 that regulates the activation of MAPK cascade after treatment of fungal cell walls component, chitin. Knockout mutants of PBL27 suppressed chitin-induced activation of MAP kinases. To understand the molecular mechanism of chitin-induced MAPK activation by PBL27, we searched PBL27 interactor using Y2H and found MAPKKKa, one of 60 Arabidopsis MAPKKKs. Chitin-induced MAPK activation in the *mapkkka* mutants was clearly reduced compared with WT. On contrary, the MAPK activation induced by perception of bacterial flagellin derived peptide, flg22 and bacterial elongation factor Tu derived peptide, elf18 was enhanced in both *pbl27* and *mapkkka* mutants. These data suggest a cross-talk between MAPK cascades induced by CERK1 and FLS2/EFR-mediated pathogen recognition. The mechanism of how MAP kinase cascades are activated in chitin and flg22/elf18 signaling will be discussed.

P124 (Talk) - A Plasma Membrane-Associated Ubiquitin Ligase Regulates Plant Growth and Autoimmunity

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Plant performance depends on the balance between growth and immune responses. In Arabidopsis, plant immunity and thus defense against pathogens evolved on the cost of plant growth or vice versa. The plasma membrane-associated Arabidopisis SENESCENCE ASSOCIATED E3 UBIQUITIN LIGASE1 (SAUL1), which is a member of the plant U-box armadillo repeat (PUB-ARM) protein family, is an essential regulator of growth and autoimmunity. Here we show that *saul1* mutant plants lacking expression of the *SAUL1* gene show autoimmunity and reduced growth depending on a decrease in temperature or relative humidity. Expression of plant defense marker genes is induced in *saul1* plants. In order to understand the SAUL1 signaling pathway and the function of SAUL1 in autoimmunity and defense response, we performed a suppressor screen with *saul1* plants. We identified 12 independent alleles suppressing the *saul1* phenotype. In several lines the suppressor mutation was identified in a TIR-NBS-LRR immune receptor gene that we named *SUPPRESSOR OF SAUL1 1 (SUSA1).* Here we present data on the characterization of SUSA1 and the interplay between SAUL1 and SUSA1 that contributes to the understanding of the SAUL1 signaling network and of autoimmunity.

P123 - Antagonistic regulation of MAP kinase cascades in PRRs-mediated immunity

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Botrytis cinerea is the causing agent of the grey mold disease. This fungus kills host cells using a large arsenal of weapons that enables it to infect a broad range (>200) of host plants. Signaling pathways leading to the basal resistance against this fungus are well described, but the role of the import of sugars into host cells remains to be investigated. Several studies have reported that sugar mobilization is affected upon infection by pathogens suggesting that the plant ability to retrieve extracellular sugars may be determinant for the final outcome of the interaction. In *Arabidopsis thaliana*, apoplastic hexose retrieval is mediated by the activity of Sugar Transport Proteins (STPs). Expression analysis of the 14 STP genes revealed that only *STP13* was induced in leaves challenged by *B. cinerea*. To explore the functional role of STP13 during infection, we used a reverse genetic approach. STP13-modified plants displayed constrasting phenotypes. The correlation between *STP13* transcripts, protein accumulation, glucose uptake rate and resistance level indicates that STP13 contributes to the basal resistance to *B. cinerea*. Our work further suggests that the differential expression level of plant hexose transporters can probably change the balance of hexose fluxes toward either host living tissues or fungus cells, highlighting the importance of the competition for apoplastic hexoses at the plant/fungus interface.

P126 - VIH2 Controls the Synthesis of Inositol Pyrophosphate InsP8 and Jasmonate-dependent Defenses in Arabidopsis

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Diphosphorylated inositol polyphosphates, also referred to as inositol pyrophosphates, are important signaling molecules that regulate critical cellular activities in many eukaryotic organisms. In mammals and fungi, two distinct classes of inositol phosphate kinases mediate their biosynthesis: KCS1/IP6K- and VIP1/PPIP5K-like proteins. Recent studies in our lab show that PPIP5K homologs are widely distributed in plants and that Arabidopsis VIH1 and VIH2 are functional PPIP5K enzymes. We will report a specific induction of the inositol pyrophosphate $InsP_8$ by jasmonate and will provide evidence that steadystate and jasmonate-induced pools of $InsP_8$ in Arabidopsis seedlings depend on VIH2. We will further report a role of VIH2 in regulating jasmonate related defenses by potentiating jasmonate perception. Using *in silico* docking experiments and radioligand-binding based reconstitution assays we can show high affinity binding of inositol pyrophosphates to the F-box protein COI1-JAZ jasmonate co-receptor complex and will provide evidence that coincidence detection of jasmonate and $InsP_8$ by COI1-JAZ is a critical component in jasmonate-regulated defenses.

P127 - Overexpression of the tomato glycosyltransferase GAGT increases susceptibility to Pseudomonas syringae in Arabidopsis

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Salicylic acid (SA) is essential in the plant defence response, being crucial to establish the resistance response in many plant-pathogen interactions. Gentisic acid (GA), a metabolic derivative of SA, has also been described as a signal molecule in plant defence. GA has been found to accumulate in different plants, including Arabidopsis, during their interaction with pathogens. Application of GA induces the expression of a set of Pathogenesis-Related (PR) proteins different to those induced by SA. Moreover, GA treatment also induces resistance to RNA pathogens, in a similar way as its metabolic precursor SA. Therefore, GA and SA may play complementary roles in plant defence signalling. Both SA and GA accumulate in the form of glycoconjugates and this conjugation is catalyzed by the corresponding glycosyltransferases. We identified GAGT (Gentisic Acid Glycosyl Transferase) as the protein that conjugates GA in tomato. Transgenic Arabidopsis plants overexpressing GAGT and exogenously treated with GA displayed higher GA conjugation and, consequently, lower accumulation of free GA, which is the active form of the compound. In agreement with this observation, GAGT overexpressing plants showed increased susceptibility to inoculation with the avirulent bacterial strain *Pseudomonas syringae* pv. *tomato AvrRpm1* as well as reduced *PR1* gene expression, when compared to control plants. Together, these results confirm the role of GATG in the regulation of plant defence.

P128 - The circadian clock component LUX ARRHYTHMO regulates Arabidopsis defense through salicylic acid

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Recent studies showed that two morning clock genes regulate Arabidopsis defense independently of the key defense signaling mediated by salicylic acid (SA). To further understand the defense role of the circadian clock, we tested a mutant impaired in the evening clock gene *LUX ARRHYTHMO* in defense responses. We found that the *lux-1* mutant was compromised to both basal and R-gene mediated defense against *Pseudomonas syringae* and expression of the *LUX* gene was suppressed by *P. syringae*. We also found that *lux-1* had transiently reduced SA accumulation after infection with a virulent *P. syringae* strain. Consistent with this result, the double mutant *acd6-1lux-1* displayed suppression on dwarfism, cell death, and constitutive defense phenotypes, compared with *acd6-1*, which has been used as a convenient genetic tool in gauging the change of defense levels. We further found that two downstream targets of LUX also could modulate resistance to *P. syringae* via the SA pathway. Together our results showed that LUX regulates Arabidopsis defense, possibly through affecting SA signaling. These data further support crosstalk between the circadian clock and plant innate immunity and also reveal different molecular mechanisms underlying clock-defense crosstalk.

P129 - Pairing with the right one: What's the secret?

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Plants are often attacked by pathogens. Plant resistance (R) proteins activate immunity via recognition of pathogen effectors. Most R proteins recognise a specific effector, but some function in pairs and can recognise multiple effectors. Arabidopsis R proteins RRS1-R and RPS4 cooperate to recognise two bacterial effectors, AvrRps4 from *Pseudomonas syringae* and PopP2 from R*alstonia solanacearum*. We have identified RRS1B and RPS4B, a paralogous R gene pair linked to RRS1/RPS4, which only recognises AvrRps4. Atypically, RRS1 and RRS1B both carry a C-terminal WRKY DNA-binding domain. Like RRS1/RPS4, RRS1B/RPS4B proteins associate in complex for effector detection and defence activation. We have recently shown that these complexes detect AvrRps4 and/or PopP2 via the WRKY domain. Interestingly, inappropriate pairings (RRS1/RPS4B or RRS1B/RPS4) are non-functional. We hypothesised that specific domain/domain interactions of cognate pair partners are required for function. We carried out systematic domain swaps between the paralogous proteins to identify the domain/domain interactions that specify pairing. We propose that effector detection via the WRKY domain triggers conformational changes around the WRKY and leads to complex activation and defence.

P130 (Talk) - Proteome analysis using two nuclear proteins with unknown functions provides new insights into the rRNA biosynthesis system in plants

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The complete genome information of several plant species is available now. However, our understanding of biological processes in plants is far from complete, probably because many functions of proteins encoded in the plant genomes have not been identified yet. Here we show characterization of two nuclear-localized and sugar-inducible proteins with unknown functions, NuGWD1 and NuGAP1, by proteome analysis of complexes involving epitope tagged NuGWD1 or NuGAP1. Such analysis revealed that both of them are involved in rRNA processing although they regulate distinct steps of the processing. Taken together with results of phenotypic analysis, this suggests that sugar responses in plants include promotion of rRNA transcription and maturation through activation of a variety of steps for it. Furthermore, interestingly, our proteome analysis also revealed that the rRNA processing complex in plants includes plant-specific components, implying differences between animal and plant systems for rRNA maturation. Thus, our analysis provided new insights into the rRNA maturation system in plants and also suggested the potential of proteome analysis to reveal unknown functions of proteins and protein complexes. We will also discuss the potential of proteome analysis of protein complexes to identify new molecular links of proteins with unknown functions among signaling pathways.

P131 - The bZIP63 transcription factor is a molecular link between energy status and resistance to Pst DC3000

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The AtbZIP63 transcription factor of *Arabidopsis thaliana* participates of gene expression reprograming induced by SnRK1 kinases KIN10/11 to adjust plant to conditions that results in low energy status. The Gene Ontology (GO) functional categorization of misregulated genes in the knockdown *atbzip63-1* mutant revealed that the AtbZIP63 target genes are involved in the plant responses to abiotic, biotic and energy stress. The enrichment of biotic stress associated genes suggested that AtbZIP63 could be involved in plant defense against pathogens. Indeed, *atbzip63-1* showed increased resistance to the hemibiotrophic pathogen *Pseudomonas syringae* pv. *tomato (Pst)* DC3000, which was at least in part attributed to enhanced PTI (PAMP-triggered immunity). The atbzip63-1 showed enhanced flg22-triggered callose deposition and enhanced stomatal immunity in response to *Pst* DC3000, which could be attributed, at least partially, to the higher accumulation of the flagellin receptor *FLS2* mRNA in *atbzip63-1* leaves. Also, the accumulation of WRKY70, a transcription factor that is a positive and negative regulator of salicylic acid (SA) and jasmonic acid (JA) responses, respectively, is induced in *atbzip63-1*, suggesting that the balance between SA-JA antagonism is shifted towards SA-induced responses. The results suggest that AtbZIP63 may acts as a modulator that adjusts the balance between

energy availability and defense against pathogens, regulating key genes involved in biotic stress responses.

P132 - Functional and genetic analysis identify a role for Arabidopsis ARGONAUTE 5 in anti-viral RNA silencing

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RNA silencing functions as an anti-viral defense through the action of DICER-like (DCL) and ARGONAUTE (AGO) proteins. In turn, plant viruses have evolved strategies to counteract this defence mechanism, including the expression of suppressors of RNA silencing. *Potato virus* X (PVX) does not systemically infect *Arabidopsis thaliana* Col-0, but is able to do so effectively in mutants lacking at least two of the four *Arabidopsis* DCL proteins. PVX can also infect *Arabidopsis ago2* mutants, albeit less effectively than double DCL mutants, suggesting that additional AGO proteins may mediate anti-viral defenses. Here we show, using functional assays, that all *Arabidopsis AGO* proteins have the potential to target PVX lacking its viral suppressor of RNA silencing (VSR), P25, but that only AGO2 and AGO5 are able to target wild-type PVX. However, P25 directly affects only a small subset of AGO proteins, and we present evidence indicating that its protective effect is mediated by precluding AGO proteins from accessing viral RNA, as well as by directly inhibiting the RNA silencing machinery. In agreement with functional assays, we show that Potexvirus infection induces *AGO5* expression and that both AGO2 and AGO5 are required for full restriction of PVX infection in systemic tissues of *Arabidopsis*.

P133 - The central regulatory kinase BIK1 is rate-limiting in plant immune signaling

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Plants perceive pathogen- and damage-associated molecular patterns (PAMPs or DAMPs) through surface-localised pattern recognition receptors (PRRs). Plant PRRs are ligand-binding receptor kinases or receptor-like proteins that exist in multi-protein complexes to transduce intracellular immune signaling via phosphorylation events. The plasma membraneassociated cytoplasmic kinase BOTRYTIS-INDUCED KINASE 1 (BIK1) is an immediate convergent substrate of several different PRRs and is a key component of the plant immune system. BIK1 is also a substrate of BRI1-ASSOCIATED KINASE 1 (BAK1), an important co-receptor kinase that interacts with and phosphorylates several PRRs and is required to achieve their full signaling potential. We recently demonstrated that the calcium-dependent protein kinase CPK28 regulates BIK1 turnover to buffer immune signaling (Monaghan et al., 2014 Cell Host Microbe). We found that the amplitude of immune signaling triggered by PAMPs/DAMPs is positively correlated with the cellular level of BIK1 (Monaghan et al., 2014 Cell Host Microbe; Monaghan et al., Plant Signaling and Behavior, in press), demonstrating that BIK1 is rate-limiting in PRRtriggered immune signaling. In addition, we uncovered a complex interplay between phosphorylation and ubiquitination in BIK1 regulation, which raises interesting questions about the role of these post-translational modifications in immune homeostasis. Additional data surrounding this regulation will be presented.

P134 - 2-hydroxylation of sphingolipid fatty acids is essential for the formation of plasma membrane microdomains and innate immunity in plants

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Sphingolipids specifically possess 2-hydroxy fatty acids, and 2-hydroxylation of fatty acids are catalyzed by sphingolipid fatty acid 2-hydroxylase (FAH). To reveal the intracellular and physiological functions of sphingolipid 2-hydroxy fatty acids in plants, we produced knock-down lines of *FAH*s in Arabidopsis and rice by RNAi system. Observation of plasma membrane (PM) by using a di-4-ANEPPDHQ revealed that PM microdomains decreased in the RNAi-FAH lines. PM microdomains are small, heterogeneous, highly dynamic, sphingolipid- and sterol-enriched domains, and are believed to be important for innate immunity in plants, because a large number of defense-related proteins are present in PM microdomains. In fact, the RNAi-OsFAH was more susceptible to the infection of rice blast fungus, suggesting that sphingolipid 2-hydroxy fatty acid-derived PM microdomains are essential for plant immunity. To uncover the mechanism, we compared protein changes in detergent-resistant membrane (DRM) fraction between WT and the RNAi-OsFAH, and demonstrated that PM microdomains are required for the dynamics of a Rac/Rop small GTPase OsRac1 and respiratory oxidative burst homologs (OsRbohs) in response to chitin elicitor. Furthermore, ROS production after chitin treatment was completely suppressed in the RNAi-OsFAH. Taken together, sphingolipid 2-hydroxy fatty acid-derived PM microdomains are required for chitin-induced immunity through ROS signaling mediated by OsRac1-OsRbohB/H pathway.

P135 (Talk) - The calcium-dependent protein kinase CPK3 directly binds to Arabidopsis 14-3-3s with various affinities in a calcium- and phospho-dependent manner

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In eukaryotic cells, sphingoid Long Chain Bases (LCBs) behave as second messengers involved in various processes including programmed cell death (PCD). In plants, Fumonisin B1 (FB1), a toxin produced by the necrotrophic fungus *Fusarium moniliforme*, induces plant PCD by accumulation of dihydrosphingosine (DHS) and phytoshingosine (PHS), the two major plant LCBs. However, the LCB pathway leading to PCD in plants is still enigmatic. Recently, we showed that DHS-induced PCD is controlled by nuclear calcium in tobacco BY-2 cells and that *Arabidopsis* CPK3, a member of the plant family of calcium-dependent Ser/Thr protein kinases (CDPKs or CPKs), is a key positive regulator of LCB-mediated cell death. We found that CPK3 phosphorylates 14-3-3 proteins at their dimer interface in a PHS- and calcium-dependent manner. We also showed that CPK3 dissociates from 14-3-3s and is then degraded during PHS-induced cell death. To get further insights into this still unknown signaling pathway in plants, we investigated which 14-3-3 isoforms could interact with CPK3 in control conditions by combining biochemistry and microscale thermophoresis (Nanotemper®) approaches. We show here that CPK3 binds directly to 14-3-3s in a calcium- and phospho-dependent manner and displays a greater affinity for 14-3-3s of the non-ε group than for those of the ε-group.

P136 - Arabidopsis MPK4 regulates immunity through inducing degradation of its substrate MKS3

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Mitogen-activated protein kinase (MAPK) cascades are highly conserved signaling pathway that transduces extracellular stimuli into the nucleus in all eukaryotes. MAPK cascades play central roles in plant immunity. In response to pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and pathogen effectors, activation of MAPK cascade leads to multiple defense responses through phosphorylation and activation of varying effector proteins. In Arabidopsis thaliana, the MEKK1-MKK1/MKK2-MPK4 cascade is one of the best-characterized MAPK signaling pathways. MPK4 was shown to be a negative regulator of the immune responses. However, how MPK4 regulates the defense responses is still largely unknown, since only a few MPK4 substrates has been identified. To this end, we employed a modified version of yeast two-hybrid system to screen for MPK4 putative substrates. Here, we show that MKS3 (MAP Kinase Substrate 3) interacts with MPK4 through its N terminal domain and acts as a substrate of MPK4. Phosphorylation of MKS3 by MPK4 upon PAMPs perception leads to its degradation, thereby down-regulates the plant immunity. Our data provide new insights into the regulatory mechanism of *Arabidopsis* MPK4 cascade.

P137 - Nep1-like proteins from three kingdoms of life act as a microbe-associated molecular pattern

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Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are secreted by a wide range of plant-associated microorganisms. They are best known for their cytotoxicity in dicot plants that leads to the induction of rapid tissue necrosis and plant immune responses. The biotrophic downy mildew pathogen *Hyaloperonospora arabidopsidis* encodes 10 noncytotoxic NLPs (HaNLPs) that do not cause necrosis. We discovered that these noncytotoxic NLPs act as potent activators of the plant immune system in *Arabidopsis thaliana*. Ectopic expression of *HaNLP3* in *Arabidopsis* triggered resistance to *H. arabidopsidis*, activated the expression of a large set of defense-related genes, and caused a reduction of plant growth that is typically associated with strongly enhanced immunity. N- and C-terminal deletions of HaNLP3 pinpointed to a small central region of the protein that is required to trigger immunity, indicating the protein acts as a microbe-associated molecular pattern (MAMP). A synthetic peptide of 24 aa, derived from the conserved central region of HaNLP3 induces ethylene production, a well-known MAMP response. Strikingly, corresponding 24-aa peptides of fungal and bacterial NLPs were also able to trigger immunity in *Arabidopsis*. The widespread phylogenetic distribution of NLPs makes this protein family the first proteinaceous MAMP identified in three different kingdoms of life.

P138 - The S-domain receptor-like kinase LORE mediates lipopolysaccharide perception in Arabidopsis thaliana

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Invading pathogens are detected through conserved Microbe-Associated Molecular Patterns (MAMPs) by specific host pattern-recognition receptors (PRR) that initiate pattern-triggered immunity (PTI). Cell wall components such as lipopolysaccharide (LPS) of Gram-negative bacteria are predestined to be MAMPs. LPS consists of a lipid component, lipid A, a oligosaccharide core region and a O-polysaccharide repeat moiety. The lipid A moiety is the most potent MAMP in mammals recognized by the TLR4/MD-2 complex. Different moieties of LPS also trigger immune responses in plants, but the plant LPS perception systems are yet unknown. Here, we report about sensitive perception of the conserved core-lipid A moiety of Pseudomonas LPS in *Arabidopsis thaliana* triggering typical PTI responses[(1)]. In a forward genetic approach we identified a candidate LPS receptor, the S-domain receptor-like kinase LORE (LipoOligosaccharide-specific Reduced Elicitation). lore mutants are strongly impaired in LPS-induced responses and more susceptible to *Pseudomonas syringae* infection. Transient expression of LORE in LPS-insensitive tobacco results in gain-of-function of LPS responsiveness, thus demonstrating its function as an LPS receptor[(1)]. PTI can confer durable resistance to a broad range of pathogens. Hence, transfer of LORE to related crop species like tomato may enable engineering of broad-spectrum bacterial resistance traits.

(1) Ranf S. *et al.* Nat Immunol 16, 426-433, doi:10.1038/ni.3124 (2015).

P139 - RIN4 intrinsic disorder provides a platform to integrate its phosphoswitch capability with defense functions and explains conserved effector sites

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Arabidopsis Rpm1-INteracting protein 4 (RIN4) was recently described as a phosphoswitch regulating the two tiers of plant defense (Chung *et al.* 2014, Cell Host Microb. 16: 484). Li *et al.* (2014, Cell Host Microb. 16: 473) describe how one of these phosphorylation events initiates conformational transformations around a neighbouring proline by using a cyclophilin prolyl isomerase. We present bioinformatic and biophysical data which demonstrates that RIN4 is a largely intrinsically disordered (ID) protein. This ID platform is punctuated by Molecular Recognition Features (MoRFs, Sun et al. 2014 FEBS J. 281: 3955). ID in regulatory proteins is known to be strongly associated with post-translational modifications (phosphorylation being the most common). An ID scaffold provides an environment that is readily accessible to protein kinases and other post-translational modifications, enabling RIN4 to act as a phosphoswitch that could then control the transition between significant conformational variants. We have analysed RIN4 across plant species and find that the disordered platform as well as type III effector interacting motifs that coincide with MoRFs are the key conserved traits. This combination is consistent with evolutionary constraints operating on these MoRFs due to their functional significance in the mode of action in this important plant defense regulator indicating why they may have become the key sites targeted in RIN4.

P140 - SA mediated suppression of callus and lateral roots is dependent on 2 ARFs and on the cytokinin signalling pathway.

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Successful pathogens often induce plant developmental hormones pathways (e.g auxin and cytokinin) to suppress plant defences. As a result, diseased plants frequently display symptoms such as hypertrophy, hyperplasia and hypocotyl elongation. *Plasmodiophora brassicae*, a biotrophic protist, is the causal agent of clubroot disease of brassica. This disease is characterized by the production of galls on the roots of the plant. To produce the gall, *P.brassicae* induces accumulation of two phytohormones, auxin and cytokinin, in roots. We aim to understand how salicylic acid, which controls the main defence pathway against biotrophic pathogens such as *P. brassicae*, affects the auxin/cytokinin induced phenotype. Like galls, callus can be induced by exogenous application of auxin and cytokinin. We used *in vitro* callus formation as a simplified model for studying the crosstalk between the induction of developmental hormone signaling pathways and SA response. *In vitro* treatment with auxin and cytokinin triggered the induction of callus or lateral roots depending on the type of hormones used. Addition of SA prevented the induction of either callus or lateral roots. We then demonstrated that mutants with low cytokinin signalling and mutant of 2 specific auxin response factors displayed lateral roots in presence of SA. However, the suppression of lateral roots by SA was independent of SA signalling. We are now trying to characterize this mechanism in more details.

P141 - Quantitative Disease Resistance to Xanthomonas involves an Arabidopsis immune receptor pair and a gene of unknown fu

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While quantitative disease resistance (QDR) is a durable and broad spectrum form of resistance in plants, identifying genes underlying QDR is still in its infancy. *Xanthomonas campestris* (Xc) is a biotrophic bacteria that causes black rot disease (possibly the most important disease of crucifers), and one of the most prevalent bacterial pathogens in natural populations of *Arabidopsis thaliana*. We previously identified *RKS1*, (Resistance related KinaSe 1), which encodes an atypical kinase and confers broad-spectrum resistance to Xc. However, *RKS1* confers QDR to most but not all races of Xc (Huard-Chauveau *et al.*, 2013). We therefore explored the genetic bases of QDR in A. thaliana to diverse races of Xc. Nested Genome Wide Association mapping was used to finely map genomic regions associated with either QDR to Xcc12824 (race 2) or XccCFBP6943 (race 6). Insertional mutants were then selected for the candidate genes and phenotyped in response to Xc. Two major QTLs conferring resistance specifically to Xcc12824 and XccCFBP6943, were identified. Whereas QDR to Xcc12824 is conferred by a gene encoding for a protein of unknown function, QDR to Xcc6943 involves the immune receptor pair RRS1/RPS4. This study reveals that three genes are involved in resistance to Xc with strikingly different ranges of specificity, suggesting that QDR to Xc involves a complex network integrating multiple response pathways triggered by distinct pathogen molecular determinants.

P142 - Arabidopsis - Xanthomonas citri: a new approach to study plant molecular responses to TAL effectors

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Arabidopsis thaliana is a model organism with many tools that enable the study of molecular mechanisms in plants. Previous study showed that the non-host interaction between Arabidopsis and the Xanthomonas citri pv. citri (Xcc) enable the bacteria to populate the apoplast of the Arabidopsis leaves. Therefore, we though to use this pathosystem as a new model to study the plant molecular responses to Xcc. The main pathogenicity factors of Xcc are the TAL (transcription activator-like) effectors (PthAs), which are involved in the regulation of host genes, such as citrus *CsLOB1* (lateral organ boundaries) and *CsCyp* (Cyclophilin), resulting in the citrus canker symptoms. Here we aimed to establish if the Xcc is capable to inject PthAs into the Arabidopsis cells and regulate its genes in a PthA-dependent manner. Col-0 young plants (three week old) were deep inoculated with OD_{600} 0.1 suspensions of the wild type Xcc strain 306 and the mutant XccDhrpB2, which lack functional type-III secretion system. The presence of PthA proteins was observed in the Col-0 leaves 3 days post inoculation only with the Xcc 306. Arabidopsis genes *LOB1* and *LOB11*, and *ROC1* and *ROC3*, homologs to citurs *CsLOB1* and *CsCyp*, respectively, showed differential expression between Xcc 306 and XccDhrpB2 infections. Our results indicate Xcc indeed inject PthA effectors in Arabidopsis cells, leading to effector-dependent changes in plant gene expression.

P143 - Danger peptide signaling on depletion of the shared regulatory kinase BAK1 underlies basal pathogen resistance in Arabidopsis

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Pathogens infect a host by suppressing defense signaling induced upon recognition of microbe-associated molecular patterns (MAMPs). Despite this suppression, MAMP receptors mediate post-invasion basal resistance to limit host susceptibility, via a process that is poorly understood. The *Arabidopsis* leucine-rich repeat (LRR) receptor kinase BAK1 functions with different cell-surface LRR receptors. Plants lacking BAK1 display critical defects in MAMP signaling, cell death control, and brassinosteroid responses, but intact or even enhanced basal resistance. We report that pathogen-induced BAK1 depletion leads to defense activation through the endogenous PROPEP peptides and their LRR receptor kinases PEPR1/PEPR2. In the absence of BAK1, PEPR elicitation results in extensive cell death and prioritizing salicylate branches over jasmonate branches, in a manner independent of brassinosteroids. These *bak1* effects seem to be reinforced by Pep-induced elevation of PROPEP and PEPR accumulation. Importantly, BAK1 disruption increases extracellular release of PROPEP3 following pathogen effector-dependent induction, and renders PEPRs necessary for basal resistance. Of relevance, challenge with the fungal pathogen *Colletotrichum higginsianum* specifically lowers BAK1 accumulation, consistent with PEPR dependence of anti-fungal resistance. Our findings indicate that the PEPR pathway ensures basal resistance to virulent pathogens that deplete BAK1 during their infection attempts.

P144 (Talk) - UGT76B1 and its substrate isoleucic acid in plant defense and development

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Loss-of-function mutants of the small-molecule glucosyltransferase UGT76B1 trigger salicylate (SA)-dependent defense enhancing resistance towards biotrophic pathogens, whereas the jasmonate-dependent pathway is suppressed (von Saint Paul et al., 2011). This effect is mainly involving the SA pathway as deduced from introgression of SA- and jasmonate-related mutations. Isoleucic acid (ILA), the 2-hydroxy relative of isoleucine, was identified as a UGT76B1 substrate; in addition, UGT76B1 had an activity towards SA, which was found to be inhibited by ILA (Noutoshi et al., 2012). To further investigate the role of ILA, a GC-MS-based method for its quantification has been developed. UGT76B1 overexpression lines accumulated less unconjugated ILA (and more ILA hexoside), whereas more free ILA (and less ILA conjugate) was found in *ugt76b1-1*. Thus, ILA has been confirmed as an endogenous substrate and a possible new small molecule modulator of plant defense. Importantly, ILA was also shown to be present in a diverse array of plant species. To get further insight into ILA action, its inhibitory effect on root growth was used for a genetic approach. Ten thousand homozygous insertion lines were scored for enhanced ILA resistance revealing a diverse collection of genes putatively involved in ILA susceptibility. In addition, a genome-wide association study of 200 *A. thaliana* accessions has been initiated. Results revealing genomic regions related to ILA susceptibility will be presented.

P145 - Negative control of PTI by a PP2C-type MAPK phosphatase in Arabidopsis

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PAMP-triggered immunity (PTI) includes phosphorylation-based activation of mitogen-activated protein kinases (MAPKs), which is counteracted by protein phosphatases. MAPK activation leads to reprogramming of plant cellular activities, including changes in plant stress hormone levels. Here we report the biological role of *Arabidopsis thaliana* MAPK phosphatase AP2C as a negative regulator of plant basal resistance and defense responses to pathogenic bacteria. We studied the role of AP2Cs in regulation of kinase activities and disease resistance in plants. We correlated kinetic profiles of pathogen-induced MAPK activities in phosphatase knock out and overexpressing Arabidopsis plant lines with plant disease resistance and ethylene amounts. We found that loss of AP2C leads to enhanced plant resistance to *Pseudomonas syringae* pv. *tomato (Pto)* that correlates with enhanced pathogen-induced MAPK activities. Our data show that overexpression of the phosphatase leads to strong suppression of kinase activities, whereas in the absence of the

phosphatase kinase activities are enhanced. Taken together, this work advances current understanding on regulation of MAPK signaling in plants and highlights the regulatory role of PP2C type MAPK phosphatase in PTI.

P146 - NADPH oxidase is critical for the ulvan-induced resistance in Arabidopsis thaliana against Alternaria brassicicola

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Ulvan is a water-soluble algal polysaccharide that can induce defense responses in several crop plant species to a broad range of fungal pathogens [Stadnik & de Freitas, Trop Plant Pathol 2014; 39: 111-8]. However, mechanisms of the resistance induction are not yet fully understood. Thus, using the model system *Arabidopsis thaliana* and *Alternaria brassicicola*, the present work was aimed at studying the role of reactive oxygen species generated by NADPH oxidase in the ulvan-induced resistance. Foliar spraying of ulvan reduced the fungal colonization of host tissues and, consequently, the leaf disease severity by 90% in both wild type and *AtrbohF* plants, while increasing NADPH oxidase activity and hydrogen peroxide levels. It also tended to enhance the activity of enzymes related to the removal of reactive oxygen species such as APX, GSR, CAT and SOD, suggesting a tight control of the antioxidant system. Ulvan failed to protect *AtrbohD* mutant and wild type plants previously infiltrated with diphenyleneiodonium, both impaired in NADPH oxidase activity and hydrogen peroxide accumulation. Collectively, our results demonstrate that ulvan-induced resistance in A. thaliana against *A. brassicicola* requires reactive oxygen species by the respiratory burst oxidase homologue D (RBOHD) NADPH oxidase [de Freitas & Stadnik, Physiol Mol Plant Pathol 2015; doi: 10.1016/j.pmpp.2015.03.002].

P147 - Illuminating stomatal regulation in pathogen defence

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Stomata are specialized pores in the leaf surface whose aperture is controlled by a pair of guard cells by means of osmotically driven water transport. As natural openings in the leaf surface, stomata are major entry sites for microbes, such as bacterial pathogens. To prevent this, plants close stomata (stomatal immunity) upon the perception of pathogenassociated molecular patterns (PAMPs) such as bacterial flagellin (flg22). This can alter the outcome of infection to the disadvantage of the pathogen (Melotto *et al.*, 2006). As the outcome of flg22 perception by the Flagellin Sensing 2 (FLS2) receptor kinase is unique for guard cells, we hypothesize that FLS2 signalling involves guard cell-specific components and subcellular rearrangements. In order to shed light on the molecular regulation of stomatal closure in PAMP-triggered immunity we make use of guard-cell specific expression to investigate FLS2 signalling in a guard cell-specific context. We will present data from transient and stable approaches, e.g. showing that guard cell ROS production is not sufficient for flg22-induced stomatal closure and requires pavement cell ROS.

P148 - Novel short ORF-encoded peptides with a role in the stress response of A. thaliana

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Peptides display a broad array of functions in growth, development, reproduction and stress responses of plants. They exert their biological roles through direct interaction with pathogens or through interference with signaling transduction cascades. Nevertheless, the diversity and abundance of the peptidome is strongly underexplored. Recently, evidence is emerging on peptides directly translated from short open reading frames, in contrast to peptides derived from protein precursors such as well-studied antimicrobial and signaling peptides. Here, we aimed at identifying novel, unannotated stress-induced peptides (SIPs) that are directly translated from short open reading frames in Arabidopsis. A transcriptomic approach was employed on Arabidopsis leaf material treated with *Botrytis cinerea*, a necrotrophic fungus, and paraquat, a reactive oxygen species-inducing herbicide. 925 putative SIPs were predicted and their bioactivity was tested via a high-throughput yeast screening system in oxidative stress conditions, as well as via Arabidopsis knock-out mutants treated with (a)biotic stressors. For example, *sip1* was more susceptible to infection by the fungal pathogen *Fusarium oxysporum. Sip2* was also more susceptible to the latter pathogen and showed interveinal chlorosis. In conclusion, the presented approach enables to identify novel, bioactive peptides with a role in the stress response of plants.

P149 - Identifying core complex interactors of the Arabidopsis thaliana LSU peptides, important regulatory hubs in the plant stress response

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odel plant Arabidopsis thaliana contains four members of the LSU (response to Low Sulfur) gene family *(LSU1-4)*, encoding small proteins with 59-89% sequence similarity and homologues in several important plants and crops. Originally, LSU genes were characterized as strongly induced during sulphur deficiencies. Recent studies suggest an important regulatory role of the A. thaliana LSU peptides in development and the plant stress response. It has been proposed that LSU peptides function as important hubs for the integration and regulation of environmental signals. However, the precise molecular role of LSU family peptides still remains unclear. In order to unravel the molecular function of the *A. thaliana* LSU peptides as hubs in the plant stress response, we are following an interactomics-based approach combined with a detailed functional analysis of LSU1-4 and their *in planta* confirmed interactors. Up to now, preliminary functional analyses revealed possible redundancy between some of the LSU peptides, indicated nuclear localisation of the peptides and a role in the defence response against both necrotrophic and (hemi-)biotrophic plant pathogens. Through Tandem Affinity Purification (TAP) analyses in *A. thaliana* cell cultures we identified a core complex interactor of the LSU peptides, a GTP-binding protein. Currently, confirmation of this interaction partner in *A. thaliana* seedlings and its role in plant defence is ongoing.

P150 - A bile acid elicits receptor-like kinase-dependent defenses in Arabidopsis and reduces bacterial infection

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Crop yield loss is significantly affected by disease. Considering that the worldwide demand for agricultural products is increasing, there is a need to pursue the development of new methods to protect crops from disease. One mecanism of plant protection is through the activation of its immune system. By exogenous application, “plant activator molecules” with elicitor properties, can be used to activate the plant immune system. These defense-inducing molecules represent a powerful and often environment-friendly toolset to fight pathogens. We investigated a new molecule and show that a bile acid induces defense in Arabidopsis and reduces the proliferation of two bacterial phytopathogens. We describe the global defense response triggered by this new plant activator in Arabidopsis at the transcriptional level. Several induced genes were selected for further analysis by RT-qPCR and we describe the kinetics of their induction. Finally, we demonstrate that the activation of defense by this bile acid requires a specific receptor-like-kinase.

P151 - Global gene expression in insect galls to understand development and carbohydrate flux

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Insect galls may provide food, shelter, and/or protection to the insect. Each galling insect produces a unique gall structure on the host plant such that the gall is considered to be an exended phenotype of the galler. This predictable development resembles normal organ formation, with determinate sizes, predictable morphologies, and organized internal tissues. As such, galls offer a unique system to understand the signalling pathways that control plant development. We hypothesize that gallers hijack normal developmental pathways using an unknown chemical signal. In an attempt to understand how these structures form, we have begun to examine gene expression patterns in the *Ectodoemia sp.* gall and control tissues of *Populus granditentata*. Here we present results from a microarray that outlines major patterns in development and metabolic flux. This gall forms at the junction of the leaf blade and petiole, making it an ideal system to study not only development, but also carbohydrate source-sink dynamics. Here we present gene expression patterns as related to these topics.

P152 - Interactions among resistance genes lead to hybrid necrosis

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Immune responses mediated by Resistance (R) proteins with nucleotide binding/leucine rich repeat (NB-LRR or NLR) domains are instrumental to pathogen defense. Activation of R proteins often leads to hypersensitive response (HR), characterized by local cell death. Recently, pairwise interactions between NLR and other R loci have been identified as a cause for hybrid necrosis, an HR-like phenomenon observed in F1 hybrids between *A. thaliana* accessions. It has been proposed that such cases present an extreme example of the tradeoff between growth and activation of the immune system. Relevant to this observation, the integrated decoy model, based on pairs of NLR proteins such as RPS4/RRS1 in *A. thaliana* and RGA4/RGA5 in rice, posits that in the absence of a trigger, some NLR proteins could function as repressors that inhibit the activity of other NLR proteins. We are using CRISPR/Cas9 to systematically remove NLR loci from *A. thaliana* genomes, to identify NLR proteins that may be negative regulators of defense.

P153 (Talk) - PBL27, a member of RLCKs, directly transduces immune signal from chitin receptor to MAPK cascade in plant immunity

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Plants recognize pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs) at cell surface to induce immunity. MAPK cascades play critical role in PRRs-mediated immune responses. However, it is not known how the MAPK cascades are activated downstream of PRRs. Previously, we found that a receptor-like cytoplasmic kinase PBL27 functions as downstream signaling component of chitin receptor complex CERK1/LYK5 and regulates chitin-induced activation of MPK3 and MPK6. Here, we identified MAPKKKa as a MAPKKK that directly interacts with PBL27. The *mapkkka* mutations reduced chitin-induced activation of MPK3/6 and resistance to *Alternaria brassicicola* as found in the *pbl27* mutants, suggesting that PBL27 and MAPKKKa function in the same signaling pathway. To elucidate the molecular mechanism of how PBL27 activates MAPKKKa, we performed in vitro phosphorylation assay and found that PBL27 directly phosphorylates C-terminal domain of MAPKKKa. In addition, MAPKKKa phosphorylates MKK4 and MKK5, MAPK kinases for MPK3/6. These data suggest that PBL27 is the MAPKKK kinase that activates the MAPK cascade consisting of MAPKKKa-MKK4/5-MPK3/6 in chitin signaling.

P154 - Comprehensive Analysis of RALF Gene Family in Plant Species

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Receptor mediated signal carriers play a critical role in regulation of plant defense and development. Rapid Alkalization Factor (RALF) is an important signaling family which has a role in plant growth and development. However, only few RALF polypeptides have been identified till date, mainly because of enormous efforts required for their isolation through mutational analysis. In this study, an extensive database search yield 39, 43, 34 and 23 potential RALF genes in *Arabidopsis*, rice, corn and soybeans, respectively. RALF genes are highly conserved across the plant species. A comprehensive analysis including the chromosomal location, gene structure, subcellular location, conserved motif, protein structure and promoter analysis was performed. RALF genes from four plants under study were divided in 7 groups based on phylogenetic analysis. *In silico* expression analysis of these genes, using microarray and EST data, revealed that these genes exhibit a variety of expression pattern. Furthermore, RALF genes showed distinct expression pattern under nitric oxide (NO) stress in Arabidopsis. This suggests a role of RALF genes in plant defense regulation. Our comprehensive analysis of RALF genes is a valuable resource that further elucidates the roles of RALF family members in plants. In addition, comparative genomics analysis deepen our understanding of the evolution of RALF gene family and will contribute to further genetics and genomics studies of other monocot and dicot species.

P155 - Structure-guided design of a jasmonate receptor that uncouples endogenous hormone perception from pathogen toxin hijacking

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The plant hormone jasmonate (JA) plays a major role in regulating plant defense against numerous insect pests and necrotrophic pathogens in plants. Jasmonoyl-isoleucine (JA-Ile), the bioactive form of JA, is perceived by a co-receptor consisting of CORONATINE INSENSITIVE 1 (COI1) and JASMONATE ZIM-domain (JAZ) proteins. Despite its important role in defense against insects and necrotrophic pathogens, the co-receptor COI1 and JAZ proteins are targets of virulence factors from biotrophic/hemi-biotrophic pathogens, especially coronatine, a potent JA-mimicking bacterial toxin, illustrating the vulnerability of the host immune systems. Guided by the crystal structure of the COI1-JAZ co-receptor, we were able to make a single amino acid substitution, A348V, in the JA-binding pocket of the COI1 protein that allows for sufficient signal transduction of endogenous JA hormone, but has greatly reduced sensitivity to coronatine. Consequently, transgenic Arabidopsis plants expressing the engineered COI1A^{348V} receptor maintained a high-level of insect defense, but gained resistance to the hemi-biotrophic pathogen *Pseudomonas syringae* pv. *tomato DC3000*, which produces coronatine. Our results provides an example illustrating that modification of the host targets of pathogen virulence factors may be a broadly applicable approach to broaden the capacity of host defense against highly evolved pathogens that have developed mechanisms to subvert host cellular functions.

P156 (Talk) - From a phylogenic analysis of Calmodulin-like proteins in green lineage to

the role of CML8 in root development and responses to biotic stresses

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In their natural environment, plants are continuously exposed to fluctuating and often unfavorable conditions such as water deficit, high temperature and to biotic agents which could be harmful such as bacteria, fungi, viruses or herbivores. To survive and reproduce, plants have evolved sophisticated mechanisms to perceive and appropriately respond under these adverse conditions. It is well-known that the free calcium ion act as a key second messenger during developmental processes and stress signaling. To become informative, these $Ca2^{+}$ messages need to be decoded by $Ca2^{+}$ sensors such as calmodulin (CaM) to carry out the appropriate response. In addition to CaM, plants possess CaM-Like proteins (CMLs) that are specific to plants. Here, we first explored the genomes of 15 organisms in the green lineage going from chlorophyceae to land plants to search for CaM and CMLs homologs. This analysis shows that CaM/CML number increases from green algae to Angiosperms. CaMs are present in all plant species but some CML subgroups emerged in a concomitant manner with the development of new organs. We then investigated the role of CML8 from Arabidopsis, a CML that belongs to a subgroup only found in vascular plants whose expression strongly increases in response to biotic stresses. Using genetic approaches, we showed that CML8 is expressed in primordia of lateral roots and involved in root growth and development and in plant immune response to Pseudomonas syringae and Ralstonia solanacearum.

Development

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P157 - TEMPRANILLO regulates the age-dependent developmental pathway at different levels

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Arabidopsis plant development can be divided in three distinct phases: Juvenile phase, adult phase and reproductive phase. These developmental phases are controlled by different genetic pathways that respond to diverse environmental and endogenous stimuli including photoperiod, temperature, hormones and age. The age-dependent pathway controls the transition from the juvenile to the adult vegetative phase. The expression level of the *microRNA156* (miR156) is high during early stages of plant development, while decreases in adulthood. Most *SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE)* genes are silenced by miR156, and become active as miR156 decays in the adult phase when they in turn activate miR172. *TEMPRANILLO (TEM)* genes act as repressors of flowering. The similarity in the temporal expression pattern of miR156 and TEMs suggested a putative role of TEMs in the juvenile-to-adult transition. We found that as miR156, *TEM* genes control this developmental transition. However, TEMs affect to a greater extent the length of the adult phase, whereas miR156 shows a major role in controlling the juvenile phase. Phenotypic and expression studies indicate that TEMs regulate these developmental transitions by regulating miR156 levels and by directly acting on miR156 downstream genes. TEMs are able to repress *SPLs (SPL9, SPL3)* and miR172 expression by binding *in vivo* to their regulatory regions. Therefore, TEMs act in a miR156-dependent and -independent manner in the age-dependent pathway.

P158 - Revealing connections between polarity maintenance and cell fate in a dynamic developmental system.

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In plants, asymmetric cell divisions (ACDs) are crucial to produce cells with distinct fates. Recently, BASL was found to be required for the polarization of cells prior to asymmetric divisions of the stomatal lineage. Despite its fundamental role in ACDs, the mode of action of BASL remains unclear. To determine if BASL acts before or after division, I developed an approach that combines stage-specific protein expression and degradation during the cell cycle. I engineered a version of BASL, that is degraded during anaphase and I am testing the ability of this BASL variant to complement the *basl* mutant phenotype when expressed before or after division. In addition, to identify mutants affected in polarity establishment, I initiated a forward genetic screen in which I screened for mis-localization of polarized proteins in the leaf epidermis. This approach already led to the isolation of promising potential mutants affected either in: polarity establishment (leading to non polarized cell); maintaining a restricted polarity domain; or maintaining a proper stomatal pattern (with clusters of stomatal lineage cells or stomata in contact). Interestingly some of the candidates present incomplete cell walls and are impaired in the stomata pattern and/or polarity establishment. This new mutant set will improve our knowledge of connections between polarity establishment, cell division and cell fate decisions.

P159 - The regulation of growth and cell wall deposition by DEFECTIVE KERNEL1 (DEK1) in Arabidopsis thaliana

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Plant cell walls are composed of complex arrays of polysaccharides (~90%) and proteins (~10%) and represent the world"s largest source of renewable biomass. They provide a crucial structural function for the growing plant by harnessing the internal pressure (turgor pressure) of cells in a highly regulated manner; allowing controlled turgor-driven tissue growth whilst maintaining turgor-dependent structural support [1]. The outer cell layer of the plant, the epidermis, is thought to restrict plant growth in an analogous way and regulate final organ size. A key regulator of epidermal development is DEFECTIVE KERNEL1 (DEK1), a 240 kDa modular protein comprising a large trans-membrane domain region and a cytoplasmic calpain (cysteine protease) domain. Changes in organ size and shape are observed when DEK1 levels are altered [2]. Our studies show that plants over-expressing the calpain domain of DEK1 (CALPAIN OE) have thicker cell walls and changes in cell wall composition, most notably in the epidermis. The up-regulation of several cell wall-related genes in response to CALPAIN OE indicates that DEK1 may regulate growth through cell wall modification.

[1] Cosgrove DJ. (2005). *Nat Rev Mol Cell Biol* 6, 850-861; [2] Johnson KL et al. (2008). *Plant Cell* 20(10), 2619-2630

P160 - Computational modelling reveals a new cell division rule during plant early embryogenesis

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In plants, cell division is one of the major mechanisms that orchestrate the transition from a single cell to a large number of cells organized in tissues and organs. Several phenomenological rules have been proposed to relate the position and orientation of division plane to cell geometry.

It is generally admitted that symmetric divisions correspond to a default mechanism driven by physical constraints and that asymmetric divisions are triggered by specific signals. However, research efforts to date have mostly concentrated on symmetric divisions, in systems where cells can be assimilated to 2D shapes, or have used simplified division models. Combining confocal microscopy, image analysis, cell lineage reconstruction, and computer modelling, we investigated 3D cell division patterns during the first five generations in *A. thaliana* embryos. We showed the existence of a new rule relating the position and area of division plane to cell geometry. The rule was valid for both symmetric and asymmetric cell divisions, in various cell types and morphologies, and at all considered generations. Starting from the original apical cell, recursively applying our rule predicted the sequence of observed division patterns. An important consequence of our findings is thus that the apparently complex cell organization of the embryo could be interpreted as a self-organized structure emerging from a geometrical feedback loop between cell shape and division plane positioning.

P161 - Maternal plant vernalization exposure influences germination behavior of progeny

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The genetic basis of how seasonal environmental conditions experienced by maternal plants influences the phenology of their offspring is poorly known, despite the fact that such maternal effects on progeny are intense and ecologically important. We studied the effect of maternal-plant vernalization (Vern) at the seedling stage on the germination behavior of the progeny in 20 different ecotypes of *Arabidopsis thaliana*. We found that Vern of maternal plants very early in the life cycle increased germination of the progeny, even in ecotypes without Vern requirements for flowering. The result is unexpected because prior study correlated increased *FLOWERING LOCUS C (FLC)* expression with higher germination, yet Vern decreases *FLC*-expression in the rosette stage. In addition, in ecotypes showing an effect of Vern on progeny germination, *FLC* levels along the life cycle did not strongly correlate with germination probability. Thus, our results suggest that *FLC*-mediated Vern is uncoupled from *FLC*-mediated germination. However, we found evidence that other Vern-related genes influence germination, and this regulation may be independent of *FLC*. Preliminary data also shows that increased *VERNALIZATION INSENSITIVE 3 (VIN3*) expression during silique development of Vern plants might be related to the increase in progeny germination. Our results highlight the potential for seasonal cues early in the life cycle to influence the phenological performance of the next generation.

P162 - Redox-dependent Control of Root Architecture

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Thiol reduction proteins are key regulators of the redox state of the cell, managing development and stress response programs. In plants, cytosolic thiol reduction proteins, namely thioredoxins (TRX), glutaredoxins (GRX), and their respective reducers glutathione reductases (GR) and thioredoxin reductases (NTR), are organized in complex multigene families. In order to decipher the function of the different proteins, we have performed a comprehensive *in silico* study of the expression of all members of different classes of thiol reduction genes (TRX, GRX) in *Arabidopsis thaliana*. Tissue expression profiles and response to many biotic and abiotic stress conditions have been studied systematically. Putative candidates have been identified for the control of root development and for its regulation by environmental cues. Phenotypic analyses of some of the respective mutants are in progress. Among them, the cytoplasmic and nuclear multidomain *GRXS17*, already known to participate in heat-stress resistance, plays a critical role during root development, by regulating both the primary root growth rate and the initiation speed of lateral roots. In order to better depict the function of *GRXS17* during root system development, we are pursuing structurefunction analyses, together with genetic studies. A suppressor screen of the *grxs17* root phenotypes has been done and the identification of several suppressor mutations is in progress.

P163 - Roles and regulation of cell walls surrounding plasmodesmata in organ formation and patterning

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Most plant cells have channel-like structures, named plasmodesmata, that transverse their relatively rigid cell walls to provide cytoplasm-to-cytoplasm connectivity with their neighbours. Plasmodesmata provide a pathway for molecular transport between individual cells and mediate phloem loading and unloading to communicate distant tissues. Transcription factors, metabolites, RNAs, miRNAs, and other signals have been shown to move through plasmodesmata to regulate development and the plant response to the environment. Our research aims to investigate the composition and regulation of these channels using Arabidopsis as a model organism. Specifically, we focus on the regulation of cell wall polysaccharides (mainly callose) around plasmodesmata and on the developmental consequences of changing their relative composition. In this context, we have pursued the identification of plasmodesmata-located callose hydrolases and established the role of these enzymes in the regulation of symplastic transport and in the formation and patterning of lateral root organs. More recently, we have made progress on the identification of other plasmodesmata components and, using callose modifiers, the importance of plasmodesmata regulation in the response to environmental factors that affect the formation and patterning of root secondary organs is been further dissected. Novel results on these areas will be discussed.

P164 (Talk) - COBRA-LIKE 2 plays a role in cellulose deposition in Arabidopsis seed coat mucilage secretory cells

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Recent studies identified cellulose as an important component of seed mucilage in various species. Cellulose is deposited as a set of rays that radiate from the seed upon mucilage extrusion, serving to anchor the pectic component of seed mucilage to the seed surface. Using transcriptome data, we identified COBRA-LIKE2 (COBL2), a member of the glycosylphosphatidylinositol-anchored COBRA-LIKE gene family in Arabidopsis (Arabidopsis thaliana), as coexpressed with other genes involved in cellulose deposition in mucilage secretory cells throughout the course of seed development. Disruption of the COBL2 gene results in substantial reduction in the cellulosic rays present in seed mucilage accompanied by an increased solubility of the mucilage pectic component upon. Electron microscopy and in situ quantification of light birefringence demonstrates substantially compromised crystalline cellulose deposition into the radial cell walls and the columella of the *cobl2* mutants. Measurements by Updegraff assay indicate an approximate 40% reduction in whole-seed crystalline cellulose content in the *cobl2* mutants when compared to wild-type seeds. This data establishes a role for COBL2 in deposition of crystalline cellulose into various secondary cell wall structures during seed coat epidermal cell differentiation.

165 - Manipulation of Vascular Patterning and Plant Growth through constitutive of Auxin Response Factor Activity

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Differential auxin signaling is of pivotal importance in plant tissue patterning. Combinatorial interactions of AUXIN RESPONSE FACTORs (ARFs) and Aux/IAA proteins play a central role in the regulation of such auxin responses. Short-lived Aux/IAA proteins negatively regulate ARF activity through physical interaction, mediated by shared domains III and IV. As a new tool to explore the systems properties of this regulatory network, we generated a gain-of-function ARF genotype by

eliminating domains III and IV from the functionally well-characterized ARF MONOPTEROS(MP)/ARF5. This truncated version of MP, termed MPΔ, rescued the mp loss-of-function mutant, but also displayed a number of semidominant traits affecting auxin signaling and organ patterning. In the absence of auxin application, the expression levels of many auxin-inducible genes were increased. We report, how this new tool is being used to dissect the ARF-Aux/IAA regulatory network and identifies MP/ARF5's position in it as well as downstream targets of MP/ARF5 and their roles in early in vascular development. We also report how this new genotype can be used to better understand the role of MP in vascular patterning, meristem patterning and how its influence on leaf and stem development and on adaxial-abaxial patterning in various organs is related to its role in vascular patterning.

P166 - TOWARDS ELUCIDATING THE FUNCTION OF DOCS1, A LRR-RLK GENE INVOLVED IN ROOT OUTER CELL LAYERS FORMATION IN RICE

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Development of new rice varieties more tolerant to abiotic stress is one challenge of this century. To be able to respond to an alteration of their environmental conditions, plants have first to sense the changes and then to react to them. At the organ and molecular levels, these two steps can be achieved by roots and by multicomponent signaling pathways in which protein phosphorylations play an essential role. A member of the *Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK)* gene family named *DOCS1* for *DEFECTIVE IN OUTER CELL LAYERS 1* (Huang *et al.*, 2012) determines root cell identity (ground tissues, epidermis) and number of sclerenchyma cell layers. The *c68* mutant, coding for a truncated DOCS1 protein, is sensitive to aluminum due to its disorganization of root outer cell layers structure. Aluminum can penetrate deeper in root tissues in c68 (Huang *et al.*, 2009). DOCS1 is probably a key element of a larger gene network involved in rice root outer tissues identity and specification. We are presently creating knockout lines using CRISPR to specify *DOCS1* function as *c68* is probably not a complete loss of function mutant. A yeast-two-hybrid screen is also ongoing to identify members of the *DOCS1* gene network.

Huang, C. F., et al. (2012) Plant J 69(4): 565-576.

Huang, C. F., et al. (2009) Plant Cell Physiol 50(5): 976-985.

P167 - Exploring auxin signaling during leaf morphogenesis

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The understanding of molecular mechanisms controling organ or body size and shape represents one of the main challenges in developmental biology. Plants and more specifically plant leaves exhibit interesting features for such purpose. Leaf morphogenesis results from the coordination in time and space of cellular divisions and cellular expansion. Transcription factors of the *CUP-SHAPED COTYLEDON (CUC)* genes are essential for the control of leaf shape, playing a critical role in leaf serrations. In addition, the phytohormone auxin which is a critical regulator of growth and development, is involved in the regulation and coordination of cell division and cell expansion and interacts with *CUC* genes during leaf shaping. The mechanisms of auxin signalling are based on a complex set of co-receptors exhibiting high to low affinity for auxin and an even more complex modular network of transcriptional repressors and activators tightly controlling the expression of a large set of genes. In the perspective of studying further the CUC/auxin regulatory network, we analysed auxin response maxima during early stages of teeth formation using the recently developed R2D2 ratiometric reporter[1]. We tentatively established a map of expression of transcription factors of the AUXIN RESPONSE FACTOR (ARF) family during leaf margin morphogenesis.

[1] Liao et al, 2015 – Reporters for sensitive and quantitative measurement of auxin response. Nature Methods 12, 207-210.

P168 - WDR55 target screen to study post-translational modification of translational control in plant reproductive development.

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Sexual reproduction in flowering plants, comprising flower, gametophyte and embryo development, probably builds the most complex network of highly specialised cells formed by evolution in plants. Developmental studies on reproduction in Arabidopsis have revealed the importance and function of several essential growth and cell cycle regulators. Those experiments showed that the precise coordination between growth and differentiation in such a structured network is not only based on transcription but highly controlled on a translational and post-translational level of protein interaction and

degradation. WDR55 is one of the essential proteins that were identified as necessary for reproductive development while at the same time being fundamental for growth, proliferation, bilateral symmetry and hormone signaling. Bjerkan et al. showed that WDR55 is a potential substrate receptor in a DDB1-CUL4-based E3 ubiquitin ligase complex and thus probably involved in targeting proteins for degradation. New results from our target and interactor screen indicate a functional role of WDR55 in translational control connecting hormone signaling, growth and developmental decisions during reproductive development.

Bjerkan, K.N., et al. (2012). Arabidopsis WD REPEAT DOMAIN55 Interacts with DNA DAMAGED BINDING PROTEIN1 and Is Required for Apical Patterning in the Embryo. The Plant Cell 24, 1013–1033.

P169 - Shotgun Label-Free Quantitative Proteomics of Muscadine Grape (Vitis rotundifolia) Berry

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Muscadine grapes are well known for a variety of nutraceutical and enological characteristics as well as anticancer activity. Until recently, most studies were focused on vinifera grapes, with little information available on muscadine grapes. Recent advances in mass spectrometry has enhanced our ability to identify more proteins and metabolites. The objective of this research was to investigate the proteome profile of muscadine berries using a label free separation method. Total proteins of pericarp were extracted from 4 time points of berry development. LC-MS/MS spectra were acquired from 3 biological replicates per time point by a sample-optimized gas phase fractionation method on an LTQ XL mass spectrometer (Thermo). Spectrum-peptide matching was performed with X! Tandem and GPM Cyclone. A protein database was compiled from all reviewed *V. vinifera* protein entries in UniProt and *V. vinifera* proteins predicted by the IGGP. When calculated, NSAF detected 2796 total proteins, with 515 differentially expressed. We observed that the label free method of protein identification revealed more proteins and some that were not previously reported. Berry Stage 3 indicated a major shift in the regulation of several proteins including photosynthesis, stress, PR proteins, and flavor. Further investigation on interaction network will determine the role of differentially expressed proteins associated with the biosynthesis of nutraceutical compounds.

170 - Thermospermine represses xylem differentiation by enhancing translation of the SAC51 family mRNAs

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The *acaulis5 (acl5)* mutant of Arabidopsis exhibits severe dwarf phenotype along with excess proliferation of xylem vessels. *ACL5* encodes thermospermine synthase. To know the mode of action of thermospermine in repressing xylem differentiation, suppressor mutants that reverse the dwarf phenotype of *acl5* have been isolated and named *sac. SAC51* encodes a bHLH-type transcription factor and the *sac51-d* allele has a point mutation in the 4th uORF within the 5" leader. The sac57-d allele has a point mutation in the 7th uORF of *SACL3*, a member of the *SAC51* family. These uORF sequences are also conserved in homologous genes in other plant species. By using the GUS reporter gene, we found that these mutations enhance translation of each main ORF and that the 5" leader sequence of *SAC51* and *SAC57/SACL3* is responsive to thermospermine. We further made quadruple mutants of *SAC51, SACL1, SACL2*, and *SAC57/SACL3*, and the phenotypic characterization will be presented.

P171 - Interchangeable functions of LEAFY, SHOOT MERISTEMLESS and CUP-SHAPED COTYLEDON2 in the control of leaf complexity in Arabidopsis and Medicago.

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CUP-SHAPED COTYLEDON2/NO APICAL MERISTEM (NAM/CUC2) and SHOOT MERISTEMLESS (STM) act in concert to establish and maintain meristems and boundaries. The LEAFY/UNUSUAL FLORAL ORGAN (LFY/UFO) module, which determines floral identity, also has a meristematic function. Beside their roles in meristems, the LFY, STM and CUC2/NAM pathways also contribute to the formation of compound leaves. To explore the degree of overlap and specificity between these pathways, we compared the activity of *LFY, STM* and *CUC2/NAM* in *Arabidopsis*, a species with simple leaves and in *Medicago truncatula*, a legume species with compound leaves, in which leaflet formation depends on the *LFY* ortholog *SINGLE LEAFLET (SGL1)* and not on *STM*-related genes. Overexpression of *LFY* in *Arabidopsis* results in the formation of more complex leaves similar to plants overexpressing *STM* or plants expressing a *miR164*-resistant version of *CUC2. LFY* ectopic expression slightly rescues stm mutant. Expression of a *miR164*-resistant version of *NAM* or overexpression of *STM* in *M. truncatula* leads to more complex leaves. Remarkably, the *sgl1* leaf phenotype can be complemented by overexpression of either *STM* or *NAM*.

These data show that *Arabidopsis* and *Medicago* retain the ability to respond to both *LFY* and *STM* pathways respectively. Transcriptomic analyses are currently performed to investigate if these transcription factors use the same targets to increase leaf complexity in *Arabidopsis* and *Medicago*.

P172 - The phytohormone ABA activates flowering by promoting florigen genes expression

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A drought escape (DE) response allows some plants to adaptively shorten their life cycle to make seeds before severe stress leads to death. In *Arabidopsis* a DE response occurs under long day conditions, when photoperiod-stimulated GIGANTEA (GI) protein promotes the transcriptional activation of the florigen genes *FLOWERING LOCUS T (FT)* and *TWIN SISTER OF FT (TSF)*. The phytohormone ABA participates in this process in an unknown manner, upstream of the florigen genes. A key question arises as to how ABA-mediated signals might affect activation of the florigen genes. We find that an increase in ABA signalling results in *FT* activation, whilst the opposite occurs when impairing ABA signalling. Double mutants analyses confirm that the ABA-mediated effect on flowering requires the presence of *GI* and the florigen genes. The florigen upstream regulator *CONSTANS (CO)* is also required, suggesting a role of *CO* in the ABA-dependent florigen regulation. Neither ABA biosynthetic mutants nor ABA signalling mutants present obvious defects in *CO* transcript accumulation suggesting that ABA affects florigen upregulation via modifying CO protein activity and/or through other mechanisms, which however must be related to CO function. Interestingly, *CO* is not required for *TSF* upregulation under drought conditions, implying the existence of alternative routes for florigens upregulation. Our analyses indicate that ABA plays a key role in transducing water status information upon the florigen genes.

P173 - Characterization of an Arabidopsis mutant lacking high-nitrate-induced lateral root inhibition

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The root system architecture deeply shapes nutrient uptake. Understanding the root plasticity in response to mineral resource availability could provide insights into crop productivity improvement. In agricultural soil, nitrate is a major nutritional determinant of root morphology and biomass production. Our goal is to identify key genetic mechanisms regulating the lateral root development in response to nitrate of the model species *Arabidopsis thaliana*. During a forward genetic screen with mutants treated with ethyl methanesulfonate, *hn74* was isolated for the presence of numerous lateral roots upon high nitrate supply - a restrictive condition for the wild type. A positional cloning of the mutation, which was carried with an experimental population segregating for the root morphological trait and with polymorphic markers, identified a 2 Mb zone of interest on chromosome 4. Further *in vitro* phenotypic characterization revealed that *hn74* mutant did not undergo the negative repression on lateral root elongation exerted by high salt and osmotic conditions. Finally, higher nitrate content was found in the mutant tissues compared to wild type (1.5-fold increase) suggesting a dysfunction of N metabolism. Those results place *hn74* as a genotype of choice to study the mechanisms of lateral root growth repression exerted by high nitrate supply and the signaling pathways overlapping with other nutrient stresses.

P174 - How does injury reinitiate developmental programs during regeneration?

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The plant root is a highly organized organ with concentric tissue layers that converge at the Quiescent Center (QC)- a cluster of cells that maintains the root stem cell niche. Despite having high organization of adult growth centers within organs, plants are flexible and have the ability to regenerate lost or damaged organs -a phenomenon that begs the question: how do plants regenerate a root long after *de novo* organogenesis first occurs in the embryo? Preliminary results show that auxin signaling returns early and is necessary for regeneration. One important patterning factor in root embryogenesis is the Auxin Response Factor (ARF) *MONOPTEROS (MP* or *ARF5*). In *mp* mutant embryos, the hypophysis, which will give rise to much of the root tip, fails to form and mutants lack a root. MP expression is re-established in the root stump 3 hours after root tip excision. Interestingly, MP is expressed independently of auxin signaling. Thus, it appears that there is a parallel pathway to auxin that operates early in regeneration and potentially controls MP expression in response to injury. I will show results of a conditional knockdown of MP to test its role in root regeneration, and the results of a cell-based gain-of-function screen for upstream regulators of MP. A *spatial and temporal development program, centered on MONOPTEROS, is critical during embryogenesis. My research works towards addressing how a similar program is induced for post-embryonic patterning and tissue repair.*

P175 - Arabidopsis tRNA-derived RNA Fragments as a new source of small noncoding RNAs

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In the expanding repertoire of noncoding RNAs (ncRNAs), tRNA-derived RNA fragments (tRFs) have been identified in all domains of life. First discarded as RNA turnover by-products, there is now evidence that they are not just random degradation fragments but rather stable entities with major biological functions. Only few data report on plant tRFs. Using high-throughput sequencing technology, we retrieved tRFs from various *Arabidopsis* ncRNA libraries. Two classes of tRFs (around 20 and 35 nt long respectively) are produced after cleavage at very specific sites. Among the remarkable observations: numerous tRFs originate not only from nuclear-encoded tRNAs but also from organellar tRNAs, a few tRFs are either enhanced or repressed depending on abiotic stress or plant tissues, a few of them are strongly associated with AGO1 or AGO4. In addition, we now have evidence that organellar tRFs are generated outside the organelles. We also identified a family of *A. thaliana* endonucleases differentially expressed during plant development (e.g. two of them are expressed during seed maturation) and able to cleave tRNAs not only in the anticodon but also in the D-loop, an activity not demonstrated so far for such enzymes.

Altogether, our data suggest that some tRFs play important regulatory functions during plant development and/or in response to stress, including tRFs of organellar origin. Beyond translation, our data open new perspectives for nucleusand organelle-encoded tRNAs as major actors of gene expression in plants.

P176 - Nitrate-regulated glutaredoxins control Arabidopsis thaliana primary root growth

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Nitrogen is an essential soil nutrient for plants, and it is typically available in two inorganic forms: nitrate and ammonium. We performed transcriptional profiling of the shoots of ammonium-supplied and nitrate-supplied *Arabidopsis thaliana* plants and identified seven genes encoding class III glutaredoxins that were strongly and specifically induced by nitrate. Nitrate is known to trigger *de novo* cytokinin biosynthesis, and the expression of the identified glutaredoxins was also upregulated by cytokinin, independent of plant nitrate status. RNA silencing of four of the glutaredoxin genes resulted in plants with increased primary root length (~25% longer than wild-type), which is a classic phenotype of cytokinin-deficient plants. Crosses between cytokinin-deficient plants and glutaredoxin-silenced plants produced hybrids whose primary root length was comparable to the parental lines. Collectively, these findings suggest that nitrate, cytokinin, and class III glutaredoxins operate in a common signal transduction pathway that acts to negatively regulate Arabidopsis primary root growth. This pathway could be an important component of the “nitrogen foraging” activity of plant roots, with nitrate acting to suppress primary root growth (vertical dimension) in concert with its well-characterized stimulatory effect on lateral root growth (horizontal dimension), thereby tailoring root system architecture to maximize utilization of heterogeneously distributed nitrogen in the soil.

P177 - PROLYL 4-HYDROXYLASE 5 THAT DEFINES THE SUBSEQUENT O-GLYCOSYLATION SITES IN EXTENSINS CONTROLS POLARIZED GROWTH IN PLANT CELLS

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Root hairs are single cells that develop by tip growth and are specialized in the absorption of nutrients. Structural cell walls imposed constraints prompt utilization of new molecules to accomplish tip growth in plants. Their cell walls are composed of polysaccharides and hydroxyproline-rich glycoproteins (HRGPs) that include extensins (EXTs). Proline hydroxylation, an early post-translational modification (MPT) of HRGPs that is catalyzed by prolyl 4-hydroxylases (P4Hs), defines the subsequent *O*-glycosylation sites in EXTs, which are mainly arabinosylated. Here, we explored the biological function of P4Hs in root hair cell growth. Biochemical inhibition or genetic disruption of P4Hs resulted in the blockage of polarized growth in root hairs and reduced arabinosylation of EXTs. Secondly, we demonstrate that prolyl-4-hydroxylase 5 (P4H5), and to a lower extent P4H2 and P4H13, are pivotal for EXT-mediated root hair tip-growth. These three P4Hs are targeted to the secretory pathway, most specifically to the ER and Golgi compartments, where P4H5 forms dimers with P4H2 and P4H13 suggesting the existence of P4Hs protein complexes. Thirdly, we explored the subcellular localization and substrate specificity of the P4H5, as well as the resulting cell wall architecture in the *p4h5* mutant. We also addressed the physiological significance of the MPTs of EXTs. In particular, mutants who were deficient in Hyp-*O*-arabinosylation or in Ser-*O*-galactosylation showed shorter root hairs, due to both slower kinetics and premature growth termination. Our results demonstrate that correct *O*-glycosylation on EXTs is essential for cell wall self-assembly and hence root hair elongation in *Arabidopsis thaliana*.

P178 - The Plethora Transcription Factors - Key Players of a Secondary Meristem in Arabidopsis.

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The Vascular Cambium – a secondary meristem in plants produces secondary xylem (wood) and secondary phloem. Meristematic activity in vascular cambium ensures the production of phloem and xylem, which are essential for transportation of nutrients and water. Thus understanding the molecular mechanism behind the maintenance of meristematic state of vascular cambium and its development becomes essential. The PLETHORA (PLT) transcription factors are the central regulators of the primary meristems. Recent research works on PLT/AINTEGUEMNTA (AIL) and/or AINTEGUMENTA (ANT) genes provide the insight of stem cell maintenance in plant primary meristems and their role in phyllotaxis and rhizotaxis[1,2,3,4,5,6]. However their functional role in a secondary meristems is largely unknown and it needs to be elucidated. Therefore, we studied whether the PLT/AIL factors have a function also in the vascular cambium. Expression analysis revealed that several PLT/AIL family members are expressed in cambium, and when we generated mutant combinations from the cambium-expressed PLT/AILs, we found defects in vascular patterning and cambial cell maintenance in a few double and triple mutant combinations. Connection of the PLT/AIL factors to the other cambial regulators and its specific role will be elucidated in the future.

P179 - An ancient bacterial stress response pathway dynamically controls chloroplast function to regulate plant growth and development.

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Chloroplasts have retained elements of bacterial stress response pathways that are thought to be involved in regulating chloroplast function. Here we report on our investigation into one of these pathways, showing for the first time that it can act as a potent controller of chloroplast gene expression *in vivo*. We then use a panel of mutants to show that the pathway has an unprecedented role in the dynamic control of chloroplast activity, and that it promotes nuclear-chloroplast cooperation to regulate plant growth and developmental transitions. We discuss how this ancient bacterial pathway has evolved, and its implications for the integration of the chloroplast into the cells of photosynthetic eukaryotes.

P180 - Competition signals suppress branching via abscisic acid and auxin signaling

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Branching is an important plant architectural characteristic, affecting plant fitness in natural environments and productivity in agricultural crops and pastures. Work has demonstrated that intrinsic genetic programs are major determinants of branching, but it is also known that environmental signals regulate this central aspect of plant form. Among these environmental signals is the ratio of Red light to Far Red light (R:FR) which is perceived by the phytochromes (phy). Reduced R:FR signals impending competition from neighboring plants and elicits the shade avoidance response, which includes decreased branching. Our ongoing research has demonstrated that elevated abscisic acid (ABA) restricts axillary bud outgrowth under low R:FR, thereby contributing to the suppressed branching phenotype. We have also shown that elevated auxin signaling suppresses branching in plants grown under low R:FR and in phyB-deficient plants, though indole-3-acetic acid levels are not increased. We present evidence defining how the bud local ABA and systemic auxin pathways interact to regulate branching in response to dynamic R:FR signals characteristic of competitive environments.

P181 - An integrated approach to characterize shoot stem cell activity in Arabidopsis thaliana

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In contrast to animals, plants generate most of their tissues post-embryonically through the continuous activity of multipotent stem cell pools embedded in specialized tissues, called meristems. Despite their importance in generating the entire plant body, the molecular circuits underlying plant stem cell activity still remain poorly understood. We recently identified *HECATE* genes (*HEC*), members of the bHLH transcription factor family, to be involved in the regulation of stem cell proliferation at the Shoot Apical Meristem (SAM). Intriguingly, HEC1-driven stimulation of stem cell activity is cell type specific and independent of WUS. Since the known core regulatory network is almost fully suppressed in these plants, it follows that important alternative stem cell control pathways must exist. My project now aims to identify these so far unknown stem cell regulatory mechanisms and to understand how HEC1 connects them with the canonical WUS/CLAVATA feedback and phytohormone signaling systems. To this end I will use an integrated approach combining live cell imaging, cell type specific genomics, advanced genetics including interaction screens in yeast and plants, molecular techniques, as well as computational analysis. The integration of results from my diverse experimental approaches will allow me to build a mechanistic map, which will be an ideal starting point for mathematical modeling and will substantially advance our understanding of plant stem cell regulation.

P182 - Arabidopsis Defective Kernel 1 (DEK1) promotes and maintains plant epidermal differentiation by indirectly controlling HD-ZIP IV gene expression.

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To exert its functions epidermis needs to be produced as a continuous and intact layer. In leaves, to achieve that, epidermis concurrently produces mature pavement cells forming a perfect jigsaw that allows the blade to grow flat and correctly spaced stomatal guard cells specialized for gas exchange. Producing this complex mosaic not only demands that neighbouring cells co-ordinate their growth but also that they maintain their specialization over time. Our work shows that the Arabidopsis DEK1 protein is an important regulator of epidermis differentiation and differentiation maintenance. Plants with reduced DEK1 activity, despite showing normal growth, produce cotyledon epidermis with protodermal characteristics. Moreover, in non-embryonic tissues DEK1 is required for trichome and giant cell differentiation maintenance. These phenotypes are accompanied by a diminished expression of the differentiation-promoting HD-ZIP IV transcription factors, but not by striking changes in cell ploidy or mis-regulation of cell cycle-related genes. In severe dek1 mutants epidermal cells have a tendency to separate (Johnson *et al.*, 2005) and in the weak allele *dek1-4* cell wall junctions between epidermal cells are less uniform than in wild-type plants. Consistent with defects in the epidermal adhesion zone, mutants also display an abnormal accumulation of callose at epidermal cell boundaries. In *Arabidopsis*, the expression of *HD-ZIP IV* encoding genes has been shown to be maintained by inter-cellular signaling (San-Bento *et al.*, 2014). It is therefore possible that DEK1, by promoting cell- cell communication in the epidermis, indirectly regulates HD-ZIP IV expression and thus epidermis differentiation.

P183 - MIR390a is regulated by MONOPTEROS/ ARF5 in the primary root meristem of Arabidopsis thaliana

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The study of roots in *Arabidopsis thaliana* serves as an exquisite model to study the developmental plasticity and robustness of the plant system. Previous studies in our lab have shown that a specific small RNA pathway called the *TAS3* pathway controls the process of lateral root formation. A specialized type of small RNA, trans-acting siRNAs (tasiRNA) found specifically in plants, are produced by the TAS precursors and targets the different Auxin Response Factors (ARFs) for degradation leading to several developmental effects. miR390 is involved in the *TAS3* pathway, which is induced during lateral root initiation, sensing the effects of phytohormone auxin and triggers the local production of tasiRNAs. In order to understand how the *TAS3* pathway is activated, it is essential to identify what controls miR390 expression. To find *cis*-regulatory elements of *MIR390a*, a reporter line *pMIR390a::GUS:GFP*, that faithfully reflects the expression of miR390 at both the primary and lateral roots, was used to create nested deletion mutants. The minimal regions necessary for expression of miR390 both at the primary and lateral root were identified. These were dubbed as the Primary Root Enhancer (PRE) and Lateral Root Enhancer (LRE) respectively. Interestingly a putative AuxRE (Auxin Response Element) related TGTC element was found to be present within the PRE region. Further analysis (both *ex-vivo* as well as *in-vivo*) revealed that the transcription factor MONOPTEROS (MP)/AUXIN RESPONSE FACTOR5 (ARF5) binds specifically to the AuxRE element contained in the PRE region thus indicating a potential regulatory role of MP/ARF5 on expression of *MIR390a* at the primary root meristem. Absence of *pMIR390a::GUS:GFP* reporter expression in mp mutant background indicated that *ARF5/MONOPTEROS* is upstream of MIR390a and is necessary for its regulation hence providing further evidence towards a functional relevance of the *ARF5-MIR390a* regulation.

P184 - Fucose is required for leaf morphogenesis

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NAM/CUC (NO APICAL MERISTEM/CUP-SHAPED COTYLEDON) genes encode transcription factors of the plant-specific NAC family. In Arabidopsis CUC2 and CUC3 but not CUC1 are required for the serration of its simple leaf, as shown by the smooth leaves of *cuc2* and *cuc3* mutants. The *CUC2* specific expression pattern at the leaf margin in the sinuses of developing teeth is in part orchestrated by the *miR164*. Increased levels of CUC2 activity in the microRNA resistant *CUC2g-m4* line lead to increased and deeper margin serrations. Despite our growing knowledge of the mechanisms that govern *CUC2* expression and activity we lack an understanding of how C*UC2* shapes the leaf margin dissection. For this we employed a multidisciplinary approach to find potential targets and partners of *CUC2* in leaf development. Using a genetic suppressor screen we identified a series of mutations capable of reverting the

CUC2g-m4 leaf phenotype. Among these, a mutation in the *MUR1* locus, responsible for the synthesis of GDP-L-Fucose shows reduced leaf margin serrations. Fucose is a component of several cell wall polysaccharides and glycoproteins and has important roles in plant growth and development. Here we present the morphological and functional confirmation that MUR1 is implicated in leaf development and show that normal levels of fucose are required for proper leaf morphogenesis.

P185 - A Repressor Protein Complex Regulating Leaf Growth in the Dicot Arabidopsis

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Cell number is an important determinant of final organ size. In the leaf, a large proportion of cells are derived from the stomatal lineage. Meristemoids undergo asymmetric divisions, generating several pavement cells next to the two guard cells. However, the mechanism controlling the asymmetric divisions of these stem cells before they differentiate is not well understood. Here, we characterized PEAPOD (PPD) proteins, the only transcriptional regulators known to negatively regulate meristemoid division. PPD proteins interacted with KIX8 and KIX9, which act as adaptor proteins for the corepressor TOPLESS. D3-type cyclin encoding genes were identified among direct targets of PPD2, being negatively regulated by PPDs and KIX8/9. Accordingly, *kix8-kix9* mutants phenocopied PPD loss-of-function producing larger leaves resulting from increased meristemoid amplifying divisions. The identified conserved complex might be specific for leaf growth in the second dimension, since it is not present in Poaceae (grasses), which also lack the developmental program it controls.

P186 - Dissecting the gene networks downstream of BRANCHED1 involved in axillary bud dormancy in Arabidopsis thaliana

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We are studying the genetic control of shoot branching in Arabidopsis thaliana. Axillary buds in the rosette leaves of Arabidopsis can elongate to become branches or remain dormant for the whole life of the plant. This decision is controlled by both internal and external stimuli. We have previously characterized one key gene involved in branching regulation, *BRANCHED1 (BRC1)*, encoding a TCP transcription factor expressed in axillary buds where it represses growth. We performed a transcriptomic analysis comparing axillary buds of wild-type plants and *brc1* mutants exposed to low R:FR light, a stimulus that promotes dormancy. Genes that changed in wild-type but not in *brc1* were proposed to be *BRC1*-dependent genes, and when we compared our microarray with other available experiments comparing active vs dormant axillary buds, we identified two groups of genes, *bud activation genes* and *bud dormancy genes*, genes whose levels are higher in active or dormant buds, respectively. *BRC1*-dependent *bud dormancy* genes formed four co-regulation networks: protein degradation, auxin and ethylene signaling, sugar repressed genes and genes related with abscisic acid (ABA). We are currently analyzing the relationship between these co-regulation networks, *BRC1* and branching control. We have focused our study in three putative direct targets of *BRC1* that are represented in one of these co-regulation networks involving bud dormancy genes. Evidence of their function and regulation will be presented.

P187 - EMBRYONIC FACTOR 6 in Arabidopsis regulates zygote division and syncytial cell cycle synchronization

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Double fertilization in flowering plants creates a diploid embryo and a triploid endosperm. Activation of divisions in the zygote and the central nuclei is critical for seed formation, but how these processes are regulated remains elusive. Here, we show that EMBRYONIC FACTOR 6 (FAC6) is essential for the first division of the zygote and for the synchronization of syncytial endosperm nuclei in Arabidopsis. Both the zygote and the endosperm in fac6 mutants exhibited cell cycle arrest and over-accumulation of cyclin-GUS protein. The bi-allelic and continuous expression of FAC6 from the egg and sperm cells before fertilization to early embryos and endosperms after fertilization suggest that neither maternal-to-zygotic transition nor imprinting is involved in FAC6 regulation. Down-regulation of FAC6 led to multinucleate cells with asynchronized mitotic divisions. Biochemical studies showed that FAC6 has E3 ligase activity and is able to ubiquitinate and degrade cyclin, suggesting that FAC6 may act directly on cyclin to initiate the first division in the zygote and to synchronize mitotic cell cycling among syncytial endosperm nuclei.

P188 - Functional genomic and biochemical characterization of Arabidopsis thaliana polygalacturonases

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The plant cell wall does not only have a structural role in determining the texture and mechanical properties of plants and their organs, but is also critically involved in growth and differentiation. If the composition of the plant cell wall is fairly well known, the mechanisms underlying its biosynthesis and modifications are far from being elucidated. Among the major components of the type I plant cell wall are pectins. In primary cell wall, pectins are notably composed of 65% of homogalacturonans (HG), a linear homopolymer of a-(1-4) linked D-galacturonic acid residues. HG can be cleaved at their glycosidic bond by polygalacturonases (PGs, GH28), which can have dramatic consequences on the rheological properties of the cell wall and plant developmental. In this study, we identified two PGs of *A. thaliana*, with distinct patterns of expression. Using a multidisciplinary approach, we characterized their biochemical activities and function *in planta*. The enzymes were expressed in *P. pastoris* as secreted proteins. After purification by affinity chromatography, the biochemical characterization was realised, giving new insights into the enzymatic properties of these enzymes. Confocal laser microscopy analysis, gene expression during plant development and protein subcellular localization will be presented.

P189 - DramaQueen - a new component of mechanosensing and hormonal signalling in Arabidopsis thaliana.

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The genetic processes that control *Arabidopsis* primary root vascular patterning are still not fully understood. We have shown that both auxin and cytokinin are required for root procambial patterning and that their interaction is mutually inhibitory (Mähönen et al. Science 2006, Bishopp & Help et al. Current Biology 2011, Bishopp et al. Current Biology 2011). In order to find new components involved in procambial cell patterning, a genetic screen using a cytokinin signaling desensitized

mutant background (*cre-12*) was done. The *DramaQueen* mutant (*drq*) shows increased sensitivity to cytokinin. *DRQ* is a single copy gene in Arabidopsis and it is conserved throughout the plant kingdom, yet its function is unknown. *DRQ* is expressed in the vasculature of roots, leaves and flowers, trichomes, pollen and silique abscission zones. *DRQ* is rapidly induced by wounding, indicating a role also in stress response. We have analyzed the missense drq mutant, <e*m>DRQ-RNAi</*em> and *DRQ-OE* plants and found differences in primary root elongation rates, protoxylem phenotypes, secondary growth rates, rosette sizes etc. We speculate that *DRQ* is involved in signaling upon mechanical stimulation and regulates cell elongation and xylem development by integrating inputs from various hormonal signaling pathways through its interaction partners/targets. As a hub for hormonal signaling, plant development & plant stress, we believe that *DramaQueen* will make novel contribution to various research fields.

P190 - OsSHR1 & OsSHR2 functions in rice root cortex formation

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Optimal root development is central for plants to reach maximum growth and yield. Most of our knowledge regarding genes involved in root development has been accumulated in the dicotyledon model plant *Arabidopsis thaliana*. Roots of rice, the monocotyledon model, present several extra ground tissue layers compared to *A. thaliana*. Between epidermis and endodermis, rice possesses two outer cell layers, exodermis and schlerenchyma, and a multilayered cortex. All these tissues have specific cell identity, anatomical and molecular adaptations related to their diverse roles in root growth and function. Variation in the number of cortex cell layers depends on the rice root type and cortex is one of the key tissues for rice adaptation to submergence and tolerance to other environmental stresses. Cortex and endodermis differentiation in *A. thaliana* has been extensively studied during the last 10 years. Thereby, SHORTROOT (SHR) gene in *Arabidopsis thaliana* has been identified as a key gene required for their formations. An elegant model was developed, where SHR is transcribed in stele tissue and its protein moves to the endodermis where it activates SCARECROW (SCR) transcription. Together, *SHR* and *SCR* induce periclinal division of the ground tissue initial separating cortex and endodermis cell layers. Cortex formation in rice represents an intriguing contrast to *A. thaliana*. Variations in the number of cortex cell layers can be observed between the root types and during rice development. There should be a control mechanism for the number of cortical cell layers, and *SCR* and *SHR* rice orthologs represent good candidates. Two duplications in rice led to apparition ortholog genes of *AtSHR* (*$OsSHR_1$* and *$OsSHR_2$*) and we have addressed the question of their respective function in cortex formation in rice root. Our first results suggest that *$OsSHR_1$* and *$OsSHR_2$* are involved in cortex formation in rice. Preliminary work in A. thaliana suggests also a plausible molecular mechanism for formation of multi-layered cortex in rice root.

P191 - Control of cell wall integrity

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The cell wall of growing plant cells accomplishes a remarkable feat, resisting to very high tensional stresses imposed by the high turgor pressure of up to 10 bars, while maintaining the ability to extend. Cell wall strength is determined by the cross links between cell wall polymers, whereas cell wall extensibility reflects the ability to remove and recreate these crosslinks. Recent studies have revealed the existence of feedback signalling networks that control the chemorheological processes underlying the mechanical homeostasis of the walls of growing cells. In this presentation I will first discuss some recent insights into the architecture of plant cell walls in particular the role of pectins as part of a load-bearing network. I will then discuss how receptor kinases of the CrRLKL1 family are part of a regulatory module involved in a feedback regulatory loop that senses cell wall properties and triggers wall stiffening upon wall damage. These modules play a role in normal growth and development and in the response to abiotic and biotic stresses.

P192 - The Impact of GM Soybeans to Rhizosphere Soil Denitrification Microbial Diversity

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Denitrification is certain microorganisms under anaerobic or microaerobic conditions oxynitride as an electron acceptor to produce energy, while reducing nitrogen oxides to nitrogen dioxide or nitrogen process. Denitrification driven by denitrifying bacteria is an important part of the natural nitrogen cycle.Studies using PCR-DGGE technique and sequence amplification method to compare the impact of different soybean genetically modified soybeans PAT□ALS rhizosphere soil denitrification bacterial diversity and community structure composition. The results showe that compared the different transgenic soybean PAT, ALS and the corresponding parent PAT1-ALS1 rhizosphere soil denitrifying bacterial community composition was not significant difference. Strip position among treatments has not significant difference,but in high degree of variability in brightness area bands appear partial differential. Cluster analysis showed that ALS transgenic varieties and their respective parents gather for a cluster of ALS 1,the similarity is 81%; PAT transgenic varieties and their respective parents gather for a cluster of PAT 1,the similarity is 83%; The similarity between each treatment can achieve 71%. On shannon-wiener index (H) and evenness (EH) analysis showed that the difference was not significant (p>0.05)between two kinds of genetically modified soybeans PAT, ALS soil denitrifying bacteria diversity index (H) and evenness (EH) compared and the corresponding parental PAT1, ALS1.Therefore, after the planting of genetically modified varieties, soil denitrification bacterial diversity and community structure difference was not significant. Phylogenetic analysis results show different genetically modified varieties of denitrifying bacteria belongs to Alphaproteobacteria-Betaproteobacteria-Gammaproteobacteria Flavobacteria. There are six strips belong to Alphaproteobacteria, accounting for 50% of the total strips; three belong to Gammaproteobacteria, accounting for 25%. These conclusions show that, Alphaproteobacteria-Gammaproteobacteria are dominant group in transgenic soybeans PAT and ALS and their respective parent and local major plant varieties denitrifying bacteria in the rhizosphere soil biological communities, and Alphaproteobacteria is the most dominant denitrifying bacteria taxa, Denitrifying bacteria categories of Flavobacteria is not abundant.

In summary, the transgenic soybean planting to rhizosphere soil denitrification microbial diversity has not significant difference.

P193 - SNOWY COTYLEDON 4 is Required for Seed Development

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Chloroplasts are essential plant organelles and carry the molecular machinery for photosynthesis. Under different light conditions, the plant has variable pathways to optimize the photosynthesis output. Among many other processes this is also crucial for embryo development in seeds. Posttranslational modifications are essential to regulate signal transduction and metabolism. Prenylation is important to attach farnesyl or geranylgeranyl moieties to proteins. This kind of posttranslational modification leads to translocation of the modified protein to membranes or supports protein-protein interactions. CaaX endopeptidases have been shown to process proteins at the very C-terminus to enable attachment of farnesyl or geranylgeranyl moieties. Interestingly, in plastids the MEP (2-*C*-methyl-D-erythritol 4-phosphate) pathway provides isoprenyl that is required for such protein modifications (Gerber *et al.* 2009, *Plant Cell*). The chloroplast protein SNOWY COTYLEDON 4 (SCO4) shows homology to CaaX endopeptidases and functions in the photosynthetic acclimation to higher light intensities (Albrecht-Borth *et al.* 2013, Plant Physiology). Here we show that proper seed development depends on *SCO4* gene function.

P194 - Cool nighttime temperatures induce the expression of CONSTANS and FLOWERING LOCUS T genes for flowering

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Day length and ambient temperature are major stimuli controlling flowering time. To understand flowering mechanisms in more natural conditions, we explored the effect of daily light and temperature changes on Arabidopsis thaliana. Seedlings were exposed to different day/night temperature and day-length treatments to assess expression profiles of genes that regulate flowering time. Cooler temperature treatments increase *CONSTANS (CO)* mRNA and protein levels during the night. Nighttime *CO* induction was diminished in *flowering bhlh (fbh)*-quadruple mutants. Day/night temperature changes altered the day-length-dependent expression patterns of *FLOWERING LOCUS T (FT). FT* levels were reduced at dusk, but increased at the end of cooler nights. Dusk suppression, which occurred in younger seedlings, was alleviated in *short vegetative phase (svp)* mutants, while the increase at night continued over two weeks in long days. *FT* levels correlated strongly with flowering time across treatments. Day/night temperature changes modulate photoperiodic flowering by changing *FT* expression patterns. Cooler nighttime temperatures enhance FBH-dependent induction of CO and stabilize CO protein. When plants are young, cooler temperatures suppress *FT* at dusk through SVP function, perhaps to suppress precocious flowering. Our results suggest day length and diurnal temperature changes combine to modulate *FT* and flowering time.

P195 - Alone or together? Age or stage? Initiation of the seed maturation program in Arabidopsis thaliana

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The seed is a structure that is resistant to environmental insults, dormant and full of nutrients. All those attributes are the outcome of a developmental program known as "seed maturation". Two decades of research have identified the primary positive regulators of this process, a group of transcription factors known as the *AFL* genes *(ABA-INSENSITIVE3 [ABI3], FUSCA3 [FUS3], LEAFY COLTLEDON1 [LEC1*] and *LEC2*). The downstream effectors that lead to the synthesis of storage products and molecules that protect the seed from desiccation have also been characterized. However, what controls the timing of initiation of maturation is less well understood. Embryos develop synchronously, which means that all seeds in a silique mature at the same time. It is therefore unclear whether each seed decides to start maturation autonomously or is influenced by a silique-wide signal. And it is not known whether the process is triggered after a certain number of days after pollination or when the embryo reaches a certain developmental stage. We address these questions by monitoring the timing of embryo greening and expression of maturation-related genes in siliques segregating mutants that are developmentally slow but morphologically almost normal (*trehalose phosphate synthetase1-1, tilted1-4* and f*usca12*). Preliminary data suggest that maturation is seed-autonomous, and that starts at a particular developmental stage rather than at a specific time after pollination.

P196 - Secretory and Autophagic Pathways Crosstalk in Plants

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ZHANG Li'One of our major research programs has been focused on studying the underlying mechanisms of protein trafficking, organelle biogenesis and function in plants. Toward this goal, we have recently demonstrated that 1) The BAR-domain protein SH3P2 is essential for the formation and dynamics of autophagosome; 2) AP1 mediated membrane protein targeting from trans-Golgi network (TGN) and multivesicular body (MVB) to vacuole; 3) The MON1-CCZ1 complex is essential for vacuole biogenesis and MVB to vacuole trafficking; 4) The FYVE-domain protein FREE1 is essential for the formation of internal vesicles of MVB; and 5) FREE1 interacts with SH3P2 to regulate the autophagic pathway. Current studies address the underlying mechanisms of FREE1 function in regulating organelle biogenesis and turnover using both cellular and genetic approaches including on-going suppressor screening and characterization. An update on our research progress will be presented here. Supported by grants from the Research Grants Council of Hong Kong (GRF, CUHK/CRF/11GC4011-14R, and AoE/M-05/12).

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P197 - Role of DME Family during Seed Development in Arabidopsis

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Arabidopsis DEMETER(DME) is a DNA glycosylase expressed in the central cell of the female gametophyte, activating maternal MEDEA through demethylation process. As the expression of DME is exquisitely controlled temporarily and spatially, the maternal inheritance of a DME null allele results in seed abortion due to deregulation of DNA methylation in the developing seed and loss of gene-imprinting. In contrast to DME, DME family members Repressor of Silencing 1 (ROS1), DME-like 2(DML2) and DME-like 3(DML3) are not expressed in the central cell and do not exhibit mutant phenotypes in reproductive development. To investigate the effect of DME homologues mutation on seeds, we made DME, ROS1, DML2, and DML3 quadruple mutant plants. During the research, we found an alteration of seed abortion rate and phenotype when DME mutation is combined with mutation of other homologs.

P198 (Talk) - Disruption of PME36 activity during seed development alters hormone homeostasis and triggers a shifted compensatory mechanisms in hypocotyl

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between the degree of methylesterification (DM) of pectins and early seedling development, we analyzed the *pme36* knock-out line. As expected, *pme36* displayed a decrease in PME activity during seed maturation and a resulting increase in the DM of pectins in the mature seed. Unexpectedly, 48-hours after germination, PME activity in dark-grown hypocotyl of *pme36* was higher than in the wild-type leading to a decrease in the DM of pectins. These results imply the existence of a transitory mechanism overcompensating, later in *pme36* dark-grown hypocotyl, the absence of PME36 occurring earlier during the seed maturation stage. We found that this shifted compensatory mechanism involved transcriptional regulations, since *PME36* knock-out led to dramatic consequences for the regulation of several *PME* genes at the onset of germination. In addition, looking at auxin and jasmonate contents and assessing the expression of genes involved in the hormone signaling pathways, we found a strong, but transitory, alteration of hormone homeostasis in *pme36*. Altogether these results suggest that seedling development is likely to be controlled by a regulatory network involving crosstalk between PME activity, which is modulated through a compensatory mechanism triggered by variations in DM of pectins, and JA & IAA signaling.

P199 - A high-resolution expression map of the inflorescence stem - insights into the development of a differentiated plant organ

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Even though plant stems contribute fundamentally to the variation in architecture and growth of plant bodies, the molecular regulation of stem development has hardly been explored. An essential piece of knowledge in this context is information about genome-wide profiles of gene expression in individual stem tissue types. Similarly as for high-resolution expression maps of the root, this knowledge would be key for the formulation of global concepts in developmental and physiological terms. However, strong cell walls in mature stems hamper efficient protoplast-based profiling as done previously during similar approaches for shoot and root apical meristems. Therefore, we have initiated a project employing fluorescence-based nucleus sorting in order to get access to tissue-specific mRNA from *Arabidopsis* stems. By using a comprehensive series of tissue-specific promoters driving the expression of a nucleus-targeted fluorescent protein (H4-GFP) and subsequent nucleus sorting, we are now able to determine the transcriptomes of all major stem tissues. As a proof of principle, by taking advantage of the *APL* promoter, we identified the transcriptome of the stem phloem. From 12,000 genes being expressed in the phloem we classified 335 genes as being predominantly active in this tissue. We expect that the analysis of transcriptome remodelling during stem thickening will help deciphering cell fate acquisition and physiological adaptations during postembryonic growth processes in plants.

P200 - Function of α-xylosidase in xyloglucan metabolism, cell wall extensibility and seed germination

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We have identified a high temperature resistant germination mutant, *trg1*, as a loss of function mutant of *TRG1/XYL1* gene that encodes A-xylosidase. The seeds of *trg1/xyl1* also showed reduced dormancy and far-red light resistant germination. A-xylosidase has been shown to remove xylose residue from the non-reducing end of xyloglucan oligosaccharides (XGO), which allows further degradation of the XGOs. HPLC and MALDI/TOF MS analyses revealed the over-accumulation of XGO (XXXG) in germinating seeds, developing fruits and elongating flower stem of *trg1/xyl1*. Creep extension analysis indicated that cell wall of *trg1/xyl1* elongating internode had significantly lower elasticity and viscosity values than wild type. We found that the size of xyloglucan reduced greatly in *trg1/xyl1* mutant cell wall by gel permeation chromatography, and the size reduction may be responsible for the enhanced extensibility of the mutant cell wall. It has been shown that elongation of the cells in lower hypocotyl and the transition zone between radicle and hypocotyl is critical for Arabidopsis seed germination. *TRG1/XYL1* promoter driven GUS reporter expression was relatively abundant in radicle and upper hypocotyl, but was only a low level in transition zone and lower hypocotyl. We will discuss the contribution of A-xylosidase to cell elongation and seed germination.

P201 - Diversification of leaf shape via evolution of local growth repressors

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Understanding how form evolves requires identifying the genetic changes underlying morphological variation between species and elucidating how those changes influence morphogenesis. We investigate morphological differences between *Arabidopsis thaliana*, which has simple leaves, and its relative *Cardamine hirsuta*, which has dissected leaves comprising distinct leaflets. Using genetics and interspecific gene transfers we show that leaflet development requires leaf specific REDUCED COMPLEXITY (RCO) homeodomain protein. *RCO* evolved in the Brassicaceae family through gene duplication and was lost in *A. thaliana* genome which retained only its paralog, *LMI1 (LATE MERISTEM IDENTIY1). RCO* does not influence auxin-based patterning. We demonstrate instead, by combining confocal time-lapse imaging with MorphoGraphX software, that RCO shapes *C. hirsuta* leaves by locally repressing growth at the flanks of initialing leaflets. The potential of RCO to repress growth is conserved in *A. thaliana*. Species-specific *RCO* action with respect to its **LMI1** paralogue results from its gene expression, which is restricted to the actively growing leaf base. Thus, evolution of growth repressing factors generated leaf shape diversity by locally modifying growth patterns during leaf organogenesis.

P202 - RNA-seq gene expression analysis of Arabidopsis thaliana apical meristem time series during flower initiation

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Floral transition is an important process in the life cycle of a flowering plant to ensure its reproductive success. Some genes regulating this process are well studied, but the global changes in transcriptome in time when a vegetative meristem turns into an inflorescence are not characterized. Using high-throughput sequencing, we analyzed gene expression during *Arabidopsis thaliana* meristem development from day 7 to 16 after germination in one-day increments. Main flowering regulators have expression dynamics consistent with previous reports: the expression of negative regulator *FLOWERING LOCUS C (FLC)* decreased over the course of the time series while expression of positive regulator *LEAFY (LFY)* increased. We found a time point between 10 and 12 days after germination where *FLC* and L*FY* have parity in expression: first had already decreased but second had not yet increased. This time point is characterized by a peak in the number of differentially expressed genes. Gene Ontology (GO) enrichment analysis of these genes identified an overrepresentation of genes related to the cell cycle. Analysis of expression profiles of specific genes involved in cell cycling suggests that acceleration of rate of the divisions and partial cell cycling synchronization takes place at this point.

P203 - Role of VOZ Genes in Controlling Flowering Time and Jasmonic Acid Signaling in Arabidopsis thaliana

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Plants have evolved several strategies to respond to environmental stimuli that influence important physiological processes including flowering transition. A transcription factor network functional in leaves plays an important role in timing the flowering response in distal meristems. The

VASCULAR PLANT ONE-ZINC-FINGER PROTEIN1 (VOZ1) and VOZ2 are two key vascular transcription factors that promote flowering transition in A. thaliana. As reported earlier, we found that the *voz1voz2* double mutant flowered significantly later than the wild type specifically under long days. Consistent with previous reports, we observed that *CONSTANS* transcript level was not altered in *voz1voz2*, but the expression of *FLOWERING LOCUS T* was downregulated while that of *FLOWERING LOCUS C (FLC)* increased. Surprisingly, the *voz1voz2flc* triple mutant did not rescue the late flowering phenotype of *voz1voz2*. Together, our results suggest that VOZs redundantly promote flowering independent of *FLC*, and possibly via photoperiod pathway. Besides, transcriptome profiling revealed that several jasmonic acid (JA) responsive genes including MYC2 were downregulated in *voz1voz2*. JA-mediated root inhibition was reduced in *voz1voz2* suggesting that JA-signaling was compromised. Recombinant VOZ2 can bind to the *MYC2* promoter region in vitro. Previous studies have shown that *voz1voz2* mutant is susceptible to pathogens. We suggest that this increased susceptibility is due to dampened JA-signalling in *voz1voz2.*

P204 - ASYMMETRIC LEAVES 2-LIKE 9(ASL9), one of the AS2/LOB family gene, affects secondary growth in Arabidopsis thaliana.

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The cambium is a tissue layer of actively dividing cells, located between phloem and xylem. It is known that the cambium is responsible for the secondary growth of roots and stems. Cells in the cambium undergo asymmetric cell divisions to generate daughter cells that differentiate into phloem toward the periphery and xylem toward the center of stems and roots. To find novel genes that regulate the secondary growth, we searched for candidate genes, which are highly and specifically expressed in the cambium in *Arabidopsis thaliana* and *Raphanus sativus*. Among candidates, we found *ASYMMETRIC LEAVES 2-LIKE 9 (ASL9)* in *Arabidopsis thaliana*. Diameters of both stems and roots in transgenic plants overexpressing *ASL9* (*ASL9*-OX) were increased compared to those in wild type plants. Such an increase was further promoted when cytokinin, the phyto-hormone involved in cell division, is treated to *ASL9*-OX plants. These data suggest that *ASL9* positively regulates cell division activities of cambia and affects cytokinin responses.

P205 - Gene Regulatory Networks of the SHORT-ROOT Pathway in the Arabidopsis Shoot

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SHORT-ROOT (SHR) plays key roles in radial patterning, stem cell maintenance, and vascular development in the root. In the leaf, SHR, together with its direct target SCARECROW (SCR), also positively controls proliferative cell divisions. Also, SHR and SCR, along with SCR-LIKE 23 (SCL23), the close homolog of SCR, regulate specification of the leaf bundle sheath (known as equivalent to the endodermis). To further elucidate its regulatory roles in the shoot, we first identified the genes affected by SHR in the shoot using inducible transgenic plants (pSHR::SHR-GR in *shr-2*). Also, we performed comparative transcriptomic analysis between dark-grown Columbia wild-type (Col WT) and *shr-2* hypocotyls. Our genome-wide expression analyses reveal that SHR directly regulates expression of genes involved in cell wall modification. With yeast-two hybrid (Y2H) assays, we also found that SHR interacts with SCL23, whose expression is directly regulated by SHR, similar to the mode of transcriptional control of SCR by SHR. Furthermore, we reveal a novel feedback loop, in which the downstream target SCL23 in turn controls SHR protein movement and SHR mRNA abundance to fine-tune SHR levels for its proper activity/function. Therefore, we propose that SHR as master regulator controls radial patterning and vascular development in the Arabidopsis shoot, similar to its role in the root, and that SHR also directly regulates cell wall modification genes in the Arabidopsis shoot.

P206 - The Arabidopsis leaf developmental transcriptome: Multi-dimensionally coordinated shifts of transcriptional programs along the whole life span of leaf

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Leaves harvest light energy and fix CO2, serving as the only practical source of foods on earth. Leaves, during their lifespan, undergo developmental and physiological shifts, ending with senescence and death. Exploring transcriptional regulatory programs of the entire leaf lifespan could provide a unique opportunity for gaining insights into how processes for biogenesis and degeneration during the lifespan are timely coordinated. Our transcriptome data revealed substantial reprogramming for functional transitions from biogenesis and degeneration during leaf lifespan. Intriguingly, ageassociated biological processes toward senescence and death were actively coordinated by multi-layered regulatory programs including transcription factor-mediated regulation. Differential utilization of regulatory networks involving small ncRNAs and their targets along leaf lifespan and the importance of tRNA-derived small RNAs in leaf lifespan programs were also evident. Our data provide insights into the importance of chloroplast transcriptome as a key constituent in leaf lifespan programs and the coordination between nuclear and chloroplast transcriptomes.

P207 - Structural and functional investigation of Fasciclin-like arabinogalactan-proteins in Arabidopsis thaliana

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Fasciclin-like arabinogalactan-proteins (FLAs) are important plant cell wall glycoproteins proposed to play roles in cell expansion, growth and development [1]. Fasciclin domains are known to be conserved in proteins from a broad range of living organisms playing important biological roles related to adhesion [2]. Their roles and properties are yet to be fully elucidated in plants, and questions remain as to whether they function as wall biosynthesis regulators, architectural components, or both. Recent studies have revealed FLA11 and FLA12 contribute to the strength of *Arabidopsis* stems, findings that have potential applications in the forestry industry [3]. Through mutant studies, we have shown that FLA16 also contributes to stem growth and biomechanical properties in *Arabidopsis.* We are now purifying FLAs in order to characterize their glycans as part of a broader effort to establish the function(s) of these complex glycoproteins.

[1] Seifert et al. (2007) Annu Rev Plant Biol: pp137-161

[2] Johnson et al. (2003) Plant Physiol: pp1191-1925 [3] MacMillan et al. (2010) Plant J: pp689-703

P208 - Xyloglucan metabolism by α-xyloxidase controls Arabidopsis seed dormancy

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Seed dormancy and germination are controlled by the balance between two hormones, abscisic acid (ABA) and gibberellins (GA). ABA induces

and maintains dormancy while GA activates the germination process. After dormancy release, germination results from the protrusion of the radicle through the surrounding layers (endosperm and testa). Cell wall remodelling is essential in the regulation of embryo growth and envelope properties and integrates hormonal and environmental signals to control germination. A screen for *Arabidopsis thaliana* mutants affected in the hormonal control of germination identified a new mutant, *xyl1*, able to germinate on paclobutrazol, an inhibitor of GA biosynthesis. This mutant also exhibited reduced dormancy. The *XYL1* locus encodes an A-xylosidase required for the maturation of xyloglucans in the cell wall, through the trimming of xylose. These hemicelluloses are described as important regulators of cell wall properties (elasticity and rigidity). *xyl1* seed phenotypes were associated with modifications to xyloglucan composition and intracellular distribution in the embryo hypocotyl and endosperm during germination. Moreover, the phenotypes of mutants impaired in other enzymes of xyloglucan metabolism, together with the characterisation of *xyl1* transgenics, provides new insights into the role of tissue-specific xyloglucan modifications in germination processes.

P209 - "Rhizoponics": a novel hydroponic rhizotron for root system analyses on mature Arabidopsis thaliana plants

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Well-developed and functional roots are critical to support plant life and reach high crop yields. Their study however, is hampered by their underground growth and characterizing complex root system architecture (RSA) therefore remains a challenge. In the model plant *Arabidopsis thaliana*, *in vitro* culture remains the easiest and preferred method to study root development, which technically limits the analyses to young seedlings. We present here an innovative design of hydroponic rhizotrons (rhizoponics) adapted to *Arabidopsis thaliana*. The setup allows to simultaneously characterize the RSA and shoot development from seedling to adult stages, i.e. from seed to seed. This system offers the advantages of hydroponics such as control of root environment and easy access to the roots for measurements or sampling. Being completely movable and low cost, it can be used in controlled cabinets. We chose the case of cadmium treatment to illustrate potential applications, from cell to organ levels.

P210 - Regulation of CUP-SHAPED COTYLEDON and MIR164 expression during Arabidopsis thaliana leaf development

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NAM/CUC (NO APICAL MERISTEM/CUP-SHAPED COTYLEDON) genes encode transcription factors of the plant-specific NAC family. *NAM/CUC* genes are required for all types of leaf dissection across Eudicots. In *Arabidopsis thaliana*"s simple leaves, *CUC2* and *CUC3* but not *CUC1* allow formation of serrations along the leaf margin. *CUC2* and *CUC3* are expressed at the leaf margin in the sinuses of developing tooth. During leaf development, *CUC2* is negatively regulated by a microRNA, *miR164*, which expression overlaps with the one of its target. This negative regulation of *CUC2* by *miR164* is required for proper leaf shape acquisition. Using two different approaches, we wish to unravel how the specific *CUC* and *MIR164A* expression pattern are established during leaf development. We adopt a candidate-based approach to study the precise contribution of different known regulators in the establishment of CUC2"s expression pattern. This analysis is based on the morphometric study of mutant phenotypes and the quantification of reporter genes. In an unbiased approach, we look for new regulators of *CUC* and *MIR164* genes through a yeast-one-hybrid screen. The functional analysis of putative regulators is currently in progress.

P211 - Separating light-dependent and plastid-derived regulation of nuclear genes during chloroplast development in the Arabidopsis pap7 mutant

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In photosynthetic eukaryotic cells, the chloroplast is the site of photosynthesis and biosynthesis of many essential metabolites. The chloroplast proteome is composed of nuclear- and chloroplast-encoded components. Their coordinated expression requires a tight regulation that is partly executed by the chloroplast, a process called retrograde control. A well-known example is the repression of the Photosynthetic Associated Nuclear Genes (PHANGS) when plastid differentiation is chemically or genetically blocked. However, PHANGS are also known to be the target for the photoreceptor-mediated control. In order to distinguish between these two types of regulation we used the *pap7* mutant of *Arabidopsis*, that is depleted in one of the plastid RNA polymerase-associated proteins. This mutant is highly affected in plastid transcription and chloroplast biogenesis, displaying an albino phenotype that is seedling-lethal. However, young *pap7* homozygous mutant plantlets can still be grown from an heterozygous progeny and exhibit a relative normal photo-morphogenesis. Using Affymetrix microarrays, by comparison of the transcriptome data from plants grown in different dark-light conditions, we have 1) determined the extent of the PHANGS regulation, 2) identified distinct nuclear gene groups that are specific for either photo-morphogenesis and/or chloroplast biogenesis, and 3) investigated the cross-talk between chloroplasts and mitochondria.

P212 - A Genome-Wide Association mapping approach to the discovery of novel organ size regulators in Arabidopsis

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The discovery of genes responsible for the control of final organ size in plants is of key scientific and socio-economic interest. Genome Wide Association mapping (GWAS) is a powerful approach that enable high-resolution detection of these important loci. Utilizing SNP data for more than 200 sequenced Swedish Arabidopsis accessions, and focusing on petal length as a proxy for organ size, GWAS was performed. This analysis revealed a very promising locus on chromosome 4. Closer assessment of this locus revealed six genes in strong linkage disequilibrium with the associating SNPs. Analysis of gene expression by qRT-PCR revealed that differential expression of one of these genes explains >75% of the variation in petal length seen across a subset of Arabidopsis accessions. This gene encodes a transmembrane domaincontaining protein of unknown function, and work is currently underway to further understand the potential role of this gene in organ size in Arabidopsis. Accessions showing differential expression of this gene also showed significant variation in yield traits such as silique length. It is possible therefore that the role of this transmembrane protein extends beyond petal growth and that it may be a key contributor to plant yield.

P213 - Dual Fatty Acid Elongase Complex Interactions In Arabidopsis

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Very long chain fatty acids (VLCFAs) are required for the synthesis of triacylglycerols, waxes, phospholipids and sphingolipids. Fatty acyl chain length is essential for plant development in particular for membrane trafficking during cell division and cell differentiation (1-3). VLCFAs are elongated by the sequential addition of two carbons through four successive enzymatic reactions gathered in the endoplasmic reticulum within a protein complex named the elongase. The acyl-CoA dehydratase PASTICCINO2 (PAS2) is involved in the third step of elongation[3] . The *pas2* mutants show strong defects such as lost of cellular adherence, defects in division plate formation and vesicular dynamic (3-4). In order to identify new factors associated with the biosynthesis of VLCFAs, a yeast multicopy suppressor screen with an A. thaliana cDNA library was carried out in *phs1* strain. Loss of function of *PHS1*, the yeast *PAS2* ortholog, prevents growth and induces cytokinesis defects. The screen identified the PTPLA as a dehydratase-like gene involved in VLCFA elongation in both yeast and plant. Functional analysis of PTPLA in Arabidopsis demonstrated the existence of a new fatty acid elongase complex activity independent of PAS2-based complex but also uncovered unsuspected regulatory interactions between the two complexes.

[1] Roudier *et al.* Plant Cell. (2010)

[2] Markham *et al.* Plant Cell. (2011)

[3] Bach *et al.* Proc. Natl. Acad. Sci. U. S. A. (2008)

[4] Bach *et al.* J. Cell Sci. (2011)

P214 - Genetic and chemical genomic dissection of the cell adhesion mechanisms in plants

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Cell to cell adhesion in plants is mediated by the cell wal. The various components of this matrix are organized in order to create a continuum linking the cells together. However the cell wall is a dynamic compartment that participates in growth and development through its constant loosening and remodeling and it is not clear how cell adhesion is actually maintained during these events. In order to get a better understanding of the mechanisms that control cell adhesion in plants we used a combination of a forward genetic suppressor screen and a chemical genomic suppressor screen on cell adhesion deficient mutants. We have isolated a number of suppressor mutants and molecules implicated in cell adhesion. Our genetic screen led to the identification of suppressors mutated in various genes appearing as major players in the control of cell adhesion. Furthermore, our chemical genomic screen has revealed the implication of auxin transport and cell wall located enzyme in the process of cell adhesion. Based on these new elements we have established a model explaining the loss of cell adhesion in our mutants, and from this model we have inferred the existence of mechanisms that dynamically allow the maintenance of cell adhesion in plants during growth and development.

P215 - The ANGULATA7 essential gene is required for leaf development

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In a large-scale screen for EMS-induced mutants with abnormal leaf shape, we isolated the *angulata7-1 (anu7-1)* recessive mutant, which exhibits pale-green leaves with prominent marginal teeth. *anu7-1* vegetative leaves are small and exhibit reduced chlorophyll and carotenoid levels compared to wild type. Map-based cloning allowed us to identify the causal mutation of the phenotype of *anu7-1*: a G-to-A transition that is predicted to cause a G-to-E amino acid substitution in ANU7, a chloroplast-localized protein of unknown function. A 35_{pro}*:ANU7* transgene fully complemented the mutant phenotype of *anu7-1*. We also identified two insertional, embryo-lethal alleles: *anu7-2* and *anu7-3*. A number of nuclear genes encoding transcription factors were found deregulated in a microarray analysis of *anu7-1* RNA, suggesting a role for ANU7 in retrograde signalling.

P216 - Arabidopsis FHA2 plays a role in plant fertility by regulating stamen development

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The forkhead-associated (FHA) domain is involved in protein-protein interaction by recognizing a phosphothreonine epitope on target proteins. In this study, we investigated *in planta* functions of the *Arabidopsis* FHA domain 2. AtFHA2 was mainly localized in the nucleus. *Arabidopsis fha2* null mutants grew normally during the vegetative stage, but had severely reduced fertility during reproductive stage. The reduced fertility was mainly caused by defective stamen filament elongation, while female flower parts of the mutants were fertile. Additionally, the mutants had fewer stamens than the wild type and the vegetative organs of the mutants, such as cotyledons and leaves, had increased ploidy. These results suggest that AtFHA2 may play a role in a signaling pathway for the control of plant organ development.

P217 - Octopus Negatively Regulates BIN2 To Control Phloem Differentiation In Arabidopsis Thaliana

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The phloem is a vascular strand that conducts photo-assimilates and systemic signals throughout the plant to coordinate growth. To date, few molecular genetic determinants have been identified that control both specification and differentiation of this tissue. Among them, the OCTOPUS (OPS) protein was previously identified as a polarly membrane-associated protein of unknown biochemical function, whose broad provascular expression becomes restricted to the phloem upon differentiation. OPS loss of function mutants showed an intermittent phloem differentiation in the root. Here, we demonstrate a role for OPS as a positive regulator of the brassinosteroid (BR) signaling pathway. Indeed, transgenic lines overexpressing OPS (OPS-OE) display the hallmarks of constitutively overactivated brassinosteroid (BR) mutants. Physiological and genetic analyses place OPS as a positive regulator of the BR signaling pathways upstream of the key transcription factors BES1 and BZR1. Directed protein interactions with known BR signaling proteins identified the Glycogen Synthase Kinase 3 BIN2, as a potential partner of OPS. This interaction recruits BIN2 to the plasma membrane thus preventing its inhibitory activity in the nucleus. Finally, both bikinin (a potent inhibitor of GSK3) treatment and downstream dominant mutants *bes1-D* and *bzr1-D* can rescue phloem defects of ops in the root. Together our data show that OPS antagonizes BIN2 to promote phloem differentiation.

P218 - Characterization of VIGS phenotypes of Nicotiana benthamiana RabE1

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We characterized the gene expression, subcellular localization, and *in vivo* functions of a *Nicotiana benthamiana* small GTPase belonging to the RabE family, designated NbRabE1. The *NbRabE1* promoter drove strong Y-glucuronidase (GUS) reporter expression in young tissues containing actively dividing cells and in stomata guard cells. GFP fusion proteins of NbRabE1 and its dominant-negative and constitutively active mutants were all localized to the Golgi apparatus and the plasma membrane but showed different affinities for membrane attachment. Virus-induced gene silencing (VIGS) of *NbRabE1* resulted in pleiotropic phenotypes, including growth arrest, premature senescence, and abnormal leaf development.

At the cellular level, the leaves in which *NbRabE1* was silenced contained abnormal stomata that lacked pores or contained incomplete ventral walls, suggesting that NbRabE1 deficiency leads to defective guard cell cytokinesis. Ectopic expression of the dominant-negative mutant of NbRabE1 in *Arabidopsis thaliana* resulted in retardation of shoot and root growth accompanied by defective root hair formation. These developmental defects are discussed in conjunction with proposed functions of RabE GTPases in polarized secretory vesicle trafficking. We characterized the gene expression, subcellular localization, and *in vivo* functions of a N*icotiana benthamiana* small GTPase belonging to the RabE family, designated NbRabE1. The *NbRabE1* promoter drove strong Y-glucuronidase (GUS) reporter expression in young tissues containing actively dividing cells and in stomata guard cells. GFP fusion proteins of NbRabE1 and its dominant-negative and constitutively active mutants were all localized to the Golgi apparatus and the plasma membrane but showed different affinities for membrane attachment. Virus-induced gene silencing (VIGS) of *NbRabE1* resulted in pleiotropic phenotypes, including growth arrest, premature senescence, and abnormal leaf development. At the cellular level, the leaves in which *NbRabE1* was silenced contained abnormal stomata that lacked pores or contained incomplete ventral walls, suggesting that NbRabE1 deficiency leads to defective guard cell cytokinesis. Ectopic expression of the dominant-negative mutant of NbRabE1 in *Arabidopsis thaliana* resulted in retardation of shoot and root growth accompanied by defective root hair formation. These developmental defects are discussed in conjunction with proposed functions of RabE GTPases in polarized secretory vesicle trafficking.

P219 - Natural Variation in Sensitivity to a Loss of Chloroplast Translation in Arabidopsis

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We evaluate here the underlying causes of hypersensitivity to a loss of chloroplast translation in natural accessions of Arabidopsis. The response of embryos and seedlings to a loss of chloroplast translation is mediated by *ACC2,* a duplicated nuclear gene that targets homomeric acetyl-CoA carboxylase (ACCase) to plastids [*Parker et al. (2014) Plant Physiol 166: 2013-27*]. Functional *ACC2* is not required in natural environments, where heteromeric ACCase encoded in part by the plastid genome can function instead to support fatty acid biosynthesis. Accessions that are hypersensitive to a loss of chloroplast translation contain *ACC2* nonsense mutations, splicing defects, or gene rearrangements, or disruptions in another locus that remains to be identified. *ACC2* missense mutations do not explain most known cases of hypersensitivity. In the presence of functional *ACC2*, tolerance is increased by a semidominant enhancer located on chromosome 5 and by additional genetic modifiers. Several models for enhancer and modifier function are currently being evaluated. This work highlights an interesting example of a tandem gene duplication, helps to explain the range of embryo phenotypes found in mutants disrupted in essential chloroplast functions, addresses the nature of essential proteins encoded by the chloroplast genome, and underscores the value of using natural variation to study the relationship between chloroplast translation, metabolism, protein import, and plant development.

220 - POL2A and DPB2 sub-units of Arabidopsis thaliana polymerase epsilon: different roles in DNA repair and cell cycle regulation

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The mechanisms involved in the maintenance of genome integrity, notably during the S-phase of the cell cycle, are of utmost importance in plants for the faithful transmission of the genetic information. DNA Polymerase Ɛ (pol Ɛ) is a replicative polymerase responsible for the synthesis of the leading strand during S-phase which consists of one catalytic sub-unit (Pol2A) and three accessory sub-units (DPB 2, 3 and 4). In addition in yeast, Pol Ɛ is also required for the activation of the S-phase checkpoint upon replication defects such as replication fork stalling, collapse or DNA damage. In *A. thaliana* the roles of Pol2A and DPB2 have been partially characterized during development, but both mutations are lethal. So far we have shown that DPB2 over-expression (DPB2oe) severely affects plant development and cell cycle regulation: these lines display severe dwarfism and slower cell cycle progression, together with constitutive activation of DNA repair genes. All these features are shared with a partial loss of function alleles of Pol2A (*abo4*), suggesting that both lines display endogenous DNA damage. These results point to the importance of the stoichiometric accumulation of Pol e sub-units. However, DPB2oe display tolerance to all genotoxic-agents tested whereas *abo4* is tolerant to hydroxy-urea, but hypersensitive to various DNA damaging agents. Together, our results suggest that plant Pol2A could participate in the activation of an ATR-dependent S-phase checkpoint.

P221 - potent regulates postembryonic organogenesis in Arabidopsis thaliana

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Plants grow continuously and have high regenerative capacities. Both developmental mechanisms require the formation of new organs and tissues from organ founder cells (FC). Specification of FC is, therefore, crucial for plant postembryonic growth and survival. To investigate organogenesis and specification of FC we used as model system the formation of lateral roots (LR) in *Arabidopsis thaliana*. Out of an ethyl methanesulfonate screen, we identified a heritable mutation with altered postembryonic organogenesis, which we named *potent*. Although, stem cell and tissue specification is not affected in the main root of *potent*. New stem cells are not specified during LR formation, as indicated by lineage analyses and stem cell specification markers. In *potent*, many pericycle cells change their identity becoming FCs. These FCs, although are normally arrested in subsequent development, can be stimulated to undergo organogenesis by auxin treatment that results in overproduction of LRs. Furthermore, we analyzed the effect of this mutation during regeneration of roots from leaves, and also found abnormal over-reprograming of cells which were, in turn, arrested in subsequent development. This indicates a conserved functionality of *potent* in postembryonic development. Finally, we mapped the mutation by next generation sequencing and identify the affected gene. Analysis of interactors of *potent* putatively relates its function with auxin signaling.

222 - Identification of new stem cell regulators via RBR-network inference

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In plants, where cells do not migrate, the position of asymmetric cell divisions is crucial for proper tissue specification. Over the last decade, the *A.thaliana* RETINOBLASTOMA-RELATED(RBR) protein has emerged as a key regulator of state transitions in different stem cell systems. In the root stem cell niche, a well-studied example is the RBR-mediated control of asymmetric stem cell division for ground tissue formation. This process depends on an intricate regulatory network where RBR binds to, and modulates the activity of, a SHORTROOT/SCARECROW(SHR/SCR) complex, that controls transcription of a specific D-type cyclin,CycD6;1. *CycD6;1* in turn controls RBR activity via phosphorylation, thus activating SHR/SCR, and this modulation is re-set by mitosis-coupled proteolysis. Similar modules, involving RBR-mediated regulation of lineage-specific transcription factors in conjunction with D-type cyclins, have been shown to regulate also columella stem cell replenishment and stomatal lineage differentiation. Tissue-specific complementation experiments suggest that a different RBR-dependent network governs differentiation of the lateral root cap(LRC). We identified a set of candidate transcription factors and a different D-type cyclin that might be part of a LRC-specific, RBR-dependent module responsible for the control of LRC stem cell division. A deeper investigation of this module allows us to dissect roles of RBR in maintenance and asymmetric cell division of different stem cells.

P223 - Functional and biochemical characterization of Arabidopsis thaliana pectin acetylesterase during plant growth development

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In Arabidopsis, the primary cell wall is important for plant development. It composed of cellulose, hemicelluloses and pectic polysaccharides. Pectins are complex polysaccharides, rich in galacturonic acid residue (GalA) which consist of four domains: Homogalacturonan (HG), Rhamnogalacturonan-I (RG-I), Xylogalacturonan (XGA) and minor amounts of Rhamnogalacturonan-II (RG-II). The D-galacturonic acid units (GalA), can be methylesterified at the C-6 carboxyl group and/or acetylated at the O-2 and/or O-3 in both HG and RGI. HG is synthesized from nucleotide sugars and then secreted as methylesterified and acetylesterified form into the cell wall where its structure can be de-esterified by cell wall enzymes, pectin methylesterases (PMEs) and pectin acetylesterases (PAEs). However, the biological functions of pectin acetylation and PAE have not been elucidated yet. Here we report preliminary results obtained by qRT-PCR on 12 putative *Arabidopsis thaliana PAE* genes expression during plant growth. Some PAE genes were selected according to the transcriptional analysis and were characterized using a multidisciplinary approach, including *in silico* analysis, promoter activity assay and protein subcellular localization. To assess *PAE* activity, the candidate genes were further expressed in an heterologous system and the quantification of enzyme activity on different substrates have been carried out. The data will provide new insights in the role of PAE in the acetylation state of pectin.

P224 - Looking for new genes involved in root growth and development

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Roots are essential for water and nutrients acquisition in plants. Root architecture can be modulated by endogenous and environmental factors. In plants, microRNAs (miRNAs) play various developmental and physiological roles. These small RNAs regulate their targets by transcript cleavage and/or inhibition of protein translation and are known as major post-transcriptional regulators of various developmental pathways and stress responses. GWAS was performed in Arabidopsis to identify candidate genes involved in root growth and development (Meijon M et al 2013; Slovak et al 2014). Among them SNPs involving miR170 were selected as potentially modulating gravitropic responses in specific growth conditions. To analyse the role of this miRNA in such responses characterization of miR170 overexpressing lines and lines with decreased miR170 has been undertaken. Preliminary results on the impact of miR170 in plant development will be presented.

P225 - Really going for growth: control of organ size in Arabidopsis by a novel ubiquitin-mediated mechanim

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The control of organ and seed size in plants is starting to be understood at the cellular level, with current models of organ size control involving an initial period of cell proliferation followed by cell expansion and differentiation. The duration of these phases determines the final number and sizes of cells in the organ. We are studying a set of genes that contribute to determining the duration of the period of cell proliferation during organ formation in Arabidopsis, *DA1, EOD1* and *DA2*. We tracked the width and area of leaves one and three in *da1-1* and *da1 -1eod1-2* plants compared to wild type throughout growth and found that there is a prolonged period of growth in the mutants, as well as an increased rate of growth in the initial stages in the double mutant *da1-1eod1-2*. This is contrary to the previous hypothesis that growth rate was constant between the wild type and the mutants, with mutants only having a longer duration of cell proliferation. To measure the role of these mutations at a cellular level, we have generated lines with plasma membrane fluorescence to monitor cell size and rate of division in staging experiments and live-cell imaging. We have shown that EOD1 and DA2 encode RING E3 ligases that specifically ubiquitinate DA1, activating DA1 latent peptidase activity, which then cleaves EOD1 and DA2. We are currently assessing other putative targets of DA1 peptidase activity, for example TMK1 and TMK4, putative receptor proteins that promote cell proliferation.

P226 - Small but thick: the Arabidopsis hypocotyl as a model to study periderm establishment.

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The periderm is the protective tissue that during secondary replaces the epidermis once the latter cannot accommodate anymore radial growth. It acts as a physical barrier to protect the plant from loss water, gases and solutes and difficult environmental conditions (i.e. pathogens, flooding). These barriers properties are conferred by the accumulation of suberin in the cork. The periderm as the vasculature cambium is a three layers system: the phellogen or cork cambium produces inward the phelloderm and outward the cork/phellem. The periderm is a great system to study how a meristem is established and how cell differentiation occurs. The herbaceous Arabidopsis plant has been recently shown to be a valid genetic model to study secondary development. In particular working with the hypocotyl, offer several advantages 1) radial growth is not masked by ongoing elongation 2) the structural arrangement of secondary tissue is similar to trees 3) thanks to the "quantitative histology approach" is possible to follow morpho-dynamics at cellular level 4) a periderm is formed (unlike in stem). In Arabidopsis root and hypocotyl the periderm arises from the pericycle and its development is tightly connected to the loosening of the outside tissues (Endodermis, Cortex and Epidermis), which follows a predetermined pattern. However the regulatory network underlying periderm establishment and maintenance is still largely unknown. Here we present our recent data on periderm development using the Arabidopsis hypocotyl as a model.

P227 - How to convert a lateral root primordium into a shoot meristem

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Differentiated plant cells can be triggered to form an organ or an embryo, either in nature or in vitro. Our work focuses on the conversion of lateral root primordia (LRM) into shoot meristems (SM). In the system we developed, this conversion relies on the in vitro culture of primary Arabidopsis root segments bearing lateral roots switched from auxin- to cytokinin-enriched media. With 3D confocal microscopy, we characterized the histological features defining the developmental window compatible with the LRM-to-SM conversion. The competent LRM stages have initiated the establishment of the root meristem stem cell niche, but earlier or later stages do not convert into shoots. This observation suggests that the stem cell niche has an undetermined ground state whose fate can be channeled towards root development by exogenous application of auxin and towards shoot by cytokinin. The switch in organogenesis is correlated with controlled by changes in key identity genes, root and shoot meristem genes being respectively repressed and induced during the conversion. Transcript level analysis revealed a central role for the CUC2 gene in the process.

P228 - FDP transcription factor contributes to the integration of photoperiodic and endogenous flowering signals at the shoot meristem of Arabidopsis

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In Arabidopsis, the transition from vegetative to reproductive growth is controlled by several genetic pathways. The photoperiod pathway links flowering with day length. In long days, *FLOWERING LOCUS T (FT)* is activated in the leaves and its protein moves from the leaves to the shoot apical meristem (SAM) to initiate flowering. FT interacts with two closely related bZIP transcription factors (TF), FD and FD PARALOGUE (FDP), which are expressed in the SAM leading to the activation of downstream genes and to flower differentiation. In this work, we study FDP using different approaches that combine Affymetric arrays, ChIP and reverse genetic studies, including null alleles derived with CRISPR technology. Our results demonstrate that FDP promotes flowering by directly regulating *GA2ox7*, a catabolic enzyme of GA biosynthesis both in LDs and in SDs. By contrast, FD does not regulate *GA2ox7* and is expressed in different meristematic regions. We propose that these *bZIP TFs have separable functions* and that their roles are not restricted to the FT signaling pathway.

P229 - Specification and Differentiation of Abscission Zone Layers

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Abscission is the developmentally controlled shedding of plant parts, such as leaves and floral organs. Despite abscission being a long-studied subject, the question of why abscission takes place exclusively at precise sites is still open. Abscission requires the formation of specialized layers of cells that remain quiescent during organogenesis, and eventually differentiate into distinct cell-types. The molecular mechanisms behind formation of abscission layers are largely unknown. To investigate abscission zone cell specification and differentiation, we are using the model plant *A. thaliana*, where floral organs are shed during fruit maturation. We are taking both forward and reverse genetics approaches to identify key players in abscission zone development. Our initial effort has allowed the identification of genes expressed at different stages of floral development, and the visualization of distinct populations of cells in abscission layers. In particular, we have identified genes exclusively expressed in quiescent abscission zones and on the distal side of the abscission fracture. These genes are being used as markers for the characterization of sub-populations of cells in abscission zones. With this information in hand, we can now analyze abscission zone cells with cellular resolution, acquire knowledge on the developmental processes that lead to the formation of abscission zones, and device strategies for controlling abscission in economically relevant crops.

P230 - Transcriptional control of root hair growth under adverse conditions

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Plants are able to adjust their cellular growth to tune morphogenesis with environmental demands. Insights into the underlying molecular mechanism are so far restricted to the description of vast transcriptional changes correlated with modified growth. Here, we describe the identification of a GROWTH REGULATORY TRANSCRIPTION FACTOR 7 (GRTF7) during a screen for novel growth regulatory transcriptional regulators. GRTF7 is a DOF-type transcriptional regulator and its expression gradually increases with the onset of cell expansion and differentiation in the roots. Moreover, application of abscisic acid (ABA) enhances *GRTF7* expression and broadens its expression profile to meristematic cells. Ectopic expression of *GRTF7* causes growth retardation, associated with defective cell expansion. Transcript profiling after *GRTF7* induction in the root identified a significant enrichment in genes related with transport, root hair formation and stress responses being repressed by *GRTF7*. Based on these potential targets, we show that *GRTF7* directly represses ROOT HAIR DEFECTIVE-LIKE 2 (RSL2), a transcription factor essential for proper root hair expansion. Furthermore, we show that a reduction in the expression of *RSL2* is essential for the root hair growth response to ABA. Based on our results we discuss the existence of multiple parallel pathways to regulate the plasticity of root hair growth under environmental constraints.

P231 - Regulation of Early Seedling Development in Arabidopsis by Glucan Synthase-like8

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Callose, a linear Y-1,3-glucan polymer, is accumulated at the cell plate during cytokinesis, in plasmodesmata (PD), where it regulates cell-to-cell communication, in dormant phloem, where it seals sieve plates after mechanical injury and pathogen attack, and in male and female gamehtophytes. Glucan synthase-like (*GSL*) genes in Arabidopsis comprise a family of 12 members. A new allele of *GSL8, essp8*, was identified as having seedling-lethal phenotype through forward genetic screening of an EMS mutant population of Arabidopsis showing ectopic expression of seed storage proteins (*essp*). The gene responsible for the observed mutant phenotype was detected using a combination of bulked-segregant analysis, rough-mapping, and next-generation mapping (NGM). An EMS-induced point mutation was identified on an intron splicing site of *GSL8* predicted to introduce a premature STOP-codon. *essp8* seedlings exhibit several growth defects, including disruptions of root tissue patterning and morphogenesis, somatic embryo formation, incomplete cytokinesis, and increased size exclusion limit of PD. Histochemical detection of callose and cell-to-cell diffusion assays showed lack of callose deposition at the cell plate and PD, ectopic polyploidization, and significantly increased symplastic macromolecular trafficking between root cells in *essp8* seedlings. Our findings suggest that GSL8 is required for cell wall integrity, maintaining the basic ploidy level, and restriction of symplastic movement.

P232 - The DUF642 At2g41800 (TEEBE) protein is located in the cell wall at the end of mitosis and acts as a negative regulator of hypocotyl growth

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In plants, the cell wall is a complex and dynamic structure that is built by high molecular weight carbohydrates and proteins. The cell wall plays an important role during several stages of the plant life cycle, including cell division, elongation and differentiation. The DUF642 family of cell wall proteins is highly conserved in spermatophytes and may be involved in pectin structural modifications. In particular, *At2g41800* is one of the most highly induced genes during the M/G1 phases of the cell cycle, and the protein it encodes has been detected only in cell wall proteomes of cell suspension cultures. In this study, the subcellular localization of At2g41800 (TEEBE, TEB) was investigated. Callus induction and auxin treatment of *Arabidopsis thaliana* roots from *pTEB::TEB-GFP* transgenic plants were used to visualize TEB protein localization in the cell wall. Furthermore, root meristem cells were synchronized with hydroxyurea

treatments. The cell wall localization of the TEB protein in root meristem cells was confirmed by electronic microscopy. Although no alteration in root growth was observed in *teb* null mutants, hypocotyls were longer in mutant seedlings grown in continuous light. Furthermore, overexpression of TEB inhibits hypocotyl growth and induces resistance to cobtorin treatment.

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P233 - Transcriptional control of cell state transitions by PLETHORA gradient

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During their life cycle, plants continuously grow from specialized regions of undifferentiated cells, termed meristems. In the root meristem of the model plant *Arabidopsis thaliana*, a positive feedback loop between the plant hormone auxin and the PLETHORA (PLT) transcription factors creates a narrow PLT expression domain in the stem cell region. This domain is extended shootwards in the form of a protein gradient via a mechanism that involves mitotic segregation and cell-to-cell movement. The PLT gradient guides the developmental progression from stem cells to differentiating cells in a dose-dependent manner: high PLT levels specify and maintain stem cell identity, intermediate levels promote cell proliferation and low levels permit differentiation. How can a protein gradient instruct all the necessary cell state transitions to enable root growth? Here, we investigate the mechanistic basis of transcriptional control of root meristem growth by PLT gradient, using a combination of high-throughput experimental and computational approaches. We found that PLT regulated targets provide spatial context for differentiation. PLTs activate cell proliferation genes in the meristematic region and repress genes that are expressed in the elongation and differentiation zone of the root. Our analysis of the mechanisms of PLT gradient regulation will help elucidate how transcription factor gradients function to instruct cell differentiation programs.

P234 - Towards the understanding of shoot apical meristem organogenesis: isotropic growth as a major regulator of organ formation

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The complexity of aerial plant shapes largely results from the activity of the shoot apical meristem (SAM), which gives rise to all the aboveground organs of the plant. At the cellular level, the formation of a new organ involves the differential growth of the cells within the SAM tissue to promote organ emergence and to generate the final organ shape. The generation of new organs strictly depends on auxin action which, through an integration of active transport, signaling and transcriptional regulation, determines the spatio-temporal information that drives organ emergence at the SAM periphery. We previously showed that auxin promotes organ initiation by disrupting the organization of highly anisotropic cortical microtubule (CMT) arrays at the periphery of the meristem. The auxin-induced isotropy switch acts synergistically with minor reductions in cell wall rigidity occurring at the periphery of the SAM, to amplify growth rates of the bulging primordium. Here we show that the disorganization of CMT plays additional role in regulating organ initiation. We provide evidence that chemical or genetic disruption of CMT at the SAM results in a localized induction of cell wall remodeling genes that accompanies organ outgrowth. In addition, we show that cortical microtubule disorganization also affects the expression of transcriptional regulators involved in SAM developmental patterning, suggesting that isotropy might act as a major regulator of SAM morphogenesis.

P235 - The Sec14-Nodulin AtSFH1 patterns phosphoinositide distribution to control polarized membrane growth

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Root hairs provide a remarkable plant-soil interface that favors water and nutrient absorption, as well as plant-microbe interactions. Their development relies on fine-tuned molecular events that culminate in the polarized expansion of distinct root epidermal cells (i.e. trichoblasts). Phosphoinositides play a key role in root hair development by establishing signaling foci that localize cellular events, such as cytoskeleton organization and vesicle trafficking, to specific membrane sites. Our previous work in yeast demonstrates that SEC14 proteins act at the interface between phosphoinositide homeostasis and membrane trafficking by assisting phosphoinositide kinases to overcome their intrinsic inefficiency to recognize phospholipid substrates in the context of the membrane bilayer. In particular, yeast SEC14 renders phosphatidylinositol (PtdIns) vulnerable to PtdIns 4-OH kinase attack during phosphatidylcholine (PtdCho)-dependent heterotypic phospholipid exchange, generating a PtdIns(4)P pool at *trans*-Golgi membranes that is crucial for proper vesicle biogenesis. Notably, root hair development relies on AtSFH1, a multidomain protein harboring an N-terminal SEC14 domain and a C-terminal Nlj16-like nodulin domain. We will present evidence that the Nlj16 module exhibits high PtdIns(4,5)P2 binding specificity *in vivo* and will provide a mechanistic insight into how AtSFH1 couples phosphoinositide synthesis with lateral organization of PtdIns(4,5)P2 showcasing a novel mechanism of lipid microdomain formation.

P236 - Down-regulation of PECTIN METHYLESTERASE 32 expression triggers compensatory mechanisms in Arabidopsis thaliana

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In *Arabidopsis thaliana*, dark-grown hypocotyl is characterized by two distinct growth phases: a slow phase followed by a rapid acceleration which is related to changes in the expression of genes encoding enzymes involved in homogalacturonan

(HG) modifications, among which pectin methylesterases (PMEs, 66 members in Arabidospis), polygalacturonases and pectate lyases-like. Overexpression of genes encoding PME inhibitors (PMEIs), such as *PMEI4*, which is differentially, regulated during etiolated hypocotyl growth, affect pectin properties leading to delayed growth acceleration. This suggests that PME activity, which affects the degree of methylesterification of HG backbone, play a key role in the pectin dynamic and cell wall plasticity, thus controlling elongation of dark-grown hypocotyl. We characterized mutants for *PME32*, which is expressed during etiolated hypocotyl growth. Using functional genomic, biochemical, transcriptomic and proteomic approaches, we showed that the deregulation of *PME32* expression leads to surprising increased PME activity, likely to be related to compensatory mechanisms among the PMEs expressed in dark-grown hypocotyl. Moreover this compensatory mechanism could be controlled by PMEIs, affecting finally growth rate of dark-grown hypocotyl. Our study shed new light on the identification of compensatory isoforms within a large multigenic family, and the likely contribution of cell signaling components.

P237 - Finding DEMETER suppressor gene through EMS mutagenesis in Arabidopsis.

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The DEMETER (DME) gene encodes a dual-function glycosylase that functions in DNA demethylation through base excision repair pathway at all three cytosine contexts. DME expression is exquisitely controlled during female and male gametogenesis and reinforces transposon methylation in gametes. DME activates MEDEA (MEA) gene transcription, a PRC2 component that is imprinted in the endosperm and is required for seed viability. Loss-of-function dme mutant allele resulted in hypermethylation at its target loci and transposons and displayed endosperm over-proliferation causing seed abortion. Seed abortion dme phenotype is rescued by met1 methyltransferase mutant. Thus, DME and MET1 function antagonistically through DNA methylation. However, MET1 is the only DME suppressor known until today. To identify more DME suppressors, thereby, to broaden our knowledge for DME function and mechanism, we mutagenized dme-1 and dme-2 T-DNA insertional homozygous mutants with ethyl methane sulfonate (EMS). Both alleles show severe seed abortion phenotype resulting in sparse viable seeds. We counted seed abortion raio in EMS-treated dme mutant plants and candidate lines showing improved seed survival ratio were selected. MET1 region of these candidate lines was sequenced to rule out met1 mutants. Out of 17 candidate lines, 13 lines did not show any SNPs at MET1 locus. They show a variable seed abortion ratio, indicating that many different suppressors might be obtained. We will map the mutation sites and will investigate their functions. We will also investigate genetic and biochemical interaction between DME and the new suppressors. This study will shed light on the mechanism of DME demethylase during reproduction.

P238 - Phloem sieve element regulates periclinal cell divisions in Arabidopsis root vasculature via mobile transcription factors

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Plant growth and development are crucially dependent on positional information from neighboring cells, mediated by mobile regulatory molecules. In Arabidopsis, the interaction of hormones auxin and cytokinin is essential for the radial organization of the root vasculature consisting of central xylem axis, two phloem poles, and intervening procambium. It has been proposed that xylem controls non-cell-autonomously periclinal divisions occurring in the phloem/procambium by activating cytokinin production. However, whether phloem has a regulatory role as well has remained unclear. Plasmodesmata (PD) are plant specific nanochannels that enable the movement of molecules directly from cell to cell. In Arabidopsis, PD apertures can be regulated in a spatially and temporally specific manner with a mutated CALLOSE SYNTHASE 3 (cals3m) that enhances callose deposition at PD resulting in inhibition of symplastic transport. To investigate the requirement of symplastic connection during vascular development, we inhibited trafficking by icals3m, and observed a reduction of cell files in phloem/procambium after expressing icals3m in the early protophloem sieve element (SE). Subsequently, we identified a family of transcription factors that move from the SE to the surrounding cells, and which promote periclinal cell divisions in phloem/procambium. Thus, our results indicate that not only xylem but also the SE has an essential role as an organizing center during vascular development.

P239 - A Myb-related transcription factor plays an important role for the FT transcription.

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In *Arabidopsis thaliana*, FLOWERING LOCUS T (FT) protein represents a major component of florigen. Molecular mechanism of the transcriptional activation of floral-meristem identity genes via FT at the shoot apical meristem has been well studied. However, it remains largely unknown how *FT* transcription is up-regulated in leaves by the crucial flowering promoter, CONSTANS (CO), in a day-length dependent manner. Here, we aimed to identify other transcriptional activators of *FT* other than CO, in the photoperiod-dependent pathway. One of the interesting candidates is a novel flowering regulator, FE, encoding a Myb-related transcription factor. The *fe-1* mutant, that harbors a single amino acid substitution, exhibited late-flowering phenotypes under the long-day (LD) conditions. This LD-specific flowering phenotype was caused by the down-regulation of *FT* expression in addition to the impairment of FT protein transport from leaves to the shoot apex. We constructed a heat-inducible system of FE and performed detailed expression analysis of *FT* by qRT-PCR and *GUS*-reporter assays. Our experiments revealed that a significant *FT* up-regulation after heat-treatment is observed and this up-regulation depends on CO protein activity. These results implied that FE and CO has some relations in the activation of *FT* transcription.

P240 - IPX (IRREGULAR PHYLLOTAXIS), Encoding a Novel Protein Containing SMC Domain, Regulates Phyllotaxis and Embryo Development with RBR1

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Phyllotaxis, the arrangement of leaves on a plant stem, mainly affects the architecture of plants. Phyllotactic patterns can be altered by the perturbation of genes regulating SAM (Shoot Apical Meristem) organization. Here, we characterize a recessive T-DNA insertion mutant, *ipx (irregular phyllotaxis)*, which shows irregular phyllotatic pattern and abnormal development of SAM. *ipx* shows growth retardation and also has defects in embryo development such as arrested embryos and premature desiccation of immature seeds. *IPX* is expressed in actively dividing tissues such as SAM, RAM, vasculature, etc. *IPX* encodes a novel protein containing SMC (Structural Maintenance of Chromosomes) domain. We found RBR1 (RETINOBLASTOMA-RELATED PROTEIN 1) as an interacting partner of IPX through SMC domain by yeast two-hybrid assay. Some target genes of RBR1 and PRC (Polycomb Repressive Complex) are up-regulated in *ipx*. *RBR1* controls nuclear proliferation in the female gametophyte and regulates stem cell maintenance in root. *IPX* might be involved in cell cycle regulation with *RBR1*. Additionally, *ipx* shows defects in auxin flow and ectopic *WUS* expression in SAM, hypocotyl, leaf, root, etc. *ipx* seems to be weak allele, so we made null mutants by targeted gene knockout techniques and confirmed the embryo-lethal phenotype of ipx knockout plants.

P241 - Evolutionary insights into the auxin-mediated root development

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In *Arabidopsis thaliana*, root development is initiated during early embryogenesis by an instructive, asymmetric cell division of an extra-embryonic cell, the hypophysis. Mutants showing aberrant hypophysis division lead to loss of the primary root and rootless seedlings. The majority of these mutants fails to establish an auxin maximum in the basal pole of the early embryo or is insensitive to auxin-mediated responses. The ARF transcription factor MONOPTEROS (MP) is detrimental for these responses as loss-of-function leads to rootlessness. Analogously, gain-of-function mutations in the co-expressed repressor BODENLOS (BDL) show the same phenotype. Downstream of MP, the mobile bHLH transcription factor TARGET OF MONOPTEROS 7 (TMO7) acts non-cell-autonomously. However, it is not known if this auxin-dependency evident in *Arabidopsis* with its stereotypic embryonic division pattern is conserved in other plant species. With orthologs from *Brachypodium dystachion* and *Gossypium raimondii*, which show entirely different division patterns during early embryogenesis, we seek to shed light on the evolutionary conservation of this signaling cascade. In *Arabidopsis* protoplasts, orthologous proteins are interchangeable and gain-of-function BDL expressed under *Arabidopsis* regulatory sequences causes loss of the primary root. Additionally, orthologs are able to rescue loss of MP in *Arabidopsis* embryos. This provides a starting point to test the evolutionary conservation of this module.

P242 (Talk) - Linking regulatory networks in vascular development

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The vascular stem cell niche or procambium is responsible for the radial growth of the plant and for the formation of new transport tissues. Imbalances in a stem cell niche result in severe growth defects and patterning abnormalities. Therefore, regulation of stem cell niches should be tight and robust. Several pathways that are involved in regulating the procambium have previously been described. In this project I aimed to connect two of these: the PHLOEM INTERCALATED WITH XYLEM (PXY) signaling pathway that is responsible for maintaining the balance between cell proliferation and differentiation in the stem, and the action of targets of MONOPTEROS (MP) in controlling the amount of periclinal cell divisions in the root. Using enhanced yeast one hybrid assays I have mapped putative direct regulatory connections that link these pathways. The complete network contains 312 genes acting in 690 binding interactions. Novel interactions between regulators of genes previously identified as key players in vascular development were uncovered. Subsequent support of key interactions linking PXY and MP regulation was estimated using gene expression data where PXY signaling was perturbed. Our data suggests direct connections exist between two major signaling pathways in procambium regulation. Further phenotypic analysis will inform the biological function of these connections in planta.

P243 - The regulation of CONSTANS stability by distinct roles of LOV domain-containing two F-box proteins, FKF1 and ZTL, and GI in photopoeriodic flowering

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Many plants measure changes in day length to coordinate their flowering with favorable seasons for maximum reproductive success. In Arabidopsis, a facultative long-day plant, the day length-dependent regulation of CONSTANS (CO) protein stability is a crucial aspect for the induction of *FLOWERING LOCUS T (FT)* gene that determines the timing of flowering. CO protein levels oscillate throughout a day, which are abundant at the end of day and are extremely low at night in long day conditions, and several photoreceptors are involved in the control of CO stability changes directly or indirectly. We have previously shown that the blue light photoreceptor FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1) that plays a vital role in floral induction binds to CO protein directly. The binding is important for the timing of CO stabilization at dusk in long days, which promotes flowering in these conditions. ZEITLUPE (ZTL) protein, a FKF1 homologue blue light photoreceptor, is known as a negative flowering regulator because its overexpression causes a delayed flowering phenotype. We recently showed a potential role of ZTL in the regulation of CO stability. ZTL physically interacted with CO protein in tobacco leaves as well as Arabidopsis plants. Moreover, CO protein was more stabilized in the morning in the *ztl* mutant compared to wild-type plants, suggesting that ZTL negatively controls CO stability in the morning of long days. We also found a protein complex between GIGANTEA (GI) that interacts with FKF1 as well as ZTL and CO. In addition, the CO protein profile in the *gi* mutant resembles that in the ztl mutant, indicating that ZTL activity on CO stability may be changed by the presence of GI protein. Our findings suggest a balanced mechanism between FKF1 and ZTL functions mediated by the interactions with GI on CO stability regulation for the precise control of flowering time.

P244 - Jasmonic acid is a downstream component in the Arabidopsis hemoglobin 2 Glb2 modulation of somatic embryogenesis

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Previous studies show that the beneficial effect of suppression of the Arabidopsis hemoglobin 2, Glb2, on somatic embryogenesis occurs through the accumulation of nitric oxide (NO) within the embryogenic cells originating from the cultured explant (Elhiti et al., 2013). Nitric oxide activates the expression of Allene oxide synthase (AOS) and Lipoxygenase 2 (LOX2), two key enzymes of the jasmonic acid (JA) biosynthetic pathway, elevating JA content within the embryogenic tissue. The number of embryos in the single aos mutant and glb2-aos double mutant declined, and was not rescued by increasing levels of NO stimulating embryogenesis in wild type tissue. NO also influenced JA responses by up-regulating PLANT DEFENSIN 1(PDF1) and JASMONATE-ZIM-PROTEIN (JAZ1), as well as down-regulating MYC2. The NO and JA modulation of MYC2 and JAZ1 controlled embryogenesis. Ectopic expression of JAZ1 or suppression of MYC2 promoted the formation of somatic embryos, while repression of JAZ1 and up-regulation of MYC2 reduced the embryogenic performance. Sustained expression of JAZ1 induced the transcription of several IAA biosynthetic genes, resulting in higher indolacetic acid (IAA) levels in the embryogenic cells. Collectively these data fit a model integrating JA in the Glb2 regulation of Arabidopsis embryogenesis. Suppression of Glb2 increases JA through NO. Elevated levels of JA repress MYC2 and induce JAZ1 favoring the accumulation of IAA in the explants and the subsequent production of somatic embryos.

P245 - Mapping the interactions between the Phosphatidylethanolamine-binding Protein (PEBP) Family and Phospholipids

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Arabidopsis thaliana PEBPs (AtPEBPs) consist of six protein members, ATC, BFT, FT, MFT, TFL1, and TSF that are localized in several cellular compartments such as the cytoplasm, nucleus, ER, plasma membrane, and vacuole. The membrane localization of AtPEBPs can be explained their phospholipid-binding ability. Recently, one member of the family, FT was reported to interact with phosphatidylcholine (PC). Here we show that AtPEBPs were capable of binding to phospholipids differentially. We used small unilamellar vesicles (SUVs) and the purified His-tagged overexpressed proteins to determine the possible protein sedimentation with SUVs. MFT, TFL1, BFT, and ATC, which participate in flowering time pathways showed the highest preferences towards phosphatidylglycerol (PG), whereas TSF preferred PC. Our molecular docking analysis with the crystal structure of TFL1 (1WKO) also showed the highest affinity towards PG. Interestingly, the positive charge of the Histidine residue was capable to form the salt bridge with the negative charge of phosphate group of phospholipids. Our biophysical analysis indicated that AtPEBPs also affected the fluidity status of the membrane. Fluorescence Polarization (FP) assay revealed that TFL1, TSF, BFT, and ATC could increase the microfluidity, whereas MFT increased the microviscosity of the artificial membranes. In summary, our data provides biochemical and biophysical evidences showing that AtPEBPs could differentially interact with phospholipids.

P246 - Regulation of floral meristem initiation through LEAFY and RAX1

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In plants, all organs are formed from pool of stem cells contained in meristems. While the shoot apical meristem (SAM) is responsible for the elongation of the stem, flowers arise from newly formed meristems at the axil of the SAM. The positional cue for the formation of floral meristems is given by auxin maxima that rapidly induce expression

of the floral identity gene LEAFY (LFY). Despite recent advances in the field of meristem formation, the chain of events linking auxin signaling to stem-cell proliferation remains poorly understood. We and others have recently shown that in addition to its role in flower formation, LFY is able to ectopically stimulate axillary meristem growth in part through induction of the transcription factor REGULATOR OF AXILLARY MERISTEM1 (RAX1). While *RAX1* is expressed in flower primordia and has been linked to axillary meristem formation, mutants in *RAX1* do not show a floral phenotype. We hypothesize that *RAX1* function in the SAM is cryptic due to redundancy of genetic pathways and aim at deciphering the molecular event linking floral identity and stem-cell formation.

P247 - A Cistus creticus WD40-repeat protein is the functional homologue of Arabidopsis thaliana TTG1 and interacts with SPL transcription factors

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Glandular trichomes are epidermal structures for the accumulation of secondary metabolites associated with plant interactions with their environment. *Cistus creticus* is a xerophytic shrub of scientific interest due to the antimicrobial and cytotoxic properties of its resin "ladano" enriched in labdane-type diterpenes secreted by glandular trichomes. In model species Arabidopsis, trichome morphogenesis is modulated by TTG1, a WD-40 protein that interacts with bHLH and MYB factors forming a stable ternary transcriptional complex (MBW). The homolog of At*TTG1* was isolated from *C. creticus (CcTTG1)* and restored the pleotropic phenotype of Arabidopsis *ttg1* mutant. Yeast two-hybrid analysis identified two SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins, putative homologs of Arabidopsis SPL3/4/5 group, interacting with CcTTG1. The interactions between CcTTG1 and CcSPLA or CcSPLB were confirmed *in planta* by BiFC analysis. The biological significance of these interactions were clarified in vivo by the reduction of trichomes and decreased expression of *GLABRA2 (GL2)* the major regulator of trichome morphogenesis, in the first internode leaves of Arabidopsis lines overexpressing the miR156 resistant isoforms of At*SPL4* and At*SPL5*. This novel molecular interplay between TTG1 and SPLs is associated with MBW complex destabilization providing new insights in trichome morphogenesis.

P248 - A ROS responsible TF regulates root growth by directly controlling expression of novel protein that modulates cell length.

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Reactive oxygen species (ROS) is one of the key molecules for controlling the plant root growth. To elucidate the ROS roles for root growth, we made time course microarray by using Arabidopsis root treated with H_2O_2. From this microarray analysis, we found one transcription factor named ROS First Response TF 1 (RFRT1), and we investigate molecular function of RFRT1 involving in ROS signaling. Although *RFRT1* expressed very weak at the root tip under the control condition, RFRT1 accumulated strongly at the root tip after H_2O_2 treatment. Root growth of *rfrt1-1* mutant showed clear insensitivity to ROS. For identifying RFRT1 target genes, we performed RNA sequencing analysis (RNAseq). As a result of RNAseq analysis, we found 5 genes as the candidates of RFRT1 target. The *RFRT1 target gene A (Target A)* which encoded for small protein related lipid metabolisms expressed in the same domain where *RFRT1* expressed. In the RFRT1 over expressor, the expression of target A was strongly up-regulated. Moreover, we found that RFRT1 directly bound to the promoter of target A both in vivo and in vitro. These results indicated that RFRT1 was a novel transcriptional activator in ROS signaling that regulate plant root growth.

P249 - Arabidopsis PIRL6 encodes a Ras-group leucine-rich repeat protein important for formation of both male and female gametophytes

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Genes with critical functions in both the male and female gametophyte are not easily identified by traditional forward or reverse genetics, because null alleles cannot transmit through either parent and thus do not persist in mutagenized populations. Here we present evidence that Arabidopsis *P*lant *I*ntracellular *R*as-group *L*RR 6 (PIRL6), a member of a novel plant-specific class of LRR proteins, functions in the formation of both male and female gametophytes. Only two candidate insertion alleles were identified in mutant populations, and RT-PCR detected *PIRL6* mRNA in homozygotes from both lines, indicating that neither was a *bona fide* knockout. In contrast, there were six insertion alleles of an adjacent overlapping gene, indicating the chromosomal region is readily accessible to insertion mutagenesis. Multiple *PIRL6* transcripts were detected by nested RT-PCR in adult plants. Sequencing of 21 leaf and root cDNAs identified five different aberrantly spliced RNA species with premature nonsense codons. Properly spliced translatable mRNA was detected only in flowers, suggesting regulation by organ-specific alternative splicing. To trigger non-lethal *PIRL6* knockdown, we introduced an RNAi construct into wild-type plants. Fourteen independent T1 transformants produced high frequencies of both aborted ovules and abnormal pollen, strongly supporting a function for *PIRL6* in both male and female gametophyte development. *Supported by NSF 0616166 & Whitman College Abshire & Perry Funds*

P250 (Talk) - Isoprenoid homeostasis in Arabidopsis thaliana

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Plant growth, architecture and fertility are controlled by hormones and light, and require the homeostasis of pathway end-products such as lipids, pigments and products of the photosynthetic activity. Among those products, hormones (such as brassinosteroids) and lipids (sterol lipids, polyprenols) are part of the isoprenoid group of compounds. These latter products are built from five carbon atoms precursors produced in large parts by the mevalonate pathway; aspects of their homeostasis are however largely missing. It is generally assumed that the enzyme 3-hydroxy-3-methylglutaryl-CoA-reductase (HMGR), is a key element of their biosynthesis. We further characterized proteome phenotypes of an Arabidopsis mutant partially deficient in the latter biochemical function (Heintz D, Gallien S, Compagnon V, Berna A, Suzuki M, Yoshida S, Muranaka T, Van Dorsselaer A, Schaeffer C, Bach TJ, Schaller H (2012) Phosphoproteome exploration reveals a reformatting of cellular processes in response to low sterol biosynthetic capacity in Arabidopsis. J Proteome Res.11:1228-39) and are currently working on genetic screens with the aim to identify regulators of isoprenoid homeostasis in plants using next-generation-mapping.

P251 - The circadian clock rephases during lateral root organ initiation in Arabidopsis thaliana.

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The endogenous circadian clock enables organisms to adapt their growth and development to environmental changes. Here we describe how the circadian clock is employed to coordinate responses to the key signal auxin during lateral root emergence. In the model plant, *Arabidopsis thaliana*, lateral roots originate from a group of stem cells deep within the root, necessitating that new organs emerge through overlying root tissues. We report that the circadian clock is rephased during lateral root development. Metabolite and transcript profiling revealed that the circadian clock controls the levels of auxin and auxin-related genes including the auxin response repressor *IAA14* and auxin oxidase *AtDAO2*. Plants lacking or overexpressing core clock components exhibit lateral root emergence defects. We conclude that the circadian clock acts to gate auxin signalling during lateral root development in order to facilitate organ emergence.

P252 - The DESIGUAL (DEAL) genes contribute to leaf bilateral symmetry

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The body architectures of most multicellular organisms consistently display both symmetry and asymmetry, which raise fundamental biological questions on their underlying molecular mechanisms. Answers to these questions are lacking for plant leaves. We performed a large-scale search for leaf mutants among 21,000 Arabidopsis lines from the Salk homozygous T-DNA collection, and found only one exhibiting leaf bilateral symmetry breaking in a strict sense, with incomplete penetrance. We dubbed *desigual1-1 (deal1-1)* this mutant, which also shows defects in flower and silique organogenesis. Bilateral symmetry is altered in all these organs of *deal1-1* in a random fashion, as a consequence of the presence of both outgrowths and invaginations, phenotypes that are more severe in adult rosette leaves. Asymmetry is apparent in *deal1-1* leaf primordia, where cell expansion has not yet started, suggesting impaired cell proliferation. There are three *DEAL* redundant paralogs in the Arabidopsis genome: the *deal1 deal2 deal3* triple mutant exhibits leaf bilateral symmetry breaking with complete penetrance. We are examining the action of the redundant *DEAL* genes and in particular their interactions with auxin-related genes, including *CUC2.*

P253 - Are MAIN and MAIL1 involved in chromatin organization?

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MAINTENANCE OF MERISTEMS (MAIN) and MAINTENANCE OF MERISTEMS-LIKE1 (MAIL1) are two closely related proteins that share a conserved domain which is also found in transposases. Both proteins are ubiquitously expressed and exclusively localized to the nucleus. Single loss of function mutants for MAIN and MAIL1 show a severe short root phenotype associated with loss of cell fate in stem cells and differentiating cells. Moreover several key developmental genes as well as transposable element genes which are mainly controlled at the chromatin level are mis-expressed in both mutants. These results suggested that MAIN and MAIL1 might be involved in the control of chromatin organization and function. Here we show results on interaction studies between MAIN and MAIL1 and proteins involved in chromatin remodeling complexes. We also show analyses of histone modification patterns of genes which are mis-expressed in *main* and *mail1* mutants.

P254 - Using Juncus (Juncaceae) as a Model System to Study the Development of Unifacial Leaf

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Unifacial leaf (leaves lack adaxial side) has evolved repeatedly in monocot but is not present in Arabidopsis or Brassicaceae. *Juncus* (Juncaceae), however, is a useful model system to study its development. It was previously shown that the leaf blade of *J. prismatocarpus* is abaxialized in terms of gene expression and *DROOPING LEAF (DL)* is responsible for the flattened leaf blade in directional growth towards the shoot apical meristem (SAM). In addition, we found that the leaf blade of *J. torreyi*, a closely related species, seems to have a narrow adaxial sector, although it belongs to a unifacial leaf subfamily. These species therefore offers a unique opportunity to study the mechanisms of unifacial leaf development and evolution. We have started a detailed study of expression patterns of key genes involved in ad/abaxial patterning and unifacial leaf development in *J. torreyi*, including *PHABULOSA* (*PHB*, adaxial identity), *AUXIN RESPONSE FACTOR3 (ARF3*, abaxial identity), *DL* (cell growth towards the SAM), and *PRESSED FLOWER* (*PRS*, marginal growth). We have also developed a novel 5-ethynyl-2’-deoxyuridine (EdU) method to analyze the cell division pattern in *J. prismatocarpu*s to determine whether *DL* plays a direct role in cell division pattern. The improved EdU method is a quick and powerful method and can be also applied to Arabidopsis to study its leaf development. Our work on unifacial leaf development is a valuable complement of current knowledge about bifacial leaf development.

P255 - A small molecule serves as a specifically root promoting agent in Arabidopsis development

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Smart plants evolve multiple organs with diversity function to adjust its immotile life. Among them is the multitasking roots, which not only act as the mouth of plants to eat and drink but also help to cope with complex heterogeneous soil environment as well as the function of anchoring. We took use of the short root phenotype of ethylene overproduction mutant eto1-2 and constitutively ethylene response mutant ctr1-1 to perform a high-throughput small chemical library screening, aiming at chemicals that promote the root elongation. We identified four chemicals with root-promoting potency from screening, then we focused on the mechanism exploration of one named PRT. The small molecule PRT promotes the root elongation in a concentration dependent manner with no effect on the development of hypocotyl. Further genetic and biochemical analysis revealed that PRT attenuates the auxin signaling in root. Combinatorial application of PRT and a known auxin biosynthesis inhibitor L-Kynurenine (the inhibitor of TRYPTOPHAN AMINOTRANSFERASE ARABIDOPSIS1/ TRYPTOPHAN AMINOTRANSFERASE RELATEDs TAA1/TARs) indicates the PRT works in the downstream of TAA1, probably by influencing the activity of auxin biosynthesis gene YUCCA or auxin metabolism process. The small molecule PRT identified in this study can serve as a root promoting agent in agriculture application.

Key Words: chemical screening, PRT, root-promoting agent

P256 - Role of Receptor-like Kinase in Root Architecture changes under Abiotic Stress conditions in Arabidopsis thaliana

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Receptor-like kinase localized at the plasma membrane of plant cell with a wide range of functions during plant growth and development(i.e. organ identity, plant micro inter action, tissue patterning, self-incompatibility and stress tolerance). The focus of our study was to determine the role of receptor-like kinase in root architecture changes under different

environmental conditions. One receptor-like kinase T-DNA insertion line (Salk_143700c) showed a clear phenotype with shorter primary root length and reduced lateral root numbers at lateral stages of development under standard growth condition for Arabidopsis. The reduction of primary root length is caused by a lower rate of elongation in the zone of elongation of root tip. A second receptor -like kinase T-DNA insertion line (Salk_071422) with the same gene showed a similar phenotype as wild-type Columbia under same conditions. This lack of shorter primary root length of the second mutant could be explained that this insertion is located at the end of the protein after the kinase domain and apparently does not affect the function of the protein.

P257 - Spermidine retards plant aging via antagonistically regulating ethylene signaling cascade

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Spermidine, a naturally occurring polyamine, is involved in regulating anti-aging processes in animals and plants. Intracellular spermidine decreases with age and exogenous application of spermidine prolongs the lifespan of several model organisms. However, little is known regarding its role in plant aging. We found that the genetically engineered increase or decrease in endogenous spermidine extends or shortens plant longevity, respectively. Intriguingly, spermidine promotes the protein degradation of ETHYLENE INSENSITIVE3 (EIN3), a core regulator of leaf senescence, through stabilizing the EIN3 BINDING F-BOX1 (EBF1) and EBF2 that retard leaf senescence when overexpression. Screening of putative regulators of EBF1/EBF2 by yeast two-hybrid system, we found that the E3 ligase, SALT AND DROUGHT-INDUCED RING FINGER1 (SDIR1), interacts with EBF1/EBF2 and mediates their proteins degradation via the ubiquitin-proteasome pathway. Moreover, spermidine decreases transcription levels of *SDIR1* that functions as a positive regulator of ethylene signal and induces premature senescence in an EIN3/EIL1-dependent manner, suggesting that spermidine extends plant longevity by finely regulating the SDIR1-EBF-EIN3 cascade. Our findings provide a novel molecular mechanism that can explain how spermidine slows the aging process in plant.

Genome and chromatin dynamics

Posters 258 to 265

P258 - Genetic basis underlying the variation of meiotic recombination frequencies in a polyploid species, Brassica napus

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Meiotic recombination is a crucial component of evolution and breeding. Although studies in *Arabidopsis thaliana* have provided important insights into the formation of meiotic crossovers (COs) in plants, little is known about the genes/mechanisms responsible for natural variation in CO rates, especially in polyploid crops. Capitalizing on our previous results, this project specifically aims to analyze whether: (i) changes in CO rate in *Brassica napus* (AACC) may correlate with gene expression changes during meiosis, (ii) CO rate can be increased in this species by knocking down an antirecombination protein that has been identified in Arabidopsis (FANCM) and (iii) recombination frequencies can be further increased by knocking down FANCM in Brassica AAC hybrids already showing extra COs. An RNA-Seq experiment revealed how much variable the meiotic transcriptome is and to what extent each of the main factor (genome, variety and ploidy) accounts for the observed variability. This expression analysis also revealed extensive polymorphism between two *Brassica napus* varieties, some of which colocalizing with previously detected QTLs for the control of CO frequencies. Homeologue-specific (i.e. A^- and C^-) TILLING mutants have been identified for *FANCM* and the null homozygous double mutants ($A^-A^-C^-C^-$), as well as their progenies, have been produced and are currently under analysis. The same strategy will be followed for AAC plants. This project will shed new light on the pending cause of CO variation within plant species, which is essential for genetics, evolution and plant breeding.

P259 - Functional characterization of SMC5/SMC6 complex in Arabidopsis thaliana

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STRUCTURAL MAINTAINANCE OF CHROMOSOMES 6 (SMC6) is a protein that jointly with SMC5 and Non SMC Elements (NSEs) constitutes a complex involved in DNA damage repair. The SMC5/SMC6 complex is conserved among eukaryotes, which suggests its vital role for cell survival. However, complex assembly seems to partially differ between species. In Arabidopsis, homologs of all yeast SMC5/SMC6 components have been identified, but only few were functionally characterized to date. We focus on characterization of duplicated SMC5/SMC6 components in *A. thaliana*. While there is accumulating evidence about importance of SMC6B function in DNA damage repair, there are only limited data concerning SMC6A. We showed that *smc6b* is sensitive to the DNA damaging agent zebularine, whereas *smc6a* not. This indicates that although both paralogs are similar at protein level, they might be functionally diversified. We will present our genetic and molecular data on functional characterization of SMC6 paralogs in Arabidopsis.

P260 - Identification and Characterization of genes involved in the control of meiotic crossover frequency

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Meiotic crossovers (COs) generate genetic diversity and are essential for proper segregation of chromosomes during meiosis in most species. Despite their significance, CO number is very low in vast majority of eukaryotes and mechanisms underlying this limitation remain poorly understood. In order to unravel the mechanisms that limit meiotic CO, a genetic screen was performed in *Arabidopsis thaliana*. This led to the identification and characterization of several genes that limit meiotic crossovers (Crismani et al.,2012; Girard et al.,2014; Séguéla et al.,2015 and Girard et al.,submitted). A novel mutant was obtained in the same suppressor screen and does not belong to the previously identified complementation groups. So, the aim of first project is to identify the causal mutation and to functionally characterize the corresponding gene. The work on different mutants with increased CO frequency showed that the meiotic CO frequency can be largely increased without having any consequences on chromosome segregation at meiosis and doesn’t affect fertility. It raises two questions: (i) why is the number of CO so low in eukaryotes while CO frequency can be increased without any immediate deleterious consequences? (ii) Is there a physical limit, that has not been reached yet, that constrains the number of CO? To address these questions, the second project aims to analyze the effect of recombination by combining all the identified mutations with increased crossover frequency.

261 - Pipeline for whole-genome analysis of heavy-ion-induced mutants in Arabidopsis thaliana

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A heavy-ion beam is an effective mutagen and has been used for mutation breeding and molecular genetics. For the effective use of the heavy-ion beam, the knowledge of the mutation spectrum at the whole-genome level is valuable. In the whole-genome re-sequencing of heavy-ion induced mutants, total mutation candidates, including base substitutions, duplications, in/dels, inversions, and translocations, are detected by using three algorisms (SAMtools, Pindel and BreakDancer). The outputs from

these algorisms contain many false-positives, which have to be removed by visual checking with a genome browser. However, the visual checking is very time-consuming. We found that the false-positives were attributed to mainly two factors: 1) mismapped reads near repetitive sequences such as microsatellites and 2) genetic polymorphisms between the reference genome on the database and that of the laboratory line. By utilizing this information for false positives, we constructed an original pipeline for mutation and false-positive detection. When read data of multiple mutants are input, the pipeline outputs the list of candidate mutations with marks on false-positives. The pipeline enabled to reduce the numbers of candidate mutations to approximately 11% of that originally listed by those mutation-detection algorisms. Our pipeline will accelerate genome-wide mutation study.A heavy-ion beam is an effective mutagen and has been used for mutation breeding and molecular genetics. For the effective use of the heavy-ion beam, the knowledge of the mutation spectrum at the whole-genome level is valuable. In the whole-genome re-sequencing of heavy-ion induced mutants, total mutation candidates, including base substitutions, duplications, in/dels, inversions, and translocations, are detected by using three algorisms (SAMtools, Pindel and BreakDancer). The outputs from these algorisms contain many false-positives, which have to be removed by visual checking with a genome browser. However, the visual checking is very time-consuming. We found that the false-positives were attributed to mainly two factors: 1) mismapped reads near repetitive sequences such as microsatellites and 2) genetic polymorphisms between the reference genome on the database and that of the laboratory line. By utilizing this information for false positives, we constructed an original pipeline for mutation and false-positive detection. When read data of multiple mutants are input, the pipeline outputs the list of candidate mutations with marks on false-positives. The pipeline enabled to reduce the numbers of candidate mutations to approximately 11% of that originally listed by those mutation-detection algorisms. Our pipeline will accelerate genome-wide mutation study.

P262 (Talk) - Methylome variation within and between genetically identical Arabidopsis thaliana individuals

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Heritable epigenetic marks, such as cytosine methylation, can be sources of phenotypic variation. In order to understand their role in evolution, it is important to ascertain the intrinsic variability and stability of such marks. Previous studies in *Arabidopsis thaliana* have described and quantified the stability and emergence of DNA methylation variability across multiple generations. The degree of epigenetic variation within an individual plant as well as between individuals from the same generation has, however, remained elusive. We have compared the methylomes of (i) different organ types and (ii) a series of leaves from the same individual as well as of the corresponding leaves from different individuals. Organ type had the greatest effect on methylome variation, while analogous leaves from different individuals were the most similar. Importantly, there appears to be an ordered progression among a series of consecutive leaves from the same plant. Since these were harvested at the same time point, these methylation polymorphisms reflect changes in leaf identity, rather than temporal progression of the entire plant. We have also been able to show the effect of methylated transposable elements on gene expression at a organ specific level. In conclusion, we have been able to identify unique patterns that change systematically with leaf development and organ identity, and provide insights on the variability of epigenetic marks in short time scales.

P263 - Comprehensive characterization of genomic rearrangement in heavy-ion induced Arabidopsis mutants by wholegenome re-sequencing

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Heavy-ion irradiation has been accepted as an efficient technology for mutation breeding and molecular genetics. A noted character of heavy-ion irradiation is that mutation frequencies and mutation spectrum can be changed by selecting its linear energy transfer (LET). Beams with LET of 30 keV/μm induced small deletions (1 to 51 bp) with high frequency, while beams with LET of 290 keV/μm caused larger deletions (1 kbp to several hundred kbp) with chromosomal rearrangement. To comprehensively characterize the mutations induced by high-LET beams, the genomes of three Ar-ion induced *Arabidopsis* mutants (LET = 290 keV/μm, 50 Gy) were re-sequenced in the M3 generation. Total mutations, including base substitutions, duplications, in/dels, inversions, and translocations, were detected using three algorithms (SAMtools, Pindel, and BreakDancer). Averages of 30 homozygous mutations and 49.7 heterozygous mutations were detected per genome. All mutants had genomic rearrangements. Of the 22 DNA fragments that contributed to the rearrangements, 19 fragments were responsible for the intrachromosomal rearrangements, and multiple rearrangements were formed in the localized regions of the chromosomes. The interchromosomal rearrangements were detected in the multiply rearranged regions. These results indicate that the heavy-ion beams led to clustered DNA damage in the chromosome, and that they have great potential to induce complicated intrachromosomal rearrangements.

P264 - Repair of DNA Damage Induced by the Cytidine Analog Zebularine Requires ATR and ATM in Arabidopsis

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DNA damage repair is an essential cellular mechanism that maintains genome stability. Here we show that the non-methylable cytidine analogue zebularine induces DNA damage response in Arabidopsis. This effect is independent of DNA methylation changes. In contrast to genotoxic agents with cell cycle stage-independent damage induction, zebularine-induced damage occurs specifically in the course of strand synthesis during DNA replication. The signalling of this damage is mediated by additive activity of ATAXIA_TELANGIECTASIA MUTATED AND RAD3-RELATED (ATR) and ATAXIA_TELANGIECTASIA MUTATED (ATM) kinases, which cause post-replicative cell cycle arrest and increased endoreduplication. The repair requires a functional STRUCTURAL MAINTENANCE OF CHROMOSOMES (SMC) 5-SMC6 complex and is accomplished predominantly by synthesis-dependent strand annealing type of homologous recombination. Here, we provide a novel insight into the response mechanism coping with the genotoxic effects of zebularine and identify several components of the zebularine-induced DNA damage repair pathway.

P265 (Talk) - Homoeologous alleles regulations and their contributions in Arabidopsis allotetraploids development

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The merging of diverged genomes in hybrids has created novel combinations of homoeologous alleles, providing new opportunities for their interactions that could contribute to the development of hybrids. In an interspecific cross between autotetraploids *Arabidopsis thaliana* (At4) and *Arabidopsis arenosa* (Aa), heterotic phenotypes (increased biomass, starch accumulation and disease resistance) have been observed in the resynthesized tetraploid hybrids (Allo). While associations between phenotypes and molecular changes have been reported upon genome hybridization, detailed mechanisms leading to such changes remain unclear. Here we reported preferential protein-DNA and protein-protein interactions between homologous proteins in the CHE-CCA1 regulatory node of the circadian pathway, providing a mechanistic model to explain altered alleles regulation in the *Arabidopsis* allotetraploids. In addition to the clock regulators, we observed different induction kinetics of several WRKY transcription factors that are important for plant defense. Like the components in the circadian pathway, the presence of the homoeologous WRKYs in the allotetraploids could provide new interactions among the WRKYs, altering their targets regulation and defense response signaling. Data presented here are expected to advance our understanding of the molecular bases contributing to the modulations of the clock and defense pathways in plant hybrids.

Secondary metabolism

Posters 266 to 277

P266 - Impact of flavonoids on germination and protein carbonylation of Arabidopsis seeds submitted to oxidative stress

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Flavonoids are polyphenolic secondary metabolites synthesized by plants during their development and in response to various biotic and abiotic stresses. They influence the agronomic and nutritional qualities of seeds. The Arabidopsis *transparent testa (tt)* mutants affected in seed coat pigmentation are efficient tools to assess the roles of flavonoids in seed biology (Rajjou L., Debeaujon I., 2008, CR Biology 331, 796-805). Here we investigated the impact of flavonoid seed composition on their tolerance to oxidative stress using tt mutant seeds submitted to UV-B radiations and artificial ageing. In this work: 1) the germination of non-dormant control and stressed seeds was assessed and compared ; 2) protein carbonylation, which is an irreversible oxidation process leading to a loss of function of the modified proteins, was analyzed in control and stressed seed lots by a proteomics approach ; 3) flavonoid seed composition was analyzed (UPLC-MS). The relationships / correlations between these three parameters will be presented. Our results emphasize in particular the important role of testa flavonoids in embryo protection against oxidative damages induced by UV-B radiation.

P267 (Talk) - Change in secondary metabolism in Arabidopsis thaliana Pinoresinol reductase mutants.

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Arabidopsis thaliana has two Pinoresinol reductases, Pinoresinol reductase 1 (PR1) and Pinoresinol reductase 2 (PR2). These enzymes are employed in lignans biosynthesis pathway, PR1 converts (+)- and (–)-Pinoresinol to (+)- and (–)Lariciresinol whereas PR2 reduces only (–)-Pinorésinol. These lignans have a strong antioxidant activity like SDG (Secoisolariciresinol diglucoside). To understand the connections between lignans and other secondary metabolism pathways, two single homozygous mutants *pr1* and *pr2* were crossed to obtain the double mutant. The functional deficiency of different mutants was checked by RT-qPCR. Plants were cultivated in hydroponic system, and floral stalk, roots and rosette were harvested separately while seeds were collected at maturity stage from other plants. Combining ^{1}H-NMR, LC/MS and GC/MS allowed to characterize metabolites in the different organs. Furthermore, lignin content and composition were determined on roots and floral stalk. Results on *pr1* and *pr2* mutants indicated a strong reorganization of secondary metabolism, with important alteration in various molecule families like glucosinolates, flavonoids, phenylpropanoid. Deep modifications of lignin were also shown. The biological function of lignans is little known *in planta* and may be underestimated in view of the many changes observed in this study.

P268 - In Planta Function of Three Rice Genes Encoding Carotenoid Cleavage Dioxygenase in Transgenic Arabidopsis and Rice Plants

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Carotenoid cleavage products (CCPs) are apocarotenoid compounds derived from carotenoid substrates by catalysis through the carotenoid cleavage dioxygenases (CCDs). CCPs have biologically diverse functions in plants, acting as hormones, pigments, flavors, and defense compounds. The types of apocarotenoids are highly dependent on the enzymatic action of the CCD family and the specific position of the double bonds cleaved on the carotenoids. In our study, the *in vivo* function of three rice CCD genes together with a *Arabidopsis* CCD4 gene have been characterized in transgenic *Arabidopsis* and rice plants. To make sure the effect by transgenes, single-copy insertions of the four CCD genes were determined by TaqMan PCR and Southern blot analysis, respectively. Overexpression of the four CCD genes could restore to reduce the total carotenoid content at the similar level of wild-type Col-O when complemented into *Arabidopsis atccd*4 knockout line. A decreased level of total carotenoids was evident in transgenic rice leaf tissues by introduction of transgenes with the altered composition of apocarotenid volatile compounds including b-ionone and b-cyclocitral as CCPs. Interestingly, two carotenoid cleaved products (b-ionone and b-cyclocitral) could give agricultural values of biotic/abiotic stress-resistance with enhanced flavor to crops. These agronomical benefits of diverse apocarotenoids will heighten expectations for the use of plant CCDs for future crop engineering as well as promising targets for increasing the total amounts of provitamin A carotenoids by suppression of CCDs expression.

P269 - Secondary metabolites of model Brassicaceae species in response to a microbe-associated molecular pattern.

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Plants in nature are exposed to a broad spectrum of microbial pathogens and consequently forced to evolve efficient and robust immune system. One of the unique components of plant immunity is synthesis and accumulation of low-molecular compounds classified as secondary metabolites. Among these, of special interest are tryptophan derivatives present in the model Brassicaceae species *Arabidopsis thaliana*. Similar to other defense responses, biosynthesis of these compounds can be triggered by recognition of microbe-associated molecular patterns (MAMPs). In this study we investigated conservation of defensive secondary metabolites produced by Brassicaceae plants in response to a bacterial MAMP flg22. We used comparative metabolomic approach to identify compounds that are induced with flg22 treatment in seedlings of Arabidopsis and four related species. Among induced compounds, we detected several Trp-derivatives known from Arabidopsis. However, we observed variability in the occurrence and in the fold of flg22-induced concentration changes of these compounds between the tested species. Furthermore, we found a number of common or species-specific undefined MAMP-inducible compounds, which may have a function in the immune responses of Brassicaceae plants.

P270 - Effect of AP2/ERF Transcription Factor on gene expression of Carotenoid Biosynthetic Pathway

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The accumulation of various secondary metabolites, including carotenoids, phenylpropanoids and alkaloids has been implicated in light signaling. It has been demonstrated that the biosynthesis of phytochemicals made from different pathway could be controlled concomitantly by single transcription factor and/or substrate-sharing. The present study aimed at identifying the key regulator of phytochemical biosynthesis in the light responses in rice. The rice seedlings grown under blue light have high contents of carotenoids and phenolic compounds compared to those grown under white, green and red light. Microarray analysis displayed the differential expressions of a large number of phytochemical biosynthesis genes and transcription factor genes under blue light. The promoters of several phytochemical biosynthesis genes might be trans-activated with various types of transcription factors that were up-regulated under blue light. Ectopic expressions of these rice transcription factors in Arabidopsis resulted in neither growth inhibition nor visible phenotype alterations except TF13 transgenic plant, which showed the outgrowth phenotype and significant transcript increases of geranylgeranyl pyrophosphate synthase, phytoene synthase and z-carotene desaturase of Arabidopsis. TF13 is an AP2/ERF transcription factor, which has typical AP2/ERF domain and C-terminal EDLL activation domain. Gel retardation assays suggested that a TF13 binds the promoter of Arabidopsis phytoene synthase at two separate sites in the -1169/-1010 region. These results provide possible evidences that TF13 contribute to the blue light-induced carotenoids accumulation in rice.

P271 - Substantial Reprogramming of the Eutrema salsugineum (Thellungiella salsuginea) Transcriptome in Response to UV and Silver Nitrate Challenge

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Cruciferous plants synthesize a large variety of tryptophan-derived phytoalexins in response to pathogen infection, UV irradiation, or high dosages of heavy metals.The major phytoalexins of *Eutrema salsugineum (Thellungiella salsuginea)*, which has recently been established as an extremophile model plant, are probably derivatives of indole glucosinolates, in contrast to Arabidopsis, which synthesizes characteristic camalexin from the glucosinolate precursor indole-3-acetaldoxime. To study the transcriptional response of *E. salsugineum* to UV irradiation and silver nitrate we performed comprehensive RNAseq and microarray analysis. Most transcripts (respectively 70% and 78%) were significantly differentially regulated and a large overlap between the two treatments was observed (54% of total). While core genes of the biosynthesis of aliphatic glucosinolates were repressed, tryptophan and indole glucosinolate biosynthetic genes, as well as defencerelated *WRKY* transcription factors, were consistently upregulated. The putative *Eutrema WRKY33* ortholog was functionally tested and shown to complement camalexin deficiency in *Atwrky33* mutant.

P272 - The role of the cardenolide biosynthetic enzymes in planta, e.g. Arabidopsis thaliana

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The conception of the existence of well-defined biosynthetic pathways is outdated. This holds particularly true for the formation of small natural products (SNAPs). The catalytic efficiencies described for enzymes involved in the formation of SNAPs are very low. Many enzymes catalyse not only their reported “natural” reaction but also alternative reactions. In several cases, substrate as well as product promiscuity were demonstrated. Cardenolide biosynthesis is a good example to explain how paradigms have to be messed up. According to the text book, early steps in cardenolide biosynthesis are supposed to be catalysed by 3Y-hydroxysteroid dehydrogenase (3YHSD), Ketosteroidisomerase (KSI; [1, 2]) and progesterone 5Y-reductases (P5YR). When analysing the corresponding genes and enzymes (3YHSDs, P5YRs) in the genera *Digitalis, Erysimum, Medicago, Catharanthus* and *Arabidopsis* it became clear that small gene families exist that encode for 3YHSDs or P5YRs [3, 4]. Furthermore, some reactions are common for several classes of these enzymes that are not typical for cardenolide biosynthesis [5, 6].

Refs. Herl et al., 2009; Meitinger et al., 2015; Munkert et al., 2011, 2015; Bauer et al., 2012; Geu-Flores et al., 2012.

P273 - Function of a glutathione-S-tranferase in Arabidopsis immunity and glucosinolate metabolism

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Induced defense responses in plants usually involve biosynthesis of antimicrobial metabolites and their targeted secretion at the site of pathogen contact. Our former study on the model plant Arabidopsis revealed a novel pathogen triggered metabolism pathway for indole glucosinolates [1]. This pathway requires at least two enzymatic components: CYP81F2 P450 monooxygenase and PEN2-myrosinase. CYP81F2 is essential for the pathogen induced accumulation of 4-methoxyindol- 3-ylmethyl glucosinolate, which in turn is activated by PEN2 for antifungal defense. In addition, our former analysis suggested contribution of glutathione to the PEN2/CYP81F2-defence pathway [1]. This finding prompted us to investigate in detail the mechanisms underlying this putative glutathione immune function. Here we report on the Arabidopsis glutathione-S-transferase that is crucial for the pathogen triggered indole glucosinolate metabolism. We provide evidence that this particular glutathione transferase constitutes an indispensable component of the PEN2/CYP81F2 immune pathway and mediates resistance towards biotrophic, hemibiotrophic and necrotrophic fungal pathogens.

References: 1. Bednarek P., Piślewska-Bednarek M., Svato A., Schneider B., Doubsky J., et al. (2009) A Glucosinolate Metabolism Pathway in Living Plant Cells Mediates Broad-Spectrum Antifungal Defense. Science 323, 101-106.

P274 - Functional analysis of dehydroascorbate reductase in optimal and oxidative stress conditions in Arabidopsis

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Glutathione (GSH), the tripeptide (non protein) thiol (γ-glutamyl cysteinyl glycine) is the most important low molecular weight thiol in plant cells. Among its most well-known functions is the regeneration of ascorbate from dehydroascorbate (DHA) in the ascorbate-glutathione pathway, a reaction that can occur chemically or be catalyzed by several types of enzymes. The latter notably include specific reductases (DHARs), which are encoded by three genes in Arabidopsis. We have analysed the function of these three genes by production of double and triple mutants and their functional analysis in optimal and oxidative stress conditions. Among the latter, we have used ozone, reagents that generate oxidative stress inside the cells, and the oxidative stress mutant, *cat2*. Overall, the results provide no evidence that expression of any of the three genes is required for growth of Arabidopsis in optimal conditions. In oxidative stress conditions, our analyses point to some redundancy between different isoforms of DHAR, while at the same time suggesting that DHAR activity is required to optimize defense signalling, notably in the activation of salicylic acid pathway in response to pathogens.

P275 - Glucosinolate degradation products: their physiological effects on Arabidopsis thaliana

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Glucosinolates are one of the most important groups of sulfur- and nitrogen-containing secondary plant metabolites, characteristic of the Brassicales. To date, more than 130 different glucosinolates have been identified among plants and this huge diversity is due to the complex biosynthetic pathway. Glucosinolates are metabolized by specific enzymes called myrosinases. This enzymic decomposition by myrosinases depends on the presence or absence of special cofactors such as additional proteins, metal ions and pH. The substrate and the enzyme are separated from each other but upon tissue disruption they come into contact and the chemical reaction can lead to a variety of biologically important degradation products such as thiocyanates, isothiocyanates, nitriles and epithionitriles. These degradation products play a key role in plant defense responses against pathogens, pests, insects and herbivores, however some of them are also subject of great interest in medical research owing to their dietary cancer preventive effects in humans. In order to better understand the biological effects of glucosinolate hydrolysis products we have established and standardized an *in vitro* system which allows us to evaluate their effects on *Arabidopsis thaliana*. Several mutant lines were exposed to glucosinolate hydrolysis products to investigate their dose-dependent behavior. The remarkable results will be presented and discussed.

P276 (Talk) - DELLA Proteins Promote Anthocyanin Biosynthesis via Sequestering MYBL2 and JAZ Suppressors of the MYB/bHLH/WD40 Complex in Arabidopsis thaliana

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anin accumulation is recognized as a visible biomarker of plants that are suffering from environmental stresses. However, the molecular mechanisms underlying stress-induced anthocyanin biosynthesis remain unclear. Previous studies showed that the expression of anthocyanin-specific genes is regulated by the conserved MBW complex, which is composed of the MYB, bHLH and WD40 subunits in higher plants. MBW activity is repressed by MYBL2 and the JAZ family, which competitively bind to bHLH and MYB/bHLH, respectively. Here, we found that MYBL2 and JAZs mediate the gibberellin acid (GA)-inhibited anthocyanin biosynthesis in *Arabidopsis.* Pull-down and dual-luciferase assays showed that the master regulators (DELLAs) of GA signaling directly sequester MYBL2 and JAZ repressors, leading to the release of bHLH/MYB subunits and subsequently the formation of active MBW complex, which then activates the anthocyanin biosynthetic pathway. The JAZ-DELLA-MYBL2 module identified here also plays an important role in Jasmonate (JA)- and abiotic stress-induced anthocyanin biosynthesis. Furthermore, the DELLA protein RGA accumulates upon plant exposure to JA or abiotic stresses. Altogether, our data reveal that DELLA-promoted anthocyanin biosynthesis is mediated at least in part by MYBL2 and JAZ regulatory proteins. This study provides new insight into the coordinated regulation of plant growth and defense through metabolic pathway reconstruction.

P277 - The role of the Class I acyl-CoA-binding protein family in Arabidopsis cuticle formation

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The *Arabidopsis thaliana* Class I acyl-CoA-binding protein family was shown here to function in cuticle formation. This was achieved using T-DNA insertional mutants in Arabidopsis. The *acbp* mutants showed fewer wax crystals on the stem/leaf surfaces and displayed disrupted cuticle layers in scanning and transmission electron microscopy, respectively. Cuticular wax and cutin monomer composition was found to be reduced in the mutants in comparison to the wild type when analyzed with gas chromatography (GC) and GC-mass spectrometry (MS). Mutant tissue also had consistent reduction in the expression of genes involved in the biosynthesis of cuticular wax and cutin. To further investigate the spatial expression of *AtACBP, AtACBP* promoter::*Y-glucuronidase* gene fusions were generated. Resultant transgenic Arabidopsis plants demonstrated GUS expression on the surfaces supporting its role in cuticle formation. Our findings imply that the Class I ACBP family participates in Arabidopsis cuticular wax accumulation by trafficking very-long-chain acyl-CoAs to which recombinant forms are known to bind.

Reproduction

Posters 278 to 301

P278 - DUET/MMD1 Regulates Male Meiotic Gene Expression in Arabidopsis

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Meiosis produces haploid cells essential for sexual reproduction. In yeast, entry into meiosis activates transcription factors which trigger a transcriptional cascade that results in sequential co-expression of early, middle and late meiotic genes. However, these factors are not conserved, and the factors and regulatory mechanisms that ensure proper meiotic gene expression in multicellular eukaryotes are poorly understood. Here, we report that DUET/MMD1, a PHD finger protein essential for Arabidopsis male meiosis, functions as a regulator of meiotic gene expression. We find that DUET-PHD binds H3K4me2 in vitro, and show that this interaction is critical for function during meiosis. We also show that DUET is required for proper microtubule organization during meiosis II, independently of its function in meiosis I. Remarkably, DUET protein shows stage-specific expression, confined to diplotene. We identify two genes, *TDM1* and *JAS*, with critical functions in cell cycle transitions and spindle organization in male meiosis, as DUET targets, with *TDM1* being a direct target. Thus, DUET regulates microtubule organization and cell cycle transitions during male meiosis, and functions as a direct transcription activator of the meiotic gene *TDM1*. Expression profiling showed reduced expression of a subset comprising about 12% of a known set of meiosis preferred genes in the duet mutant. Our results further point to a role for histone modifications in the control of meiotic gene expression in plants, and suggest that transcription of meiotic genes is under stagewise control in plants as in yeast.

P279 (Talk) - A conserved role for CUP-SHAPED COTYLEDON transcription factors during ovule development

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Plant sexual reproduction depends notably on the development of the gynoecium that contains the ovules. Early ovule development relies on differential growth of placental structures controlled by the concerted actions of hormones and key regulators. Among these regulators the *CUP-SHAPED COTYLEDON* genes play a crucial role in gynoecium patterning and ovule development. Indeed, CUC1 and CUC2 control the initiation of ovule primordia via an auxin-dependent mechanism. Here we highlight an additional role for CUC2 and CUC3 in ovule individualisation. While *CUC1* and *CUC2* are broadly expressed in the medial-tissue of the gynoecium, *CUC3* is specifically expressed between ovule primordia at early stages. Consistent with this expression pattern, we show that the *cuc2cuc3* double mutant specifically harbors defects in ovule separation, producing fused seeds sharing the seed coat. These results highlight a partially redundant role for CUC2 and CUC3 in ovule individualisation. Functional analyses in *Cardamine hirsuta* show that NAM/CUC3 transcription factors also control ovule development in this species. In situ hybridization expression studies in more phylogenetically distant species reveal a conserved NAM/CUC3 expression pattern between ovule primordia. Taken together these results highlight an ancient role for NAM/CUC3 transcription factors in ovule separation and shed light on the conservation of regulatory mechanisms in the development of innovative structure.

P280 - Epigenetic marks of germline genes in somatic tissues of Arabidopsis thaliana

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Plants display distinct vegetative and reproductive phases, and the male and female germ lines originate in flowers from the cells of a previous somatic lineage. This developmental switch is associated with germline specific or preferential expression of a subset of genes that are normally repressed in a plant. The gene regulatory mechanisms involved in such long-term suppression and short-term activation in plant germline remain unclear. We used whole genomic analysis to explore the nature of epigenetic marks that are likely associated with long-term gene repression in the non-germline cells. Our study showed that the majority of germline genes are associated with repression-related epigenetic histone modifications in one or more non-germline tissues, among which H3K9me2 and H3K27me3 are the most widespread repression-related marks. Further, our data indicated that the repressive epigenetic mechanisms differ between male and female germline genes, and the diverse states of epigenetic marks are present in different non-germline tissues. Some germline genes also have activation-related marks in non-germline tissues, and the proportion of such genes is higher for female germline genes. Thus, our study shows that epigenetic control of gene expression is likely to be a dominant mechanism for repressing germ line genes in somatic tissues, paving the way for discovering additional marks in future large-scale genomic studies.

P281 - The LARP6c protein is required for pollen tube guidance and male fertility in Arabidopsis thaliana.

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Angiosperms" male gametophyte, the pollen grain, consists in a large cell containing both a vegetative nucleus and the two sperm cells.After pollen grain hydration on the stigma, the growth of a pollen tube through the pistil allows sperm cells delivery into the ovule. Pollen tube growth and guidance until fertilization require an extensive communication between male and female. This process involves the secretion of female- but seemingly also male-emitted peptides, small proteins, amino acids but also hormones, nitric oxide,... LA and Related Proteins (LA and LARPs) constitute a family of RNA binding factors with members scattered in protists, fungi, plants and animals. They classify into five evolutionarily distinct subfamilies. Most LA and LARPs contain a LA motif and a subfamily specific RNA Recognition Motif 1. LARP6 subfamily members also share a specific domain denoted LSA (LA and S1-Associated). Human LARP6 binds type-I collagen mRNAs at their 5'UTRs and coordinates their subcellular localization and translation. Arabidopsis has three LARP6 proteins, two of them (b and c) possess a PAB interacting Motif 2 (PAM2), so named for its ability to bind Poly(A) Binding Proteins (PAB). We found that AtLARP6c is a male specific protein dispensable for pollen tube germination and growth but necessary for pollen tube guidance to the ovule. Our data support that AtLARP6c is involved in the post-transcriptional regulation of genes involved in transport and/or secretion processes.

P282 (Talk) - GA Signaling Playing a Role in Arabidopsis Male Meiotic Cytokinesis

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Environmental factors influence plant development and in particular the reproductive system is sensitive to extreme temperature conditions which may cause reduction in fertility. Previously we reported that a short period of cold stress evokes the formation of diploid pollen by interfering with the organization of the radial microtubule arrays at the telophase II stage, and consequently leading to incomplete or irregular cytokinesis. A subpopulation of binuclear and polynuclear microspores then form which eventually develop into diploid or polyploid pollen grains. However, the involved molecular regulators and signaling pathways are still unknown. Here, we show that GA signaling plays a role in male meiotic cytokinesis. By cytological and genetic approaches, we found that Arabidopsis GA signaling mutants show cytokinesis defect in male meiocytes similar to those observed in cold-treated wild type Columbia-0 plants. Moreover, male sporogenesis in the GA mutants was not overly sensitive to cold suggesting that cold-induced defects in male meiotic cytokinesis may be mediated by GA signaling. In conclusion, we propose that GA signaling contributes to the establishment of cytokinesis in male meiosis and potentially plays a role in cold-induced diploid male gametes formation.

P283 - Mini Zinc Finger 2: a new adaptator peptide involved in floral development

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In plants, flowers are produced by the activity of floral meristems which differ from vegetative meristems in their determinate fate. Termination of stem cells in floral meristem is critical for reproductive success, and involves a complex crosstalk between genetic program and hormonal pathways. MIni zinc Finger family (MIF) from *Arabidopsis thaliana* encodes small proteins involved in the regulation of floral development and hormonal signaling pathways (Hu and Ma 2006). MIni zinc Finger proteins can't be defined as transcription factors because they do not bind to DNA. Their ability to control physiological events is linked to their unique domain, the zinc finger, which confers to MIF the capacity to interact with other proteins. Constitutive over-expression of *MIF2* caused pleiotropic developmental defect such as a bushy phenotype and a dramatic alteration of flower development. Gene expression analysis during flower development revealed that *MIF2* is involved in the floral termination genetic network and regulates indirectly the expression level of the meristem organizing centre gene *WUSCHEL*. Using Bimolecular Fluorescent Complementation experiments we demonstrated the interaction between *MIF2* and *KNUCKLES* which is known to be a repressor of WUS during floral termination (Payne et al. 2004). Phenotypic data, gene expression and BiFC suggest that *MIF2* acts as a small adaptator protein during floral development, regulating genes expression by interaction with transcription factor.

P284 - Auxin production couples endosperm development to fertilization

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In flowering plants seed development is preceded by a double fertilization event, whereby two male sperm cells fuse with the female gametes, egg cell and central cell. The fertilization of the egg cell results in the developing embryo, while that of the central cell gives rise to the triploid endosperm, whose function is to nourish and support the embryo. Even though seed endosperm has an unparalleled role for human nutrition, the molecular bases for its development are yet to be understood. Our results indicate that increasing auxin levels after fertilization drive the replication of the central cell in *Arabidopsis*. We show that external auxin is sufficient to trigger central cell division when applied to unfertilized ovules and that this autonomous replication is dependent on the MADS-box transcription factor AGL62. Moreover, mutants impaired in either auxin biosynthesis or signalling show severe endosperm defects, indicating that this hormone is necessary for its correct development. Interestingly, we observe that the auxin-driven autonomous endosperm results in the removal of the epigenetic modifiers Polycomb Group (PcG) proteins, in a manner similar to that which is triggered by fertilization. PcG proteins establish the trimethylation of lysine 27 on histone 3 and they exert a block on central cell division that has to be lifted upon fertilization. Our work suggests that auxin may be the factor necessary for alleviating the PcG repression on endosperm development.

P285 - Role and function of Arabidopsis thaliana WIP proteins

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One of the characteristic of living organisms is the ability to reproduce themselves. Sexual reproduction is a mechanism that increases the potential diversity of a species and then its positive selection. To reproduce some plants species use a type of sexual organ named flower. The sex of this organ is determined by the presence or absence of two different types of reproductive tissue. In many species the development of theses tissues is controlled by a network of genes. Cucumis melo sex determination genetic model can be explained by the modulation of 2 genes. The “gene A” or CmACS-7 (boualem and al 2008) that induces growth arrest of stamen (the male reproductive tissue) and the ”gene G” or CmWIP1 (martin and al 2010) that induces growth arrest of pistil (the female reproductive tissue). Both of these genes were identified by the laboratory of AbdelHafid BENDAHMANE and during my thesis I am trying to understand in which biological process cmWIP1 is involved to inhibit pistil development. To achieve this goal the lab has decided to give me the opportunities to use the power of genetic screens in Arabidopsis thaliana to unravel genetic interactors of CmWIP1. Crop species plants that are mutated in theses cmWIP1 genetic interactors should harbor female organ inside each of their flowers. Then the introgression of theses alleles in crop species may increase fruit and/or seed yields.

P286 - High throughput analysis of Arabidopsis seed lipid and protein content by Near-Infrared Reflectance Spectroscopy in four recombinant inbred lines.

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Plant seeds constitute a key component of both human and livestock diets, as seed storage compounds are mainly composed of protein, oil and starch. The increasing demand of plant-derived products for nutritional and industrial applications highlights the urgent need to develop new methodologies to increase the overall seed oil and protein content. Although most of the biochemical steps involved in lipid and protein biosynthesis are known, the genetic factors that control the lipid/protein ratio in the seeds have to be identified. In order to do so, a QTL approach to study lipid and protein metabolisms in Arabidopsis seed has been implemented. Quantitative genetic relies on statistical links between phenotype and genotype, meaning genotyping and phenotyping of thousands samples. In Arabidopsis, genotyping is not a limiting factor, whereas high-throughput phenotyping can be an obstacle. Thus, the potential of Near Infrared Spectroscopy (NIRS) for the simultaneous analysis of total lipid, and protein content of Arabidopsis seeds was studied. Our results demonstrated that NIRS is a powerful non-destructive, high-throughput method to assess lipid and protein content in Arabidopsis seed. NIRS was further used in quantitative trait loci (QTL) analyses in order to identify genetic factors governing natural variability in these traits in Arabidopsis thaliana, using four recombinant inbred line populations (Ct-1 x Col-0, Cvi-0 x Col-0, Bur-0 x Col-0, Bay-0 x Shahdara).

P287 - Isolation and Functional study of Pollen Tube Transcripts Upregulated by Pollination

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Transcriptome profiling has been used to identify genes expressed in the *in vitro* elongating pollen tubes, however, little is known of the transcriptome of *in vivo*-grown pollen tubes, due to the obstacle of collection of pollen elongating within the solid maternal gynoecium. By using a pollen specific promoter (*ProLAT52*) to generate an epitope-tagged polysomal-RNA complexes that can be affinity purified, we obtained mRNAs undergoing translation (the translatome) of in *vivo*-grown pollen tubes from self-pollinated gynoecia of *Arabidopsis thaliana*. Translatomes of pollen grains as well as in vivo and *in vitro* cultured pollen tubes were assayed by microarray analyses, revealing over 500 transcripts specifically enriched in *in vivo* elongating pollen tubes. Functional analyses of several *in vivo* mutants of these pollination-enhanced transcripts exposed partial pollination/fertilization and seed formation defects in siliques. Cytological observation confirmed the involvement of these genes in specialized processes including micropylar guidance, pollen tube burst, repulsion of multiple pollen tubes in embryo sac and pollen tube elongation. In summary, the selective immunopurification of transcripts engaged with polysomes in pollen tubes within self-fertilized florets has identified a cohort of pollination-enriched transcripts that facilitated the identification of genes important in *in vivo* pollen tube biology.

P288 - The developmental basis of Arabidopsis lyrata and Arabidopsis arenosa hybridization barriers

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The presence of post-zygotic hybridization barriers in higher plant species secures reproductive isolation and prevents genetic exchange between diploid populations. In higher-ploidy populations though, hybridization barriers have been described to be partially lifted to allow gene flow between species. However, the developmental basis of post-zygotic hybridization barriers as well as the causal genetic regulatory network resulting in species barriers remains to be identified. Here, we describe a post-zygotic hybridization barrier in interspecies diploid *Arabidopsis lyrata* and *A. arenosa* crosses, where most seeds are not viable. Preliminary results show that reciprocal interploidy interspecies crosses between *A. arenosa* and *A. lyrata* produce viable seeds, indicating that the post-zygotic block is lifted. Moreover, we observe that the direction of the interploidy cross is crucial for bypassing the hybrid barrier. We are currently investigating embryo- and endosperm development in interspecies crosses and the hypothesis that an increased maternal dosage reduce the abortion rate in hybrid crosses. Furthermore, we seek to identify major developmental checkpoints resulting in hybrid barriers and to understand these phenotypes in light of developmental gene regulatory networks.

P289 (Talk) - Autophagy’s Link to Self-Incompatibility in the Brassicaceae

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Self-incompatibility (SI) in flowering plants is a reproductive barrier resulting in self-pollen rejection to prevent inbreeding depression. In the Brassicaceae, SI is controlled by the pistil through the failure to deliver factors required for pollen hydration and germination. SI is initiated by the allelic binding of the pollen S-locus Cysteine Rich/S-locus Protein 11 (SCR/SP11) ligand to the pistil S Receptor Kinase (SRK). Downstream components include the ARC1 E3 ubiquitin ligase, which is proposed to ubiquitinate and inhibit Exo70A1, following SRK activation. With compatible pollinations, Exo70A1, as part of the exocyst, is responsible for directing secretory vesicles to the stigmatic papillar plasma membrane under the pollen grain. Inhibition of Exo70A1 results in blocked resource delivery to pollen, causing pollen rejection. Recent work from our research group has implicated autophagy in the sequestration of secretory vesicles during the SI response. Thus, we hypothesize that a breakdown in autophagy will compromise SI pollen rejection. *Arabidopsis lyrata* SCR, *SRK*, and *ARC1* transgenes have been transformed into *A. thaliana* Col-0 and autophagy mutants to reconstitute SI, and the phenotypes of these transgenic lines are currently being assessed. Autophagy and vesicle markers are also being used to visualize cellular responses following the addition of SI pollen. Overall, these studies will aid in understanding the role of autophagy in the pistil to reject SI pollen.

P290 - PATRONUS1 is Expressed in Meiotic Prophase I and Interacts with OSD1 to Link Centromeric Cohesion and Cell Cycle Progression in Arabidopsis

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Retention of sister centromere cohesion during meiosis I and its dissolution at meiosis II is necessary for balanced chromosome segregation and reduction of chromosome number. *PATRONUS1 (PANS1)* has recently been proposed to regulate centromere cohesion after meiosis I, during interkinesis, and PANS1 protein interacts with components of the Anaphase Promoting Complex (APC/C). We show here that PANS1 protein is found mainly in prophase I of meiosis, with its level declining late in prophase I during diplotene. We demonstrate that, in addition to premature loss of centromere cohesion during interkinesis, *pans1* mutants show partially penetrant defects in centromere cohesion during meiosis I. We also determine that *pans1* shows synthetic lethality with *Omission of Second Division 1 (osd1),* which encodes a known inhibitor of the APC/C that is required for cell cycle progression during mitosis, as well as meiosis I and II. Our results indicate that PANS1 and OSD1 are part of a network linking centromere cohesion and cell cycle progression through control of APC/C activity.

P291 - Reproductive failure in Arabidopsis thaliana under transient carbohydrate limitation

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The impact of transient carbon depletion on reproductive growth in *Arabidopsis thaliana* was investigated by transferring long photoperiod-grown plants to darkness and returning them to a light-dark cycle. After two days of darkness, carbon reserves were depleted in reproductive sinks and RNA *in situ* hybridization of marker transcripts showed that carbon starvation responses had been initiated in the meristem, anthers and ovules. Treatments of two or more days resulted in a bare-segment phenotype on the floral stem, with 23-27 aborted siliques. These resulted from impaired growth of very immature siliques, and abortion of mature and immature flowers. Depolarization of PIN1 protein and increased DII-VENUS expression pointed to rapid collapse of auxin gradients in the meristem and inhibition of primordia initiation. After transfer back to a light-dark cycle, flowers appeared and formed viable siliques and seeds. A similar phenotype was seen after transfer to sub-compensation point irradiance or CO_2. It also appeared in a milder form after a moderate decrease in irradiance, and developed spontaneously in short photoperiods. We conclude that *Arabidopsis thaliana* inhibits primordia initiation and aborts flowers and very young siliques in C-limited conditions. This curtails demand, safeguarding meristem function and allowing renewal of reproductive growth when carbon becomes available again.

P292 - Characterization of DEFECTIVE SEED MUTANT (DSM1), a novel gene implicated in carbon resources allocation

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In the search for new genes involved in seed development and storage compound accumulation we have identified three *Arabidopsis* allelic mutants. We have mapped the mutations to a small and constitutively expressed gene we named *DSM1 (DEFECTIVE SEED MUTANT 1)* that encodes a peptide whose function has not been previously characterized. The mutant shows pleiotropic phenotypes such as reduced germination rate, stunted growth, reduction in seed set and production of wrinkled/twisted seeds. A first analysis of the mutant has revealed an excessive starch granules accumulation in roots, vegetative tissues and seeds. Mutant seeds are depleted in fatty acids whereas they accumulate more storage proteins (both globulins and albumins) when compared to wild-type, suggesting a possible role for *DSM1* in regulating carbon resources allocation in *Arabidopsis. Dsm1* mutants also show cuticle defects that might be interpreted as a problem to acquire epidermal cell fate or a general defect in some functions of the secretory pathway. Indeed, DSM1 protein does accumulate in the endoplasmic reticulum, thus implying that its molecular role is linked to the function of this key subcellular compartment. We are currently performing more experiments to figure out if *dsm1* mutants show defects in the secretory machinery and to further investigate DSM1 function in regulating *Arabidopsis* metabolism.

P293 - Revival of self-incompatibility in Arabidopsis thaliana

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Self-incompatibility is the genetic system which prevents selfing and accordingly promotes outcrossing. In Brassicaceae, SI reaction is initiated by interaction between the male specificity factor SP11/SCR and the female specificity factor SRK. While *Arabidopsis thaliana* is a self-compatible, predominantly selfing species of Brassicaceae, it is believed that it had originally SI system to obligate outcrossing, because disrupted *SP11/SCR* and *SRK* exist in the *A. thaliana* genome. Although we have shown that the 213-bp inversion in the *SCR* coding sequence can be responsible for the loss of self-incompatibility in *A. thaliana* (Tsuchimatsu et al 2010), a detail of its historical contribution is still conclusive. To dissect gene-disruptive mutations in *SCR* conferring the evolutionary loss of self-incompatibility in *A. thaliana*, we conducted functional, physiological, genic and genetic analyses of self-incompatibility in genus *Arabidopsis*, by taking advantage of trans-specific sharing of *S*-haplotypes between *A. thaliana* and *A. halleri*, a self-incompatible close relative of A. thaliana.

P294 - The pollen-expressed cysteine rich peptide CR3 involves in pollen germination and pollen tube growth

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In plant reproduction, pollination is the first step in bringing together the male and female gametophytes, and a number of factors function at interaction and communication between pollen and pistil. Among them, cysteine-rich peptides (CRPs) act as the key for ligand-receptor interactions, and, for example, *S*-locus cystein rich protein (SCR) which is the male determinant factor of self-incompatibility and LURE which guides pollen tubes to ovule for successful fertilization have been identified. In this study, we focused on the pollen-expressed CRPs, named CR3, and elucidated how CR3 functions in the plant reproduction through various analyses. By RT-PCR in sepal, petal, stamen and pistil of Col-0 buds, the *CR3* expression was observed predominantly in the stamen. On microscopic observation of pollination, GFP-fused CR3 seemed to exist at inside of pollen grain and pollen tube, not at pollen surface, and it was further supported by *in situ* hybridization using the *CR3*-specific RNA probe. To reveal a biological function of CR3, *in vitro* pollen tube observation was conducted using the phosphorothioate antisense CR3 oligonucleotide, for the inhibition of translation of *CR3*. The translationally CR3-repressed pollen grains exhibited a lower rate of pollen germination, shorter pollen tube and a higher rate of tube tip burst. These results suggest that CR3 is one of key CRPs for successful plant reproduction and involves in pollen germination and pollen tube growth.

P295 - NIP4;1 and NIP4;2 are aquaporins specifically expressed in pollen grains and pollen tubes involved in reproduction in Arabidopsis thaliana

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In Angiosperms, reproduction involves processes where water and solutes transport is tightly regulated. In plants with dry stigmas, water and non-polar molecules are transported into pollen from the stigma. Then, additional transport allows cytosolic adjustment of ions and maintenance of turgor pressure during tip growth. Aquaporins may mediate this transport during pollen germination and/or tube growth. In Arabidopsis thaliana, only 4 aquaporin genes are expressed in mature pollen and tubes: *TIP5;1, TIP1;3, NIP4;1* and *NIP4;2*. Here, we proposed that NIP4;1 and NIP4;2 mediate water and solute transport during pollen development, germination and tube growth. They have 84% amino acidic identity, but they displayed different expression patterns. While *NIP4;1* showed low expression levels in mature pollen, *NIP4;2* peaked during tube growth. NIP4;1$_{pro}$:GUS was active in mature pollen and pollen tubes, whereas NIP4;2$_{pro}$:GUS only in pollen tubes. Simple *nip4;1* and *nip4;2* mutants and double amiRNA knockdowns showed distorted segregation ratios, reduced number of seeds, pollen germination rate and tube length. Lines expressing EGFP-tagged *NIP4;1* and *NIP4;2* under their own promoters rescued the mutant phenotypes. Fusion proteins were localized in the plasma membrane and intracellular vesicles of pollen and pollen tubes. Thus, we propose NIP4;1 and NIP4;2 are aquaporins of mature pollen and pollen tubes respectively, playing partially overlapping roles in pollen development and pollination.

P296 - Negative regulatory roles of DE-ETIOLATED1 (DET1) in flowering time in Arabidopsis

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Arabidopsis flowers early under long days (LD) and late under short days (SD). The repressor of photomorphogenesis *DE-ETIOLATED1 (DET1)* delays flowering; *det1-1* mutants flower early, especially under SD, but the molecular mechanism of DET1 regulation remains unknown. Here we examine the regulatory function of DET1 in repression of flowering. Under SD, the det1-1 mutation causes daytime expression of *FKF1* and *CO*; however, their altered expression has only a small effect on early flowering in det1-1 mutants. Notably, DET1 interacts with GI and binding of *GI* to the *FT* promoter increases in det1-1 mutants, suggesting that DET1 mainly restricts GI function, directly promoting FT expression independent of *CO* expression. Moreover, DET1 interacts with MSI4/FVE, which epigenetically inhibits *FLC* expression, indicating that the lack of *FLC* expression in *det1-1* mutants likely involves altered histone modifications at the *FLC* locus. These data demonstrate that DET1 acts in both photoperiod and autonomous pathways to inhibit expression of *FT* and *SOC1*. Consistent with this, the early flowering of *det1-1* mutants disappears completely in the *ft-1 soc1-2* double mutant background. Thus, we propose that DET1 is a strong repressor of flowering and has a pivotal role in maintaining photoperiod sensitivity in the regulation of flowering time.

P297 (Talk) - Molecular Genetic Analysis of LORELEI Function in Pollen Tube Reception by the Arabidopsis Female Gametophyte

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In angiosperms, the pollen grain forms a pollen tube and extends through the transmitting tract to reach the female gametophyte, where it releases two sperm cells and completes double fertilization. In *Arabidopsis thaliana* lorelei (lre) mutant, most female gametophytes fail to induce pollen tube reception and thus remain unfertilized. *LORELEI (LRE)* encodes a putative glycophosphatidylinositol (GPI)-anchored membrane protein and is hypothesized to localize in the plasma membrane of synergid cells of the female gametophyte, as GPI-anchored proteins are covalently linked to a GPI anchor in the ER and localize in the plasma membrane using this lipid moiety. Wild-type LRE fused to YFP (LRE-YFP) rescues pollen tube reception and seed set defects in *lre* mutant and hence it is fully functional. The fusion protein localizes in an unknown organelle within the synergid cell and in the plasma membrane-rich filiform apparatus (FA) of the synergid cells, the first point of contact between the pollen tube and the female gametophyte. We will provide an update on our progress in answering the following questions using the functional LRE-YFP fusion protein: i) is LRE a GPI-anchored membrane protein? ii) is GPI anchor essential for LRE function? iii) is the intracellular localization in the unknown organelle essential for LRE function? iv) is the ectodomain of LRE alone sufficient for LRE function? v) between pollen tube and synergid cell, which one is essential for LRE function?

P298 - Developmentally regulated auxin production for proper embryo development in Arabidopsis.

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Plant reproduction relies on well-defined and coordinated series of cell division and cell differentiation of the zygote. The zygote and later the embryo are embedded into maternal-derived integuments, whose influence on embryo development remains elusive. The plant hormone auxin is known to play a crucial role in defining the embryonic body axis. And dynamic transport of the hormone by auxin efflux carriers PIN proteins is involved in specifying embryonic shoot and root poles. Moreover TAA1/YUCCA-dependent auxin biosynthesis pathway has also an essential function during embryo development, as assayed by the embryonic phenotypes of certain combinations of loss-of-function mutations in these genes. Local auxin production, with feedback on auxin transport, influences different steps of embryo development. An increase of auxin production in the maternal integuments followed by auxin transport from those integuments to the zygote is important for the first asymmetric division and specification of the apical embryonic pole. At later globular stages, a new auxin source in apical cells of the embryo triggers polarization of the auxin transport to the basal pole for a proper formation of a root. Altogether these data will propose a model integrating the dynamic behavior of auxin production, and its influence on the hormone transport, for the proper development of the embryo.

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P299 - Different causes of pollen lethality induce reproductive barriers between Arabidopsis thaliana natural accessions

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Sexual reproduction involves the production of both male and female gametes. In predominantly selfing plants like *Arabidopsis thaliana*, a defect in the production of gametes of either sex potentially leads to a loss of fitness and ultimately to a full sterility. This deleterious phenotype can sometimes be observed in crosses between different natural accessions of the same species. This phenomenon, also called genetic incompatibility, limits gene flow and may ultimately lead to speciation events. In a series of systematic reciprocal crosses between distant accessions of *Arabidopsis thaliana*, we highlighted a male sterility in Shahdara (Sha, from Tadjikistan) x Monte-Rosso (Mr-0, from Italy) F1

hybrids. The fertility of the reciprocal F1 indicated the Sha cytoplasm is involved in the sterility of the ShaxMr-0 F1. Previous genetic analyses identified two nuclear loci involved in this phenotype. Our recent results suggest that the male sterility observed in the ShaxMr-0 hybrid is based on the combination of two different genetic mechanisms, each inducing pollen lethality:(1) a cytoplasmic male sterility, killing pollen carrying Mr-0 alleles at restorer loci and (2) several cytoplasm-independent hybrid male sterilities, or pollen-killers, killing pollen with Sha alleles when produced by a plant heterozygous at the considered loci. We propose that the association of both effects participate to the elaboration of genetic barriers between Shahdara and Mr-0 natural accessions.

P300 - Florigen-induced transposon silencing in the shoot apical meristem in rice

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Floral induction is a crucial developmental step in higher plants. Florigen, a mobile floral activator that is synthesized in the leaf and transported to the shoot apex, was recently identified as a protein encoded by *FLOWERING LOCUS T (FT)* and its orthologs; the rice florigen is Heading date 3a (Hd3a) protein. The 14-3-3 proteins mediate the interaction of Hd3a with the transcription factor OsFD1 to form a ternary structure called the florigen activation complex on the promoter of *OsMADS15*, a rice *APETALA1* ortholog. However, crucial information, including the spatiotemporal overlap among FT-like proteins and the components of florigen activation complex and downstream genes, remains unclear. Here, we confirm that Hd3a coexists, in the same regions of the rice shoot apex, with the other components of the florigen activation complex and its transcriptional targets. Unexpectedly, however, RNA-sequencing analysis of shoot apex from wild-type and RNA-interference plants depleted of florigen activity revealed that 4,379 transposable elements (TEs; 58% of all classifiable rice TEs) were expressed collectively in the vegetative and reproductive shoot apex. Furthermore, in the reproductive shoot apex, 214 TEs were silenced by florigen. Our results suggest a link between floral induction and regulation of TEs.

P301 - AP1G-mediated dynamic vacuolar organization is critical for pollen development and pollen tube reception

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Double fertilization is a complex process involving gametophytic development, pollen tube growth, guidance and reception, and gamete recognition and fusion. Pollen tubes perceive female cue to grow directionally toward the embryo sac, whose entrance induces programmed cell death (PCD) of a synergid, which in turn signals pollen tube discharge. Except for surface receptors such as FERONIA (FER), little is known regarding cellular components mediating this process. We report here function of adaptor protein 1 (AP1) in pollen development and pollen tube reception. *AP1Gs* encode the U subunit of the heterotetrameric AP1, which is critical for both anterograde and retrograde trafficking by mediating protein sorting at the *trans*-Golgi network/early endosome (TGN/EE). We show that functional loss of *AP1Gs* impaired the dynamic organization and acidification of vacuoles during pollen development, resulting in complete male gametophytic lethality. Pollen tube reception was also compromised such that tubes failed to arrest and discharge, resulting in partial female gametophytic lethality. We further demonstrated that pollen tube entry-induced synergid PCD is compromised by functional loss of *AP1Gs* due to defects in vacuolar dynamics and acidification. Indeed, mutation of vacuolar proton pump or genetic interference of vacuolar trafficking both compromised pollen tube reception. Our results demonstrate that AP1-mediated vacuolar dynamics and acidification, likely through sorting of key ion transporters, is critical for both pollen development and pollen tube reception.

Post-transcriptional / post-translational regulations

Posters 302 to 338

P302 - Arabidopsis PIP1 aquaporin expression depends on two major PIP2 isoforms

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Plasma membrane intrinsic protein (PIP) aquaporins are abundant membrane proteins, which are considered to play a role in regulating plant water status especially regarding transcellular water flow. *Arabidopsis* PIPs are comprised of two phylogenetic subgroups with five PIP1 and eight PIP2 members. PIP1 expression is dependent on PIP2;1 and PIP2;2, two major and abundantly expressed PIP2 isoforms; loss of PIP2;1 and PIP2;2 led to a parallel repression of PIP1 protein level in roots and leaves. The reduced PIP1 level was not due to repression of *PIP1* transcription or *PIP1* mRNA translation, since neither total transcripts nor ribosome-associated transcripts (assessed by translatome analysis from PIP2;2-expressing cells) were significantly altered. Therefore, the repression of PIP1 proteins takes place at the post-translational level. Translational fusions of two major PIP1 isoforms, PIP1;1 and PIP1;2, with EGFP- or HA-tags were used to complement the corresponding *pip1* mutants in an otherwise wild-type or *pip2;1 pip2;2* mutant background to investigate whether these PIP1s had been affected. Quantitative analysis of these lines indicated that both PIP1 isoforms had been repressed in *pip2;1 pip2;2*. Possible pathways of PIP1 degradation as deduced from the EGFP-tagged versions will be discussed. The dependence of PIP1 expression on PIP2;1 and PIP2;2 may be a means to more tightly or quickly regulate PIP1- and PIP2-related membrane water permeability.

P303 - Investigation of translational regulation and molecular mechanisms involved during the NB-LRR-mediated defense response in plants

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One major layer of plant immunity is conferred through NB-LRR proteins which recognize specific proteins encoded by pathogens and activate multiple signaling pathways. Translational repression of viral mRNAs upon NB-LRR response activation has already been described. In many cases, translational control occurs through the formation of cytosolic foci called Processing Bodies (PBs), where the translationally repressed RNAs are directed. PBs are dynamic and reversible structures composed by numerous proteins ensuring the decapping, the degradation or the storage of the viral RNAs. Using molecular imaging techniques and transient transformation of fusion PB proteins in *Nicotiana benthamiana*, we are studying the biogenesis and composition of PBs. With the use of a PB quantification assay, we find that NB-LRR activation induces a robust increase in the number of cellular PBs. Transcriptional reprogramming of endogenous genes has also been previously described. However, little is known about post-transcriptional gene regulation of this response, such as translational control of endogenous genes. Thus we used Translating Ribosome Affinity Purification followed by RNAseq (TRAPseq) as a mean to evaluate translational regulation of host mRNAs upon NB-LRR signaling activation, in *Arabidopsis thaliana*. Translatome analysis has revealed extensive translational regulation of hundreds of genes, and we have studied the effects on NB-LRR-mediated resistance of several novel candidate genes.

P304 (Talk) - Salt stress reveals new role of AGO1 in miRNA biogenesis pathway at both, co-transcriptional and posttranscriptional levels

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MicroRNAs control gene expression at the post-transcriptional level, by cleavage of mRNA targets or by inhibiting their translation. We applied the mirEX qPCR panel to test the influence of salt stress on *MIR* genes expression in *A. thaliana* cells. Our data indicate that the profile of 40% of all pri-miRNAs was significantly changed under salt stress conditions. Data obtained from the qPCR and northern blot analyses shows that the majority of pri-miRNA and miRNA follows in the same direction (both are up or down regulated) under stress conditions. These results indicate that those *MIR* genes are transcriptionally regulated. Selected examples were confirmed using ChIP technique and GUS system. We also observed *MIR* genes with down-regulation of pri-miRNA but increased level of mature miRNA (e.g. *MIR*161, *MIR*173). Such expression profiles indicate posttranscriptional regulation of miRNAs biogenesis. We have shown that miR161 and miR173 are more stable in salt stress condition and that AGO1 is responsible for this phenomenon. Additionally, we found that AGO1 is present not only in cytoplasm but also in nuclei. Using ChIP we have shown that AGO1 co-localizes on *MIR*161 and *MIR*173 genes and its level is increased under stress conditions. Parallely, we observed that RNA Pol II drops off during the transcription of these *MIR* genes. Our results indicate that AGO1 beside the last step of miRNA pathway plays also important role in co-transcriptional regulation of *MIR* gene expression.

P305 - The Roles of Arabidopsis CDF2 in Transcriptional and Posttranscriptional Regulation of Primary MicroRNAs

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The precise regulation of microRNA (miRNA) transcription and processing is important for eukaryotic development. Plant miRNAs are first transcribed as stem-loop primary miRNAs (pri-miRNAs) by RNA polymerase IIøthen cleaved in the nucleus into mature miRNAs by Dicer-like 1 (DCL1). We identified a cycling DOF transcription factor, CDF2, which interacts with DCL1 and regulates the accumulation of a population of miRNAs. CDF2 binds directly to the promoters of some miRNAs and works as a transcription activator or repressor for these miRNA genes. CDF2 binds preferentially to the pri-miRNAs regulated by itself and suppresses DCL1-mediated pri-miRNA processing. Genetically, CDF2 works in the same pathway as miR156 or miR172 to control flowering. We conclude that CDF2 regulates a group of pri-miRNAs at both the transcriptional and posttranscriptional levels to maintain proper levels of their mature miRNAs to control plant development. CDFs were identified to play roles in the blue light signaling. The regulation of miRNA abundance by CDF2 sheds light on the roles of miRNAs in the light signaling pathways.

P306 - Ferredoxin-NADP+ oxidoreductase is post-translationally modified in response to changing environment

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The changing environment challenges plants and requires rapid adjustment of plant metabolism. Shifts in enzyme activity, one of the fastest and most efficient metabolic responses, can be achieved through post-translational modifications (PTMs), which are known to affect the activity, interaction, as well as localization of proteins. Recent studies have shown that, besides protein phosphorylation, also acetylation, methylation and glycosylation of chloroplast proteins, are important in the regulation in plastid metabolism. Ferredoxin-NADP$^+$ oxidoreductase (FNR) is an important mediator linking the light reactions of photosynthesis to carbon assimilation. We have shown that both *A. thaliana* FNR isoforms existing as two distinct forms with different pI are partially N^A-acetylated. Additionally, the N-terminus of the distinct isoforms possessed alternative transit peptide cleavage sites. Both isoforms were found to contain acetylation of a conserved lysine residue near to the active site, while no evidence for *in vivo* phosphorylation was gained. Experimental evidence and structural modeling show that the membrane attachment of FNR or its direct interaction with ferredoxin is not affected by the identified modifications. However, the amounts of differently modified FNR forms change upon transfer from darkness to light, which implies involvement of the modifications on the regulation of FNR function.

P307 - Endogenous substrates and importance during dark stress of Arabidopsis XRN4 revealed by the polyA+ and polyARNA degradomes

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XRN4 (in plants) and XRN1 (in yeast and animals) are 5’ to 3’ exoribonucleases that function in cytoplasmic mRNA decay. An RNA degradome approach called Parallel Analysis of RNA Ends (PARE) was used to identify, on a global scale, the substrates of XRN4 and gain insight about the biological impacts of the enzyme. Analysis of the polyA+ and polyA- RNA degradomes separately showed that deadenylated transcripts were prominent substrates of XRN4, consistent with the current models of mRNA turnover. XRN4 was known to degrade the 3’ products of miRNA-guided cleavage of select target mRNAs. PARE confirmed and expanded on this showing that most 3’ cleavage products of miRNA targets with increased accumulation in *xrn4* are not deadenylated. Those RNAs that overaccumulate in *xrn4* mutants as precisely decapped decay intermediates were identified by comparison to data from C-PARE, a technique to determine cap sites. Among decapped XRN4 substrates were transcripts with Conserved upstream ORFs and those elevated in the Nonsense-mediated mRNA Decay (NMD) mutant *upf1*, providing evidence for the involvement of XRN4 in NMD. The deadenylated and polyadenylated decapped substrates of XRN4 were enriched for transcripts that encode photosynthesis and chlorophyll-binding functions. This led to the finding that XRN4 deficiency results in oversensitivity to prolonged darkness and is characterized by elevation of a sugar-starvation marker and decreased chlorophyll content in dark-grown plants.

P308 - SUMOylation at the crossroad between plant defences and temperature acclimation

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SUMOylation is a post-translational modification consisting in the establishment of an isopeptide bond between a SUMO protein and a lysine lateral chain of target proteins. 2 SUMO canonical isoforms are found in *Arabidopsis thaliana*: SUMO1 and SUMO2. They are almost identical (89% identity on protein sequence) and act redundantly. SUMOylation requires the sequential action of Activating (E1) and Conjugating (E2) enzymes. Importance of SUMOylation is underlined by the embryo-lethality of E1 or E2 mutants. For some targets, the action of a 3rd enzyme, named E3 ligase, is required. In *Arabidopsis*, only 2 E3s have been characterized: SIZ1 and HPY2. Interestingly, both *siz1* and *sumo1* amiR-*SUMO2* mutants display an autoimmune phenotype characterized by Salicylic Acid (SA) accumulation, spontaneous cell death, enhanced *Pseudomonas* resistance, defence marker gene expression (e. g . PR1/2), leading to dwarfism and partial sterility. In fact, this phenotype is similar to mutants with high levels of SNC1, an auto-active TIR-NB-LRR whose role is to enhance the SA defence responses. The phenotype of these high levels of SNC1 mutants are known to be totally temperature-dependent: plants grown at elevated temperature (here 28°C) are fully recovered. The dwarf phenotype of both *siz1* and *sumo1* amiR-*SUMO2* mutants are

SA dependent. We here show that at elevated temperature, while *siz1* mutants display a recovery like high levels of SNC1 mutants, *sumo1 amiR-SUMO2* mutants die. Unlike the dwarfism, this dying phenotype cannot be rescued in SA deficient plants. SIZ1-independent SUMOylation seems to be important for early stage development in elevated temperature. We also show that SIZ1 does not directly control SNC1 expression nor SNC1 stability: SIZ1 acts downstream of SNC1 and seems to act at the level of the PAD4/EDS1 hub of the SA pathway. We here uncouple SIZ1-mediated and SIZ1-independent SUMOylations.

P309 - Post-translational regulation of the root nitrate uptake transporter NRT2.1 in Arabidopsis Thaliana.

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In *Arabidopsis, NRT2.1* gene encodes the main component of the root NO_{3}^{-} High-Affinity Transport System (HATS). Due to the strong correlation generally found between root NO_{3}^{-} influx and *NRT2.1* mRNA level, it has been postulated that transcriptional regulation of *NRT2.1* is a key mechanism for modulation of the HATS activity. However, we showed that the abundance of NRT2.1 protein at the plasma membrane in response to light, sugars or high nitrogen supply is only slowly affected, unlike *NRT2.1* mRNA level or NO_{3}^{-} HATS activity, which showed much faster changes. Furthermore, the constitutive expression of *NRT2.1* under the control of a *35S* promoter did not prevent HATS activity in the roots to be down regulated in response to repressive N or dark treatments that strongly reduce *NRT2.1* transcription and NO_{3}^{-} HATS activity in the wild type plants. In addition, changes in abundance of NAR2.1 closely followed those of NRT2.1, and thus could not explain the close-to-normal regulation of the HATS in the *35S::NRT2.1* transformants. These results confirmed that post-translational regulatory mechanisms are involved in the regulation of NRT2.1 activity. In order to elucidate this level of regulation, we recently started a phosphoproteomic approach combined with NRT2.1 phospho-peptides quantification. Some preliminary results will be presented.

P310 - The nucleolar GTPase Nucleostemin-like 1 plays a role in plant growth and senescence by modulating ribosome biogenesis

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Nucleostemin is a nucleolar GTP-binding protein that is involved in stem-cell proliferation, embryonic development, and ribosome biogenesis in mammals. Plant nucleostemin-like 1 (NSN1) plays a role in embryogenesis, and apical and floral meristem development. In this study, we identified a nucleolar function of NSN1 in the regulation of ribosome biogenesis. GFP-fused NSN1 localized to the nucleolus, which was primarily determined by its N-terminal domain. Recombinant NSN1 and its N-terminal domain (NSN1-N) bound to 25S and 18S ribosomal RNAs (rRNAs) *in vitro*. Recombinant NSN1 expressed GTPase activity, which was diminished by mutations in GTP-binding motifs. *NSN1* silencing in *Arabidopsis thaliana* and *Nicotiana benthamiana* led to growth retardation and premature senescence. NSN1 interacted with Pescadillo and EBNA1 binding protein 2 (EBP2), which are nucleolar proteins involved in ribosome biogenesis, and with several ribosomal proteins. NSN1, NSN1-N, and EBP2 co-fractionated primarily with the 60S ribosomal large subunit *in vivo*. Depletion of NSN1 delayed 25S rRNA maturation and biogenesis of the 60S ribosome subunit, and repressed global translation. NSN1-deficient plants exhibited premature leaf senescence, excessive accumulation of reactive oxygen species, and senescence-related gene expression. Taken together, these results suggest that NSN1 plays a crucial role in plant growth and senescence by modulating ribosome biogenesis.

P311 - Structural determinants for pri-miR156a processing in ambient temperature-responsive flowering time control

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Plant miRNAs are playing an important regulatory role in plant development. MiR156 is important regulator for flowering time control in the response to ambient temperature in Arabidopsis. In this study, we performed structure-function analaysis of Arabidopsis pri-miR156s in ambient temperature responsive-flowering time. All region of upper stem is important and the upper stem region closer to the miR156/miR156* duplex is more important for pri-miR156a processing at 23°C. Additional pairing and non-pairing mutation in lower stem had effect to repress and enhance miR156 processing, respectively. NMR analysis revealed that less stable base-pair of second cleavage site of pri-miR156a is more effective to pri-miR156a processing. Moreover, we proposed the key role of second stem from miRNA/miRNA* duplex of pri-miR156a and pri-miR172a on the region where first cleavage occurs for ambient temperature-responsive flowering. Our finding provides a clue to decode the information embedded in the sequence of miRNA in ambient temperature-responsive flowering.

P312 - Identification and biochemical characterization of a new class of tyrosine phosphatase in Arabidopsis thaliana.

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The study of tyrosine phosphorylation in plants has been neglected due to the lack of classic tyrosine kinases and tyrosine phosphatases. However, thanks to advanced phosphoproteomics studies, it has been revealed that the abundance of phospho-tyrosine residues in plants rivals humans. This strongly suggests that in plants tyrosine phosphorylation is as important as in humans, yet we know nothing about the players responsible of these events. The *Arabidopsis thaliana Rhizobiales*-like phosphatase 2 (AtRLPH2) is a novel protein phosphatase, not found in mammals which, according to bioinformatics analysis, clusters with the serine/threonine specific phospho-protein phosphatase (PPP) group. AtRLPH2 has not been characterized however, we have demonstrated *in vitro* that the recombinant AtRLPH2 surprisingly behaves like a tyrosine phosphatase. AtRLPH2 is not affected by the classic PPP inhibitors but in turn it was shown to be inhibited by the specific tyrosine phosphatase inhibitor, sodium orthovanadate. Moreover, AtRLPH2 dephosphorylates phospho-tyrosine peptides having essentially no activity towards phospho-serine/threonine residues. To support the phospho-tyrosine phosphatase nature of the bacterially expressed AtRLPH2, the endogenous AtRLPH2 from *Arabidopsis thaliana* was purified by immunoprecipitation and was indeed shown to preferentially dephosphorylate tyrosine phosphorylated peptides. Furthermore, a 56 kDa protein, which is heavily tyrosine phosphorylated and is commonly used in human phospho-tyrosine phosphatase studies (GST-Fer), was also readily dephosphorylated by the endogenous AtRLPH2. This is the first example of a supposed serine/threonine specific phosphatase that behaves as a tyrosine phosphatase in plants. To gain a better understanding of AtRLPH2 significance and targets, a phosphoproteomics study is currently being undertaken to compare tyrosine phosphorylated peptides from wild type (WT) and *atrlph2* knock out plant lines. Finally, a preliminary phenotypical study has shown that seeds lacking AtRLPH2 germinate faster than WT plants under standard conditions suggesting AtRLPH2 involvement in repressing the initiation of germination.

P313 - Diverse Roles of Chloroplast-Targeted RNA-Binding Proteins in the Growth, Development, and Stress Response of Arabidopsis thaliana

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The chloroplast genome harbors only 130-150 genes in its own circular genome, but recent studies on the chloroplast proteome have revealed that thousands of nuclear-encoded proteins are targeted to chloroplasts and play essential roles in chloroplast gene expression, biogenesis, and function. Despite the increasing understanding on the functional roles of nuclear-encoded RNA-binding proteins (RBPs) in chloroplasts, the physiological and cellular function of a majority of chloroplast-targeted RBPs remain largely unknown. To understand the functional roles of nuclear-encoded chloroplast-targeted RBPs in plant growth, development, and stress responses, we determined the functional roles of a CRM-domain protein CFM4 and a S1-domain protein SDP1 in *Arabidopsis thaliana*. The loss-of-function *cfm4* and sdp1 mutants displayed delayed growth, abnormal chloroplast biogenesis, and reduced photosynthetic activity under normal and stress conditions. These abnormal phenotypes resulted from the impaired processing or maturation of chloroplast rRNAs. Importantly, CFM4 and SDP1 possessed RNA chaperones activity that aids in correct folding of RNA substrates during these cellular processes. Collectively, these results suggest that nuclear-encoded chloroplast CFM4 and SDP1 play important roles in plant growth, development, and stress responses by participating in chloroplast RNA metabolism. [Supported by grants from NRF and Next-Generation BioGreen21]

P314 - Transcriptome and translatome have distinct roles in the regulation of Arabidopsis seed germination

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Dormancy is a complex evolutionary trait that temporally prevents seed germination. High throughput analyses of transcriptomes have led to significant progress in the understanding of the molecular regulation of this process but the role of post-transcriptional mechanisms has received little attention. We have studied the dynamics of mRNAs association with polysomes and compared the transcriptome to the translatome in dormant and non-dormant seeds of *Arabidopsis thaliana* during their imbibition at 25°C in the darkness, a temperature preventing germination of dormant seeds only. DNA microarrays analysis revealed that 4670 and 7028 transcripts were differentially abundant in dormant and non-dormant seeds in the transcriptome and in the translatome, respectively. Using SeedNet network, in which co-expressed genes associated with germination or dormancy have been clustered in 3 topographic regions, we show that transcripts associated with polysomes in dormant seeds are localized randomly and not only within the "dormancy" region as predicted by the network. This clearly highlights the discrepancy between transcription and translation in the context of seed germination. Analysis of functions of polysome-associated transcripts in dormant and non-dormant seeds allowed to reveal actors of seed dormancy and germination. In conclusion our results demonstrate that the regulation of germination is more related to changes in the translatome than to a transcriptional regulation.

P315 - Discovery of arginine citrullination in Arabidopsis thaliana

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Protein citrullination is a post-translational modification implicated in an increasing number of biological processes in animals including histone modifications, transcriptional regulation as well as a number of pathologies. It occurs when arginine side chains are deiminated and converted into an amino acid citrulline, a process carried out by Ca2+-dependent peptidylarginine deiminases (PADs; EC 3.5.3.15). However, to-date citrullination has not been reported in higher plants. Here we show, firstly, that the *Arabidopsis thaliana* proteome does contain citrullinated proteins and secondly, that the citrullination signature changes in response to cold stress. Using proteomics, we noted that out of 14 citrullinated proteins, seven are DNA- or RNA-binding proteins and two are annotated as protein-binding indicating roles in cell signalling and/or regulation. Based on the cold-induced expression in Arabidopsis agmatine iminohydrolase that conceivably operates as PAD, we also predict that specific citrullination events occur in response to low temperature stimuli.

P316 - Stability of VNI2 protein is regulated by RING finger proteins

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A NAC domain transcription factor VNI2 has been isolated as an interacting factor with another NAC domain transcription factor VND7, a master regulator of xylem vessel formation. VNI2 negatively regulates the xylem vessel differentiation by inhibiting VND7 activity. Here, to understand how VNI2 is controlled during the differentiation, we screened interacting factors with VNI2 by using yeast two-hybrid system. When full length VNI2 was used as a bait, a cDNA encoding a RING finger protein was isolated. The RING finger protein is known to be a member of a family and act as an ubiquitin E3 ligase by forming complex with other proteins. VNI2 lacking the C terminal region containing a PEST motif is unable to bind to the RING finger protein. We generated a mutant line, which seems to reduce in the ubiquitin-E3 ligase activity. VNI2 protein is more stable in the mutant than that in wild type plants. Furthermore, discontinuous xylem vessels are observed in cotyledon of the mutant. These results suggest that the RING finger proteins regulate stability of the VNI2 protein, and the control of the VNI2 protein stability plays important roles in normal xylem vessel formation.

P317 - Regulation of the Arabidopsis translation machinery by the TOR (Target of Rapamycin) kinase.

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The evolutionary conserved TOR (Target Of Rapamycin) kinase regulates growth and development in response to environmental cues, including nutrients and hormones. Indeed, in animal and yeast, the TOR signaling pathway controls essential biological outputs like mRNA translation, ribosome biogenesis, autophagy and primary metabolism. We first show that TOR inactivation results in a decrease in polysome abundance. Next we performed a proteomic and phosphoproteomic study of the ribosomal fraction in response to TOR inactivation, together with transcriptomic and translatomic analyses. Thus we identified ribosomal protein families which expression is affected by the decrease in TOR activity. In animal and yeast cells, TOR controls the phosphorylation of the ribosomal protein S6 (RPS6) through activation of the S6 kinase. The phosphoproteomic analysis of ribosomal proteins also indicated that TOR inactivation in Arabidopsis seems to lead to a decreased phosphorylation status of RPS6. In conclusion this study revealed putative targets of the TOR kinase in the plant ribosome fraction which can be controlled either at the transcriptional, translational or phosphorylation levels.

P318 - A role for the DENTICULATA genes in the regulation of gene expression

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In Arabidopsis, loss-of-function alleles of a number genes encoding ribosomal proteins (*rp*) cause weak developmental phenotypes and synergistically interact with mutations in the *AS1 (ASYMMETRIC LEAVES 1)* and *AS2* genes. AS1 is a MYBdomain protein and AS2 a plant-specific nuclear protein belonging to the LATERAL ORGAN BOUNDARIES family. The rp as double mutants exhibit strong alterations in leaf dorsoventrality, an observation that points out the possible involvement of translational regulation in plant development. We isolated Arabidopsis mutants exhibiting pointed and dentate leaves, which we termed *denticulata (den)*; these mutants were induced by EMS and fast neutrons and found to fall into 19 complementation groups. We positionally cloned five *den* mutations, all of which resulted to be alleles of genes encoding ribosomal proteins. Map-based (positional) cloning has traditionally been the preferred strategy for identifying the causal genes underlying the phenotypes of mutants isolated in forward genetic screens. Massively parallel sequencing technologies are enabling the rapid cloning of genes identified in such screens. To identify the remaining 14 *DEN* genes, we are using a combination of linkage mapping and whole-genome sequencing.

P319 - Ribosome biogenesis requires RRP7 and NOP53 in Arabidopsis

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We identified *mas2 (morphology of argonaute1-52 suppressed 2)* alleles as informational suppressors of *ago1-52*, a hypomorphic allele of *AGO1*. The *MAS2* gene was positionally cloned and found to encode the Arabidopsis ortholog of NKAP (NF-kappa B activating protein), a conserved protein involved in transcriptional repression in animals. A yeast two hybrid assay with MAS2 as a bait identified 14 interactors, two of which are the putative orthologues of proteins known to participate in ribosome biogenesis in *Saccharomyces cerevisiae*: Ribosomal RNA Processing Protein 7 (RRP7) and Nucleolar Protein 53 (NOP53). The Arabidopsis *rrp7-1* and *nop53-1* insertional mutants exhibit pointed and reticulated leaves, which are traits shared by many mutants defective in ribosome biogenesis. We obtained transgenic plants expressing the NOP53:GFP and RRP7:GFP translational fusions and found that both are nuclear proteins. We also obtained double mutant combinations of n*op53-1* or *rrp7-1* and loss-of-function alleles of genes required for ribosome biogenesis and the microRNA pathway; the phenotypes of most of these double mutants resulted to be synergistic.

P320 - Silencing of green fluorescent protein gene present in plastid genome of Nicotiana benthamiana

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RNA interference (RNAi) is a conserved biological process to regulate the activity of genes by causing the destruction of specific mRNA molecules in many different organisms. The regulation of RNA silencing generally takes place in cytosol following micro RNA or small interfering RNA (siRNA)-loaded RNA-induced silencing complex binding to the target mRNA. Although certain small non-coding RNAs have been identified in chloroplasts, it remains largely unknown whether subcellular organelles such as chloroplasts are also regulated by RNAi. To investigate whether the RNA silencing is processed in chloroplast, we experimented *GFP* gene silencing in leaves of two different *Nicotiana benthamiana* transgenic plants, encoding *GFP* gene inserted in nuclear or plastid genome, respectively, by Agrobacterium-mediated transient expression assay. Microscopy established that the exogenous *GFP* overexpression induces disappearance or decline of the fluorescence from the both cytosol and chloroplast, and siRNAs derived from GFP (siGFP) were identified with total RNA from the infiltrated leaves by small RNA gel blot assay. These results imply that the RNA silencing event may be subsumed under the gene expression regulation of chloroplast. Due to none of component unidentified in chloroplast, which functions in RNA silencing mechanism, further studies are in progress to examine whether the siRNA of *GFP* is generated in or transferred into chloroplast, then it is able to induce the gene silencing.

P321 - Post-transcriptional regulation of the paternal patterning cue SHORT SUSPENSOR

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In *Arabidopsis thaliana*, the suspensor is a mostly extra-embryonic structure that supports embryo development but also contributes to the embryonic root. It emerges from the basal daughter cell after the first zygotic division. The decision between embryonic development of the apical daughter cell and extra-embryonic cell fate in the suspensor is regulated by the YODA (YDA) MAPKK Kinase pathway. Upstream of YDA acts SHORT SUSPENSOR (SSP), a membrane-bound pseudo-kinase, which is regulated in a remarkable manner on both the transcriptional and translational level. *SSP* is solely transcribed in sperm cells, but is not translated there, at least not on a detectable level. After fertilization, paternally inherited *SSP* transcript is translated in the zygote, where the protein transiently activates the YDA pathway. So far, the mechanism how the accumulation of SSP protein in sperm cells is prevented and how this block is removed in the zygote is unclear. Here we provide evidence that transposable elements in the 5'UTR region of *SSP* seem to mediate the translational block in sperm cells. We show that this translational regulation is connected to the recently described epigenetically activated small interfering RNA (easiRNAs) pathway. Furthermore, we found that precocious translation of *SSP* transcript variants in sperm cells seems to affect sperm fusion competence.

P322 (Talk) - Degradation of BES1 mediated by adaptor protein BIP5 controls the balance between plant growth and stress responses in Arabidopsis

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BES1 is a central transcription factor in the Brassinosteroid (BR) signaling pathway that controls the expression of thousands of genes involved in plant growth and development. Despite extensive characterization of BES1 in carrying out BR-related gene expression, little is known regarding how levels of BES1 protein are regulated. By screening for BES1 interacting partners, we have identified BES1 Interacting Protein 5 (BIP5) as an adaptor that delivers BES1 to the proteasome and autophagy machinery for degradation. Degradation of BES1 might provide a switch by which plants can shut down growth when unfavorable environmental conditions are encountered. In support of this hypothesis, knockdown of BIP5 in *BIP5 RNAi* plants results in accumulation of BES1 protein and susceptibility to drought or nutrient deprivation. Cell biological studies reveal that BES1 is targeted to the autophagy pathway during stress conditions through interaction with ATG8, a ubiquitin-like protein that directs autophagosome formation and cargo recruitment. In addition, we found that BIP5 is a substrate of the GSK3-like kinase BIN2, a negative regulator in the BR signaling pathway. Phosphorylation of BIP5 by BIN2 promoted its interaction with ATG8, thereby targeting BES1 for degradation. Our results revealed a new mechanism by which plants coordinate growth and stress responses by regulating BES1 protein levels through integration of BR signaling and autophagy pathways. This research is supported by NSF (IOS1257631).

P323 - Role of RING E3 ubiquitin ligases in B. cinerea flower infection

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Flowers are susceptible to pathogen attacks affecting fruit yield and quality. *Botrytis cinerea* is an air borne plant fungal pathogen able to infect and rot a broad spectrum of economically important crops. For a long time *B. cinerea* has been studied using *A. thaliana* leaf infection assays. However, in several crops B. *cinerea* infection takes places in flower rather than leaves. Although *B. cinerea* wreaks havoc at flower no studies have been carried out in such tissues from a molecular point of view. In this study we have developed a flower defense genes selection work flow using gene ontology (GO), plant ontology (PO) and public gene expression databases. From these data we have chosen as working candidate a RING E3 ubiquitin (ub) ligase that shows high transcription up regulation under *B. cinerea* infection. In order to further characterize the molecular role and regulation of this E3 Ub ligase in pathogen defense responses, we performed Y2H and TAP screens and found five proteins that might be involved in pathogen defenses responses, representing SUMO E3 ligase homologs, homeotic and Myb transcription factors, two disease resistance proteins. The main objective of this study is to establish the molecular role of the E3 Ub ligase candidate and its target in plant-pathogen responses at flower level by phenotype analysis, *in vitro* interactions and ubiquitination assays, offering a novel approach to study plant-pathogen responses.

P324 - New insights into the regulation of the vacuolar antiporters NHX1 and NHX2 activity by pH and phosphorylation

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The tonoplast-localized K^+,Na^+/H^+ exchangers NHX1 and NHX2 of *Arabidopsis* are involved in the accumulation of K^+ into the vacuole of plant cells, thereby increasing the osmotic potential, water uptake and the turgor pressure necessary for cell expansion and growth. These proteins are also required for vacuolar remodelling during stomatal movements. Phosphoproteomic analyses and preliminary results from our laboratory strongly support that NHX1 and NHX2 function is regulated by phosphorylation of their C-terminal domain by CBL-interacting protein kinases (CIPKs). Functional and *in vitro* phosphorylation assays demonstrating a differential regulation of NHX1 and NHX2 by CIPKs will be presented. We have also identified by phylogenetic analysis and computational modelling of the NHX1 protein various structural domains and amino acid residues putatively involved in ion transport, cation coordination, and pH sensing of NHX1. Point-mutation alleles for these relevant residues have been generated to analyse their impact in the biochemical activity and pH dependence of NHX1 by functionality tests in yeast and *in vitro* ion transport assays. Our results point to the fine-tuning of NHX1 and/or NHX2 activity in response to developmental and environmental cues. In addition, we expect to unravel the biochemical mechanisms for pH sensing and regulation of these critical K+ transporters of *Arabidopsis*.

P325 - Light signals regulating alternative splicing in Arabidopsis

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Light is a source of energy and also a regulator of plant physiological adaptations. We recently show that light/dark conditions affect alternative splicing of a subset of Arabidopsis genes preferentially encoding proteins involved in RNA processing. The effect requires functional chloroplasts and is also observed in roots when the communication with the photosynthetic tissues is not interrupted, suggesting that a signaling molecule travels through the plant. We are now trying to identify the nature of the light signals that communicate the chloroplast status to the nuclei of leaf and root cells. By using different approaches we want to elucidate the roles of sugars as possible mobile signals and also the involvement of the spliceosome and the RNA polymerase II as possible targets and/or effectors of these light signaling pathways.

P326 - Comprehensive analysis of tRNA-derived small RNAs biogenesis in plants

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In the last few years different classes of endogenous small non-coding RNAs were identified and described in diverse species with the use of advanced high-throughput sequencing technology (NGS) and computational analyses. Among them, one originating from canonical tRNA molecules seems to be the most evolutionary conserved and thus represented in all three domains of life. tRFs (tRNA-derived fragments) of 15-26 nucleotides in length have long been regarded as random byproducts of tRNA biogenesis or degradation processes, but emerging evidence demonstrates that they are precisely generated through a specific cleavage pattern. Using *Arabidopsis thaliana* as a model organism, we aim at identification and characterization of the components involved in tRNA and microRNA biogenesis pathways that may play a role in generation of tRFs. The RNAs sequences used for this analysis were obtained using sRNA libraries from over 30 A. thaliana mutant lines carrying mutations in genes associated with tRNA and microRNA biogenesis. Interestingly, bioinformatic analysis of our NGS data revealed the existence of small RNAs deriving not only from mature tRNAs but also from tRNA flanking regions embedded within longer noncoding transcripts synthesized by RNA polymerase II.

This work was supported by grants from the National Science Center UMO-2011/03/B/NZ2/01416 and UMO -2013/11/N/NZ2/02511.

P327 - MAS2, the Arabidopsis ortholog of human NKAP, regulates 45S rDNA transcription

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We conducted a screen for suppressors of *Arabidopsis thaliana ago1-52*, a hypomorphic allele of *AGO1 (ARGONAUTE1)*, a key gene in microRNA pathways. We identified nine extragenic suppressors as alleles of *MORPHOLOGY OF AGO1-52 SUPPRESSED 2 (MAS2)*. Positional cloning showed that *MAS2* encodes the putative ortholog of NKAP (NF-kappa B activating protein), a conserved eukaryotic protein involved in transcriptional repression and splicing in animals. The *mas2* mutations behave as informational suppressors of *ago1* alleles that cause missplicing. *MAS2* is a single-copy gene whose insertional alleles are embryonic lethal. The artificial microRNA *amiR-MAS2* partially repressed *MAS2* and synergistically interacted with a loss-of-function allele of *AtNuc-L1*, which encodes NUCLEOLIN1, which affects epigenetic control of 45S rDNA expression. 45S rDNA promoters were hypomethylated in *amiR-MAS2* plants, indicating that MAS2 negatively regulates 45S rDNA expression. In yeast two-hybrid assays, MAS2 interacted with splicing and ribosome biogenesis proteins, and fluorescence *in situ* hybridization showed that MAS2 co-localized with 45S rDNA at the Nucleolar Organizer Regions. Our results thus reveal a key player in the regulation of rRNA synthesis in plants.

P328 - Arabidopsis Proteomics: Concurrent Understanding of Diurnal Protein Levels and Post-Translational Modifications

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The circadian clock is a series of molecular encoded negative feedback loops that precisely regulate global gene expression in a coordinated 24 hour cycle. As a result, the circadian clock is responsible for controlling numerous physiological processes central to plant growth and survival. To date, the vast majority of circadian clock studies have relied on the changing transcriptome to infer molecular connections between the circadian clock and observable plant phenotypes. Recent findings have shown however, that a disconnect between changing transcripts and proteins exists in circadian clock mutants, indicating that clock regulatory mechanisms beyond gene expression may also be important to its far reaching biological influence. In light of this, protein level changes and post-translational modifications (PTMs) have been considered as alternative points of regulation. In particular, reversible protein phosphorylation, which represents the most prolific of the known PTMs, has been characterized to regulate upwards of 70% of proteins in eukaryotes, including core-clock proteins in plants. Using a variety of quantitative proteomics approaches we endeavored to uncover new input and output components of the circadian clock through concurrent assessment of the changing *Arabidopsis thaliana* rosette proteome and phosphoproteome over a 24 hour time-course.

P329 - Secondary structure of target mRNAs impacts the strength of microRNA regulation in Arabidopsis.

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In both plants and animals, micro(mi)RNAs have emerged as key regulators of gene expression. To date, the degree of sequence complementarity between a miRNA and its target mRNA is thought to be the decisive factor in target recognition and the strength of silencing, thus constituting the predominant basis of many miRNA target prediction programs. However, of the multitude of computationally predicted targets, typically only a subset appears functionally relevant, implying that there are factors beyond complementarity influencing miRNA regulation. Using the *Arabidopsis* miR159 system, we have shown that of the eight conserved *MYB* target genes who have bioinformatically analogous miR159 binding sites, only *MYB33* and *MYB65* are strongly regulated. Unlike poorly regulated targets, *MYB33/65* contain highly conserved nucleotides flanking their miR159 binding sites, which are predicted to form highly similar, highly conserved RNA secondary structures with two stem-loop regions (SL1 and SL2). Disruption of SL1 and SL2 in *MYB33* without altering the miR159 binding site resulted in strong perturbation of miR159 regulation. Conversely, compensatory mutations to restore SL1 and SL2, but not the wild-type sequence, re-established strong regulation of *MYB33*, demonstrating that these local RNA secondary structures confer strong silencing. Currently, we are trying to understand the mechanism by which these structures facilitate miR159 regulation and whether RNA-binding proteins are involved.

P330 - Validation of SUMO conjugates in Arabidopsis mesophyll protoplasts

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The covalent attachment of SUMO (small ubiquitin-related modifier) to target proteins constitutes a mechanism of protein regulation and modulates many biological processes in eukaryotes. The characterization of SUMO targets is a key factor for understanding SUMO biological role. This process has two critical steps: the identification of putative SUMO conjugates and their validation as bona fide SUMO targets. This second step can be approached by *in vitro* SUMO conjugation assays, which may be compromised by difficulties in the expression of recombinant proteins in bacterial expression systems, or by transient expression in homolog systems such as *Arabidopsis* protoplasts. The transient expression in protoplasts, in addition to overcome the limitations of soluble protein expression, also provides a valuable biological system to analyze the dynamics of SUMO conjugation of specific targets in response to hormones or other physiological stimuli. We will present a detailed protocol for analyzing SUMO conjugation in *Arabidopsis* mesophyll protoplasts.

P331 - Circadian control of seasonal rhythms in the Arabidopsis proteome

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Plants respond to seasonal cues in order to anticipate the expected environmental changes that accompany the changing seasons. A key environmental cue in this regard is the duration of daylight i.e. the photoperiod. In the EU FP7 TiMet project, we have been examining the wide-ranging effects that photoperiod has on metabolism and growth. One example of this is in the regulation of translation, with protein synthesis proceeding more rapidly in the light than in the dark. For transcripts whose rhythmic expression is under the control of the circadian clock, it is therefore expected that the timing of transcript expression will determine how protein synthesis changes with photoperiod. In order to quantitatively test this hypothesis, we integrated several genome-scale transcript and protein datasets, allowing integrated analysis of ~2400 genes. By combining measured transcript dynamics and measured relative rates of total protein synthesis in the light vs. the dark we successfully predicted changes in protein levels across photoperiods, demonstrating that this post-transcriptional mechanism contributes significantly to the photoperiodic adjustment of the Arabidopsis proteome. This finding was further corroborated by examination of transcript-specific changes in polysome loading in the light and dark. These results demonstrate a significant role for the circadian clock in mediating photoperiod responses in Arabidopsis far beyond well known examples such as flowering time.

P332 - CULLIN neddylation is regulated during seed development in Arabidopsis thaliana

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Cullin-RING E3 ligases (CRLs) are the most abundant class of E3 ubiquitin ligases in plants. CRLs regulate different aspects of plant development from hormone and light signaling to organ growth. CRLs are activated by modification of their cullin subunit with the ubiquitin-like protein Nedd8 (neddylation) and deactivated by Nedd8 removal (deneddylation). CRL deneddylation is carried out by the COP9 signalosome (CSN), an eight-subunit complex conserved during evolution. Plants lacking the CSN accumulate CRLs in their neddylated form and are impaired in several developmental pathways, which culminate in growth arrest at the seedling stage. We have monitored the cullin deneddylation/neddylation status from embryo to early seedling development in wild-type and *csn* mutants. We show that the majority of cullin proteins is progressively neddylated during the late stages of seed maturation and becomes deneddylated upon seed germination. This developmentally regulated shift in the cullin deneddylation/neddylation ratio correlates with changes in free Nedd8 levels and does not occur in *csn* mutants, in which cullins are constitutively neddylated. The balance of deneddylated vs neddylated cullin seems also to be constant during vegetative growth, since, under our experimental conditions, both embryos and seedlings have the same cullin deneddylation/neddylation ratio. Taken together, our results indicate that the balance of deneddylated *vs* neddylated cullin is tightly regulated during plant growth and development.

P333 - Regulation of the nonsense-mediated mRNA decay (NMD) pathway and its involvement in snoRNA homeostasis

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The nonsense-mediated mRNA decay (NMD) pathway is a eukaryotic post-transcriptional RNA surveillance mechanism that has a great impact on the plant transcriptome. NMD affects the stability of both aberrant transcripts and normal protein-coding mRNAs. The affected transcripts have cis-elements that can be recognized as pre-mature termination codons. These elements include features that are abundant in normal mRNAs such as upstream open-reading frames (uORFs). The regulation of the NMD pathway is not well understood. We found that the *Arabidopsis* NMD factor UPF3 is regulated by a negative feedback loop that targets its own transcript for degradation by NMD. We then studied the physiological significance of this feedback loop for the overall regulation of plant NMD by expression of a de-regulated form of *UPF3*, which could not be targeted by NMD. A moderate increase in *UPF3* expression resulted in over-degradation of certain transcripts and inhibited NMD of other transcripts, including genuine NMD targets. This indicates that delicate balancing of *UPF3* transcript levels by its feedback loop plays a crucial role in NMD buffering. We also found that small-nucleolar RNAs (snoRNAs) constitute a major group of transcripts whose levels are indirectly affected by NMD. Most snoRNAs are involved in modifying ribosomal RNAs and spliceosomal small-nuclear RNAs. Our findings also highlighted differences between plant and animal cells with respect to NMD regulation and impact on snoRNA levels.

P334 - Natural variation among Arabidopsis thaliana accessions in expression of miR826 and corresponding alkenyl glucosinolate synthesis

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ccurring polymorphisms within *MIRNA* genes have the potential to affect biogenesis efficiency of their mature microRNA (miRNA) and consequently contribute to phenotypic diversity among accessions within a given species. To illustrate the linkage between *MIRNA* genes and corresponding phenotypic diversity, we analyzed a polymorphic miRNA that targets a biosynthetic gene in the production of glucosinolates, the main chemical defense compounds of *Arabidopsis thaliana*. In the glucosinolate biosynthesis pathway, a 2-oxoglutarate-dependent dioxygenase (AOP2) is responsible for converting methylsulfinylalkyl glucosinolates to alkenyl glucosinolates. The *AOP2* transcript is targeted by miR826, the gene of which shows sequence variation among natural accessions. In this study, we found that *MIR826* genes from nineteen different accessions can be divided into two groups according to their sequences. The sequence variation between these two groups causes different levels of mature miR826 expression. *AOP2* expressions among these accessions also vary between the two groups, but interestingly the miR826 and *AOP2* expression levels show a positive correlation. Furthermore, alkenyl glucosinolate levels among these accessions do not correlate with *AOP2* expression level. Our result exemplifies that MIRNA gene polymorphisms are able to affect mature *miRNA* biogenesis and corresponding target expression.

P335 - Identification of circular RNA in Arabidopsis

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Non-coding RNA varies, in term of function, length, and structure. Circular RNA, a type of non-coding RNA, has been rediscovered by comprehensive analysis using next generation sequencing. In animals, some functional circular RNAs have been identified and there are some reports regarding circular RNA biogenesis. Few circular RNAs have also been observed in plants; however, no functions have been reported for these. We conducted RNA-seq in seedlings, leaves, buds and flowers of *Arabidopsis* using RNase R, an exonuclease that digests linear RNA only. We detected circular RNA candidates through computational analysis and confirmed experimentally that they are circularized. This work will enable us to explore new functional circular RNAs and this initial step will help us to understand how circular RNA contributes to tissue-specific gene regulation.

P336 - AtKBR1 limits the levels of KNOX homeodomain transcription factors in Arabidopsis

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KNOTTED1-like homeobox (KNOX) proteins such as *Arabidopsis thaliana* SHOOTMERISTEMLESS (STM) or BREVIPEDICELLUS (BP/KNAT1) interact via their MEINOX domain with BEL1-like homeodomain proteins (BLH). KNOX-BLH heterodimerization affects subcellular localization and target site specificity of these transcription factors involved in regulation of developmental processes such as patterning or meristem maintenance. Expression of *STM* is strictly controlled: it is limited to meristematic cells and excluded from developing leaf primordia. Curiously STM and KNAT1 were shown to move from cell to cell via plasmodesmata, and it is unknown how meristem boundaries can be maintained despite STM mobility. We identified AtKBR1 (KNOX-BINDING and REDUCING 1) as an interaction partner of certain KNOX and BLH proteins. The small, plant specific AtKBR1 harbors a predicted coiled-coil domain and specifically binds to the KNAT1 MEINOX domain. AtKBR1 accumulates in the nucleus, but the interaction between AtKBR1 and KNOX proteins takes place in the cytoplasm. The expression domains of *AtKBR1, STM* and *KNAT1* overlap in the shoot apical meristem. Notably, AtKBR1 protein is partially instable in the meristem, moves to adjacent regions, and suppresses ectopically expressed KNOX protein levels by a yet unknown mechanism. Based on these observations we propose that AtKBR1 functions to regulate STM and KNAT1 protein presence at the boundaries of the shoot apical meristem.

P337 - Functional characterization of two nutrient-induced kinases

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The transmembrane receptor kinase family is the largest protein kinase family in Arabidopsis, and it contains the highest fraction of proteins with yet uncharacterized functions. SIRK1, a receptor kinase, has been identified in sucrose-induced conditions (Niittyla et al, 2007, MCP) and was shown to be involved in regulation of aquaporins, sugar transporters (Wu et al, 2013, MCP). In the proposed model, receptor-like kinase SP3 has been found as one of interaction partners and phosphorylation target of SIRK1 under sucrose-induced condition (Wu et al, 2013, MCP).

Aim of project: Using a combination of single and double knockout mutants (*sirk1, sp3* and *sirk1/sp3*), phospho-profiling and activity measurements, we aim at dissecting the functions of the sucrose-induced kinase complex.

Procedure of experiment: Roots of single and double knockout mutants, harvested under sucrose-free and sucrose-resupplied conditions, were used for microsome preparation and phosphopeptides enrichment. Enriched phosphopeptides were measured by mass spectrometry (Q-exactive). Phosphopeptides were identified and quantified by Maxquant. Data analysis based on cRacker, Perseus. Method of kinase assay was from Wu et al, 2013, MCP.

Results: Sucrose can induced kinases activities of SIRK1 and SP3 in root. Phosphorylation profiling contributes to find phosphorylation targets of SIRK1 and SP3 under sucrose-starvation and resupply condition. SIRK1, SP3 and their complex are involved in regulating transport process and have functions in cellular signaling. In vitro kinase assays can help us find direct targets of SIRK1 and SP3.

P338 - Identification and characterization of novel factors involved in regulatory mechanism on COP1 and/or COP1-regulated processes via a genetic screen

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CONSTITUTIVE PHOTOMORPHOGENESIC 1 (COP1) possesses E3 ubiquitin ligase activity, and is evolutionally conserved from plants to human. COP1 plays a key role in multiple developmental processes including photomorphogenesis, flowering, circle clock, root development, stomatal opening, shade avoidance, brassinosteroid signaling, and cold acclimation in *Arabidopsis*. While mammalian COP1 mediates the carcinogenesis and tumorigenesis. To explore the novel regulators of COP1 and/or COP1-regulated development, we performed a genetic screen for mutants that suppressed *cop1-6* constitutive photomorphogenic phenotype in darkness. Via a detailed genetic characterization, we obtained 41 extragenic recessive *cop1* suppressors that may define 15 different novel candidate *cop1* suppressor loci, as well as 51 extragenic dominant *cop1* suppressors. COP1 SUPPRESSOR 1 (CSU1) is the first extragenic recessive *cop1* suppressor molecularly characterized from this genetic screen. Mutations in CSU1 almost completely suppress *cop1-6* de-etiolated phenotype in darkness. CSU1 colocalizes with COP1 in nuclear speckles and negatively regulates COP1 abundance. Moreover, CSU1 is able to ubiquitinate COP1 *in vitro* and is essential for COP1 ubiquitination *in vivo*. Collectively, CSU1 functions as an E3 ubiquitin ligase and targets COP1 for ubiquitination in maintaining its homeostasis in dark-grown seedlings. Identification and characterization of remaining COP1 suppressors could therefore further shed light on the molecular mechanisms on COP1 in organisms ranging from fungi to humans.

Translational biology and biotechnologies

Posters 339 to 351

P339 - The NE1 and NE2 proteins regulates nitrogen use efficiency in Arabidopsis and Rice.

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Development of genetic varieties with improved nitrogen use efficiency (NUE) is essential for sustainable agriculture. However, achieving this goal has proven difficult due to the fact that NUE is a complex trait encompassing multiple physiological and developmental processes. We thought to tackle this problem by taking a Systems Biology approach to identify candidate target genes. First, we used a supervised machine-learning algorithm to predict a NUE gene network in *Arabidopsis thaliana*. Second, we used network statistics to rank candidate genes and identified NE1, a scaffold protein, as the most central and connected gene in the NUE network. We found NUE decreased in plants overexpressing the NE1 gene as compared to wild-type plants under nitrogen (N) limiting conditions. No difference was observed for the *ne1* mutant plants. However, NUE increased as compared to wild-type plants under low nitrate conditions in double mutant plants in *ne1* and its closely related homolog *ne2*. Expression of the nitrate transporter genes *NRT2.1* and *NRT2.4* increased in the *ne1ne2* double mutant as compared to wild-type plants, with a concomitant 65% increase in nitrate uptake, under low nitrate conditions. Similar to Arabidopsis, we found that mutation of the *NE1/NE2* ortholog gene in rice, *OsNE1*, increased NUE as compared to wild-type rice plants. Our results suggest a conserved mechanism exist in Arabidopsis and rice to modulate NUE. NE gene family members (*NE1/NE2* in Arabidopsis and *OsNE1* in rice) are at the center of the NUE network acting as negative regulators of *NRT2.1/NRT2.4* gene expression, nitrate uptake and nitrogen use efficiency. These results point to NE genes as prime targets for future biotechnological strategies to improve NUE in crops.

P340 - Genetic basis for Non-Host Resistance in Arabidopsis against a Brassica-infecting race of Albugo candida revealed by transgressive segregation

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Albugo candida is an obligate biotrophic pathogen that causes white blister rust and comprises many physiological races that each specialize on distinct Brassicaceae host species. *Albugo candida* races that colonize Brassica crops do not colonize Arabidopsis. The genetic basis for this non-host resistance (NHR) was intractable, since no Arabidopsis lines are susceptible. We hypothesized that resistance in different Arabidopsis accessions might be explained by the presence of non-overlapping sets of *Resistance (R)* genes, that might show transgressive segregation in recombinant inbred lines. We screened Arabidopsis MAGIC lines, derived from 19 parents, all of which resist *Brassica juncea*-colonizing race Ac2V, and identified rare individuals that show different levels of susceptibility. We found two lines, MAGIC329 and MAGIC23, are fully susceptible to Ac2V. We crossed these lines to each MAGIC parent. We screened F2 populations derived from these crosses with Ac2V and obtained segregation ratios ranging from 3:1 to 255:1, suggesting one to four dominant R genes. This approach enabled us to clone three new White Rust Resistance (*WRR*) genes effective against *Brassica juncea*-infecting race Ac2V in Arabidopsis. All three genes encode TIR-NB-LRR type of R-proteins. We also carried out transformation of all the *WRR* genes into *Brassica juncea* and *Brassica oleracea*. Thus, in this example, we showed that NHR is a consequence of "Effector-triggered Immunity" mechanisms.

P341 (Talk) - Using a low temperature degron cassette exploiting the N-end rule to efficiently control protein abundance and activity in multicellular organisms

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Efficient control of protein abundance and activity *in vivo* presents a powerful tool for biotechnology and genetics. Here, we demonstrate that an N-terminal degron cassette consisting of a primary destabilizing amino acid acording to the N-end rule, followed by a temperature labile version of mouse dehydrofolate reductase (DHFR), can be robustly used in various eukaryotic multicellular organisms such as *Drosophila, Nicotiana benthamiana* and *Arabidopsis* to dynamically control protein stabilization allowing for on-demand accumulation and complementation of deletion mutant phenotypes. We show the temperature dependent accumulation of a wide variety of different target proteins ranging from the commonly used reporters Y-glucoronidase (GUS) and green fluorescent protein (GFP) to the transcription factor CONSTANS (CO) and the WD40 protein TRANSPARENT TESTA GLABRA1 (TTG1) in plants, as well as GFP and tobacco etch virus (TEV) protease in *Arabidopsis* and *Drosophila*. We currently focus on an in-depth demonstration of the power and the possibilities offered by this approach through stable expression of the highly cytotoxic proteins *Diphtheria* toxin A-chain (DT-A) and the bacterial ribonuclease Barnase (BAR) in *Arabidopsis* as well as intensively fine-tuning the degron cassette using synthetic peptide array technology. Overall, our low temperature degron offers a powerful, fast, and versatile tool for efficient control of protein accumulation in multicellular organisms.

P342 - Identification and characterization of Arabidopsis thaliana seed development homologous genes in Camelina Sativa

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Seeds are rich sources of nutrients (vitamins, proteins, minerals, oil..) and represent a large portion of human diet and animal livestock feed. The rapid growth of the world population and continuing use the dwindling fossil energy resources and its environmental consequences incite the search for green and sustainable resources to meet the increased demand of food, raw materials and energy. Oilseed crops are being increasingly used as resources for biofuel production, replacing the conventional fossil fuel. Thus, the engineering of new oilseed crops with increased seed size and oil content is of major economic importance. *Arabidopsis thaliana* seed development is well documented with the characterization of most of it regulatory genes. However, little is know about seed regulatory genes of *Camelina Sativa*, an emerging oilseed crop for protein and oil production. The aim of this project is the use of high sequence homology between Arabidopsis and Camelina to identify and characterize Camelina seed regulatory genes for future biotechnological use.

P343 - Translation of knowledge from Arabidopsis to Pooideae : toward an improvement of Nitrogen Use Efficiency

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New cereal varieties with improved Nitrogen Use Efficiency need to be developed to increase grain yield while reducing the usage of fertilizers. This can be achieved by genetically enhancing the plant capacity to uptake and assimilate nitrate from the soil and its capacity to remobilize nitrogen from the leaves. Many molecular and genomic data have been acquired in Arabidopsis, whereas most physiological and agronomical studies have been done using Pooideae species (wheat and barley) and other cereals (rice, maize). We are using the Pooideae model species *Brachypodium distachyon* together with barley to transfer molecular knowledge from Arabidopsis to cereals (Girin *et al.*, J. Exp. Bot. 2014). A part of our work aims at characterising members of the NRT2 family of nitrate transporters. Physiological functions have been assigned to several NRT2 proteins in Arabidopsis, and homologs have been isolated in most studied species. Characterisation of the expression of Brachypodium *NRT2* genes, together with physiological and functional studies, led to the identification of a possible major high affinity transporter involved in root nitrate uptake. Additionally, we are deciphering the molecular mechanisms involved in gene regulation in response to nitrate availability. The focus is made on homologs in Brachypodium and barley of the Arabidopsis NLP7 transcription factor, described as a major regulator of the Primary Nitrate Response. Recent results and approaches will be shown.

P344 (Talk) - From the Arabidopsis model plant to the Medicago root hair model system: role of Mechanosensitive channels in legume symbiosis

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Root hairs not only have a major role in water and nutrient uptake but they also play a central role in the establishment of the nitrogen-fixing symbiosis in Legume plants. In our approach we took advantage of the knowledge acquired in Arabidopsis to address an issue specific to Legume plants. In this study we specifically question the role of mechanosensitive channels in the early interaction between the model Legume plant *Medicago truncatula* and its bacterial partner *Sinorhizobium.* During the early symbiotic interaction between Medicago and its microsymbiont, the infection is initiated by bacterial adhesion to root hairs and will lead to hair curling entrapping the bacteria. The hypothesis of the involvement of physical sensors during these early events is worth considering. In plants, the molecular nature of protein mediating mechanoperception is still elusive. Mechanosensitive ion channels belonging to the MscSL (Mechanosensitive Smallconductance Like) family represent suitable candidates to be involved in this process. MscSL proteins have been recently characterized in Arabidopsis in our laboratory. The *in silico* analyses of the Medicago genome identified six homologs putatively addressed to the plasma membrane. Two of them are expressed in root hair. Preliminary results showed that one of these provides a channel activity dependant on membrane tension. The functional characterization of these root hair channels during the early steps of symbiosis is in progress.

P345 (Talk) - Arabidopsis protoplast regeneration sheds light on specific developmental switches

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Plant tissues cultured *in vitro* have the ability to proliferate and form new organs. We study the successive steps involved in the development of flowering shoots starting with protoplasts originating from Arabidopsis plantlets (Chupeau et al., 2013, Plant Cell 7:2444). Briefly, protoplasts resume mitosis, shortly after cell wall digestion, in the presence of an exogenous auxin, leading to the formation of microcalli. Gradual exogenous cytokinin addition in the culture medium sustains further callus growth and the initiation of shoot meristems. We will present data that (i) describe the complexity of protoplast *in vitro* cultures and how the resulting calli evolve over time and (ii) document the action of factors promoting specific developmental switches, in particular organogenesis. Furthermore, we have developed methods for high content screening based on Arabidopsis protoplast phenotypes and thereby identified bioactive compounds that promote mitotic activity or alter nuclear DNA content at the onset of protoplast proliferation. Our results provide novel insights into cellular functions involved in plant growth and development, potentially leading to improved protoplast regeneration methods. Such methods have recently gained renewed interest as site-directed nucleases and oligonucleotide-directed mutagenesis have been used to create valuable alleles in several species, including crops, yielding cultivars now reaching global markets.

P346 - The Finnish National Plant Phenotyping Infrastructure (NaPPI)

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The National Plant Phenotyping Infrastructure is located at the University of Helsinki, Viikki campus and aims at addressing the specific Nordic challenges in food, feed and fiber production. The borealic nature will have its unique responses to the climate change while the food and protein feed production is constantly maintained at the edge of the cultivation zones. Also the forest productivity for timber and pulp will need to be maintained. The aim of NaPPI is to supplement the existing research infrastructures on the different omics- technologies (transcriptomic, genomic, proteomic, metabolomic) with multianalysis of plant phenotypes. The analysis parameters include visible light for morphological and fitness assessment, pulse amplitude modulated fluorometry, and thermal imaging. The phenotyping platform consists of a small unit for model plants such as Arabidopsis, and a large unit for crops (legumes, cereals, potatoes, berries) and trees (spruce, pine, birch, poplar). Together these “omics”-platforms facilitate the move towards systems approaches and enhance both basic plant research, and translation towards plant breeding, and crop production sciences. These

high throughput facilities are part of a distributed infrastructure together with the University of Eastern Finland high precision unit and will be available for the whole public plant research community in Finland both at universities and at research institutes as well as among the commercial plant breeders.

P347 (Talk) - The Arabidopsis QQS orphan gene modulates carbon allocation across species

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Little is known about the functional significance of the species-specific orphan genes. The *Arabidopsis thaliana QQS*-orphan-gene modulates carbon allocation to protein and starch[1,2]. Ectopic expression of *QQS* increases protein content in soybean leaf and seed[2], in multiple high-/low-protein soybeans, and in rice[3]. *QQS* alters its transcript level under stresses and in mutants of genes involved in all sorts of stresses, suggesting that *QQS* may integrate primary metabolism and environmental perturbations, adjusting adaption to abiotic and biotic stresses[4]. The *QQS* protein binds to a transcriptional regulator in Arabidopsis and its soybean and rice homologs[3]. Overexpression of QQS interactor in Arabidopsis mimics *QQS*-overexpression phenotype, increasing protein content and decreasing carbohydrate[3]. The data reveal the skeleton of a previously undefined network in which *QQS* participates, and indicate QQS exerts its effect via an interaction with this transcription factor conserved across eukaryotic species. Deficiency in dietary protein is globally one of the most severe health problems; this insight provides a new strategy to modulate protein levels in crops. Our research presents *QQS* as a model plant orphan gene regulating plant metabolism, and illustrates an example of how basic research in Arabidopsis be applied in agriculture.

References
1 Li et al, Plant J (2009).
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4 Arendsee et al, Trends Plant Sci (2014).

P348 (Talk) - Expert curation of Arabidopsis proteins in UniProtKB/Swiss-Prot

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The UniProt KnowledgeBase (UniProtKB) provides a single, centralized, freely available resource for protein sequences and functional information. For Arabidopsis, our main targets for manual annotation are proteins with some functional characterization and most of them are now included in UniProtKB/Swiss-Prot. Besides harvesting, interpreting, standardizing and integrating data from literature and numerous resources, curators are also checking, and often correcting, gene model predictions. Our annotation program has been collaborating with Model Organism Databases (MODs) for a long time, and we provided TAIR with all the gene model corrections that we introduced on the bases of our trans-species family annotation. We are also completing the knowledgebase by importing missing information from EnsemblPlants. The UniProt consortium is also actively involved in GO annotation and manual annotation has been added to more than 4500 plant proteins. Experimental peptides from high-throughput proteomics experiments that uniquely match the product of a single gene are used to generate annotations describing post-translational modifications and protein processing events. UniProtKB serves as a central hub for biomolecular information with access to more than 100 other resources, such as nucleotide sequence database, 3D protein structure databases, InterPro or MODs.

P349 - Arabidopsis hairy roots for the production of heterologous proteins

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Hairy roots have some advantages over other plant systems to produce fully functional recombinant proteins: fast growth, genetic stability, ease of maintenance in liquid hormone-free medium and ability to secrete proteins. *Arabidopsis thaliana* hairy roots that produce GFP have b een established. The transformation protocol and the growth conditions were optimized. The use of hypocotyls of two day-old seedlings, ATCC 15834 *Rhizobium rhizogenes* bacteria, B5 medium supplemented with 5% sucrose and gentle shaking were important factors for establishing and growing *Arabidopsis thaliana* hairy roots. The concentrations of GFP in the culture media of two independent transgenic lines were 167 ± 22.5 mg/l and 159.9 ± 28.6 mg/l after one month. The doubling times were 7.69 and 7.87 days and the growth rates (μ) 0.088 $days^{-1}$ and 0.105 $days^{-1}$, respectively. *Arabidopsis thaliana* hairy roots have kept growing and have been stably producing GFP for over a year. The His-tag attached to GFP was also less degraded in the culture medium than it was when produced by turnip hairy roots. *Arabidopsis thaliana* hairy roots are therefore a promising recombinant protein expression system able to take advantage of the numerous *Arabidopsis* genetic resources.

P350 - SPINN (Saclay Plant INNovation), a project for an innovation accelerator dedicated to plant science laboratories in Paris area

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Developing more innovations from basic research is required to tackle societal and environmental challenges. Academic research teams working in plant sciences represent almost 700 persons in Paris area. The research activities widely cover all the fields of application of plant biology (feed and food, agriculture, agronomy, cosmetics, health, environment, bioenergy, biomaterials, etc.). They possess a wide panel of expertise, technical platforms and cutting edge technologies to develop innovative projects in those fields. However, public-private partnerships and industrial innovations remain limited.

Our project aims at developing a dedicated structure, namely SPINN (Saclay Plant INNovation) to facilitate the partnerships for building and carrying innovative projects. SPINN will prospect for new industry needs in research or services in plant sciences, while promoting the panel of scientific skills, competencies and technological platforms of the teams of the Saclay Plant Sciences (SPS) Laboratory of Excellence. SPINN will also provide additional services to industry like consulting and training. Overall, SPINN will contribute to the excellence of research, formation and innovation in Plant Sciences. This project is financially supported by the 3BCar Carnot Institute (http://www.instituts-carnot.eu/fr/institut-carnot/3bcar) and the Saclay Plant Sciences (SPS) LabEx (https://www6.inra.fr/saclay-plant-sciences_eng/).

P351 - Genome-wide identification of novel regulators of the secondary growth in storage roots

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Vascular plants undergo the secondary growth that makes stems and roots to be thickened in the radial direction. This secondary growth is promoted by the cell division activities in the cambium. Thus, the control

of cambium development and its cell division activities have significant effects on biomass production of vascular plants. Several transcriptional regulators and signaling components that control the secondary growth in stems have been identified. Some plants develop storage roots that increase root biomasses through radial growth. Many of these are also important agricultural crops. To understand the nature of their growth, we investigated root development in radish (*Raphanus sativus*). As anticipated, we found that radial root growth in radish is affected by cell division activities in the cambium. We successfully identified putative radial growth regulators by comparing with cambium-enriched genes based on cell-type specific root expression map in Arabidopsis. We were also able to ascribe variations in radial growth activities among radish inbred lines to the variations in cytokinin responses. These preliminary investigations suggest that the cambium is the center of radial growth in radish roots. To identify genes involved in the root radial growth at a genome-wide level, we established Laser Capture Microdissection (LCM)-mediated dissection of root tissues in radish. We collected cambium as well as two tissues inside and outside the cambium, respectively, extracted RNA, and constructed strand-specific Illumina sequencing libraries. Such experiments were performed in three different stages of root development and in two inbred lines with distinctive radial growth behaviors. Analysis of a compendium of gene expression data will allow us to identify novel radial root growth regulators and to understand how radial growth in relations to cytokinin signaling is controlled at a molecular level.

Hormone signaling

Posters 352 to 431

P352 - Gene Expression Analysis of Phytohormone-related Gene After Incision Treatment in Arabidopsis Flowering Stem.

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Previously, we reported that the differential gene expression between incised and non- incised Arabidopsis flowering stems and their changes in expression during reunion process. Genes that were up-regulated during the reunion process include those involved in cell division, cell wall modification, phytohormone-related gene and transcription factors (TFs) in 7days after incision. We revealed that auxin, ethylene and jasmonic acid (JA), contributed to the control of tissue reunion process in upper and lower part of incised stem by inducing the expression of *ANAC071* and *RAP2.6L*, respectively. Gene expression analyses also show that expression of those TFs were up-regulated within 3 h after incision. We also found that some of JA related genes were up-regulated at the non-incised region as well as incised region of flowering stem 30 min after incision. On the other hands, ethylene biosynthesis gene, *ACS2*, was up-regulated exclusively in incised region, 30 min after incision. We also analyzed expression of genes determined to be responsive to tissue-reunion, including those involved in cell division and TFs, within 24 h after incision.

Ref: Asahina *et al*, 2011, PNAS. Pitaksaringkarn *et al*, 2014, Plant Biotech. Pitaksaringkarn *et al*, 2014, Plant J. Asahina and Satoh, 2015, J Plant Res.

P353 - The sweet apical dominance

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Apical dominance is a developmental process by which the shoot apex inhibits the outgrowth of axillary buds on the same shoot. The hormonal network underlying this process has been extensively studied and a central role has been attributed to auxin. However, auxin treatment is not able to fully restore the apical dominance in decapitated plants in various species including *Arabidopsis thaliana*. It has been recently shown in pea that sugar availability was better correlated with bud release than auxin depletion in stem. Experiments on isolated *in vitro*-cultivated nodes of rose, pea and arabidopsis have also demonstrated that sucrose was involved in a disaccharide signalling pathway. These observations led us to carry out a set of experiments to dissect the role of sugars during the control of bud outgrowth in *Arabidopsis thaliana*. It appeared that multiple sugar signalling pathways are involved in such regulation during which the HEXOKINASE 1 seems to play an important role. Indeed, HXK1 overexpressing line presents a very high branching pattern and insensitivity to auxin while the Knock Out line fails to branch as the wild type. Moreover, the involvement of HXK1 in this process was more related to its signalling activity than to its catalytic role in glycolysis. These results give a new framework in the understanding of the control of bud outgrowth by sugars and open the way to future discoveries about the interplays between sugar and hormonal signalling pathways.

P354 - Mutants in RES-oxylipin signaling

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Biologically active oxylipins comprise the well-studied cyclopentanon jasmonic acid as well as reactive electrophile species (RES)-oxylipins like 12-oxo-phytodienoic acid (OPDA) which possess an A,Y-unsaturated carbonyl structure. Several components of the jasmonic acid signaling pathway have been identified while only little is known on signal transduction mediating the response to RES-oxylipins. We showed that TGA transcription factors are involved in the response to RESoxylipins. To find additional signaling factors we initiated a screen for mutants with altered expression of RES-oxylipin responsive genes using plants expressing the luciferase under the control of the GST6 promoter. Three mutants were selected for further characterization. One mutant with constitutively higher luciferase activity (*coe3*) and two mutants with lower activity in response to treatment with RES-oxylipins (*nr1, nr2*) were isolated. The induction of RES-oxylipin-responsive genes by different RES compounds was analysed in the mutants. In addition, tolerance to stress was tested. The *coe3* mutant is more tolerant to drought while *nr2* is more sensitive which is in agreement with a role of OPDA in the response to drought. An untargeted metabolomic approach revealed an altered glucosinolate profile of *nr2*.

P355 (Talk) - CLE6 expression recovers gibberellin deficiency to promote shoot growth in Arabidopsis

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Small peptides act as local signals during plant development, but few studies have examined their interaction with phytohormone signaling. Here, we show that application of gibberellin (GA) to Arabidopsis shoots induces substantial accumulation of transcripts encoded by *CLE6,* a member of the *CLAVATA/ESR-RELATED (CLE)* gene family, in the root stele, followed by promotion of organ growth by *CLE6* in GA-deficient plants. The long-distance effect of GA4 was demonstrated by the observation that its application to the shoot apex of the GA-deficient mutant *ga3ox1/ga3ox2* rescued the short-root phenotype. Microarray analysis was used to identify root-expressed genes that respond to systemic application of GA, and *CLE6* was selected for further analysis. *CLE6* was highly expressed in roots at the young seedling stage, and *CLE6* promoter activity was strong in hypocotyls and roots, especially in root stele cells at branch points. Application of CLE6 peptide had no obvious effect on the growth and development of GA-deficient mutant plants. Nonetheless, the fact that ectopic over-expression of *CLE6* in the GA-deficient mutant promoted root growth and branching, petiole elongation, bolting rate and stem length showed that *CLE6* expression partially compensates for the GA deficiency. Reciprocal grafting of GA-deficient mutant plants to *35S::CLE6* transformants complemented the shoot phenotype associated with GA deficiency, demonstrating the systemic effect of CLE6 from root to shoot. These data suggest that root-expressed *CLE6* is systemically involved in shoot growth under GA action in Arabidopsis.

P356 - Transcript profiling of cytokinin-induced cambium activation in Arabidopsis roots

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Cambial cell divisions lead to formation of secondary vasculature and radial growth of plant organs. In Arabidopsis roots, cambium activates from procambium soon after the primary development. Plant hormone cytokinin is required for the root secondary growth, but the signalling cascades resulting in cytokinin-induced cambium activation are not well elucidated. The quadruple *isopentenyltransferase (ipt)* mutant *ipt1,3,5,7* defective in cytokinin biosynthesis lacks active root cambium (Matsumoto-Kitano *et al.*, 2008). Accordingly, microarray analysis of one-week-old *ipt1,3,5,7* roots showed decreased expression of genes involved in the cytokinin response, xylem development as well as cambial markers compared to the wild type. The genome-wide transcriptional events during cambium activation of cytokinin-treated *ipt1,3,5,7* roots were studied in a time-course microarray experiment, which yielded abundant changes in gene expression and enriched biological processes. These results were supported by RNA sequencing, which also revealed additional cytokinin-responsive genes and gene-specific transcript levels. The most prominent cambial regulators will be characterized with loss-of-function mutants and expression pattern analysis using histological markers. The results obtained will facilitate understanding of cambium signalling dynamics at the whole-genome level.

Matsumoto-Kitano *et al.* (2008) Proc. Natl. Acad. Sci. USA 105, 20027–20031.

P357 - Cooperation of the DOF protein DAG1 and the DELLA protein GAI in the negative regulation of the AtGA3ox1 gene

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In *Arabidopsis thaliana* the gibberellic acid (GA) is an important positive regulator of seed germination. The transcription factor DOF AFFECTING GERMINATION1 (DAG1) is a repressor of seed germination and acts downstream of PHYTOCROME INTERACTING FACTOR-LIKE 5 (PIL5) by directly repressing the GA biosynthetic gene *AtGA3ox1*. PIL5 induces the expression of several downstream repressors, including the DELLA gene *GA INSENSITIVE 1 (GAI)*, which negatively regulates GA metabolism. We showed that inactivation of *GAI* results in increased expression of the *AtGA3ox1* gene, and by chromatin immunoprecipitation experiments, we demonstrated that GAI indeed cooperates with DAG1 in repressing *AtGA3ox1*, and that it directly interacts with DAG1. In order to identify other targets of DAG1 we also performed a ChIP-Seq experiment, using the transgenic line 35S-DAG1-HA. Our data contribute to the understanding of the molecular mechanisms underlying the seed germination process mediated also by DAG1.

P358 - The Post-Transcriptional Regulation of the Arabidopsis NRT1.1/NPF6.3 Transceptor Enlightens Its Role in NitrateDependent Lateral Root Development.

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NRT1.1/NPF6.3 is a major nitrate (NO_3^-) transceptor (dual function of NO3-transporter and receptor/sensor) in *Arabidopsis*. As a sensor, NRT1.1 was shown to repress lateral root (LR) development specifically in the absence of NO_3^-. Krouk et al. (Developmental Cell 2010, vol. 18-6.) suggested that this regulation is due to NRT1.1’s unexpected auxin-transport function that is inhibited by NO_3^-. In the absence of NO_3^-, NRT1.1would transport auxin out of LR primordia (LRP), preventing auxin accumulation and thus stopping LR development (at the *Emergence* stage specifically). On the other side, at NO_3^- concentration > 0.5mM, NRT1.1 auxin-transport function would be inhibited by NO_3^-, so that auxin would accumulate in LRP which could then complete their development. However, a few observations seem not to concur well with this model. First, even though *NRT1.1* expression is strongly induced by NO_3^- in LRP, NRT1.1 monitors LR development exclusively in the absence of NO_3^-. Second, although the *NRT1.1* promoter is activated in LRP at a very early stage of development (from Initiation on), LRP development is only sensitive to NO_3^- at a much later stage (*Emergence*). To address these discrepancies, we investigated NRT1.1’s potential post-transcriptional regulations in the context of LR development. We showed that NRT1.1 is subjected to a complex post-transcriptional regulation at both mRNA and protein levels. These results enlighten the role of NRT1.1 in NO_3^--dependent LR development.

P359 - Auxin biosynthesis and its regulation

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The main endogenous auxin, indole-3-acetic acid (IAA), is produced by the conversion of tryptophan into indole-3-pyruvic acid (IPyA) by a small TAA1/TAR family of three aminotransferases and IPyA is then directly converted into IAA by the YUC family of 11 flavin-monooxygenases. With the essential components of this two-step pathway characterized, the rising challenge is to decipher the regulatory mechanisms controlling the activity of this metabolic route.

TAA1 shows extremely specific and dynamic expression patterns suggesting tight spatial and temporal regulation of *TAA1* transcription. In order to find upstream regulators of *TAA1*, a yeast one-hybrid screen has been completed and several transcription factors (TFs) have been confirmed by transient expression. We are utilizing GFP-tagged recombineering lines to check for changes in *TAA1* expression patterns upon conditional expression of the candidate TFs in stably transformed plants. The goal is to identify specific TFs that directly regulate *TAA1* expression, contributing to the establishment of embryo polarity, meristem maintenance, and flower formation.

We are also investigating the poorly understood IPyA-independent routes of auxin biosynthesis and are taking advantage of forward, reverse, and chemical genetic approaches. This work is aimed at addressing the contribution of the previously postulated parallel routes to plant development.

P360 - DORNRÖSCHEN-LIKE: floral meristem initiation, founder-cell transcriptome and auxin integration

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The positional signals underlying founder cell specification in the inflorescence (IM) or floral meristem (FM) remain enigmatic. We have exploited the dynamic expression of *DORNRÖSCHEN-LIKE (DRNL)*, which marks IM or FM lateral organ founder cells, as a tool to address spatial and temporal mechanisms of floral body plan establishment; we present data from three alternative approaches: 1) The gene regulatory network associated with the earliest stages of FM initiation in the IM has been elucidated from *DRNL::GFP*-expressing cells in the *ap1 cal* mutant background following FACS and RNA-Seq. The composition of this transcriptome will be presented and includes polarity genes and the phyllotaxy regulator *AHP6*, a direct target of DRNL. 2) The combined mutation of five genes within this transcriptome completely abolishes FM initiation, which defines a regulatory FM initiation node. 3) Phylogenetic shadowing of orthologous *Brassicaceae DRNL* promoters and functional analysis of the *Arabidopsis DRNL* promoter have identified three highly conserved enhancer elements that individually, cooperatively or redundantly control aspects of *DRNL* expression in embryos and flowers. A 100-bp enhancer sequence containing two inverted-repeat auxin response elements autonomously marks lateral organ initiation in the IM and sepal founder cells. The evolutionary conservation of this sequence suggests that auxin response at the level of founder cells is embedded within a higher-order transcriptional complex.

P361 - Profiling of Plant Signaling Peptides using Tandem Mass Spectrometry

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In animal systems, cell-cell communication events are mainly mediated by peptides. However, unlike animals, most of the plant signaling peptides are still undiscovered. The major challenges for the detection of signaling peptide in plants is due to their low concentration, dynamic expression and most of the plant peptides are production from the hydrolysis of precursor proteins. To date, the MS-based proteomics approach was developed to detect and quantify the peptides in a highly sensitive and efficient manner. However, it is still difficult to detect the signaling peptides, because the enzymes for hydrolyzing proproteins are mostly unknown, all of the possible peptide cleavage rules need to be considered during the peptide identification. The use of non-enzyme specific database-based peptide identification can result in high false identification rate, thus reduce peptide identification sensitivity. In this study, we analyzed several factors in peptide identification, including mass accuracy and matching score. We discovered that the score distributions of truth- and false-positive peptide hits can be well separated by correlating the 1st and sub-ranked matching scores. With the use of liquid chromatography-tandem MS analysis of 285 known peptides, the number of correctly assigned cellular peptides was 25% enriched with the use of new algorithm in compared with the use of best matching score.

P362 - A calcium-dependent protein kinase is regulated by a protein phosphatase 2A (PP2A) via its B subunit

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Arabidopsis calcium-dependent protein kinases (CPKs) are important early components in calcium mediated signal transduction pathways. Protein dephosphorylation by protein phosphatase 2A (PP2A) is another crucial mechanism that regulates various signaling events in plants. We identified a PP2A protein phosphatase regulatory subunit B as an interactor of a CPK by yeast two-hybrid screen and bimolecular fluorescence complementation *in planta.* Biochemical analysis of the CPK in a *pp2ab* mutant and wild type plants proved that PP2A alters the activity of the calcium-dependent protein kinase. Transcriptome analysis of the *pp2ab* mutant showed upregulation of genes involved in plant immunity hinting at a role of this phosphatase as a co-regulator of a defense pathway. The possible involvement of this PP2A regulatory subunit B in plant immunity could further be illustrated by the altered abundance of a phytoalexin in the *pp2ab* mutant. Further work will analyze possible posttranslational modifications involved in the PP2A-CPK interaction and the biochemical regulation of the calcium-dependent protein kinase.

P363 (Talk) - Arabidopsis MAP KINASE1 negatively regulates ROP activity through ROP BINDING PROTEIN KINASE1 in an auxin dependent manner

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Mitogen-activated protein kinase (MPK) cascades are conserved mechanisms of signal transduction across eukaryotes. Despite the importance of MPK proteins in regulating signaling events, roles for many Arabidopsis MPK proteins are unknown. To identify MPK proteins involved in auxin responses, I surveyed insertional alleles in *MPK* genes and found that *mpk1* mutants displayed auxin hypersensitivity in cell expansion assays. Specifically, *mpk1* displays auxin hypersensitivity in cotyledon expansion, root hair elongation, and pavement cell morphology assays. Intriguingly, MPK1 appears to influence auxin responses independently of the nuclear auxin signaling system. We hypothesize that MPK1 interacts with ROP BINDING PROTEIN KINASE 1 (RBK1) to regulate the activity of the RHO OF PLANTS (ROP) small GTPases to regulate cell expansion in an auxin dependent manner. Additionally, *rbk1* insertional mutants display auxin hypersensitivity, similar to *mpk1*. A combination of phosphorylation assays and pull downs will reveal if either of these kinases directly phosphorylate or interact with ROPs. Understanding the role of MPK1 in Arabidopsis will further our understanding of MAPK functions and begin to reveal roles for novel negative regulators of auxin responses.

P364 - Regulation of growth by auxin in Arabidopsis

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Seed plants create remarkable shapes and patterns and respond to stimuli by tropisms while their cells are unable to move relatively to each other. Organ shapes and movements are thus dictated by controlling the orientation of cell division and subsequent cell enlargement. A canonical example of cell elongation is the reaction of stem segments to the application of auxin, termed also the acid growth. This system was extensively explored in the second half of the 20th century, using various model organisms and the by-then-available physiological and pharmacological approaches. The contemporary literature attributes the rapid auxin-induced stem elongation partially to the ABP1-TMK dependent signaling module and partially to the TIR1/AFB dependent pathway. Here we present an analysis of Arabidopsis cell wall acidification in response to auxin, and we investigate the signaling pathways underlying auxin-induced cell elongation in the light of the new discoveries in the field of auxin signaling. We unambiguously show that only one of these pathways is responsible for the rapid stem elongation.

P365 - Polysome Profiling Reveals Translational Regulation of Ethylene Signaling in Arabidopsis

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To accumulate more EIN3 (ETHYLENE-INSENSITIVE3) proteins in response to ethylene, Arabidopsis reduces the production of EBF1 (EIN3-BINDING F BOX PROTEIN 1) and EBF2 (EIN3-BINDING F BOX PROTEIN 2). Such regulation requires EIN2 (ETHYLENE INSENSITIVE 2), an essential component in ethylene signal transduction. Meanwhile, to avoid the high risk brought by over-accumulated EIN3, a negative feedback is established to transcribe more *EBF* mRNAs by EIN3 itself. Polysome profiling followed by RT-PCR directly shows the conditions of mRNAs being translated. By applying this assay, we revealed a new level of regulation of ethylene signaling, translational repression. We determined lower translation level of *EBF*s in response to ethylene indicated by fewer *EBF* mRNAs associated with ribosomes, and the repression depends on the presence of EIN2 but not EIN3. The 3’UTR of *EBF* mRNA mediated the translational repression by ethylene since over expressing *EBF1* 3’UTR released *EBF1/2* translation to comparative levels with or without ethylene treatment. We assumed the abundant *3’UTRs* here acted as sponges absorbing repressors called by ethylene signal. Moreover, *ein2* and *ein3 eil1* mutants displayed imbalanced polysomal profile indicating them playing a more general role in ribosome assembly to influence translation in vivo.

P366 - New E3 Ubiquitin ligases involved in jasmonate signalling in Arabidopsis thaliana

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E3-ubiquitin ligase protein complexes (UBC) have emerged as signalling hubs in the regulation of plant growth, development and defence. UBCs recruit the target substrate for ubiquitination and subsequent degradation by the proteasome. A well-known example of an UBC is the jasmonate (JA) receptor complex SCFCOI1. After binding the JA-Ile hormone, the SCFCOI1 targets the JAZ repressors for degradation by the 26S proteasome, thereby activating the downstream JA signalling cascade. Likewise, RING-type ubiquitin ligases, such as RGLG3/4, have recently been linked to JA signalling. Therefore and considering the huge variety and diversity of predicted UBCs, it is expected that additional E3-ubiquitin ligase proteins may be involved in the fine-tuning of JA responses. To identify new JA-related E3-ubiquitin ligase proteins we combined transcriptome mining with tandem affinity purification of protein complexes (TAP). Several

E3-ubiquitin ligases that are co-regulated with the JAZ genes in different conditions were selected as bait proteins for TAP analysis. Here, we present the JA-modulated Arabidopsis Toxicos in Levadura 23 (ATL23), a RING-E3 ubiquitin ligase, that interacted with several prey proteins such as, NIT1, NIT2, RD21, and ERMO2, most of which with yet unknown role in JA signalling. ATL23 was also found to interact with the 17, 24, 29 and 30 E2 subunits of UBC complexes. Functional characterization of ATL23 and its potential roles in JA responses is ongoing

P367 - Two wheat Glycogen Synthase Kinase 3 / SHAGGY -like kinases act as negative regulators of brassinosteroid signaling

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Glycogen Synthase Kinase 3 / SHAGGY-like kinases (GSKs) are non-receptor serine/threonine kinases. Plant GSKs are involved in hormonal signaling networks and are required for growth, development, light as well as stress responses. Knowledge on their biological function in Liliopsida resp. *Poaceae* is still limited as most functional studies have so far been conducted on *Arabidopsis* GSKs. Two GSK homologs named *TaSK1* and *TaSK2* and their homoeologous gene copies have been identified and characterized in the hexaploid wheat genome. A phylogenetic analysis of land plant GSKs indicated that *TaSK1* and *TaSK2* belong to GSK clade II, the *Arabidopsis* members of which are all involved in brassinosteroid (BR) signaling. The role of TaSK1 and TaSK2 in BR signaling has been evaluated by expressing a gain-of-function mutation of TaSK1 and TaSK2 in *Arabidopsis*. Phenotypic analysis, investigation of BR target gene expression in transgenic lines, and partial rescue of severe phenotypes using a GSK chemical inhibitor provided strong evidence for an involvement of TaSKs in BR signaling. The physiological effects of brassinosteroids on wheat growth and development were explored and indicated a role of BRs in embryonic development and seedling growth.

P368 - Role of AtRLP44 in brassinosteroid-mediated cell wall signaling

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Communication between the extracellular matrix and the cell interior is essential for all organisms as intrinsic and extrinsic cues have to be integrated to coordinate development, morphogenesis, and behavior. This applies in particular to plants, the growth and shape of which is governed by cell wall deposition and remodeling. In this context, the biophysical properties of the cell wall are constantly monitored and information must be relayed to the cell interior in order to finetune the physico-chemical properties of the cell wall for optimal growth responses. However, very little is known about the molecular components and signaling mechanisms involved in these processes. AtRLP44 was identified as a key component of brassinosteroid-mediated cell wall signaling. RLP44 alone is sufficient to activate brassinosteroid-regulated gene expression. However, it is not clear how RLP44 activates signaling. Interaction with the regulatory receptor-like kinase BAK1 has been demonstrated but its functional significance is unclear. During this thesis, the effect of RLP44 on composition, motility, and signaling output of the brassinosteroid receptor complex and its downstream targets will be assessed. In addition, we aim to analyze the contribution of previously identified phosphorylation sites in the cytoplasmic tail of RLP44 on its function in cell wall signaling using transgenic complementation analysis. Taken together, these approaches are expected to reveal the mechanism by which the newly discovered RLP44-mediated cell wall signaling pathway is integrated with well-known brassinosteroid hormone signaling.

P369 - Identification and characterization of a candidate receptor for the plant natriuretic peptide AtPNP-A

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The focus of our research is to elucidate the mechanism of action of plant signaling peptides, in particular plant natriuretic peptides (PNPs) comprising a novel class of hormones that share some sequence similarity in the active site with their animal analogues regulating salt and water balance. One aspect of understanding the function of these compounds is their potential biotechnological applications in conferring increased stress tolerance to plants. Since the molecular mode of action of PNPs still remains unclear, comprehensive protein-protein interaction studies have been undertaken to identify physiologically relevant interactants of the *Arabidopsis thaliana* PNP (AtPNP-A) that actings as a systemic regulator of abiotic and biotic stress responses. Specificity of the interactions was verified with surface plasmon resonance and physiological significance of the interaction was assessed in functional assays. Interestingly, one of the identified binary interactants of AtPNP-A, termed AtPNP-R1, shows guanylyl cyclase activity *in vitro* – a feature that is diagnostic for several mammalian receptors of NPs. Moreover, suppressed response of *atpnp-r1* mutant plants to the AtPNP-A dependent net water influx into mesophyll cell protoplasts confirmed that AtPNP-R1 functions in the transduction of the AtPNP-A signal. Taken together, the results suggest that AtPNP-R1 is one of the receptors for AtPNP-A.

P370 - Brassinosteroids promote anthocyanin biosynthesis under low nitrogen conditions in Arabidopsis

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Brassinosteroids (BRs) are steroid plant hormones that regulate many developmental and physiological processes in pants including responses to stress. However, little is known about BR regulation of plant responses to mineral nutrients. Our recent studies indicate that BR can induce plant tolerance to low nitrogen (N) stress in *Arabidopsis* and enhance low N-induced anthocyanin biosynthesis, which seem to be both mediated by the BR-activated transcription factor BZR1. BZR1 physically interacts with PAP1, a key transcription factor that controls anthocyanin biosynthesis in Arabidopsis, and enhances PAP1’s transcriptional activation activity on its target genes in anthocyanin biosynthesis. BZR1 can also bind the promoters of some of the anthocyanin biosynthetic genes but the binding appears to be not specific, suggesting that BR promotes anthocyanin biosynthesis under low N mainly through BZR1-PAP1 interaction. As a conclusion, our study indicates that BR is capable of promoting low N-induced anythocyanin biosynthesis, which may help plants survive the low N stress conditions.

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P371 - EIN3 Negatively Regulates Fertility through Inhibiting Pollen Germination in Arabidopsis

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Ethylene is a gaseous plant hormone, which plays important roles in plant development and response to biotic and abiotic stresses. EIN3 (Ethylene insensitive 3) and EIL1 (EIN3-like 1) are key transcription factors in ethylene pathway, which are tightly regulated by SCF complex containing F-box protein EBF1/EBF2 (EIN3-binding F-box protein 1/2) through the 26S proteasome-mediated protein degradation pathway. Previous studies found that a weak allele of *ebf 1ebf2* is infertile, and this could be rescued by loss of *EIN3* function, indicating EIN3 may negatively regulates plant fertility. However, the mechanism remains unclear. Recently, we found the length of stamens was much shorter than that of pistils in *ebf1-3 ebf2-2*, resulting in the gynoecium protruding from the unopened floral buds.

Besides, the petals of *ebf1-3 ebf2-2* was smaller than wide-type Col-0. All these defects were rescued by *ein3* mutation. Using Alexander staining and in vitro pollen germination assays, we found the pollen development is normal in *ebf1-3 ebf2-2*, but pollen germination is defective. When pollinated with Col-0, normal seeds can be produced in *ebf1-3 ebf2-2*. Our data indicated that EIN3 negatively regulates fertility may through inhibiting pollen germination.

P372 - A dominant mutation of HT1 kinase, which leads to a deficiency in high CO2-induced stomatal closure, induces accumulation of HT1 protein

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HT1 (HIGH LEAF TEMPERATURE 1) is the first component in the stomatal CO_2 signaling pathway to be isolated by forward genetic screening. The HT1 gene encodes a protein kinase expressed mainly in guard cells, and the recessive mutations, *ht1-1* and *ht1-2*, cause decrease or loss of HT1 kinase activity, respectively. As a result, the *ht1* recessive loss-of-function mutants exhibit CO_2 hypersensitive stomatal closure. We have isolated or obtained seven *ht1* mutant alleles, including the *ht1-1* and *ht1-2*, and we found that the six recessive *ht1* mutants (*ht1-1, ht1-2, ht1-4, ht1-5, ht1-6* and *ht1-7*) had mutations on highly conserved residues in the catalytic domains required for the kinase activity. In contrast, the dominant mutant, *ht1-3*, had a missense mutation affecting a non-conserved residue (R102K), and had widely opened stomata due to CO_2 insensitivity. We demonstrated that the dominant mutation did not affect kinase activity, but increased the amount of HT1 protein at the post-transcriptional level. We also showed that the amount of HT1 protein is kept at a low level in order to retain the ability to adjust the stomatal apertures in response to environmental changes. Interestingly, both of the loss-of-function and gain-of-function *ht1* mutants showed normal ABA responses, but completely altered CO_2 responses. These results strongly suggest that HT1 has a crucial and specific function in stomatal CO_2 response. We will discuss possible CO_2 signaling pathways mediated by HT1.

P373 - Small Peptides Of The INFLORESCENCE DEFICIENT IN ABSCISSION Family Are Involved In Root Cap Development

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The development and function of the plant root involve precisely controlled cell division patterns and cell separation events. Lateral root primordia have to penetrate several different cell layers during emergence and are dependent on timely activation of the cell separation machinery. INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), a small peptide ligand and the leucine-rich repeat receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2) control floral organ abscission and serve as a signaling module regulating cell separation during lateral root emergence in *Arabidopsis thaliana* [1, 2]. Other small peptides of the IDA-LIKE (IDL) family are expressed in the root apical meristem. One of these genes is expressed strongly in leaves and in the QC of the root tip. Preliminary data shows that a mutation in this gene results in a deviating cell division pattern in the root. Alterations in the division pattern of the root tip are seen as early as in the germinating seed. Mature roots of 10 days old seedlings continue to have defects in the cells underlying the QC region and show a generally unordered root cap structure. Assuming a generally similar structure for the active IDL peptide compared to IDA, a synthetic peptide of 12 amino acids with a central hydroxyproline is currently tested for its competency to rescue the mutant phenotypes described. Constructs harboring the IDL locus have been generated and introduced into the idl mutant and will be used to independently confirm the anticipated results. The target receptor(s) for IDL is currently unknown but receptors with similarity to HAE are tested in transient assays for response to application of the synthesized peptide. One of these receptors is showing a mutant phenotype very similar to the *idl* mutant root phenotype. RNA-Seq was performed on root tissue of 10 days old seedlings and a list of differentially expressed genes is currently screened for candidates potentially affected by IDL.

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P374 - Nuclear import receptors affect the transcriptional and developmental output of the BODENLOS-MONOPTEROS module

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The basic body pattern of A*rabidopsis thaliana* is established during early embryogenesis. The initiation of the primary root depends on the Auxin Response Factor *MONOPTEROS (MP)* and the degradation of its inhibitor, the Aux/IAA *BODENLOS (BDL)*. This degradation is triggered by the phytohormone auxin. Here we report that a mutation in a nuclear transporter of the *IMPORTIN A* class (*IMPA*) alters the penetrance of root initiation defects caused by the *bdl* gain-of-function allele.

impA6-1 mutant plants appear phenotypically normal and do not show a reduced level of bdl protein. Localization studies of bdl:3xGFP revealed no qualitative difference in the subcellular localization to wild-type. We used fluorescence recovery after photobleaching (FRAP) to study the effect on bdl:3xGFP import and transient expression in protoplasts to confirm that the inhibition of *MP* by *bdl* is reduced in the *IMPA6* knockdown background. In addition, we performed massspectrometry to confirm the broad range of Importin A interactors, and calculated mathematical models of auxin signaling to compare the effect of a delay in bdl import versus a reduced nuclear accumulation of bdl cofactors. This report establishes the relevance of the fast nuclear import of Aux/IAA proteins for their function in inhibiting ARF transcription factors.

P375 - Integration of Cell Wall Signaling and Growth Regulation in Arabidopsis thaliana

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Almost all plant cells are surrounded by a rigid, yet dynamic, extracellular structure, the cell wall. In the absence of cell migration, plant cells grow by modifying their walls. After expansion, cell wall integrity is consolidated through deposition of new cell wall material and the activity of cell wall remodeling enzymes. Expression and activity of these enzymes are controlled by growth hormones such as auxin, gibberellic acid and Brassinosteroids (BRs). Because of this, it has been assumed that the state of the cell wall is monitored to relay information to the cytosol and to elicit an adequate response when cell wall integrity is compromised. Recently, such a mechanism was shown to be triggered by modification of the pectin component of the cell wall, leading to an activation of the BR signaling pathway. Cell wall-mediated activation of BR signaling depends on receptor-like protein 44 (RLP44), which interacts with the BR receptor complex and is sufficient to activate BR signaling. Thus, integration of cell wall and BR signaling most likely occurs at the level of the BR receptor complex. BR hormones are perceived by the receptor-like kinases BRI1 and BAK1, both of which were shown to interact with a component of the recently identified cell wall signaling pathway. We want to study dynamics and regulation of complex formation between the relevant proteins during development, as well as reveal the consequences of these interactions on the activity of the BR receptors.

P376 - Integration of light and jasmonate signaling in Arabidopsis seedling development

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Plant growth and development is often regulated by the interaction of environmental factors such as light and various phytohormones, including jasmonates (JAs). However, the molecular mechanisms underlying the integration of light and JA signaling remain largely unknown. Arabidopsis FAR-RED INSENSITIVE 219 (FIN219)/JASMONATE RESISTANT 1 (JAR1) has

been shown to participate in phytochrome A-mediated far-red (FR) light signaling and interacts with different light signaling components. Our recent studies have revealed that FIN219/JAR1 regulates a number of transcription factors in FR light. The *fin219* null mutant was less sensitive than the wild type to various concentrations of methyl jasmonate (MeJA) under low and high FR light. High FR light reduced the sensitivity of Arabidopsis seedlings to MeJA. Intriguingly, FIN219 levels in response to MeJA showed a negative feedback regulation under FR light. Further evidence indicates that loss-of-function mutants of some bHLH TFs affected by FIN219 show altered responses to MeJA in the regulation of hypocotyl and root elongation. In addition to functional roles in FR light, FIN219/JAR1 also shows a mutual regulation with the blue light photoreceptor cryptochrome1 via physical interaction. Further regulatory relationship between both proteins under blue light and MeJA conditions will be presented in the meeting.

P377 - Activity and localization of cysteine-rich receptor-like kinases

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Cysteine-rich receptor-like kinases (CRKs) represent a subgroup of the receptor-like protein kinase (RLK) transmembrane proteins, consisting of 44 members in *Arabidopsis thaliana*. CRKs are defined by their extracellular domain containing two copies of the domain of unknown function 26 (DUF26) with its conserved cysteine motif C-X8-C-X2-C. Large-scale phenotypic screening of *crk* T-DNA insertion lines suggests roles for these proteins in reactive oxygen species (ROS) signalling and stress responses. Further evidence comes from gene expression analysis, where altered *crk* expression is observed in response to various abiotic and biotic stresses, and from the potential for redox regulation in their extracellular domain. However, their function on the molecular and biochemical level remains largely unknown. Here we investigate the *in vitro* kinase activity of selected CRKs. We also show the subcellular localization of CRKs *in planta* using fluorescently tagged proteins. These results provide a preliminary framework for the biochemical context of CRK protein function.

P378 - Characterization of the role of the Arabidopsis truncated haemoglobin

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Plant hormones are important regulators of plant growth, development and the responses to biotic and abiotic stresses. They play a central role in the determination of cellular programs by integrating environmental and endogenous developmental signals and interact at multiple levels. We are characterizing two Arabidopsis E3-ubiquitin ligases potentially involved in jasmonate signalling that were used in a Tandem Affinity Purification (TAP) screening to identify new interacting proteins. One of these targets is GLB3, which corresponds to the Arabidopsis truncated haemoglobin. In plants, three types of haemoglobins have been identified, symbiotic, non-symbiotic and truncated, which have been divided in three phylogenetic groups, where class 1 and 2 are closely related to human and animal globins and class 3 to bacterial truncated haemoglobins. The function of truncated haemoglobins in plants is unknown. To get insight into the possible function of GLB3, we generated Arabidopsis plants with loss- or gain-of-function of *GLB3* and performed a transcriptomic analysis which revealed a role in iron homeostasis. Furthermore, we performed TAP and yeast two-hybrid screenings using GLB3 as bait and several GLB3-interacting proteins related to salicylic acid and nitric oxide signalling pathways were identified. Further detailed phenotypic characterization of Arabidopsis lines will enable to unravel the importance of truncated haemoglobins for plant growth, development and defence.

P379 - Phytosulfokine receptor 1 (PSKR1) has multiple innate gateways for intracellular communication

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The phytosulfokine receptor 1(PSKR1) has multiple roles in cellular development. Recently PSKR1 has been implicated as a switch molecule that shifts plants from deploying resources in growth and development to defence strategies. We have shown that the intracellular domain of PSKR1 has both kinase and guanylate cyclase activity that can be switched by changes in calcium levels at physiological relevant levels. Here we show that recombinant PSKR1 is capable of autophosphorylation at serine, threonine and tyrosine residues. We analysed recombinant PSKR1 by mass spectrometry and revealed specific phospho sites. Using phosphomimetics we show that the phospho state of key residues impacts on the kinase and also the guanylate cyclase activity of PSKR1. Hence the pattern formed by intracellular phosphorylation sites within PSKR1 contributes to its catalytic function that can be further regulated by intracellular changes in cGMP and calcium. We speculate that these multiple levels of control contribute to the ability of PSKR1 to switch resource deployment.

P380 - Overexpression of the partial PHYB gene suppresses bri1-5

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Brassinosteroids (BRs) control virtually every aspect of plant growth and development. BRs act alone or with other exogenous and endogenous signals including auxin and light. To screen for the novel player involved in BR signaling in Arabidopsis, we employed cDNA overexpression strategy. We created a cDNA library to be expressed under the 35S overexpression promoter, and introduced into a weak *brassinosteroid insensitive 1 (bri1)* mutant. The mutant dubbed *bri1*

-5 with long petiole (*blp*) was identified to display bigger stature especially in hypocotyl and petiole length relative to *bri1*

-5. Sequence analysis of the rescued transgene revealed that *blp* consisted of a chimeric DNA consisting of a 3" half of *PHYB*, 2 bp insertion, and a part of a chloroplast ribosomal RNA. Re-introduction of chimeric DNA into *bri1-5* recapitulated blp phenotype. The *blp* phenotypes being similar to *phyB* mutants led us to examine both the *PHYB* transcript and protein levels in the *blp 35Spro:PHYB* doubly homozygous line. Lower levels of both transcripts and proteins of *PHYB* suggested that introduction of the chimeric gene interfered with the stability of *PHYB* transcripts. Our results highlight that overexpression mutagenesis facilitates functional genomics to decipher a function of Arabidopsis genome.

P381 - The Arabidopsis Zinc Finger Protein 3 interferes with ABA and light signaling in seed germination and plant development

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Seed germination is controlled by environmental signals, including light and endogenous phytohormones. Abscisic acid (ABA), the plant hormone

is known to play important role in various aspects of plant development. In addition, ABA plays a pivotal role in mediating some aspects of physiological responses to environmental stresses. ABA inhibits, whereas gibberellin promotes, germination and early seedling development, respectively. Here, we report that ZFP3, a nuclear C2H2 zinc finger protein, acts as a negative regulator of ABA suppression of seed germination in Arabidopsis (Arabidopsis thaliana). Accordingly, regulated overexpression of ZFP3 and the closely related ZFP1, ZFP4, ZFP6, and ZFP7 zinc finger factors confers ABA insensitivity to seed germination, while the zfp3 zfp4 double mutant displays enhanced ABA susceptibility. Reduced expression of several ABA-induced genes, such as RESPONSIVE TO ABSCISIC ACID18 and transcription factor ABSCISIC ACID-INSENSITIVE4 (ABI4), in ZFP3 overexpression seedlings suggests that ZFP3 negatively regulates ABA signaling. Analysis of ZFP3 overexpression plants revealed multiple phenotypic alterations, such as semidwarf growth habit, defects in fertility, and enhanced sensitivity of hypocotyl elongation to red but not to far-red or blue light. Analysis of genetic interactions with phytochrome and abi mutants indicates that ZFP3 enhances red light signaling by photoreceptors other than phytochrome A and additively increases ABA insensitivity conferred by the abi2, abi4, and abi5 mutations. These data support the conclusion that ZFP3 and the related ZFP subfamily of zinc finger factors regulate light and ABA responses during germination and early seedling development.

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P382 - Role of PLD isozymes and NADPH-oxidases in salicylic acid mediated stomatal movement

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Salicylic acid (SA) is a hormone associated with responses to abiotic and biotic stresses. Stomatal movement is a fast and effective mechanism of plant adaptation to environmental changes, controlled by a cascade of signalling events including the production of lipid-derived second messengers and of reactive oxygen species (ROS). We investigated the role of phospholipase D (PLD) in the control of stomatal movement by SA. The stomatal opening by light was inhibited in wild-type plants by an inhibitor of phosphatidic acid production by phospholipase D activity. In *Arabidopsis thaliana*, PLDs are encoded by a multigenic family. The opening was impaired in *pldA* deficient line (but not in *pldY* or *pldΓ* mutant plants). Interestingly, SA inhibits the stomatal opening by light in WT plants but no longer in *pldA* deficient line. As for stomata closure of already open stomata, we have shown that it was induced by SA. SA induced rapid increase of PLD activity in *A. thaliana* tissues. The product of PLD is phosphatidic acid that has NADPH-oxidase binding ability. NADPH-oxidases RbohD and RbohF are superoxide producing enzymes. Using the *rbohD, rbohF* and *rbohDF* deficient lines we discovered the major role of *rbohD* isozyme in SA-mediated ROS formation in tissues and stomatal closure. The upstream localization of PLDA over NADPH-oxidase RbohD in the SA-mediated cascade in guard cells was shown. Our data points out at the crucial role of PLDA and RbohD in SA-mediated reactions in guard cells.

P383 - Gene regulatory network of the LBD transcription factors controlling lateral root development in Arabidopsis

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The *LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL)* gene family encode a class of transcription factors harbouring a conserved plant-specific LOB domain and play roles in lateral organ development in plants. While developmental roles of several *LBD* genes have been identified in leaf polarity establishment, embryo development, male gametophyte development, and particularly in lateral root (LR) development in Arabidopsis, the regulatory networks of the *LBD* genes with hormone signaling are largely unknown. Here, we show that the *AUX1* and *LAX (Like-AUX1)3* auxin influx carriers are required for auxin signaling that activates *LBD16* and *LBD18* to control LR development. Our genetic and molecular data suggested that *LBD16* and *LBD18* regulate LR initiation and primordium development with *AUX1* and primordium development in part with *LAX3* and that *LBD18* controls LR emergence downstream of *LAX3*. Our results also indicated that *LBD18* and *AUXIN REXPONSE FACTOR(ARF)19* form an interlocking transcriptional positive feedback loop for LR emergence. This study revealed part of complex gene regulatory networks that control LR development in Arabidopsis. This work was supported by grants from the Next-Generation BioGreen 21 Program (PJ01104701), RDA and the Basic Science Research Program (2013R1A1A2062335) through the NRF of Korea, Republic of Korea.

P384 - The role of the MAPKs and protein phosphatase type 2C AP2C in pathogen-induced salicylic acid production

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Pathogen infection initiates numerous defense responses in plants, such as Arabidopsis, including activation of mitogen activated protein kinases (MAPKs) and production of plant stress hormones, such as ethylene and salicylic acid. Phosphorylation-based activation of MAPKs is regulated by protein phosphatases. PP2C-type protein phosphatases AP2Cs dephosphorylate MAPKs on phospho-T residue thereby abolishing kinase activity. Arabidopsis MAPKs MPK3, 4 and 6 play a role in regulation of stress hormone production, thus, we aim to explore the role of AP2C phosphatases in regulation of kinase activities, ethylene and salicylic acid production in plants during pathogen infection. We will present the data on the analysis of free and conjugated salicylic acid by chromatography performed on a reverse phase HPLC column in plant lines modified for AP2C or MAPKs. We are also implementing a method for measurement of the salicylic acid amounts in Arabidopsis plants using luminescence-based bioassays in bacteria. Applying this method we were able to observe changes in salicylic amounts produced in plants after pathogen-induced stress. This ongoing work leads towards the better understanding of the role of PP2C-type MAPK phosphatases in regulation of MAPK signaling and stress hormone production.

P385 - Overexpression of the partial PHYB gene suppresses bri1-5

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Brassinosteroids (BRs) control virtually every aspect of plant growth and development. BRs act alone or with other exogenous and endogenous signals including auxin and light. To screen for the novel player involved in BR signaling in Arabidopsis, we employed cDNA overexpression strategy. We created a cDNA library to be expressed under the 35S overexpression promoter, and introduced into a weak *brassinosteroid insensitive 1 (bri1)* mutant. The mutant dubbed *bri1*

-5 with long petiole (*blp*) was identified to display bigger stature especially in hypocotyl and petiole length relative to bri1

-5. Sequence analysis of the rescued transgene revealed that *blp* consisted of a chimeric DNA consisting of a 3" half of *PHYB*, 2 bp insertion, and a part of a chloroplast ribosomal RNA. Re-introduction of chimeric DNA into *bri1-5* recapitulated *blp* phenotype. The *blp* phenotypes being similar to *phyB* mutants led us to examine both the *PHYB* transcript and protein levels in the *blp 35Spro:PHYB* doubly homozygous line. Lower levels of both transcripts and proteins of *PHYB* suggested that introduction of the chimeric gene interfered with the stability of *PHYB* transcripts. Our results highlight that overexpression mutagenesis facilitates functional genomics to decipher a function of Arabidopsis genome.

P386 - Nitrate sensing and uptake in Arabidopsis are enhanced by ABI2, a phosphatase inactivated by the stress hormone abscisic acid

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Living organisms sense and respond to changes in nutrient availability to cope with diverse environmental conditions. Nitrate (NO3&*minus*;) is the main source of nitrogen for plants and is a major component in fertilizer. Unraveling the molecular basis of nitrate sensing and regulation of nitrate uptake should enable the development of strategies to increase the efficiency of nitrogen use and maximize nitrate uptake by plants, which would aid in reducing nitrate pollution. NPF6.3 (also known as NRT1.1), which functions as a nitrate sensor and transporter, the kinase CIPK23, and the calcium sensor CBL9 form a complex that is crucial for nitrate sensing in Arabidopsis thaliana. We identified two additional components that regulate nitrate transport, sensing, and signaling: the calcium sensor CBL1 and protein phosphatase 2C family member ABI2, which is inhibited by the stress-response hormone abscisic acid. Bimolecular fluorescence complementation assays and in vitro kinase assays revealed that ABI2 interacted with and dephosphorylated CIPK23 and CBL1. Coexpression studies in Xenopus oocytes and analysis of plants deficient in ABI2 indicated that ABI2 enhanced NPF6.3-dependent nitrate transport, nitrate sensing, and nitrate signaling. These findings suggest that ABI2 may functionally link stress-regulated control of growth and nitrate uptake and utilization, which are energy-expensive processes.

P387 - The role of cell wall signaling in the maintenance of cellular mechanics

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All plant cells are enclosed by a polysaccharide-rich cell wall, which protects against physical damage and pathogen intrusion. However, the normally rigid cell wall needs to undergo controlled loosening to enable the anisotropic growth, which is typical for plants. To maintain structural integrity during growth, the state of the cell wall is constantly monitored by cellular surveillance mechanisms. Recently, it was shown that altering cell wall state triggers activation of the well-characterized brassinosteroid (BR) hormone signaling, however, the consequences on cell wall properties are not known.

In addition, disrupting cell wall homeostasis was demonstrated to cause aberrant cell morphology and irregular cell division planes. Here, the effect of cell wall changes on cell geometry will be analyzed by following cell division with fluorescent marker proteins and morphometric analysis in the presence or absence of functional cell wall feedback signaling. In addition, using tissue and cell type- specific induction of cell wall alterations, the effect of local changes in cell mechanics on i) the activation of cell wall/BR signaling and, and ii) the cell morphology and positioning of the cell division plane in the affected cells and neighboring tissues will be studied. In order to understand the compensatory changes induced by these signaling pathways, transcriptome analysis of wild type and cell wall signaling mutants in response to cell wall stress will be combined with cell wall profiling. Together, these approaches are expected to allow a better understanding of how growth is controlled by cell wall feedback signaling in plants.

P388 - EBF1/2 mRNA 3'-Untranslated Region (3'UTR) Relays the Ethylene Signal via EIN2-mediated Translational Repression in Arabidopsis

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Ethylene is gaseous phytohormone that plays a vital role in plant growth and development. ETHYLENE INSENSITIVE 2 (EIN2) is an essential signal transducer in ethylene signaling pathway, which linking ethylene perception on ER to transcriptional regulation in the nucleus. In the presence of ethylene, the C-terminal end of EIN2 (CEND) will be cleaved and then shuttled into nucleus to active two master transcription factors EIN3 and EIN3-LIKE 1 (EIL1) in an unknown mechanism. Interestingly, ethylene also induces CEND to form discrete and prominent foci in the cytoplasm, although the function of such cytoplasmic portion remains unexplored. Here, we report a novel mechanism of EIN2-mediated ethylene signaling, whereby EIN2 imposes the translational repression of *EIN3 BINDING F-BOX PROTEIN1 (EBF1)* and *EBF2* which are responsible for the proteasomal degradation of EIN3/EIL1. We find that the *EBF1/2* 3’untranslated regions (3’UTRs) mediate EIN2-directed translational repression, and identify multiple PolyU motifs as functional cis-elements within the 3’UTRs. Furthermore, we demonstrate that ethylene induces EIN2 to associate with 3’UTRs and target *EBF1/2* mRNAs to cytoplasmic processing-body (P-body) through interacting with multiple partners, including EIN5 and PAB proteins. Our study illustrates the translational regulation as a key step in ethylene signaling, and represents the first demonstration of mRNA 3’UTR functioning as a "molecular sensor" to relay plant hormone signaling.

P389 - Screening for Arabidopsis genes involved in regulation of auxin metabolism and root development

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Auxin (indole-3-acetic acid, IAA) has a fundamental role as signalling molecule during plant growth and development. Formation of local auxin gradients and auxin maxima/minima are very important for these processes, in turn regulated by auxin biosynthesis, degradation, conjugation and transport. To get a better understanding of auxin metabolism and its role during root development, we have developed a genetic screen to identify novel genes involved in auxin metabolic pathways.

EMS mutagenesis was performed on the auxin reporter lines DR5::VENUS and DII-VENUS (Brunoud et al. 2012, Nature 482), and mutant lines with changes in reporter gene expression or with a visible mutant phenotype were selected for high-throughput liquid chromatography tandem mass spectrometry (HPLC-MS/MS) profiling of IAA and IAA metabolites (precursors, catabolites and conjugates). The method combines a simple one-step purification protocol based on in-tip micro Solid-Phase Extraction with ultra-rapid LC-MS analysis, and was optimized for screening of IAA metabolites in samples containing minute amounts (<10mg) of Arabidopsis thaliana tissue. Multivariate data analysis was then performed on the data set to identify mutants that were altered in their IAA metabolite profile. The method has so far identified several mutant lines with altered IAA metabolite profiles, and we are now in the process of characterizing the mutant phenotypes and identifying the mutated genes using next-generation sequencing.

P390 - PolyU Motifs in EBF1 3' UTR Are Necessary and Sufficient for EIN2-Directed Translational Inhibition

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The gaseous phytohormone ethylene is essential for many processes of plant growth and development. With ethylene treatment, EIN2 (ETHYLENE INSENSITIVE 2) undergoes a cleavage to produce a C-terminal (EIN2 CEND) fragment and enter into nuclear. Our research revealed that EIN2 CEND also associates with *EBF1/2* (EIN3 BINDING F-BOX 1/2) mRNA 3’UTR and form granules in cytoplasm to suppress the translation of *EBF1/2* mRNA. In order to identify the *cis*-elements in *EBF1/2* mRNA 3’UTR that respond to EIN2 and mediate translational repression, a dual-construct translation analysis system in tobacco leaves was established. 35S promoter drived GFP combined with or without different kinds of 3’UTRs are working as reporters while a 35S promoter drived mCherry is referred as an internal control. The alternations of GFP fluorescence are analyzed to evaluate the function of different 3’UTR fragments. We found a 7-8 nt successive uredines in the loop of stem loop structure (defined as polyU motif) were shared in the fragments that can mediate translational repression. Surprisingly, when all the seven poly U motifs in EBF1 3’UTR were deleted, translational repression was released. Then, a novel 3’UTR sequence only consisting of three repeated stem loop structures mentioned above was designed and it had the same function as full length 3’UTR. Similarly, phenotypes of *Arabidopsis* transgenic lines confirmed that polyU motifs are necessary and sufficient for EIN2 mediated translational silencing.

P391 - Regeneration of vascular tissue in wounded inflorescence stems of Arabidopsis.

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In our studies we established conditions allowing design Arabidopsis as a "small tree", with all features mimicking vasculature in woody plants. This gives new possibility to answer still open questions on the auxin-mediated canalization concept. We analyzed auxin signaling pathway mutants to test the auxin signaling requirement on tissue polarization and auxin-regulated canalization during regeneration of vascular tissues in wounded regions of Arabidopsis stems. According to the results, regeneration around a wound occurred very fast in control plants. Elevated auxin response and formation of auxin transport channels involving tissue repolarization was observed in 2 days after wounding (DAW) in *pPIN1::PIN1-GFP* and *DR5::GFP* lines. In mutant lines with elevated auxin response, faster regeneration including the channel formation and vasculature reconstruction was observed, whereas in auxin signaling mutants the regeneration around a wound was only fragmented and the whole process takes much longer period. These observations provided a strong support for the canalization hypothesis of vasculature formation and regeneration. It visualized the formation of auxin channels expressing the polarized PIN auxin transporters and showing elevated DR5 auxin response, which precede formation of new vasculature.

P392 - A MAPK cascade pathway is involved in the regulation of root growth and development in response to ABA in Arabidopsis thaliana

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Mitogen-activated protein kinase (MAPK) cascade is evolutionarily conserved signal transduction module involved in transducing extracellular signals to the nucleus for appropriate cellular adjustment. This cascade consists essentially of three components, a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK connected to each other by the event of phosphorylation. We report here the characterization of a MAPKKK named AAK1 (ABA-associated protein kinase) in *Arabidopsis*, regulating abscisic acid (ABA) responsive root growth and development. T-DNA insertion mutants of AAK1 showed insensitive to ABA in both root growth and stomatal closure. The effect on root growth was due to enhanced cell elongation in the elongated zone and cell division from root tip to elongated area. Bimolecular fluorescence complementation (BiFC) analysis showed that MPK3, MPK6 and AAK1 interact with MKK5, AAK1 also interacts with MKK4. *mpk3, mpk6* and *mkk5* single mutant seedlings have similar mutant phenotypes to *aak1*. AAK1 localized in the plasma cytoplasm, and was shown to active MKK5 by protein phosphorylation, this activation of MKK5 by AAK1 is an ABA-activated protein kinase. The activity of MPK6 was increased by ABA in wild-type seedlings, and the activity of MPK6 by ABA was impaired in *aak1* and *mkk5* mutants, but not *mkk4*. In addition, the expression levels of many ABA-responsive genes were altered in the *aak1*. These data clearly suggest AAK1-MKK5-MPK6 cascade function in ABA regulated primary root growth and development.

Key words: MAPK, abscisic acid, root development, signal transduction

P393 (Talk) - Rational design of a ligand-based antagonist of jasmonate perception

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(+)-*7-iso*-Jasmonoyl-L-isoleucine (JA-Ile) regulates developmental and stress responses in plants. Its perception involves the formation of a ternary complex with the F-box COI1 and a member of the JAZ family of co-repressors and leads to JAZ degradation. Coronatine (COR) is a bacterial phytotoxin that functionally mimics JA-Ile and interacts with the COI1-JAZ coreceptor with higher affinity than JA-Ile. On the basis of the co-receptor structure, we designed ligand derivatives that spatially impede the interaction of the co-receptor proteins and, therefore, should act as competitive antagonists. One derivative, coronatine-O-methyloxime (COR-MO), has strong activity in preventing the COI1-JAZ interaction, JAZ degradation and the effects of JA-Ile or COR on several JA-mediated responses in *Arabidopsis thaliana*. Moreover, it potentiates plant resistance, preventing the effect of bacterially produced COR during Pseudomonas syringae infections in different plant species, such as *Nicotiana benthamiana* or tomato. Furthermore, COR-MO can also reduce the rice blast symptoms of infection caused by *Magnaporthe oryzae*. In addition to the utility of COR-MO for plant biology research, our results underscore its biotechnological potential for safer and sustainable agriculture.

P394 - Isolation and identification of novel genes involved in gravitropism by using eal1 enhancer mutants

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eal1 (endodermal-amyloplast less 1) has one single-amino-acid deletion of the GRAS domain in SHR (SHORT-ROOT) which is one of transcription factors essential for formation of endodermis. *eal1* forms endodermis-like cell layer, which appears not to fully develop in shoots. *eal1* hypocotyls exhibit very weak but significant gravitropism. In the present study, we isolated six *eal1* enhancer mutants, *ene1-ene6* (enhancer of *eal1*), to find novel genes involved in gravitropism. We have performed hyper-gravity centrifugation to amplify the gravitropic phenotype of hypocotyls, and it enabled to select *ene* easily. Compared to *eal1* hypocotyls, the gravitropic response of *ene2* slightly reduced and that of the others drastically reduced. In roots, *ene1* and *ene2* exhibit normal gravitropism as with WT and *eal1*, while *ene3, ene4, ene5* and *ene6* exhibit decreased gravitropism. To detect causative genes for ene mutants, we applied NGS (Next Generation Sequencing). Then, we found that *ene3*

is an allele of *arg1 (altered response to gravity 1)* which is known as gravitropismrelated gene. This indicates that our method successfully identified genes associated with gravitropism. Further, we identified 3 genes as strong candidates for *ene2*. These genes have not been reported in gravitropism. Identification of the novel genes including *ene2* would help to comprehend the mechanism of gravitropism.

P395 - Discovery of protein kinase for protein tyrosine phosphorylation in plants

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In plants, recent studies have identified Tyr-phosphorylated proteins in Arabidopsis and rice by phosphoproteomic screening, implying that plants have a Tyr phosphorylation signal pathway like in mammals. However, protein kinases (PKs) that catalyze Tyr-phosphorylation in plants in vivo are largely unknown. Here, we demonstrate that CDPK-related PKs (CRKs) have high Tyr autophosphorylation activity and that they can phosphorylate Tyr residue(s) on substrate proteins in Arabidopsis. To identify PKs for Tyr phosphorylation, we examined the autophosphorylation activity of 759 PKs using an Arabidopsis protein array based on a wheat cell-free system. In total, we identified 38 PKs with Tyr autophosphorylation activity. The CRK family was a major protein family identified. A cell-free substrate screening revealed that CRKs phosphorylate beta-tubulin (TBB) 2, TBB7 and certain transcription factors such as ERF13. All five CRKs tested showed Tyr auto/trans-phosphorylation activity and especially two CRKs, CRK2 and CRK3, showed a high ERF13 Tyr phosphorylation activity. A transient expression assay revealed that Tyr16/207 sites in ERF13 were phosphorylated by CRK3 and that Tyr phosphorylation of endogenous TBBs occurs in CRK2 overexpressing cells. Furthermore, CRK knockout mutants show reduced Tyr-phosphorylation of TBB proteins. These results suggest that CRKs have Tyr kinase activity, and these might be responsible for much of the protein Tyr-phosphorylation *in vivo*.

P396 - Reciprocal regulation between salicylate- and jasmonate-responsive transcription factors in plant immunity

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Activation of plant immunity involves transcriptional reprogramming orchestrated by the immune hormones salicylate (SA) and jasmonate (JA). Antagonism between SA and JA fine tunes timely expression of immune genes, but the molecular mechanisms remain elusive. Here we report that reciprocal regulation between SA and JA signaling is regulated by the transcription cofactor NPR1 and JAZ repressors. SA-induced NPR1 suppressed JA-dependent gene expression by physically interacting with MYC transcription activators and promoting their ubiquitination. Conversely, like NPR1, JAZ repressors unexpectedly functioned as positive regulators of SA-dependent immunity by removing WRKY transcription repressors from SA-responsive promoter motifs. These findings reveal the dynamic transcriptional network underlying not only antagonistic but also cooperative interactions between the SA and JA signals that orchestrate plant immune responses.

P397 - The MAX1 Gene Encodes a Carlactone C-19 Oxidase in Arabidopsis

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Strigolactones (SLs) are plant hormones that inhibit shoot branching, and are parasitic and symbiotic signals toward root parasitic plants and arbuscular mycorrhizal fungi, respectively. SLs are biosynthesized from a precursor, named carlactone (CL), which is derived from carotenoid. However, no downstream pathway of CL has been elucidated. We found previously an extreme accumulation of CL in the *Arabidopsis max1* mutant, which exhibits increased lateral inflorescences due to SL deficiency, indicating that CL is a probable substrate for MAX1 (CYP711A1), a cytochrome P450 monooxygenase. To elucidate the enzymatic function of MAX1 in SL biosynthesis, we incubated CL with a recombinant MAX1 protein expressed in yeast microsomes. MAX1 catalyzed consecutive oxidations at C-19 of CL to convert into a carboxylated metabolite, named carlactonoic acid (CLA). We also identified endogenous CLA and its methyl ester, named methyl carlactonoate (MeCLA), in *Arabidopsis* plants using LC-MS/MS. Although an exogenous application of either CLA or MeCLA suppressed the growth of lateral inflorescences of the *max1* mutant, MeCLA, but not CLA, interacted with AtD14 protein, a putative SL receptor of *Arabidopsis*, as shown by differential scanning fluorimetry and hydrolysis activity tests. These results indicate that not only known SLs but also MeCLA are biologically active in inhibiting shoot branching in *Arabidopsis*.

P398 - Cell-type-specific cytokinin distribution within the Arabidopsis primary root apex

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Cytokinins (CKs) play a crucial role in many physiological and developmental processes at the levels of individual plant components (cells, tissues and organs) and by coordinating activities across these parts. High-resolution measurements of intracellular CKs in different plant tissues can therefore provide insights into their metabolism and mode of action. Herein, we applied fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with solid-phase micro-extraction and an ultra-high sensitivity mass spectrometry (MS) method for analysis of CK biosynthesis and homeostasis at cellular resolution. The newly developed method was validated by series of control experiments, establishing that protoplast isolation and cell sorting procedures did not greatly alter endogenous CK levels. The MS-based method facilitated the quantification of all the well-known CK isoprenoid metabolites in four different transgenic *Arabidopsis thaliana* lines expressing GFP in specific cell populations within the primary root apex. Our results revealed the presence of a CK gradient within the *Arabidopsis* root tip, with a concentration maximum in the lateral root cap, columella, columella initials and quiescent centre cells. This distribution, when compared with previously published auxin gradients, implies that the well-known antagonistic interactions between the two hormone groups are cell-type-specific.

P399 - AtAIRP2, an Arabidopsis RING E3 Ubiquitin Ligase, Promotes Degradation of CAS1 to Positively Regulate ABA Signaling during Seed Germination

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The ubiquitin-mediated 26S proteasome pathway is a principal mechanism for protein catabolism to regulate plant growth and development. The ubiquitination is an enzymatic cascade of E1, E2, and E3 Ub ligase. In general, E3 Ub ligases determine the specificity of substrate proteins. Here, we identified an E3 ligase, AtAIRP2 (for *Arabidopsis* ABA-Insensitive RING protein 2), and CAS1 (for a Candidate of AtAIRP2 Substrate protein 1). The *AtAIRP2* over-expressing transgenic and *atairp2* loss-of-function mutant plants exhibited hypersensitive and hyposensitive phenotypes, respectively, toward ABA at germination stage. We identified CAS1, an interacting protein of AtAIRP2, through a yeast two-hybrid screening. The stability of CAS1-myc recombinant protein was enhanced by MG132, and degradation of CAS1-myc recombinant protein was promoted by AtAIRP2. The *CAS1* knock-down transgenic plants showed more delayed growth at germination and seedling stages as compared to wild-type plants on ABA-supplemented medium. In addition, knock-down of *CAS1* suppressed the ABA insensitive germination phenotype of *atairp2* mutant plants. Overall, these results suggest that AtAIRP2, a C3HC4-type RING E3 Ub ligase, is involved in the positive regulation of ABA signaling at germination stage by stimulating CAS1 turn-over. This work was supported by grants from the National Research Foundation (Project No. 2014R1A2A2A01003891 funded by the Ministry of Education, Science, and Technology, Republic of Korea).

P400 - Can ABA Binds to and Affects Guard Cell Channels Activity?

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Abscisic acid (ABA) is a key hormone and has important regulatory roles in plant development and physiological processes. To date, there is no evidence for direct interaction between ABA and ion channels although ABA is known to affect ion channels in stomata. Here, we examine the effects of ABA on the outward K+-channel of *Vicia faba* guard cells by patch-clamp. Membrane depolarization to values positive of the Nernst potential for K^+ (E_K) activated a 21pS channel (111mM K^+ in : 11mM K^+ out). Addition of 20–100μM of (±)-ABA to the bath medium increased the K+-channel activity resulting in sustained opening times while the (-)-*cis,trans*-ABA had no effect. Importantly, this ABA-dependent effect was reversed upon ABA washout. The electrophysiology data suggests a membrane-delimited effect of ABA most likely on the K^+-channel itself. We have identified an ABA-binding motif in *Arabidopsis thaliana* guard cell outward rectifying K^+-channel (GORK) [AT5G37500] that resembles the ABA-binding site of the ABA-START receptors. This putative ABA-binding site was assessed by homology modeling and molecular docking. Importantly, we showed binding of (±)-ABA to a truncated recombinant GORK protein that contains the predicted ABA-binding site by immunoassay. We observed absorbance measurement that is significantly higher than the controls and consistent across replicates. Currently, we are investigating the effect of ABA on GORK expressed in the mammalian HEK cells by electrophysiology.

P401 - In vivo isotope labelling for auxin metabolic studies

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The phytohormone auxin (Indole-3-acetic acid; IAA) plays a fundamental role in various processes of plant growth and development. Crucial for auxin action is the interplay between its transport, biosynthesis, degradation and conjugation. In order to study the pathways of auxin biosynthesis and degradation, sensitive methods for analyzing steady state levels of IAA and its metabolites have been recently developed (Novák et al. 2012). The use of heavy isotope labelling of intermediates in conjunction with mass spectrometry analysis has proven to be a powerful tool for monitoring fluxes through IAA metabolic pathways. Such methods for *in vivo* labelling have been used to identify IAA biosynthetic intermediates and IAA degradation products. We performed a feeding experiments using stable labelled indole in *Arabidopsis thaliana* to investigate auxin metabolism. Incorporation of the ^{15}N from labelled precursor into most abundant IAA biosynthetic intermediates (tryptophan, indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM) and indole-3-acetonitrile (IAN)) as well as metabolites IAA-aspartate (IAAsp), IAA-glutamate (IAGlu), 2-oxoindole-3-acetic acid (oxIAA), IAA-glucose and oxIAA-glucose) was monitored by mass spectrometry. Obtained results provide us important information about the rates of IAA biosynthesis and degradation and the relevance of individual IAA precursors, catabolites and conjugates. Combined with mutant line screening, the developed approach can improve our knowledge about auxin metabolic pathways in *Arabidopsis thaliana*.

P402 - Learning the Language of the Chloroplast: Retrograde Signals That Regulate Stomatal and ABA responses

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The chloroplast can be an environmental sensor for the cell, communicating with the nucleus via retroagrade signals during biogenesis and operation to change the expression of up to thousands of genes. Recent advances have identified retrograde signals and pathways ranging from carotenoid derivatives, phosphoadenosines such as PAP, tetropyrroles and heme together with reactive oxygen species and proteins that build a communication network to regulate gene expression, RNA turnover and splicing. However, retrograde signaling pathways have largely been viewed as a means for bi-lateral communication between organelles and nuclei, ignoring the potential for interaction with hormone signaling regulating plant form and function. The impact of new findings on the processes by which organelle communication is initiated, transmitted and perceived to regulate not just chloroplast processes, but intersect with hormonal signaling altering physiological responses will be considered. Specifically, genetic manipulation of the retrograde signal, PAP, enables ABA-mediated stomatal closure conferring drought tolerance in wild-type and ABA insensitive mutants; likely via a distinct, XRN mediated transcriptional pathway in guard cells. PAP also acts as an ABA agonist in seed germination independent of ABI1. Thus, chloroplast-nuclear communication mediated by PAP regulates stomata closure and germination implicating this metabolite as a secondary messenger.

P403 - Exploring defence signalling pathways: identification of novel regulators of combinatorial stress responses

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Plant responses to multiple biotic/abiotic stresses employ finely tuned regulatory mechanisms, largely orchestrated by phytohormones. In particular, salicylic acid (SA) plays a central role in defense responses against biotrophic and hemibiotrophic pathogens, whereas jasmonic acid (JA) and ethylene (ET) are usually associated with defense against necrotrophic pathogens and herbivorous insects. In addition, abscisic acid (ABA) is the main signalling molecule of abiotic stress responses. To coordinate the complex interactions occurring during multiple stresses, an intense cross-talk among the regulatory networks is necessary. To obtain new insights into the selective capacity of plants to adapt to combinatorial stresses, their response to multiple phytohormones was explored. In particular, the effect of ABA, SA on JA responses was analyzed using a collection of 350 *Arabidopsis thaliana* accessions. This collection represents a population of globally collected plants that have been genotyped for around 250000 SNPs, allowing for genome-wide association (GWA) studies. The obtained data, reflecting a great natural variation in response to the mimicked combinatorial stresses were used to identify novel genes playing roles in the adaptation of plants to different stress conditions.

404 - The controlled in vivo accumulation of oligogalacturonides by the OG-machine: a tool to study DAMPs in the growth-defence tradeoff

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Pathogen infection is able to trigger an innate immune response in plants, that is often accompanied by a reduction of growth. Plants balance the costs associated to activation of defense responses by limiting biomass production. We hypothesized that oligogalacturonides (OGs), endogenous damage-associated molecular patterns (DAMPs) produced by the action of microbial polygalacturonases (PGs) and able to elicit multiple defense responses, modulate plant growth during infection. We have recently shown that transgenic Arabidopsis plants that express a fusion protein between a fungal PG and a plant PG-inhibiting protein (PGIP) and named "OG-machine" (OGM), accumulate OGs in the apoplast and display enhanced defense responses and resistance to pathogens, but also a significant reduction of growth. We have therefore used the OGM to dissect the mechanisms that regulate growth and defence induced by OGs. Plants expressing the OGM in specific Arabidopsis tissues or in mutant backgrounds impaired in different defense-related pathways have been generated and their growth has been analyzed after induction of the transgene. Cross-talk of the responses induced by the OGM and growth-related hormones, in particular auxins, has also been investigated. The results of these analyses, and a model of the interactions between defense responses induced by DAMPs during infection and growth-related effects caused by these molecules will be presented.

P405 (Talk) - Cytokinin Response Factor 6 is a key regulator of cytokinin and oxidative stress

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Cytokinin Response Factor 6 (CRF6) is a cytokinin (CK) responsive AP2/ERF transcription factor that via CK signaling plays a key role in the inhibition of dark-induced senescence. CRF6 expression is also induced by oxidative stress and it is a direct transcriptional target of ANAC transcription factors in the mitochondrial retrograde signaling pathway. Here we show that CRF6 functions in oxidative stress tolerance and identify downstream transcriptional targets of CRF6 in response to both CK and oxidative stress. WT and *crf6* plants were treated with CK or H_2O_2 and transcriptome changes were analyzed. Comparison of differentially expressed genes revealed CRF6-dependent transcripts, of which a larger proportion of such transcripts were found to be repressed, rather than induced. Moreover, many repressed genes also show decreased expression in 35S:CRF6 overexpressing plants. Together this suggests that CRF6 functions largely as a transcriptional repressor. Interestingly among the H_2O_2 repressed - CRF6 dependent transcripts was a set of six genes associated with different CK processes: (signaling) *ARR6, ARR9, ARR11, AHP1*, (biosynthesis) *LOG7*, and (transport) *ABCG14*. Efforts are underway to determine direct binding of CRF6 to the promoters of these target genes and the role that CRF6 is playing in CK regulation of abiotic stress.

P406 - Deciphering Root System Architecture Plasticity to Hormones using Genome-Wide Association Mapping

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As sessile organisms, plants have to adapt their growth and development to challenging environments, and therefore have evolved complex sensing and signaling mechanisms that allow them to integrate both internal and external signal information. Root System Architecture (RSA) exhibit great amount of developmental plasticity, and represent ideal model for studying developmental plasticity in plants. Increasing evidence suggests that the environmental effects are often mediated through mechanisms involved in hormone signal transduction. These root developmental plasticity responses are genetically determined. However, recent evidence suggests there is significant natural variation for root traits. Thus, natural variation of hormonal root plasticity can be used to map the alleles responsible for regulation of root traits. Here, we have conducted extensive RSA quantification of 10 root traits of 192 Arabidopsis accessions on transient hormone treatments, including auxin (IAA), cytokinin (CK), abcisic acid (ABA) and control. Using clustering and principal component analysis we identified specific groups of accessions that have common or diverse response to a particular hormone. Using genome-wide association (GWA) mapping we identified over 100 significantly associated SNPs in all of the conditions tested. We are currently selecting the most meaningful candidate genes, based on the sequence polymorphism and statistical significance of associations. We will test the phenotypic consequences in loss of function lines and conduct detailed gene characterization with regard to their involvement in root development.

P407 (Talk) - Transcriptome and metabolome analysis of developing seeds of Arabidopsis nced mutants deficient in abscisic acid biosynthesis

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The importance of ABA in dormancy induction is now well acknowledged, but its signaling network in developing seeds still remain elusive, even if it has been well established that dormancy is triggered by embryonic and not maternal ABA. The family of five genes encoding the 9-*cis*-epoxycarotenoid dioxygenase (NCED) has been proved to play a key role in the regulation of ABA biosynthesis. *NCED3* was reported as the only *NCED* gene highly expressed in vegetative tissues in response to water stress. We later showed that *NCED5, NCED6* and *NCED9* genes were spatio-temporally regulated in Arabidopsis seed tissues. To get new insights into the ABA regulatory role in dormancy induction during seed development on the mother plant, we designed multiple mutants, which are ABA deficient in specific seed tissues, but not in maternal vegetative parts. Transcriptome analysis of developing seeds revealed more than 5000 genes up or down-regulated in multiple *nced* mutants compared to wild type. These included members of gene families belonging to the ABA core signaling network. Major differences in metabolite composition were observed during late seed maturation. A comparative analysis of transcriptome and metabolome data has been carried out.

P408 - Cytokinin regulated vascular patterning in Arabidopsis primary root involves RNA methylation

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The highly organized vasculature in *Arabidopsis* primary root represents a robust model to study cell patterning. Earlier, we have shown that phytohormones cytokinin and auxin regulate vascular patterning in an interactive manner. The current project is focused on search for novel factors that may participate in cytokinin and auxin signaling pathways and modify vascular patterning in the *Arabidopsis* primary root. In search for those factors, we use a variety of approaches such as forward genetics screens for misexpression of specific markers. One such marker gene for altered vascular patterning is *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6)*, a negative regulator of cytokinin signaling that is induced by auxin and expressed in a specific cell type in the primary root (Mähönen et al. 2006; Bishopp et al. 2011). Based on this approach, we have recently identified two loci that are likely to be involved in RNA methylation. Here, we introduce a mutant initially identified by an expanded *AHP6* expression domain in a cytokinin hyposensitive background. This novel mutant is also characterized by a distinct shoot developmental phenotype. We aim at identifying the RNA modifications corresponding to our newly identified loci, and study how these modifications affect cytokinin responsiveness and development in *Arabidopsis* root and shoot.

409 - Investigating the role of the Ptranscriptional control of auxin in the gravity sensing-cells in Arabidopsis during graviresponse

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Plants are able to grow and adapt to their environment via tropic responses to external stimuli such as gravity. The ability to respond to changes in orientation allows plants to modify their growth to maintain specific patterns of growth with respect to gravity. An important component of the control of growth angle with respect to gravity is the fact that the magnitude of graviresponse varies with the angle of displacement from the vertical. The mechanisms governing changes in the magnitude of gravitropic response are still unknown. It has recently been shown that altering auxin response specifically within the gravity-sensing cells can affect graviresponse in the shoot (Roychoudhry et al. 2013, Sato et al. 2014). We are investigating whether the transcriptional responses to auxin via the TIR1/AFB-Aux/IAA-ARF system play a role in the control of the magnitude of the response in the Arabidopsis root gravity-sensing cells or statocytes. Using both free response gravitropism assays and a constant gravitropic stimulus feedback system (Mullen et al. 2002), transgenic lines with altered auxin response in primary and lateral root gravity-sensing cells have been studied. Initial results indicate that transcriptional responses to auxin within statocytes do play a role in regulating the magnitude of the gravitropic response. We are working to understand the mechanistic basis of this phenomenon.

P410 - Heat-Induced Male Sterility is Reversed by Cytokinin, Mediated by Sucrose and Expression of Putative Sucrose Transporter AtSweet7

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High temperatures during flowering are known to reduce fertility and reproductive success in many plant species due to male sterility. This effect is considered to involve reductions in pollen production, viability, release from the stigma, or growth of pollen tubes. The molecular and hormonal mechanisms behind this reduction in reproductive success by heat are not well understood. Existing evidence indicates that cytokinins are essential to pollen production under normal growth temperatures in several plant models. Further, specific to non-permissive high temperatures, sugars have been implicated in reproductive success in tomato. We show here that exogenous application of cytokinins, as well as of sucrose, were able to substantially improve fertilization and fruit set under fertility-limiting high temperatures in Arabidopsis thaliana. In bean grown under high temperature conditions during flowering in the field, pod production was also significantly increased by cytokinin application. The molecular mechanism of this capacity for cytokinin is proposed to involve sugar movement to and accumulation in the flowers. Consistent with this explanation, an Arabidopsis knockout line at sucrose transporter AtSweet 7 showed reduced recovery of heat fertility by exogenous cytokinin treatment compared to wild type.

P411 - Ethylene Inhibits Cell Proliferation of the Arabidopsis Root Meristem

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The root system of plants plays a critical role in plant growth and survival, with root growth being dependent on both cell proliferation and cell elongation. Multiple phytohormones interact to control root growth, including ethylene which is primarily known for its role in controlling root cell elongation. We took advantage of genetic, pharmacological, and molecular tools to evaluate the role of ethylene in regulating cell proliferation at the primary root meristem of Arabidopsis. Our results indicate that ethylene negatively regulates root-cell proliferation primarily through the classical ethylene receptor-CTR1-EIN2-EIN3/EIL signaling pathway. In addition, we identify a minor role for the two-component signaling pathway in this process, based on genetic analysis of receptor kinase activity as well as mutants involving the type-B response regulator *ARR1*. Because cytokinin induces ethylene biosynthesis, we also examined the role of ethylene in mediating the effects of cytokinin in inhibiting cell proliferation at the root meristem. Our results indicate that ethylene signaling contributes to but is not required for cytokinin to inhibit activity of the root meristem. Ethylene-dependent gene expression at the root meristem suggests that auxin-dependent and independent mechanisms may play roles in mediating the effects of ethylene on cell proliferation. These results support a model in which multiple phytohormones, including ethylene, interact to regulate cell proliferation at the root apical meristem.

P412 - Identification of plant hormone transporters by a new approach

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Plant hormones are endogenous bioactive small compounds that induce various physiological responses at low concentrations. It has been considered that most of the plant hormones are mobile. However, it is largely unknown how the transport of hormones is regulated. We recently developed a functional screening system to identify plant hormone transporters based on their activities using the yeast two-hybrid systems to detect hormone dependent formation of the hormone receptor complexes. By this approach, we identified NPF4.6, a member of Arabidopsis NRT1/PTR FAMILY (NPF) proteins as an abscisic acid (ABA) transporter. We also found that some other NPF proteins could also transport ABA, gibberellin (GA) and/or jasmonoyl isoleucine (JA-Ile). To determine the in vivo functions of the NPFs, we are currently investigating the phenotypes of the mutants defective in the transporters. We are also conducting large scale screening against Arabidopsis cDNA libraries using the yeast two-hybrid systems with the receptor complexes to look for novel GA and JA-Ile transporters other than NPFs.

P413 - Characterization of GH3 Acyl-Acid Amido Synthetases from Arabidopsis thaliana

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Plant hormones play an essential role in plant growth and development as well as responses to biotic and abiotic stresses. Regulation of hormone levels is acheived through both the regulation of synthesis and degradation. Inactivation or activation of hormones through conjugation is another process that can be utilized to control hormone activity. One family of enzymes known to be involved in regulating plant hormones through conjugation is the GH3 family of acyl-acid amido synthetases in *Arabidopsis thaliana*. Various family members have been shown to conjugate amino acids to jasmonic acid (JA), the auxin indole-3-acetic acid (IAA), and salicylic acid (SA). The hormone and amino acid substrates for several GH3 family members are unknown. Work in the Jez lab indicates that GH3.15 conjugates glutamine to indole-3-butyric acid (IBA), a precursor of IAA. Primary root length and lateral root density of knockout and overexpression lines of GH3.15, grown in the presence of IBA, show hypersensitivity and resistance respectively. Our data suggest GH3.15 is involved in the regulation of IBA.

P414 - A novel targeted metabolomic approach in plant hormone analysis

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Plant hormones are highly bioactive signalling molecules, which are acting as chemical messengers involved in physiological processes such as plant growth and development including flowering, seed germination, senescence and various stress responses. The occurrence as awell as the levels of these compounds strongly depend on plant organ, plant age, developmental stage and environmental conditions. As characteristic for the substances of hormonal nature, they are typically present in plant tissue only in minute concentrations. Thus, their direct quantification in very complex plant extract poses a difficult analytical task. Plant hormones as extremely large family of diverse compounds could be divided into several structurally different groups such as purine and indole derivatives, plant steroids, lipid-based substances and terpenoid carboxylic acids. In this study, we present a new method based on ultra-high liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for targeted profiling of more than 100 analytes, members of the main classes of plant hormones like cytokinins, auxins, brassinosteroids, gibberellins, jasmonates and abscisic acid. This plant-based metabolomic approach involves primary metabolites of bioactive hormones as well as their biosynthetic precursors playing indispensable role in intricate signalling network leading to the regulation of various biological processes in plants. We believe that this generalized analytical screening method can be very useful tool for phytohormonal studies dealing with their inter- and intra-cellular communications in plants.

P415 - Characterization of a higher order mutant of cytokinin degradation and inactivation enzymes

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Cytokinins are responsible for major physiological processes including senescence where nutrients are invested from source to sink tissues to ensure propagation of next generations. Cytokinin signalling of this process is crucial so in leaves an enormous decrease of cytokinin levels signalize to favour nutrient shift to sink organs. The decrease in *Arabidopsis thaliana* leaves is caused by cytokinin dehydrogenases CKX2 and CKX5. However, no senescence delay was observed in the double knock-out mutant where significantly increased levels of cytokinin glucosides were detected. Cytokinin inactivation during senescence is catalysed by UDP-glycosyltransferase UGT85A1 and therefore a triple mutant *ckx2/ckx5/ugt85a1* was prepared and characterised. The *ckx2/ckx5/ugt85a1* mutant was assessed for phenotype modifications, cytokinin content, transcriptome and proteome analysis under normal conditions and in response to induced senescence, light stress and exogenous cytokinin treatment, respectively. Even though our results suggest that neither UGT85A1 nor CKX2 or CKX5 are the crucial key regulators in senescence process of *A. thalina* leaves our results show how *A. thaliana* modulates the cytokinin metabolism to maintain the proper homeostasis during senescence.

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P416 - A MAPK cascade pathway is involved in the regulation of root growth and development in response to ABA in Arabidopsis thaliana

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Mitogen-activated protein kinase (MAPK) cascade is evolutionarily conserved signal transduction module involved in transducing extracellular signals to the nucleus for appropriate cellular adjustment. This cascade consists essentially of three components, a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK connected to each other by the event of phosphorylation. We report here the characterization of the ABA-associated stress responsive MAPK family member named AAK1 (ABA-associated protein kinase) in *Arabidopsis*, regulating abscisic acid (ABA) responsive root growth and development. T-DNA insertion mutants of *AAK1* showed insensitive to ABA in both root growth and stomatal closure. The effect on root growth was due to enhanced cell elongation in the elongated zone and cell division from root tip to elongated area. Bimolecular fluorescence complementation (BiFC) analysis showed that MPK3, MPK6 and AAK1 interact with MKK5, AAK1 also interacts with MKK4. *mpk3, mpk6* and *mkk5* single mutant seedlings have similar mutant phenotypes to *aak1*. AAK1 localized in the plasma cytoplasm, and was shown to active MKK5 by protein phosphorylation, this activation of MKK5 by AAK1 is an ABA-activated protein kinase. The activity of MPK6 was increased by ABA in wild-type seedlings, and the activity of MPK6 by ABA was impaired in *aak1* and *mkk5* mutants, but not *mkk4*. In addition, the expression levels of many ABA-responsive genes were altered in the aak1. These data clearly suggest AAK1-MKK5-MPK6 cascade function in ABA-regulated primary root growth and development.

Key words: MAPK, abscisic acid, root development, signal transduction

P417 - Jasmonates and chemical biology: a short story

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Jasmonic acid (JA) and its derivatives, collectively named as jasmonates, represent clear examples for the participation of small signaling molecules in modulating the plant"s behavior towards different biotic and abiotic stresses. JA mediates resistance towards wounding, necrotrophs, and herbivores attack. Although classical genetic approaches uncovered numerous components involved in JA perception and signaling pathway, there are open questions to fully understand the multiple roles of diverse jasmonates in regulating numerous biological responses. Moreover, the cross-talk between JA and other phytohormones is not fully understood. To find answers, a chemical genetic approach is used in this study to create conditional mutants and therefore circumvent the limitations of classical genetics such as redundancy, lethality, and pleiotropy of gene functions. A transgenic *Arabidopsis thaliana* line, reporting the expression of a JA-responsive marker gene (VSP1p::GUS), is used to screen several chemical libraries (1460 compound) to identify bioactive compounds capable of modulating the JA-responsive marker gene"s expression. Four novel compounds showed to be selectively inhibiting JA signaling under our experimental conditions. The target identification experiments indicated the possible target(s) at or downstream of the hormonal perception and signaling. Unraveling the mode of action and target(s) of these compounds may help in identifying new component(s) of JA signaling pathways.

P418 (Talk) - Subcellular localization and interaction of Zmphot1 and ZmNPH3-like proteins

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Phototropism has been noted since before the century, however, the detailed mechanism is still unclear. We previously showed that the tip region about 0-2 mm of maize coleoptile is the specific site for low fluence blue-light (LBL) perception as well as formation of asymmetric

indole-3-acetic acid (IAA) distribution. It is generally accepted that the localization of Zmphot1 determines the site of light perception. To clarify the involvement of Zmphot1 in the tip specific LBL-induced responses, we examined its distribution in detail. Interestingly, Zmphot1 proteins were detected in relative evenly down to 10-mm regions from of coleoptiles, suggesting that Zmphot1 could perceive unilateral blue-light at relatively wide regions. Therefore, factors other than Zmphot1 might be responsible for determining the tip specific LBL perception and following phototropic curvature. From micro-array and immune-blot analyses, we found a tip specific expressing protein, ZmNPH3-like, which seems to be one of substrates of Zmphot1. We further investigated subcellular localization of Zmphot1 and ZmNPH3-like proteins tagged with fluorescent proteins and their interaction. Transient expressions of these proteins in onion epidermal cells showed that both proteins were localized on plasma membrane. In addition, it is possible that both proteins are interacted each other. We will discuss involvements of Zmphot1 and ZmNPH3-like protein in tip specific blue light perception and phototropism.

P419 - Identification of novel auxin signaling components in root development using GWAS

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The shape of root system (Root System Architecture: RSA) plays a critical role in plant survival in different environments. RSA is determined by processes that are strongly regulated by the phytohormone auxin. However, auxin is involved in almost every process of plant growth and development, and the genetic mechanisms by which RSA is modified independently of these other processes are not well understood. To elucidate which mechanisms allow plasticity of root traits despite their regulation by auxin, we used natural variation in Arabidopsis thaliana and conducted a Genome Wide Association Study (GWAS). Our GWAS identified a member of an exocytotic gene family, *EXOCYST*, to be associated with a root gravitropism-related trait. We showed that this *EXOCYST* gene exclusively regulates the dynamic localization of the auxin efflux carrier PIN-FORMED 4 in columella cells, and thereby controls RSA. Furthermore, natural variation of the *EXOCYST* gene was associated with drought resistance, indicating an adaptive value in drought conditions. All together, our study using GWAS revealed a novel auxin signaling component which regulates root morphology and allows natural variation of RSA.

P420 - Functional analysis of DGE1, DGE2 and DTL genes involved in gravitropism in Arabidopsis thaliana

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To identify novel genes involved in signaling process in gravity sensing cells (statocytes) during gravitropic response, we have performed comparative transcriptome analysis between WT and mutants (*sgr1/scr, sgr7/shr and eal1*) Arabidopsis stems. Based on the results, we found that genes encoding DLLs (DGE1/LAZY Like proteins; DGE1, DGE2, DTL) are involved in gravitropic response of inflorescence stems, hypocotyls and primary roots. Furthermore, we indicated that *DLLs* are mainly expressed in statocytes and probably have a functional role in a process between gravity perception and formation of asymmetric auxin signal. Here, we demonstrated that DLLs expressed under the control of statocyte specific promoter are rescuing gravitropic phenotype of *dlls* triple mutant. DLLs proteins share sequence similarity, although they contain no known functional domais and/nor motifs. We focused on conserved 14 aa sequence at the C-termini (CDL, Conserved C-terminal in DGE1/LAZY1), and found that CDL is necessary for the function of DLLs. The result indicates that CDL would be a novel functional motif in the DLLs protein family.

421 - Abscisic acid sensor RCAR7, specific regulator of protein phosphatase coreceptors

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Abscisic acid (ABA) is an important phytohormone, that is involved in the adaptation to biotic and abiotic stresses, such as cold and drought stress. The Regulatory Component of ABA Receptor (RCAR) family consists of 14 members divided into 3 subfamilies. The RCARs provide a low affinity binding pocket for ABA (> 1mM). Magnesium ions-dependent protein serine/threonine phosphatases of type 2C (PP2C) of clade A interact with RCAR. In presence of ABA, the RCAR and clade A PP2Cs accumulate a heteromeric receptor complex, which inhibit the PP2C phosphatase activity at low ABA levels (<

0.1mM). The objective is to elucidate the contribution of the 14 RCARs and 9 PP2Cs in ABA responses; the interaction between individual PP2Cs and RCARs was studied via yeast interaction analyses, in vitro enzymatic assays, and in vivo studies. Distinct regulatory differences among individual RCAR and PP2C combinations are observed.

P422 - Signalling pathways mediating seed dormancy show distinct cell type specificity which is modulated by the environment

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The breaking of seed dormancy is one of the major decisions in the angiosperm lifecycle. Genetic and hormonal factors influencing this decision have been identified, yet the site(s) within the embryo where this binary fate switch is regulated remains unknown. Using 3D image analysis, the abundance of key dormancy regulatory proteins were quantified at singlecell resolution in *Arabidopsis* embryos. A majority of these components are found to be highly enriched in the embryonic radicle of primary dormant seeds. Within the radicle, the cell types where dormancy-promoting abscisic acid (ABA) signalling factors are present is distinct from those containing germination-promoting hormone gibberellic acid (GA) components. This spatial displacement between antagonistic signals suggest distinct cell types play opposing roles in the decision to terminate dormancy, and that non-cell autonomous signalling mediates this switch. Blocking the germination of seeds with far-red light stimulates the spatial re-localization of both GA and ABA signalling components to novel cellular locations. This suggests the role of distinct cell types in mediating the decision to germinate is not determinate, and that cells can reassign their function in response to the environment.

P423 - Phosphoproteomic analysis towards understanding the evolution of ABA signaling pathway in land plants.

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ABA is a major phytohormone which is critical for plant"s responses to environmental conditions. The major ABA signaling pathway consists of three core components; receptor, protein phosphatase (PP2C) and protein kinase (SnRK2). Recently, our phosphoproteomic analysis successfully revealed SnRK2-dependent phosphorylation network in Arabidopsis. Here we hypothesized that the ABA signaling system could be conserved in land plants, for example Arabidopsis and *Physcomitrella patens*. We enriched phosphopeptides from WT and ABA hypersensitive/insensitive mutants of *P. patens*, and then the peptides were analyzed with a LC-MS/MS system. Our data covered a total of 4,698 phosphopeptides from 2,007 proteins with 6,545 phospho-sites. Then ABA-responsive phosphopeptides in *P. patens* were identified and compared with Arabidopsis data. Our study suggested that the core pathway of ABA signaling are partially conserved between Arabidopsis and *P. patens*, as well as that *P. patens* has developed its own signaling network. Now

we are trying to select phosphopeptides which were conserved and potentially important for ABA signaling system in plants.

P424 - An oxidation of the oligogalacturonides inactivates their activity as Damage-Associated Molecular Patterns (DAMPs)

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Transgenic Arabidopsis plants expressing a chimeric protein constituted by a fungal PG (FpPG) and a PGIP from common bean (PvPGIP2), named "OG-machine" (OGM plants), accumulate oligogalacturonides (OGs) in their tissue and exhibit enhanced resistance to a variety of pathogens, thereby providing direct evidence for the function of OGs as in vivo elicitors of the plant defense responses. We have found that OG preparations, obtained from leaf strips of OGM plants by incubation in a chelating agent solution, contain both typical OGs and modified OGs. Further analyses showed that the oligomers corresponding to modified OGs are characterized by a galactaric acid residue at the reducing end, leading to the conclusion they are oxidized OGs. Oxidized OGs were purified to homogeneity and tested for their ability both in inducing the defense responses and in antagonizing auxin responses. In all experiments, oxidized OGs are inactive compared with the corresponding typical OGs. We have also extracted from OGM plants an enzyme activity capable of oxidizing standard OG preparations. We believe that this activity is a key element for controlling a too prolonged existence of OGs in the tissue and avoiding an exaggerated activation of plant defences.

P425 - Characterization of putative nuclear targets of MAPKs activated after flagellin22 treatment

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The MAPK signaling pathway plays a central role in stress responses in the model plant *Arabidopsis thaliana*. Plants sense external stress stimuli, perceived by their surface receptors and convert them into intracellular responses. MAPKs regulate a large number of diverse substrates by phosphorylation. A quantitative phosphoproteomics approach was carried out to find putative targets that are phosphorylated in response to the bacterial peptide flagellin22. We report here the current state of characterization of 3 putative targets (*PT*). To clarify the role of *PT1, 2* and *3* in response to pathogen attack, we tried to figure out which kinases are able to interact with PT1, 2, 3 by yeast-two hybrid and bimolecular fluorescence complementation (BIFC) analysis. On confirming the specific kinases as binding partner, we will check whether the respective kinases can in fact phosphorylate the targets by performing *in vitro* kinase assays. To validate the phosphosites identified in the large-scale phosphoproteomics screen, we will mutate the MAPK motif PxSP and perform kinase assays and complementation studies *in planta*. Furthermore, to assess the involvement of PT1, 2, 3 in the immune response, pathogen assays were carried out on *pt1, 2* and *3* T-DNA insertion mutants using *Pseudomonas syringe DC3000* as the pathogen. Genetic studies and complementation studies of mutants are being performed to elucidate the roles of *PT1, 2* and *3* in the regulation of gene expression during a pathogen attack.

P426 - Regulation of auxin distribution by a ROP effector and a Ca2+ sensor dependent microtubules stability switch

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Pattern formation in plants depends on polar transport and formation of local maxima and gradients of the phytohormone auxin. Remarkably, auxin self regulates its own transport and accumulation, implying a tight link between auxin signaling and mechanisms that regulate its distribution. Previously, we have identified the ICR family of ROP (Rho of Plants) effectors and found that a member of the family, designated ICR1, is required for recruitment of PIN auxin efflux transporters to the plasma membrane. Remarkably, ICR1 expression is induced by auxin but it is degraded at the site of auxin maximum formation at the root tip. The degradation of ICR1 is induced by high auxin concentrations and depends on TIR1/AFB regulated auxin induced gene expression. We will show that ICR1 is a microtubules (MTs) binding protein. Mutant complementation assays indicate that MTs binding is required for ICR1 function. Through its interaction with ROPs, ICR1 recruits and stabilizes MTs in focal plasma membrane domains. The interaction of ICR1 with MTs is negatively regulated by an auxin-induced Ca^{2+} binding protein, called CMI1, which is highly expressed at the site of ICR1 degradation at the root meristem. Our data indicate that CMI1 is part of an auxin-$Ca2^{+}$ regulated feedback loop that leads to ICR1 degradation, MTs destabilization and local auxin maximum formation.

P427 - Arabidopsis Long Primary Root (LPR) mediates ethylene regulation of plant root growth

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Roots are important organs for plants to absorb water and nutrients from soil. Growth and development of plant roots are modulated by environment factors and internal hormone cues. However, how these factors coordinate to regulate plant root growth and development remain largely unknown. In a recent phosphoproteomic study, we identified a previously uncharacterized protein, which we named Long Primary Root (LPR), with significant phosphorylation state changes under nitrogen starvation and recovery conditions. LPR contains a eukaryotic Serine/Threonine kinase domain at its N-terminus. Its T-DNA insertional mutant *lpr* has longer primary roots whereas its over-expression line has shorter roots when compared with the wild type plants. Furthermore, *lpr* mutation caused low sensitivity to ACC, a precursor of the plant hormone ethylene, reduced root hair growth, but expanded elongation zone of roots. These results suggest that LPR may mediate nitrogen-regulated plant root growth through the phytohormone ethylene. More experiments will be conducted to understand LPR function and elucidate how nitrogen and ethylene corporately regulate plant root growth and development.

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P428 - Expression of an acyl-CoA-binding protein, ACBP6, in Arabidopsis phloem

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Arabidopsis acyl-CoA binding proteins (AtACBPs) contain a conserved acyl-CoA-binding domain which facilitates the binding of long-chain acyl-CoA esters and they have been shown to function in plant stress responses and development. The 10-kDa AtACBP6 represents the smallest amongst the six in the AtACBP family and confers freezing tolerance in transgenic Arabidopsis rosettes and flowers, possibly by its interaction with phosphatidylcholine (PC), as recombinant AtACBP6 has been identified to bind acyl-CoA esters and PC. In eukaryotes, the 10-kDa isoform transports acyl-CoA esters, maintain an intracellular acyl-CoA pool and regulates lipid metabolism. *AtACBP6* encodes a cytosolic protein expressed in floral organs besides other tissues in qRT-PCR analysis, and plays a combinatory role together with AtACBP4 and AtACBP5 in pollen and seed development. As AtACBP6 shares homology with the phloem ACBPs of rice and cucumber, its potential role in long-distance lipid

transport was investigated. Arabidopsis expressing *AtACBP6pro::GUS* exhibited strong GUS activity in the vascular tissues, flowers, embryos as well as the stem cuticle. Immunoelectron microscopy using anti-AtACBP6 antibodies confirmed AtACBP6 localization in the phloem, especially in the companion cells, sieve elements and plasmodesmata, indicating its potential in systemic trafficking. Fatty acid profiling on the wild-type and acbp6 phloem exudates supported a lipid-associated role of AtACBP6 in the phloem.

P429 - DELLA, IDD and SCL3 cooperate in the gibberellin feedback system

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DELLA protein is a key negative regulator of gibberellin (GA) signaling. Although possessing strong transactivation activity, DELLA lacks a DNA-binding domain. Therefore, a model has been proposed in which DELLA acts as a transcriptional coactivator with another transcription factor or factors containing a DNA-binding domain. Here, using yeast hybrid screenings, we identified five members of INDETERMINATE DOMAIN (IDD) protein family which bind physically to both DELLA and the promoter sequence of the GA-positive regulator *SCARECROW-LIKE 3 (SCL3)*, which previously was characterized as a DELLA direct target gene. Transient assays using Arabidopsis protoplasts demonstrated that a luciferase reporter controlled by the *SCL3* promoter was additively transactivated by REPRESSOR of ga1-3 (RGA) and IDDs. Phenotypic analysis of transgenic plants expressing AtIDD3 (one of the 16 IDDs in the *Arabidopsis* genome) fused with the plant-specific repression domain (SRDX) supported the possibility that AtIDD3 is positively involved in GA signaling. In addition, we found that SCL3 protein also interacts with IDDs, resulting in the suppression of its target gene expression. In this context, DELLA and SCL3 interact competitively with IDD proteins to regulate downstream gene expression. From these results, we propose that a coregulator exchange system between DELLA (as coactivator) and SCL3 (as corepressor) regulates the expression of their downstream targets to control the GA signaling pathway.

P430 - A novel thiol-reductase activity of Arabidopsis YUC6 confers drought tolerance independently of auxin biosynthesis

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YUCCA (YUC) proteins constitute a family of flavin monooxygenases (FMOs) with an important role in auxin (IAA) biosynthesis. We report that Arabidopsis plants overexpressing *YUC6* display enhanced IAA-related phenotypes and exhibit improved drought stress tolerance, low rate of water-loss and controlled ROS accumulation under drought and oxidative stresses. Co-overexpression of an IAA conjugating enzyme reduced IAA levels but drought stress tolerance was unaffected, indicating that the stress-related phenotype is not based on IAA overproduction. YUC6 contains a previously unrecognized FAD- and NADPH-dependent thiol-reductase activity (TR) that overlaps with the FMO domain involved in IAA biosynthesis. Mutation of a conserved cysteine residue (Cys-85) preserved FMO but suppressed TR activity and stress tolerance, whereas mutating the FAD and NADPH binding sites, that are common to TR and FMO domains, abolished all outputs. We provide a paradigm for a single protein playing a dual role, regulating plant development and conveying stress defense responses.

P431 - Auxin-regulated cell division pattern during lateral root initiation in Arabidopsis Thaliana

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In plants, cell division pattern is crucial for body plan determination, and auxin has been proposed to be an important external regulator in this process. Here we investigate how such division pattern is spatially and temporal regulated through auxin signaling using lateral root initiation as a model system. The transmembrane kinases (TMKs) are a subfamily of the regulatory leucine-rich repeat (LRR) receptor-like kinases that play important roles in plasma membrane based auxin signaling. Here we show that TMKs-based auxin signaling is important for the proper cell division pattern during lateral root initiation. During lateral root development, auxin will accumulate at the future lateral root initiation sites, this initial auxin flow can active TMKs complex to regulate the activity of the downstream RHO-RELATED PROTEN FROM PLANTS 2 (ROP2); this is achieved through direct interaction with ROP (RHO OF PLANTS) GUANINE NUCLEOTIDE EXCHANGE FACTORS (GEFs). Due to the presence of auxin gradient during this process, this signaling pathway will result in the polarized distribution of active ROP2 within cells, which may eventually contribute to the polarized localization of PIN-FORMED 1(PIN1) and PIN-FORMED 3(PIN3) in the dividing cells. PIN1 and PIN3, which function as auxin efflux, can further enhance the polarized auxin signaling through accumulating auxin at sites where they are located. In this way, auxin can act as a self-organizing signal, which can amplify its own signaling through this positive-feedback loop. Our work here has illustrated how TMKs-based auxin signaling can spatially activate ROP2, which then establish the polarized auxin signaling by regulating the PIN1 and PIN3 distribution.

Natural variation and evolution

Posters 432 to 453

P432 - Habitat-associated life history and stress tolerance variation in A. arenosa

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Local adaptation is crucial for the reproductive success of organisms, especially sessile ones. In plants, earlier flowering and loss of perenniality have often been associated with a weedy lifestyle in human-associated habitats. Here we study *Arabidopsis arenosa*, a close relative of *A. thaliana* that colonized diverse habitats throughout central Europe. We found that populations from ruderal sites (railways) are rapid cycling without vernalization, while populations from sheltered outcrops in hill/mountain regions remain vernalization responsive and perennial. We compared the transcriptomes of an early flowering (railway) and a late-flowering (mountain) accession. Railway populations present low *Flowering Locus C (FLC)* expression, paralleling findings in *A. thaliana*. In addition, we found constitutive expression differences in heat stress response pathways. Genomic data showed one heat shock factor bears marks of divergent selection. Consistent with this, railway populations were more heat stress tolerant than mountain populations. The transcriptomic response of the mountain accession to vernalization was six times stronger than that of the railway accession, though cold-responsive genes had a higher constitutive expression in the railway line. Our data suggest that beyond flowering time, other traits like heat-stress tolerance and cold-acclimation are also under selection in wild populations of *A. arenosa*, fitting with the habitat differences in which they are found.

P433 (Talk) - Genome-wide expression QTL (eQTL) analysis in interaction with mild drought stress

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Plants have to be able to adjust rapidly to changes in environmental conditions such as nutrient and water availability and minimize the negative effects of the adverse growth conditions. Growth trait varies quantitatively between individuals and is significantly influenced by genetic variation and its interaction with the environment, outlining natural variation between individuals. Identification of the genetics underlying gene expression variation may improve our understanding of phenotypic variation due to genetic factors regulating transcript accumulation. Thus the objective of our work is to apply genome-wide quantitative molecular genetics to a quantitative trait *a priori* more directly linked to the source of variation (gene expression under cis-regulation), studied in interaction with drought stress. Although cis- and trans- acting factors affect gene expression and responses to environment, the extent to which they respond to abiotic stresses in plants remains still unclear. To this aim a combination of our unique high-troughput phenotyping robot (Phenoscope), RNA-seq, targeted fine-mapping and association genetics was used to pinpoint a significant number of eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity in *Arabidopsis*. Toward decoding how regulatory variants modulate transcript accumulation recent results and perspectives of complex genetic designs (diallel, star-like crosses) will be presented

P434 - Deciphering interaction between leaf senescence and nitrogen remobilization into seeds using natural variation in Arabidopsis thaliana

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Sequential leaf senescence and monocarpic leaf senescence are developmental processes observed respectively at vegetative stage and reproductive stage. Both facilitate nitrogen (N) and carbon (C) remobilization, the former from leaf to leaf, the second from the whole plant vegetative tissues to the fruits and seeds. N and C resource allocation is controlled by genetic and environmental factors. Previously, studies on natural variation of *Arabidopsis thaliana* revealed differences between accessions for leaf senescence phenotypes (Balazadeh et al. 2008) as well as for N remobilization efficiency related traits (Masclaux-Daubresset et al. 2011). In order to better understand seed filling regulation in relation to leaf senescence and resource allocation, a quantitative genetics approach was undertaken (Chardon et al. 2014). For that purpose, three Arabidopsis recombinant inbred line populations were used to map QTL (Quantitative Trait Loci) for ten traits relative to leaf senescence, nitrogen allocation and seed filling. The use of common markers across the three different maps allowed us to compare directly the QTL detected in a single consensus map. QTL meta-analysis approach was used to identify interesting regions (metaQTL) where QTL of several traits co-localized. MetaQTL were compared to the positions of candidate genes well known to be involved in senescence process and flowering time. Finally, investigation of correlation between Seed N% (N concentration in seeds) and yield in the three populations reveled that leaf senescence might disrupt the usual negative correlation reported between these two traits.

P435 - The evolution of microRNA827 targeting in plant kingdom

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Several early evolved miRNAs regulate similar physiological processes through downregulation of the conserved gene homologs in different plant species. MicroRNA827 (miR827), a phosphate-starvation induced miRNA, is one of conserved miRNAs which predates the divergence of gymnosperms and angiosperms. The previous studies demonstrated the importance of miR827 in regulating phosphate transport, however, through targeting two different kinds of SPX-domain coding genes, *AtNLA* and *OsSPX-MFS* in *Arabidopsis thaliana* and rice, respectively. To understand the evolution of miR827-mediated gene repression, we analyzed the mature miR827 sequences from different species but didn’t find critical variations that would change the preference of targeting. We next predicted the potential targets of miR827 in more than 40 plant species. Intriguingly, we found that miR827 targets to *SPX-MFS* homologs similar to rice in all the species examined, except the species of Brassicaceae, in which miR827 matched perfectly with the target sequences on *NLA* homologs as *A. thaliana*. In addition, *AtSPX-MFS1* has a potential target site in its transcript variants but with lower accessibility to miR827 than *AtNLA* according to the degradome analyses. These results suggest that during Brassica speciation *NLA* gained the miR827 target sequence while *SPX-MFS* lost it gradually, possibly under adaptation pressure.

P436 (Talk) - Identification of regulatory genes involved in central metabolism using Genome Wide Association and Knock-Out analysis

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Enzymes constitute the metabolic machinery for primary carbon and nitrogen metabolism, which provides building blocks for growth. Analysis of genetic diversity for enzyme activities offers a powerful approach to identify genes underlying the metabolism regulatory network. Thus, we performed Genome Wide Association (GWA) analyses on circa 360 Arabidopsis accessions for 21 enzyme activities and 13 metabolites in two independent experiments. A detailed comparison between both experiments allowed us to select for SNPs that showed high LOD-score associations and were confirmed in both experiments. Some candidate SNPs co-localized with coarse-mapped QTL in the *Ler x Cvi* biparental population (e.g. acid Invertase and UGPase). In addition, many strongly associated SNPs were in trans to enzyme structural genes, suggesting that they represent trans-regulatory QTL. GWA validation was carried out through analyses with a panel of 79 Knock-Out lines. The analyses confirmed for several genes that they are involved in the regulation of enzyme and/or metabolite levels in central metabolism. The present study provides the highest defined QTL dataset for primary metabolism enzymes to date and break new ground in understanding the genetic regulation of central metabolism.

P437 - Decoding the complexity of quantitative natural variation in Arabidopsis thaliana

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Most phenotypic traits of agronomical, ecological and economical interest are of quantitative nature, revealing the complex interplay of multiple genetic factors contributing to phenotypic variation, as well

as their interaction with the environment. To date as an alternative to generating laboratory-induced mutants, it is relatively popular to use naturallyoccurring variation among genetically distant *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map quantitative trait loci (QTLs) and try to resolve them at the gene level. However, some of the main issues of these approaches reside in phenotyping throughput and accuracy. To this aim we use our unique hightroughput phenotyping robot (the *Phenoscope*) to study Arabidopsis shoot growth in response to drought stress. The Phenoscope allow us to apprehend the complexity of the genetic architecture of growth, which is a very integrative trait, by mapping small- and medium-effect QTLs in several independent segregating populations. Using a combination of fine-mapping, complementation approaches, and association genetics, we further investigate genes and causative polymorphisms underlying QTLs giving us access to where selection is happening in the wild. A particular case will be presented from the QTL to the nucleotide.

P438 (Talk) - Mapping structural variants as quantitative traits in Arabidopsis populations

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The impact of structural variation on complex traits is unclear; it is difficult, at the level of whole populations, to catalogue common structural variants (SVs) that affect heritability. An allied problem is that of classifying SVs into tandem copy number variants (CNVs), translocations, inversions, deletions etc. This contrasts with the mapping of SVs in individuals, where it is possible to use accurate but expensive long-read sequencing. Consequently there is a need for efficient methods to map and classify SVs in populations. Here, using low-coverage sequence from an *Arabidopsis Thaliana* population sample, we show how to map SVs as quantitative traits using read mapping anomalies as phenotypes. The method allows to jointly call SVs in a population and to identify SVs derived from other genomic locations, such as translocations and duplications. We demonstrate the method by identifying 6502 SVs, of which 25% are translocations, in 488 Arabidopsis genomes from the MAGIC recombinant inbred population sequenced at 0.3x coverage. A set of 37 predicted SVs (31 translocations and 6 inversions) have been validated by PCR at 81% success rate. The expression of genes within predicted SVs is significantly dysregulated and in some cases completely silenced. Furthermore, structural variation is associated with physiological traits and accounts for some heritability not explained by orthodox genetic variation.

P439 - Molecular Mechanism of Epi-stomaty in an Amphibious Plant, Ranunculus trichophyllus (Ranunculaceae)

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Epi-stomaty, having stomata only in adaxial side but no stomata in the abaxial side of the leaf, is frequently appeared in wet-land- or aquatic plants. Epi-stomaty would be evolutionary beneficial for such plants since epi-stomatous plant would be more resistant to pathogen and transpire more efficiently than hypo- or amphi-stomatous plants. To elucidate the molecular mechanism of epi-stomaty, we selected *Ranunculus trichophyllus* var. kadzuensis as a model plant. *R. trichophyllus* var. kadzuensis is an epi-stomatous plant distributed in the wetlands of South Korea. We examined the expression pattern of stomata developmental genes in the leaves of *R. trichophyllus* using *in-situ* RNA hybridization technique. Surprisingly, *SPEECHLESS*, which is known to be a key regulator inducing stomatal development, is expressed only in the adaxial side of *R. trichophyllus*. In contrast, *STOMAGEN*, which participates in post-translational regulation of *SPEECHLESS*, does not show differential expression in adaxial and abaxial side. Thus, the differential transcription of *SPEECHLESS* would be the cause of epi-stomaty of R. trichophyllus. Epi-stomaty is a good example supporting evo-devo concept; evolutional adaptation through the developmental alteration. Thus, further elucidation of the molecular mechanism of the epi-stomaty of *Ranunculus* will provide an insight into evo-devo process.

P440 - Hybrid necrosis in local populations of Arabidopsis thaliana

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Hybrid necrosis, a common type of hybrid incompatibility in plants, has been proposed to be evolved as a by-product of natural selection for disease resistance. Since the discovery of the first causal gene underlying hybrid necrosis in Arabidopsis, several cases among different accessions have been characterized. However, less is known of hybrid necrosis in local populations. Therefore, we made 900 F1 crosses using 64 genetically different Arabidopsis individuals collected near Tuebingen, Germany. From these, three of the F1 hybrids showed mild symptoms of hybrid necrosis including reduced growth, cell death and necrotic lesions, when grown below 20°C. Further crosses showed that hybrid necrosis was relatively common in these populations. Using linkage analysis, amiRNA approach and genomic complementation, we concluded that in all cases the interaction between *ACD6 (ACCELERATED CELL DEATH 6)* alleles was necessary and sufficient for the necrosis. Previously, it has been shown that ACD6 also causes necrosis in hybrids among inbred accessions and that the causal change is in the transmembrane domain of this ankyrin repeat protein. Metabolic analysis comparing hybrids and parental lines revealed changes in secondary metabolites during activation of defence response. Future efforts shall be directed to further investigate how different *ACD6* alleles interact and induce pathogen responses and how and why they are maintained in natural populations.

P441 - Modulation of ambient temperature-dependent flowering time in Arabidopsis thaliana by natural variation of FLOWERING LOCUS M

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Plants integrate seasonal cues such as temperature and day length to optimally adjust their flowering to the environment. We identified the *Arabidopsis* accession Killean-0 (Kil-0), which showed accelerated flowering time in a range of environmental conditions. By combining classical marker-based mapping with next generation sequencing-based approaches (Pool-Seq and RNA-Seq), we identified *FLOWERING LOCUS M (FLM)* as a high-confidence candidate gene. *FLM* was recently described as an important regulator of ambient temperature-regulated flowering. The *FLM* transcript was strongly down-regulated in Kil-0 and sequencing of the Kil-0 FLM locus identified a 5.7 kb insertion in the first intron. By screening a large set of accessions, we identified nine further accessions with this polymorphism, which all showed a down-regulation of *FLM* combined with an early flowering phenotype when compared to the Col-0 reference. By combining information from transgenic lines, T-DNA insertion lines, and additional ecotypes that all exhibited large length polymorphisms in the first intron, we showed that not only the length but also the position of the insertion might control *FLM* abundance. Furthermore we observed that the intronic regions harbor essential elements for the expression and relative abundance of two dominant *FLM* splice forms. We thus conclude that modulation of *FLM* abundance is an important mechanism for the adaptation of plants and flowering to changing ambient temperature.

P442 - Gene module multiplication drives pathway expansion in plants

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The survival of species depends on their ability to adapt to new environments. Adaptive innovations require genetic material, mainly provided by gene duplications, which can lead to new or alternative pathways. However, the principles behind the emergence of such pathways are not understood. Here, we show that approximately one third of the genes of a plant"s genome participate in hundreds of alternative pathways, which were primarily generated by single gene duplications. We determined the copy number of alternative pathways by searching for sets of genes, termed gene modules, that occur multiple times in gene co-function networks of eight plant species. We found that plants employ a genetic copy-and-paste principle to increase the number and diversity of gene modules. Our findings demonstrate that gene module multiplication has provided the capacity for plants to increase their repertoire of cellular functions.

P443 - Natural variation of effect of GA20ox1-overexpression in Arabidopsis thaliana

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Natural genetic variation has been studied in different fields for understanding developmental processes in *Arabidopsis thaliana*. But little is known about how genetic background influences the penetrance of a transgene which results in improved growth. Here, we examined natural variation on the effect of an overexpression of *GA20ox1*, which encodes a rate limiting enzyme in GA biosynthesis pathway, in *A. thaliana*. In order to have a wide range of genetic variability 17 accessions were selected according to their geographic origin. The variability of growth related phenotypes was first analyzed at organ, cellular, and hormone level in these accessions. Then transgenic lines were generated in those 17 accessions and phenotyped. We found that these transgenics show 76% of penetrance in leaf area. At hormone level, bioactive GA and product of GA20ox1 were highly accumulated in all transgenic lines. From a transcriptome analysis, we identified differentially expressed genes, DE, (accessions-specific and common between accessions) that were enriched photosynthesis, secondary metabolism, hormone metabolism, transcription factor, and cell wall pathways. From the correlation analysis, we could identify DE genes that are correlated with penetrance. Further analysis on the relation between phenotype and transcript level is under process.

P444 - A Genome Wide Association Study identified new candidate genes involved in Zn homeostasis

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cy is a common micronutrient deficiency in crops, affecting yield and nutritional quality. Zn deficiency in soil relates to Zn deficiency in humans, which is a widespread problem in most of the developing world. Better knowledge on the plant Zn homeostasis network will contribute to the selection or genetic engineering of new crop varieties, which are more efficient with low Zn availability and/or better in Zn loading into harvested plant parts. We used a Genome Wide Association approach to identify candidate Arabidopsis genes underlying natural variation in ionomic profile (Ca, Cd, Co, Cu, Fe, K, Mn, Mo, Ni, P, and Zn) upon exposure to low Zn supply. To this effect, a comprehensive set of almost 360 well-genotyped, natural Arabidopsis accessions was grown in replicates on hydroponic solution either with normal Zn supply (2 µM ZnSO4) or deficiency inducing Zn supply (0.05 µM ZnSO4). Shoots (rosettes) and roots were collected and used for ionomic profiling. Both mineral concentration data as well as the residuals from regression of Zn deficiency on Zn sufficiency data were used to identify genomic regions associated with Zn homeostasis. Among the genes residing at these regions are heavy metal transporters, ferritin and transcription factors, which are currently subjected to validation experiments. Preliminary data already revealed a potential metal chaperone to be involved in controlling the Zn concentration. The function of this gene is currently being studied.

P445 - The evolution of light signaling modules in land plants

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Across the whole plant kingdom phytochrome (PHY) photoreceptors play an important role during adaptive and developmental responses to light. In seed plants light-activated PHYs accumulate in the nucleus, where they regulate downstream signaling components, such as phytochrome interacting factors (PIFs). PIFs are transcription factors that act as repressors of photomorphogenesis; their inhibition by PHYs leads to substantial changes in gene expression. The nuclear function of PHYs in non-seed plants (e.g. ferns and mosses), however, has so far remained elusive. Here we have identified putative target genes of PHY signaling in the moss *Physcomitrella patens* and found homologs of genes that are regulated by PIFs in *Arabidopsis thaliana*. Phylogenetic analyses revealed that an ancestral PIF-like gene was already present in the earliest land plants or their charophyte relatives. Putative PIF homologs in the genome of *P. patens* resemble *A. thaliana* PIFs in their protein domain structure, molecular properties and physiological effects, albeit with noticeable differences in the motif-dependent interaction with PHYs. Our results suggest that PIFs from *P. patens* are involved in PHY signaling. The PHY-PIF signaling node that relays light signals to target gene promoters has been largely conserved during land plant evolution, with evidence of lineage-specific diversification.

P446 - Natural Variation of Photosynthetic Responses to Low Temperature

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Low, non-freezing temperatures affects plant growth and performance. The tolerance to low temperature varies between and within species, depending on additional environmental factors and genotype. In order to precisely quantify even small differences in plant cold responses we use decreases in the light-use efficiency of photosystem II (PSII) electron transport. This can be conveniently measured using chlorophyll fluorescence imaging, a non-invasive, rapid technique that allows high-throughput measurements. With a unique, automated phenotyping system, based upon chlorophyll fluorescence imaging and built by our group, we can follow the progression of cold stress on a population of plants over time. We tested a collection of *Arabidopsis thaliana* accessions for differences in their response to low temperature and identified a large variation in PSII efficiency, especially in low temperatures compared to standard conditions. The collection is densely genotyped, this enables us to link the phenotypic variation in cold-responses to genetic regions. We identified approximately four dozen genetic loci with a significant effect in low temperature response. Most of these Quantitative Trait Loci (QTL) are stable over time, but a few are diurnal or depend on the length of the cold treatment. The large number of loci points to a complex regulation of low temperature-adaption among naturally occurring Arabidopsis accessions.

P447 - Silencing and ratio changes of homeologs of the allopolyploid Arabidopsis kamchatica studied by new bioinformatic workflows HomeoRoq and SIGN

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Genome duplication with hybridization, or allopolyploidization, occurs commonly in wild and crop plants, and is considered to be a strong force for generating new species. However, genome-wide quantification of homeolog expression ratios was technically difficult because of the high homology between homeologous gene pairs. We have focused on the allotetraploid *Arabidopsis kamchatic*a derived from *A. halleri* and *A. lyrata*, which has one of the widest climatic niches in the genus *Arabidopsis* encompassing lowland hot sandy shores to alpine regions. We first assembled the two diploid parental genomes of *A. halleri* and *A. lyrata*, then generated a synthetic allotetraploid, mimicking the natural allopolyploid *A. kamchatic*a. To quantify the homeolog expression ratio using RNA-seq obtained from polyploids, we have developed a new bioinformatic workflow named HomeoRoq. Our new statistical test identified 226 homeologs (1.11% of 20 369 expressed homeologs) with significant ratio changes after cold treatment including *RD29B* and *COR15A*. Next, we have developed a method to detect silenced genes based on the distribution of ratio, which does not require arbitrary threshold values. Although polyploidization has been thought to induce silencing, we found that about a quarte of silenced genes in parental diploid were de-silenced in synthetic polyploid *A. kamchatic*a. This methods detected a large number of silenced genes also in polyploid crop species.

P448 - Natural variation of flowering time due to cis-regulatory evolution of FLOWERING LOCUS T

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Flowering time is a crucial trait modulated by seasonal changes, which strongly influences fitness. In *Arabidopsis thalian*a, *FLOWERING LOCUS T (FT)* promotes floral transition in response to long day photoperiod as well as in response to ambient temperatures in short days. Whereas *FT* expression in the leaves is restricted to the phloem companion cells, the FT protein moves to the shoot apical meristem to trigger the floral transition through the induction of floral meristem identity genes. Quantitative trait loci contributing to differences in flowering time have been mapped to the *FT* promoter. A 5.7-kb long region upstream of the *FT* coding region is necessary to drive *FT* expression under long days. It contains a proximal promoter region and a distal enhancer that are both highly conserved within different Brassicaceae species and required for the photoperiodic induction of *FT*. We are investigating how underlying cis-regulatory changes and structural variation at *FT* can affect *FT* expression and potentially drive adaptation. We defined a list of candidate cis-elements based on phylogenetic shadowing and found accessions with single nucleotide polymorphisms in these elements. We also revealed three different structural variants of *FT* based on insertions and deletions. We are currently assessing the allele-specific expression of *FT* in different F1 hybrids from various parental accessions to reveal *cis*-regulatory effects on *FT* expression.

P449 - Genome-wide association study identifies genes for photosynthetic acclimation to increased growth irradiance in Arabidopsis

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Natural variation for the response of photosynthetic light use efficiency (Φ_{PSII}) to a step-wise increase in growth irradiance was observed for Arabidopsis. This variation was examined in detail following a genome-wide association study (GWAS) for multiple time-points of the Φ_{PSII} response to increased growth irradiance, using a non-destructive chlorophyll fluorescence imaging technique, in combination with single-nucleotide polymorphism (SNP) data sets. Underlying candidate genes were prioritized by listing genes close to SNPs with high association scores using their linkage-disequilibrium (LD) regions, and constructively searching *in silico* databases for annotations, gene function characterizations, and gene expression patterns that matched the studied trait. A reverse genetic, T-DNA insertion mutant approach was followed to confirm the involvement of the high priority candidate genes. For such genes, common haplotypes were analysed to account for the observed genetic variation. Functional analysis identified natural alleles of these genes to cause the effect on Φ_{PSII} response. Family mapping in an F2 population involving two accessions with contrasting phenotypes, complemented the GWAS results, revealing epistatic interactions between two quantitative trait loci (QTLs). This research is a comprehensive demonstration of an experimental pipeline to successfully identify natural alleles underlying QTLs detected in GWAS for a complex trait such as photosynthesis efficiency.

P450 - Local adaptation of the growth strategies across the range of A. thaliana

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Life history and growth strategies are assumed to play an important role in the adaptation of organisms. *A. thaliana* is present in many environments across the north hemisphere. We investigated whether, and how, the maintenance of natural variation in the growth strategies of *A. thaliana* could reflect adaptation to the local environment. Drawing on the resources from the 1001 Genome project, 451 sequenced accessions with known climatic conditions at the collection point were phenotyped for growth-related traits (lifecycle duration, biomass allocation, RGR, allometric exponent) and physiological traits (net photosynthetic rate, transpiration rate, specific leaf area). The analysis revealed that growth strategies are constrained by a trade-off between the duration of the lifecycle and the rate of growth, as reflected by a decrease in the allometric exponent with flowering time. GWAS and genomic selection scans identified a region on chromosome 2 that contains genes involved in the secondary metabolism, and which are associated with both the variation of the allometric exponent and the precipitation and temperature during summer. Our results suggest that the genes modulating growth strategies through the secondary metabolism mediate the adaptation to the summer conditions. Interestingly, our findings also suggest that there is selection for allometric exponents out of the three-quarter-power law observed between species when accessions come from dry and hot environments.

P451 (Talk) - Epistasis Unravelled with a Novel Chromosome Substitution Mapping Set in Arabidopsis thaliana

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Arabidopsis thaliana has been widely used in a diverse number of mapping populations. The general aim for those populations is to explain phenotypic variation using genetic information. For quantitative traits this might be very difficult due to either many small effect loci that add up to a large additive effect, or due to non-additive interactions between multiple loci, referred to as epistasis. The recent development of chromosome substitution lines through repression of crossovers and haploid induction in Arabidopsis hybrids implies a major step forward in unravelling complex trait variation (Wijnker et al. 2012). The derived homozygous lines contain a combination of the intact chromosomes present in the founding parents. In Arabidopsis with five chromosomes, a complete chromosome substitution mapping set consists of 2^5 = 32 lines. The distinguishing advantage of these genetically simple, completely balanced chromosome substitution mapping sets is that they offer a design in which all possible contrasting sets of genotypes can be assessed for any kind of genetic effect. Here we present the genesis of such a new type of population for the exploration of largely elusive genetic concepts like epistasis, GxE, epigenetics and their importance to phenotypic trait variation. Finally, statistical work can be built upon results from this new population for unravelling the genetic basis underlying epistasis and complex trait variation.

P452 (Talk) - The complex evolution of the CYSTEINE-RICH RECEPTOR-LIKE KINASEs (CRKs) as mediators of signaling specificity

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The CRKs are a large group of receptor-like protein kinases (RLKs) in *Arabidopsis* with 44 members. *CRK* expression is responsive to stress and our previous analysis of a *crk* mutant collection revealed phenotypes related to ROS signaling. Results suggest an intricate signaling network of CRKs in order to survive in a complex environment. CRKs contain the plant-specific DUF26 (domain of unknown function 26; PF01657) in their ectodomain. DUF26, hallmarked by a conserved cysteine motif, is also found in the plasmodesmata-located proteins (PDLPs) and cysteine-rich receptor-like secreted proteins (CRSPs). Higher plants encode a large number of DUF26 proteins. Gene family expansion and the impact on the evolution of function are a major challenge in understanding the roles of gene families. To understand why so many DUF26 genes are maintained in plants after duplication events and why expansions are so strikingly diverse in different plant lineages we have identified CRKs, PDLPs and CRSPs from more than 20 plant species covering most plant lineages. DUF26 originates with the first land plants and two DUF26s appear in the lycophytes. Intriguingly, in genes with two DUF26, the first and the second domain have differentiated into specific forms with unique sequence context surrounding cysteines. Variation within DUF26 domains between the different phylogenetic subgroups of the CRKs may have functional and structural importance for the extracellular domain of the CRKs.

P453 - Functional analysis of flowering time gene FRIGIDA natural alleles in Arabidopsis thaliana

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Allelic variation at *FRIGIDA (FRI)* contributes to most of the flowering time variation of *Arabidopsis thaliana* in the wild. *FRI* delays flowering by promoting the expression of the floral repressor *FLOWERING LOCUS C (FLC)*. Multiple studies have defined two main classes of *FRI* alleles: the first includes winter-annual accessions carrying the functional wild type allele (*FRI-wt*), and the second class involves early flowering summer-annual accessions that encode truncated FRI proteins. Two most frequent null *FRI* alleles in nature are the ones present in the lab strains Columbia (*FRI-Col*) and Landsberg erecta (*FRI-Ler*). The variation in flowering time among Arabidopsis accessions in these two allelic classes leads us to test the effects of various *FRI* alleles in a common genetic background. We transformed multiple *FRI* alleles into the Col-0 strain and quantified their flowering time and *FLC* expression. We demonstrate that the *FRI-Ler* allele is able to induce *FLC* expression, delay flowering time and confer sensitivity to vernalization in the presence of a functional *FLC* allele, as opposed to the true null *FRI-Col* allele. Nevertheless, the *FRI-Ler* allele revealed reduced effect when compared to the fully functional *FRI-wt* allele, in part due to its reduced expression levels. We also found *FRI* allele from Wa-1 accession (*FRI-Wa-1*) is non-functional, although it encodes a full-length FRI protein. The amino acid substitution in *FRI-Wa-1* may provide us insight in FRI protein function.

Abiotic stress

Posters 454 to 536

P454 - Molecular analysis of signal transduction mechanisms involved in plant-organic pollutant interactions

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Plants have been exposed for millions of years to a myriad of natural chemical stressors related to biotic and abiotic stresses. Moreover, in the last decades, anthropogenic-related xenobiotics have broadly spread throughout the environment, thus resulting in strong ecological and evolutionary pressure on the dynamics of plant communities. It has emerged that xenobiotic effects involved major disruption of signalling pathways, besides the more evident target-related perturbations at biochemical and physiological levels. A major difficulty is therefore to separate direct xenobiotic effects from indirect effects related to stress-induced damages. These two different responses can however be disconnected by using low doses of xenobiotics or their less-active degradation products, which elicit significant responses. Numerous secondary entities generated under mild chemical stress may represent relevant signals that develop efficient responses through metabolic and gene expression changes. Moreover, as described in animals, yeast and bacteria, rapid primary sensing of xenobiotic compounds involving mechanisms of perception and transduction is likely to exist in plants. We are using multidisciplinary approaches at different scales in *Arabidopsis thaliana* (physiological, metabolomic and transcriptomic analysis, functional genomics, mutant analysis) in order to obtain detailed biochemical and structural characterization of sensor-related cellular components.

P455 - RNA-Seq Analysis of the Effect of Kanamycin and the ABC Transporter AtWBC19 on Arabidopsis thaliana Seedlings Reveals Changes in Metal Content

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Plants are exposed to antibiotics produced by soil microorganisms, but little is known about their responses at the transcriptional level. Likewise, few endogenous mechanisms of antibiotic resistance have been reported. The *Arabidopsis thaliana* ATP Binding Cassette (ABC) transporter AtWBC19 (ABCG19) is known to confer kanamycin resistance, but the exact mechanism of resistance is not well understood. Here we examined the transcriptomes of control seedlings and *wbc19* mutant seedlings using RNA-seq analysis. Exposure to kanamycin indicated changes in the organization of the photosynthetic apparatus, metabolic fluxes and metal uptake. Elemental analysis showed a 60% and 80% reduction of iron uptake in control and *wbc19* mutant seedlings respectively, upon exposure to kanamycin. The drop in iron content was accompanied by

the upregulation of the gene encoding for FERRIC REDUCTION OXIDASE 6 (FRO6) in mutant seedlings but not by the differential expression of other transport genes known to be induced by iron deficiency. In addition, *wbc19* mutants displayed a distinct expression profile in the absence of kanamycin. Most notably the expression of several zinc ion binding proteins, including ZINC TRANSPORTER 1 PRECURSOR (ZIP1) was increased, suggesting abnormal zinc uptake. Elemental analysis confirmed a 50% decrease of zinc content in wbc19 mutants. Thus, the antibiotic resistance gene *WBC19* appears to also have a role in zinc uptake.

P456 - CAX1 co-segregates with Cd tolerance in the metal hyperaccumulator Arabidopsis halleri and plays a role in limiting oxidative stress in Arabidopsis

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Arabidopsis halleri is a model species to study the adaptation to extreme metallic conditions. In this species, cadmium tolerance seems to be constitutive and the mechanisms underlying the trait are still poorly understood. A previous QTL analysis, performed on a *A. halleri* x *A. lyrata* back-cross population (BC1), identified the metal-pump *HMA4* as the major genetic determinant for Cd tolerance. However, although necessary, *HMA4* alone is insufficient for determining this trait. After fine-mapping, *CAX1*, encoding a Ca^{2+}/H^{+} antiporter, was identified as a candidate gene for the second QTL of Cd tolerance in *A. halleri*. BC1 individuals displaying the *A. halleri* allele for *CAX1* locus showed significantly higher *CAX1* expression compared to the ones displaying the A. lyrata allele and a positive correlation between *CAX1* expression and Cd tolerance was assessed. We showed that this QTL is conditional and was only detectable at low external Ca concentration. CAX1 expression in *A. halleri* was higher in both roots and shoots compared to the related Cd sensitive species *A. lyrata* and *A. thaliana*. Moreover, the loss of function of *CAX1* in *A. thaliana* led to higher Cd sensitivity at low Ca concentration, higher sensitivity to methyl-viologen and stronger accumulation of ROS upon Cd treatment. Together, our results strongly support the involvement of *CAX1* in Cd tolerance, most probably by limiting ROS accumulation.

P457 - Arabidopsis microRNA expression regulation in a wide range of abiotic stress responses

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Arabidopsis microRNA expression regulation was studied under drought, heat, salinity, Cu excess/deficiency, Cd excess and S deficiency stresses. A home-built RT-qPCR mirEX platform for the amplification of 289 miRNA transcripts was used to study their response to the stresses. Small RNA sequencing and Northern hybridization were performed to study the expression of mature miRNAs. In the case of drought, heat and salinity, we observed broad induction of the level of primary miRNAs that was not observed at the level of miRNAs. In the case of heavy metal contaminations or deprivation of a specific micro- or macroelement, the transcriptional response of pri-miRNAs was limited but also not predictive to the level of the mature miRNA, pointing to an essential role of posttranscriptional regulation of miRNA expression. New abiotic stress responsive miRNAs were discovered. MiR319a/b, miR319b.2 and miR400 have been found to be responsive in several abiotic stresses and can be regarded as general stress-responsive microRNAs. A new target for miR319b.2 -TBL10 has been experimentally confirmed. However, its expression level under different abiotic stresses is unchanged in comparison to control conditions. In the promoter region of the *TBL10* we found numerous stress-responsive elements. We suggest that transcriptional induction resulting in the increase of transcript levels is downregulated by the increase of the miR319b.2 ultimately resulting in a stable level of TBL10 mRNA.

P458 (Talk) - Characterizing the Role of Heat Stress-Inducible Small Open Reading Frames (sORFs)

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Abiotic stresses such as heat stress, significantly affect crop yield and product quality, and ultimately reduce the opportunities to increase crop production for rapidly increasing population. Recent developments in molecular biology and the availability of complete genome sequence of numerous plant species provide a sound platform for characterizing the function of individual genes involved in stress response/tolerance. Besides the already characterized genes, presence of novel small open reading frames (sORFs) encoding small peptides(less than 100 amino acids residues) and their involvement in stress tolerance is an emerging and interesting field. In Arabidopsis, 7,901 sORFs have been reported. Among these, a number of genes are predicted to be involved in abiotic stress tolerance. The expression of AT4, which is predicted to encode two small peptides (AT4a & AT4b), is upregulated in response to heat stress. Arabidopsis plants overexpressing AT4 exhibited heat tolerance. Further characterization of AT4 would reveal the role of small peptides in heat stress response.

P459 - Reil1-1 reil2-1 double mutant "in the grip" of cold stress: Morphological and physiological phenotypes

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The Rei1 and Reh1 proteins are yeast paralogs that are part of the maturation machinery of the 60S eukaryotic large ribosomal subunit. Deletion of Rei1 or of both yeast paralogs causes cold sensitivity. Reduced growth of the yeast deletion mutants at low temperature is associated with an aberrant polysome profile that is characterized by accumulation of free 40S and 60S subunits. A. thaliana has two Rei1-like (REIL) homologs, REIL1 and REIL2. We discovered that loss of REIL2 causes reduced growth in the cold. The reil1-1 reil2-1 double mutant is even more cold-sensitive but, in contrast, has only a marginal growth defect at optimal temperature. This surprising match of growth defects between yeast and A. thaliana led us to investigate whether the function of REIL genes is conserved and whether the reil genes may have acquired new functions in a plant. In the presented work we focused on the characterization of the reil1-1 reil2-1 mutant. The cold sensitivity was explored at constant cold and by temperature shift experiments. The *reil1-1 reil2-1* mutant is an extreme dwarf but still viable at constant 10°C and arrests growth when shifted from 20°C to 10°C as was demonstrated by detailed morphometric analyses. The growth arrest is not accompanied by loss of cellular integrity in the cold. Electrolyte leakage analyses of non-acclimated and cold-acclimated mutant plants compared to *A. thaliana* Col-0 wild type (WT) revealed identical freezing tolerance as determined by LT_{50} values determined after cold acclimation to both, 4°C or 10°C. Exposure of *A. thaliana* to two subsequent cold (10°C) stresses separated by an intermittent period at optimal temperature revealed that the first cold stress primes the growth responses of the WT to the second stress. The *reil1-1 reil2-1* was priming deficient. In summary, our data suggest that the reil1-1 reil2-1 has a growth defect that is linked to the acclimation process to mild cold (10°C). In order to understand the underlying mechanism for which the double mutant is deficient and the implied link to ribosomal function we initiated an integrated systems analysis of short- and long-term, transcriptomic and metabolomic responses to 10°C

cold which we plan to complement by comparative analyses of ribosome composition and activity.

References Stefanie Schmidt, Frederik Dethloff, Olga Beine-Golovchuk, Joachim Kopka (2013) The REIL1 and REIL2 proteins of Arabidopsis thaliana are required for leaf growth in the cold. Plant Physiology 163: 1623-1639 (DOI:

10.1104/pp.113.223925).

Schmidt S, Dethloff F, Beine-Golovchuk O, Kopka J (2014) REIL proteins of Arabidopsis thaliana interact in yeast-2-hybrid assays with homologs of the yeast Rlp24, Rpl24A, Rlp24B, Arx1 and Jjj1 proteins. Plant Signaling and Behavior 9: e28224 (DOI: 10.4161/psb.28224).

P460 - Heat-Induced ribosome pausing triggers mRNA co-translational decay in Arabidopsis thaliana.

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The reprogramming of gene expression in heat stress is a key determinant to organism survival. Gene expression is downregulated through translation initiation inhibition and release of free mRNPs that are rapidly degraded or stored. In mammals, heat also triggers 5'-ribosome pausing preferentially on transcripts coding for HSC/HSP70 chaperone targets, but the impact of such phenomenon on mRNA fate remains unknown. Here, we provide evidence that, in *A. thaliana,* heat provokes 5'-ribosome pausing leading to the XRN4-mediated 5'-directed decay of translating mRNAs. We also show that hindering HSC/HSP70 activity at 20°C recapitulates heat effects by inducing ribosome pausing and co-translational mRNA turnover. Strikingly, co-translational decay targets encode proteins with high HSC/HSP70 binding scores and hydrophobic N-termini, two characteristics that were previously observed for transcripts most prone to pausing in animals. This work suggests for the first time that stress-induced variation of translation elongation rate is an evolutionarily conserved process leading to the polysomal degradation of thousands of "non-aberrant" mRNAs.

P461 - New cytonuclear combinations lead to different phenotypic responses under salt stress conditions in Arabidopsis thaliana

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In plants, mitochondria and plastids are mainly known as sites of respiration and photosynthesis, but also contribute to stress perception and tolerance. These organelles have semi-autonomous genomes that need to import a major part of their proteome from the nucleus. The proper functioning of plant cells needs optimal interactions between products from both organelle and nuclear genomes, and therefore these genomes have evolved to finely tuned coadaptation. In order to understand how disrupting cytonuclear co-adaptation impacts the phenotype, cytolines, lines containing the nuclear genome of an *A. thaliana* natural accession and the cytoplasmic genome of another were constructed as part of the Cytopheno project (ANR project, https://www6.inra.fr/cytopheno). A core collection of 8 accessions, chosen to cover nuclear and cytoplasmic genetic diversity, was used to construct 56 cytolines. In this study we address the impact of a disruption of cytonuclear coadaptation on the plant response to salt stress at early stages of development. Among all abiotic stresses plants can be facing, soil salinity is a major issue due to the accumulation of toxic ions, mainly Na+ and Cl-, in leaf tissue causing physiological and biochemical alterations. We will show that new nucleo-cytoplasmic combinations modify the response of plants to salt in early stages of their life. Our observations suggest that cytonuclear coadaptation participate in the elaboration of abiotic stress tolerance.

P462 - A forward genetic approach to seed longevity uncovers the role of hormones, light and seed coat

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Seeds constitute the main system for plant propagation but a major limitation is their loss of viability during storage, so called aging. In order to identify novel determinants of seed longevity we have screened an "activation-tagging" mutant collection of Arabidopsis for tolerance to accelerated aging of seeds. This method mimics natural aging and we isolated five dominant mutants with increased seed longevity (*isl1/5*). We have already characterized *isl1-1D* (over-express transcription factor gene *ATHB25* that up-regulates gibberellin biosynthesis; Bueso et al, 2014, Plant Physiol 164:999) and *isl2-1D* (over-express *RSL1* gene, encoding an ubiquitin ligase that targets ABA receptors to down-regulate abscisic acid signalling; Bueso et al, 2014, Plant Sci 215:110 and Plant J 80:1057). We describe now *isl3-1D* and *isl5-1D* that over-express two homologous genes encoding transcription factors, CDF4/DOF2.3 and COG1/DOF1.5, respectively. Both factors have been shown to attenuate light responses mediated by phytochromes and now we found that they act by increasing auxin biosynthesis. Phytochrome mutants have increased seed longevity and, similarly to *isl1-1D, isl3-1D* and *isl5-1D* mutants, display a seed coat modification at the outer layers, with increased suberin and reduced permeability. Mutants defective in suberin biosynthesis have reduced seed longevity, pointing to an important role of this sealing phenolic compound to protect seeds during aging.

P463 - Screening Corn (Zea mays) Varieties for Tolerance to Drought

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[(1)] Talladega College, Talladega, Alabama, USA [(2)] Tuskegee University, Tuskegee, Alabama, USA Screening Corn (Zea mays) Varieties for Tolerance to Drought Jamaya Carter[1], Desmond Mortley[2]

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Drought is the most limiting factor for corn production and is high among the criteria considered by farmers when selecting varieties. Also availability of varieties tolerant to drought is still a problem that needs to be addressed. Due to issues with climate change rainfall patterns have been disrupted and are no longer reliable sources of moisture to produce a complete crop and corn needs about 635 mm of water from planting to harvest for optimum production. Breeding for drought tolerant maize have been performed in many countries, and hybrid together with genetically modified varieties are available. In the US there are numerous maize drought tolerant varieties such as AQUAmax hybrid developed by Pioneer, Droughtgard GM by Monsanto & Agrisure Artesian by Syngenta. We hypothesize that drought tolerant plants will perform better in terms of grain yield than drought susceptible plants. Therefore, the objectives of the study are to 1) compare water use, grain yield and growth performance of several corn varieties under well watered and water stressed conditions, and 2) profile the expression of genes associated with drought tolerance during grain filling. Ten accessions will be grown in a randomized complete block design with three replications in a greenhouse and field. Each replication will consist of three -row plots 5m long and 1 m wide. In all plots water will be applied through furrow irrigation. Drought stress will be imposed by reducing irrigation by half from the 10 fully expanded leaf stages to two weeks after flowering. Leaf samples will be collected at different development stages for RNA fingerprinting to gain insight into the mechanism of drought tolerance in maize. Varieties will be assessed based on plant survival/population, photosynthetic rate, stomatal conductance and transpiration, total leaf area, silking date, plant height and ear length and grain yield. Germplasms with optimum response under water stressed conditions will be termed as drought tolerant.

P464 (Talk) - Regulation of manganese uptake and interplay with iron transport in Arabidopsis thaliana

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Manganese (Mn) is a trace element important for oxidative stress responses and essential as a cofactor of the photosynthetic machinery. Mn deficiency is a severe plant nutritional disorder while Mn toxicity can become a problem in poorly drained acidic soils. In Arabidopsis, the natural resistance-associated macrophage protein 1 (NRAMP1), has been shown to mediate Mn uptake in roots (Cailliatte *et al.*, 2010). As for all potentially toxic compounds, Mn entry within root cells must be tightly controlled. How this occurs in Arabidopsis is still unknown but is likely to involve post-translational regulation of NRAMP1. Moreover it is still not clear whether NRAMP1 provides the only route of entry of Mn across the plasma membrane. Several lines of evidence suggest that Mn can be transported along with iron (Fe) by the Fe high affinity transporter IRT1 (Vert *et al.*, 2002). To address the question of NRAMP1 and IRT1 functional redundancy, an indepth study of the double mutant *nramp1 irt1* supported by a promoter swap strategy has been undertaken. Moreover, post-translational control of NRAMP1 has been investigated by monitoring Mn-dependent NRAMP1 stability and localisation *in planta* and in yeast. The first results obtained with these approaches highlighting an unexpected and important function of NRAMP1 in Fe transport will be presented here.

P465 - Rearrangement of central metabolism and photosynthesis is linked to the dynamic phosphoproteome in abiotic stress signaling in Arabidopsis

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Salinity and oxidative stress are major factors affecting and limiting the productivity of agricultural crops. The study of biochemical and molecular responses of plants in response to these stresses is important for crop genetics and breeding. Here, we performed a far reaching quantitative analysis of the early response of *Arabidopsis* phosphoproteins and metabolites to salt and oxidative stress over time using ^{15}N-metabolic labeling and liquid chromatography and mass spectrometry (LC-MS). We found the phosphoproteomic response is more specific compared to the general response observed on the metabolite level reflected by much higher specificity especially in the very early stress phase. Despite these differences, the response on both levels still follows the same general dynamics and strategy of photosynthesis as reflected by rapid changes in the phosphorylation pattern of central carbon metabolism enzyme coinciding with the decrease of metabolites related to cell growth. Novel phosphorylation sites were found for AKIN10 and AKIN11, indicating their central roles in the stress regulated responses. Seven SnRK2 kinases showed varying levels of phosphorylation at multiple serine/threonine residues in their kinase domain upon stress, demonstrating temporally distinct modulation of the various isoforms. K^+ and Na^+ transporters showed coordinated changes in the phosphorylation pattern, indicating the importance of dynamic ion homeostasis for adaptation to salt stress.

P466 - Arabidopsis RCAR3 gene plays a major role in drought tolerance

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The phytohormone abscisic acid (ABA) plays an important role in developmental processes and stress responses. A family of soluble ABA receptors, REGULATORY COMPONENTS OF ABA RECEPTORs (RCARs)/PYRABACTIN RESISTANCE1 (PYR1) and PYR1-LIKEs(PYLs), have been identified, which enables better understanding of the signaling mechanisms of ABA. Functional analysis of one of those receptor genes, *RCAR3*, was attempted in this study. Constitutive overexpression of *RCAR3* gene (RCAR3ox) confers ABA hypersensitivity and drought tolerance. On the contrary, constitutive RNA interference of *RCAR3* (RCAR3i) resulted in resistance of germination and root growth inhibition by ABA. The stomata aperture of RCAR3i plants was reduced by approximately 20% less than the wild type plants and showed resistance to drought. Overexpression of RCAR3 enhances resistance on drought and showed higher expression levels of ABA-responsive genes than wild type plants when ABA was treated. On the contrary, RCAR3i plants displayed reduced expression of these genes. RCAR3 is localized in both the nucleus and the cytosol but it moves to the nucleus in response to ABA. These results indicated that *RCAR3* mediates plant ABA response. Histochemical assay of P_{RCAR3}:GUS revealed that RCAR3 is expressed in the internal stele layers in roots, veins and guard cells of leaves suggesting its point of action during ABA regulation. Phenotypic changes by overexpression or suppression of RCAR3 alone suggest that it functions major role in developmental processes and stress responses by ABA

P467 - Identification of a drought response gene encoding a nuclear protein involved in drought and freezing stress tolerance in Arabidopsis

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Plants have developed adaptive strategies to survive the different abiotic stresses they may be exposed to. Appreciable efforts have been made to determine which genes are involved in abiotic stress tolerance in plants and their specific roles. To identify new components involved in abiotic stress tolerance, we screened unannotated ESTs and evaluated their cold or drought response in *Arabidopsis*. We identified a drought response gene (*DRG*) encoding a 39.5-kDa polypeptide. This protein was expressed specifically in the siliques and was induced by drought stress in most tissues. When a *DRG-GFP* construct was introduced into *Arabidopsis* protoplasts, GFP signals were detected only in the nucleus. The *drg* mutant plant is more sensitive than the wild-type to osmotic stress caused by mannitol in agar plates and to drought and freezing stress in soil. Activation of the *DRG* gene restored the normal sensitivity of *drg* mutants to abiotic stress. No significant differences in drought and freezing tolerance were observed between wild-type and transgenic plants overexpressing the *DRG* gene. When *DRG* was expressed in cold-sensitive *Escherichia coli* BX04, the transformed bacteria grew faster than the untransformed BXO4 cells under cold conditions. These results demonstrate that *DRG* is a nuclear protein induced by abiotic stress, required for drought and freezing tolerance in *Arabidopsis*.

P468 - PRSD, a transcription factor implicated in seed germination under osmotic stress

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Plants have evolved to control their growth and development in response to signals from their environment. A good example is the seed germination process, which determines the end of the dormancy period and allows the plant growth. Therefore, to begin the process of seed germination, favorable environmental signals must be detected producing the silencing of seed development genes and the activation of vegetative growth genes. During stressful environmental conditions such as drought or lack of nutrients, is necessary to delay germination, for which transcription factors involved in both development and stress response are required. PRSD (Positive Regutator of Seed Dormancy) is a transcription factor involved in both salt stress *Arabidopsis thaliana*. However, its role during abiotic stress seems not completely clear. Here we investigate its role during seed germination under abiotic stress conditions, and we found that knockout lines (*prsd*) display an early germination phenotype under osmotic stress compared with wild type. In addition the molecular response during germination of *prsd* plants showed that the expression

of several genes associated with seed development and dormancy (genes *GASDs*) were decreased in this mutant under osmotic stress. These results suggest that PRSD participate as a negative regulator of germination during osmotic stress, through the regulation of genes implicated in seed development and dormancy.

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P469 - Identification and characterization of an ABA-activated MAP kinase cascade in Arabidopsis thaliana

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The mitogen-activated protein kinases (MAPKs) function in modules composed of MAPKK kinases, MAPK kinases and MAPKs that activate each other by sequential phosphorylation. Despite their key roles in plant stress signaling, only few complete MAPK cascades have been functionally characterized in plants. In particular, there is no evidence of such cascades mediating signal transduction of the important plant stress hormone abscisic acid (ABA). Here, we identified a complete and functional ABA-activated MAPK cascade, composed of the MAP3Ks MAP3K17/18, the MAP2K MKK3 and the four C group MAPKs MPK1/2/7/14. Using transient expression in mesophyll cell protoplasts, we demonstrated that the four C-group MAPKs are activated by ABA in a MKK3-dependent manner. In planta, using specific antibodies raised against MPK7, we show that ABA activation of endogenous MPK7 is blocked in *mkk3-1* and *map3k17mapk3k18* plants. Coherently, both mutants exhibit hypersensitivity to ABA and drought stress, as well as mis-regulation of a set of ABA-dependent genes. A genetic analysis further reveals that this MAPK cascade is activated by the PYR/PYL/RCAR-SnRK2-PP2C ABA core signaling module through protein synthesis of the MAP3Ks, unveiling a new mechanism for MAPK activation in eukaryotes. Our work provides evidence for a role of an ABA-induced MAPK pathway in plant stress signalling.

P470 (Talk) - Genetic dissection of the primary root growth response to low phosphate in Arabidopsis

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Phosphate (Pi) is an essential nutrient for plant growth, Pi starvation leading to a rapid growth arrest of the primary root (PR) in *Arabidopsis.* New *Arabidopsis* mutants that exhibit reduced PR growth arrest under Pi deficiency were isolated, among which many *stop1* and *almt1* alleles. STOP1 is a transcription factor that positively regulates the expression of *ALMT1,* which encodes a malate efflux transporter. Their role in the tolerance to proton and aluminium rhizotoxicities is already well documented, however this is the first description of their function in the root growth response to low Pi. We observed a STOP1-dependent induction of *ALMT1* mRNA accumulation in wild-type (WT) seedlings under Pi deficiency, whereas the expression level of *STOP1* remained stable. We also showed that STOP1 is targeted to the nucleus and functionally interacts with a ~300 bp region of the *ALMT1* promoter. Induction of *ALMT1* by Pi starvation correlated with an increased excretion of malate and citrate by WT seedling roots, whereas this excretion was significantly reduced in *stop1* and *almt1* mutants. Interestingly, the *lpr1/lpr2* double mutant, which does maintain PR growth in low-Pi conditions, excreted as much malate and citrate as did the WT. Our results shed a new light on the role played by *STOP1* and *ALMT1* in the root growth response to low-Pi environments.

P471 - Natural variation of the root morphological response to nitrate supply in Arabidopsis thaliana

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Plant mineral nutrition underpins global food security. Nitrogen (N) is the quantitatively most important nutrient required by crops, however a considerable fraction of N fertilizer gets lost as runoffs with detrimental consequences to the environment and greenhouse gas emission. Faced with those pressing societal costs, innovation has to make a step change to improve Nitrogen Use Efficiency (NUE) of plants. The goal of our research is first to identify the genetic determinism that regulate N-related root morphology in the model species Arabidopsis thaliana, then to use that information in parented Brassica crops for redesigning their root architecture and ultimately improve NUE. The focus here is on nitrate since it is the major nutritional determinant of yield and root morphology. Naturally occurring genetic variability is exploited in order to identify key genes that determine root morphology in response to nitrate supply. Our preliminary results with 24 accessions maximizing genetic diversity of the species, already showed variation for root biomass and root architecture traits and demonstrated that those traits were primarily genetically determined (De Pessemier et al., 2013. Mech. Dev. 130: 45-53). Here, we have generated a larger set of phenotypic data using a larger panel of accessions, for which association mapping tools are available. We selected also eleven accessions with contrasting root morphology and then characterized that plant material in further details.

P472 - C-TERMINALLY ENCODED PEPTIDE 3 coordinates plant development in response to environmental stress

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Complex local and systemic signalling systems enable plants to coordinate their growth, development and architecture with nutrient availability, uptake and distribution. Recently, small signalling peptides such as the C-TERMINALLY ENCODED PEPTIDE (CEP) family have been shown to be important components of these regulatory circuits (Delay *et al.* 2013 JEB 64, 5383; Imin *et al.* 3013 JEB 64 5395; Tabata *et al.* Science 2014 346, 343). We have shown that *AtCEP3* specifically responds to environmental cues such as nitrogen deficiency and salinity. Characterising mutant and over-expressing lines has revealed CEP3 negatively regulates root meristem development under stress conditions where resource reallocation is crucial. The interplay between CEP3, nutrient use and growth has been explored using restrictive nutrient assays, fluorescent microscopy and transcriptomic analyses. Overall, our data indicate CEP3 plays an important role in regulating plant development in response to plant nutrient status. Modulating CEP3 in agricultural systems may provide an opportunity to increase stress tolerance without severe yield penalties.

P473 - Route to the roots: carbon source-sink relationship in response to water deficit

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Root development is highly plastic to adapt to its changing environment. Water deficit impairs growth leading to sugar accumulation in leaves, part of which can be exported to roots via sucrose phloem transport. Phloem loading is widely described in *A. thaliana* while unloading is poorly understood. To gain information on leaf to root transport during water deficit, a soil-based culture system was developed where root growth can be monitored in 2D. Physiological measurements and $^{14}CO_2$ pulse/chase experiments indicated that, although roots were smaller after water deficit, relative ^{14}C exported to the roots increased during chase, corroborating the increase in Root/Shoot ratio noted after 30 days of water deficit. Phloem loading of sucrose occurs in leaves through the action of effluxers (AtSWEET11-12) and H^+ symporter AtSUC2. During water deficit, the transcript levels of *SWEET11-12* and *SUC2* genes were also significantly up-regulated in leaves, in accordance with the increase in carbon export to the roots already noted. Interestingly, transcript levels of *SUC2* and *SWEET11-15* were significantly higher in stressed

roots, underlying a putative role of these transporters in root sucrose apoplastic unloading. These data demonstrate that, during water deficit, plants respond to growth limitation by allocating relatively more C to the roots to maintain an efficient root system and that a subset of sucrose transporters are potentially involved in the flux of carbon to and in the roots.

P474 - Characterization of epigenetic gene-regulation through the AvSAMS1 gene derived from a poaceae wild plant Andropogon virginicus L. in Al tolerance

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Aluminum (Al) toxicity is one of the major factors limiting plant growth in acid soil. A wild species of poaceae, *Andropogon virginicus L.* shows a high Al tolerance by a combination of five mechanisms. To clarify its high tolerance mechanism, isolation of the tolerant genes from this plant were performed and S-adenosyl methionine synthase gene (*AvSAMS1*) was obtained. The *AvSAMS1*gene moreover conferred Al tolerance to Arabidopsis transformants (TF). Since SAMS synthesizes SAM (S-adenosyl methionine) which is the main methyl-residue-donor, SAMS has been suggested to be related to “epigenetic gene-regulation” under various environmental stresses. Our microarray analysis indicated that a change of gene-expression was preferentially occurred in the *AvSAMS1* expressing Arabidopsis TF rather than in a control Col-0 ecotype under Al stress. To confirm this result, DNA sequencing after bisulfite treatment was applied to several genes. Difference in DNA-methylation by Al stress was detected between the two lines in the tested genes. Moreover, change of methylation status in histone H3 by Al stress was investigated in both lines. Higher gene-induction or –repression caused by the stronger interaction with tri-methylated H3K4 or H3K9 in the *AvSAMS1* expressing Arabidopsis TF than in Col-0 ecotype was detected in some of the tested genes. This is the first report indicating that the Al tolerant gene, *AvSAMS1*, is related to an epigenetic gene-regulation under Al stress in Arabidopsis.

P475 - Optimizing chlorophyll extraction protocols and conductivity measurements using wild type Columbia ecotype plants.

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The reported data is part of a larger study to determine the role of lipoxygenases and acyl hydrolases controlled by genes *MOP9.2, LOX 4, LOX 5* and *MES 1* during light deprivation stress. We hypothesized that greater leakage and chlorophyll degradation would occur with increased light depravation stress. We report on our experiments to confirm the double knockout nature of our mutants, procedure to standardize methods for working with larger sample sizes in the dark and present data on our initial experiments conducted with wild type *Arabidopsis thaliana*. PCR confirmation and mRNA profiles were done to confirm the nature of the double knockout mutants of *Arabidopsis thaliana*. Wild type plants grown in soil for three weeks and an equal number of were randomly subjected to the dark. Entire plants were harvested at 24 hours post darkness. Ion leakage measurements were made with a conductivity meter once after holding the plants at 4C for 24 hours and again subsequent boiling for 10 minutes. Relative ion leakage of the autoclaved leaves was calculated. Ethanol extractions of chlorophyll and chlorophyll measurements of samples subject to the same conditions (including sampling time) were also carried out. Data analyses of Chlorophyll a concentrations and ion leakage measurements from dark stressed plants do not support our hypothesis. We attempt to discuss these discrepancies and proffer suggestions for future work.

P476 - Characterization of the chloride channel, AtCLCg, involved in chloride tolerance in Arabidopsis thaliana

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In plant cells, anion channels and transporters are essential for key functions such as nutrition, ion homeostasis and resistance to biotic or abiotic stresses. In Arabidopsis, members of the ChLoride Channel (CLC) family localized in the tonoplast are required for nitrate homeostasis (AtCLCa and AtCLCb) or salt tolerance (AtCLCc). We characterized AtCLCg, another member of CLC family in Arabidopsis found in the tonoplast. When grown on NaCl or KCl, *atclcg* knock-out mutants showed a decrease in biomass. In the presence of NaCl, these mutants over-accumulate chloride in shoots and sulphate in roots compared to wild-type. No difference in growth was detected in response to osmotic stress by mannitol. These results suggest a physiological function to AtCLCg in the chloride response during NaCl stress. Since AtCLCg and AtCLCc proteins share a high degree of identity (62%), and both participate in NaCl tolerance, an *atclcc atclcg* double knock-out mutant was constructed. The atclcc atclccg plants are not more sensitive to NaCl than single mutants. As the effects of both mutations are not additive, gene expression analysis were performed and revealed that: (i) *AtCLCg* is expressed in mesophyll cells and phloem while *AtCLCc* mRNA are in stomata and (ii) *AtCLCg* is repressed in the *atclcc* mutant background, and *vice versa*. Altogether these results demonstrate that both AtCLCc and AtCLCg are not redundant and form part of a complex auto-regulatory network controlling chloride sensitivity.

P477 - Influence of magnesium availability on root architecture of Arabidopsis

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The root system exhibits a high degree of architectural plasticity in response to nutrient availability in the soil. While the scarcity of certain major mineral elements triggers the proliferation of lateral roots, the depletion of magnesium (Mg) noticeably represses it in Arabidopsis Columbia-0 (Col-0) seedlings grown *in vitro*. First, microscopic analyses indicated a slowdown in the growth of pre-emerged lateral root meristems upon low Mg supply. Second, split-root systems revealed the absence of mechanism to refrain lateral root growth in unfavorable nutrient zone. The extra source of the element acquired by the plant in Mg-rich zone, stimulated lateral root outgrowth also in Mg-deprived zone. Third, the roles played by hormones in shaping root architecture were examined. Upon low Mg, founder cell specification was affected most probably through auxin mediated pathway and repression of lateral root elongation partially exerted by abscisic acid. Finally, 36 natural accessions of Arabidopsis were screened for identifying differences in root morphology. All accessions responded to Mg depletion by a lower number and length of lateral roots but some had higher foraging capacity than the Col-0 reference. Our study did not reveal a clear trade-off between primary and lateral root elongation. The diversity of morphological variation will be a useful resource to determine the genetic determinants of the root system architecture in response to Mg availability.

P478 - Subcellular localization analysis of the membrane-localized ubiquitin ligase ATL31 in Arabidopsis

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In *Arabidopsis thaliana*, *ATL31* encodes a membrane-localized protein belongs to the plant specific ubiquitin ligase Arabidopsis Tóxicos en Levadura (ATL) family. We previously reported that ATL31 is involved in growth regulation in response to balance of carbon/nitrogen nutrient availability during post-germinative development (Sato *et al.* 2009). To determine the detailed function of ATL31, we examined ATL31-interacting proteins using Arabidopsis cultured cells expressing *ATL31-FLAG*. We identified several membrane-localized proteins as novel ATL31 interactors by proteomic analysis. In this study, we will report detailed subcellular localization and physiological function of ATL31 in Arabidopsis.

Reference: Sato T, Maekawa S, Yasuda S, Sonoda Y, Katoh E, Ichikawa T, Nakazawa M, Seki M, Shinozaki K, Matsui M, Goto DB, Ikeda A, Yamaguchi J (2009) CNI1/ATL31, a RING-type ubiquitin ligase that functions in the carbon/nitrogen response for growth phase transition in Arabidopsis seedlings. *Plant J* 60: 852-864

P479 - Towards Understanding BIG's Function in Temperature Responses

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Among the stresses plants perceive from the ever-changing environment, the constant increase in global ambient temperature is considered to be one of the most severe future challenges affecting quantity and quality of crop yield. The growth related hormone auxin has been identified as one of the major players triggering plant morphological and developmental changes in response to warmth. Among these changes, hypocotyl elongation serves as a model response phenotype to high ambient temperature. However, the molecular mechanisms behind this response are still poorly understood and novel players remain to be identified. Here we present the isolation and characterization of the *opi2* mutant impaired in temperature-induced hypocotyl elongation. Mapping-by-sequencing identified a SNP in *BIG/TIR3/DOC1* as the most likely candidate for the causal mutation in *opi2*. BIG/OPI2 is encoded by a 17.5 kb gene and codes for a protein involved in polar auxin transport. While BIG/OPI2 has been associated with several auxin-related physiological processes, molecular characterization was so far hindered by the failure of successful cloning of the gene or cDNA. However, a combination of Golden Gate and Gateway techniques enabled us to clone a full length BIG fragment. In the future, generation of a variety of tagged BIG fusion constructs will in principle allow its molecular characterization. We anticipate that this will provide significant contributions to the auxin and temperature signaling fields.

P480 - The abiotic stress response data of Arabidopsis closely related species and its database provided by RIKEN BRC

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RIKEN BioResource Center (BRC) took over the project of the Sendai Arabidopsis Seed Stock Center (SASSC) and started distributing the Arabidopsis closely related species. To establish the research platform of this invaluable resource, we now collect phenotype and genotype data. At the beginning, we obtained the DNA sequences of the chloroplast genome regions, rbcL and matK as genotype data to classify the species. Then 19 species were chosen based on the classification data and seed availability, and the abiotic stress response data of the species were collected. The phenotype and genotype data are now introduced into the database that will be useful for studying environmental stress response and evolutional history of Brassicaceae. When ready, the database will be accessible from the public via our web site (http: //epd.brc.riken.jp/en/, e-mail; plant@brc.riken.jp).<

P481 - The role of the large cytoplasmic loop of IRON-REGULATED TRANSPORTER1 in the regulation of Arabidopsis iron acquisition

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Dicotyledonous plants acquire soil iron using a mechanism that consists of three principal steps: the solubilization of soil iron, its reduction from ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron and its import into the cell. The last step is achieved through the action of the bivalent metal transporter IRON-REGULATED TRANSPORTER1 (IRT1). IRT1 was shown to have a complex endomembrane trafficking behavior resulting in a regulated iron intake. Our aim is to understand how external signals and the plant developmental program affect iron acquisition, focusing on the mechanisms ensuring the presence and correct regulation of IRT1. We have discovered that members of the SORTING NEXIN protein family participate in the recycling of IRT1 at the sorting endosome. In an attempt to understand the molecular basis of IRT1 post-transciptional regulation, we are focusing on the cytoplasmically-exposed side of IRT1 and its protein environment. Functional and gene expression analyses of an IRT1-interaction partner suggest the existence of negative root-zone-specific events regulating iron acquisition.

P482 (Talk) - Selection of high temperature resistant germination mutants that have defect in abscisic acid regulation at high temperature

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Germination of winter annual seeds is inhibited by high temperature during summer, and this enables the seeds to germinate in autumn. High temperature suppresses germination of Arabidopsis seeds by up-regulating abscisic acid (ABA) biosynthesis genes (*ZEP, NCED2, 5, 9*), and the expression of gibberellin (GA) biosynthesis genes (*GA3ox1, GA3ox2*) are suppressed by ABA synthesized *de novo*. To find the genes that transduce high temperature signal to phytohormone regulation, we first selected 31 lines of high temperature resistant germination mutants from more than 32,000 M1s of ethyl methanesulfonate mutagenized populations of Arabidopsis. The seeds of nine out of the 31 lines showed normal ABA sensitivity, and required GA synthesis for germination. The seeds of the three lines (TRL14-3, TRL19-2, LE23-4) out of the nine lost induction of an ABA inducible gene, *EM6* at high temperature, suggesting that the mutants have defect in the stimulation of ABA synthesis at high temperature. TRL19-2 and LE23-4 showed increased expression of *GA3ox2* at high temperature. TRL19-2 lost induction of *ZEP, NCED2* and *NCED5* at high temperature, and induced ABA catabolism gene, *CYP707A1*, even at high temperature. LE23-4 lost induction of *ZEP, NCED2, NCED9* at high temperature, and induced *CYP707A1* even at high temperature. These results suggest that TRL19-2 and LE23-4 may have defect in the process of high temperature signaling.

P483 - Phosphate deficiency primes the jasmonic acid pathway and increases defense against herbivory.

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Plants often encounter several biotic and abiotic stresses simultaneously. Although the response of plants to individual stresses has been widely studied, much less is known about how the adaptation of plants to one stress affects its ability to adapt to another unrelated stress. Phosphate is one of the most limiting nutrients for plant growth, and plant adaptation to Pi deficiency leads to large changes at the biochemical, developmental and gene expression levels. Here we report that Pi deficiency enhances the defense of plants against herbivory via the jasmonic acid (JA) pathway. JA synthesis and the expression of marker genes associated with the JA signaling pathway were increased in the shoots of both wild type Arabidopsis grown in Pi-deficient media and in the Arabidopsis *pho1* mutant deficient in the transfer of Pi from roots to shoots. Furthermore, wounding induced greater JA accumulation in *pho1* shoots than in WT, indicating that Pi deficiency induced priming of the JA pathway. Consequently, *pho1* mutant and Pi-deficient Arabidopsis, tomato or tobacco were more resistant to herbivory compared to their Pi-sufficient controls. Comparison of pho1 with the *pho1 aos* and *pho1 coi1* double mutants revealed that induction of the JA pathway excarbated several phenotypes typically associated with Pi-deficiency in the *pho1* mutant, such as increased anthocyanin accumulation, reduced shoot growth and inhibition of primary root growth.

P484 - Chloroplast-driven ROS are key player in regulating the crosstalk between abiotic and biotic stresses

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Chloroplast is fragile as ROS are generated in response to various stresses. Apart from being toxic, mounting evidences suggest that ROS play important role in triggering retrograde signaling. Amongst singlet oxygen ($^1O_2$) has been extensively studied to delineate the entire pathway with following reasons:[1]$^1O_2$ seems unable to diffuse chloroplast envelope membrane because of their extreme short lifespan and reactivity with macromolecules; [2] identification of Arabidopsis flu mutant generating$^1O_2$in controlled manner allowed us to differentiate one particular ROS from the others;[3] photosystem II is the major place where the$^1O_2$ and thus it compels us to propose that PSII might be able to sense the level of$^1O_2$ and subsequently triggers retrograde signaling to regulate the photosynthetic activity and to activate defense response. Recent studies show that plants accumulate ROS in chloroplast upon pathogen invasion and in particular, a large portion of pathogen-responsive genes overlaps with$^1O_2$-responsive genes, suggesting that$^1O_2$-triggered chloroplast retrograde signaling seems to participate in the basal defense response. Moreover, we found that the$^1O_2$ confers significant resistance of plants to subsequently challenged adversity caused by bacterial pathogen. Our recent data suggests that chloroplast-driven ROS and the cognate retrograde signaling might play an important role in the molecular and the sub-cellular crosstalk in response to abiotic and biotic stresses.

P485 - Arabidopsis RING E3 Ubiquitin Ligase AtMNRP, which Localizes to Microtubule and Nucleus, has Positive Roles in Drought Stress Response

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To study the relationships between ubiquitination and abiotic stress responses, we previously collected 100 different T-DNA insertion mutants, in which RING-type E3 ubiquitin (Ub) ligase genes were suppressed, and screened the phenotypes of these mutants in terms of responses to abiotic stress and stress hormone ABA. One of the mutants, *atmnrp* (*Arabidopsis thaliana microtubule and nucleus RING protein*), showed a hyposensitive response toward ABA in seed germination. This mutant showed partial impairment of ABA-mediated stomatal closure and faster stomata opening than wild type. When wild type and mutant plants were subjected to dehydration, the mutant was more susceptible to drought stress than wild type plant. AtMNRP has the C3H2C3-type RING domain at its C-terminus and bacterially-expressed MBP-AtMNRP recombinant protein showed E3 Ub ligase activity *in vitro*. When the *35S:AtMNRP-sGFP* fusion construct was transiently expressed in protoplasts, florescence signal was detected in both cytosolic microtubule and nucleus. Collectively, our data suggested that a cortical microtubule- and nucleus-localized AtMNRP RING E3 is involved in drought stress response through ABA-mediated stomatal regulation. This work was supported by grants from the National Research Foundation (Project No. 2014R1A2A2A01003891 funded by the Ministry of Education, Science, and Technology, Republic of Korea).

P486 - Title: Involvement of pectin modifying enzymes in responses to trace elements and heavy metals in Arabidopsis thaliana

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Cell walls are not only a protective barrier around protoplasts but serve as signaling platform between the extracellular environment and the intracellular physiology. Primary cell walls consist of cellulose and xyloglucans embedded in a complex pectic and protein matrix. Pectins and in particular homogalacturonans (HGs) play a key role in transmitting exogenous biotic and abiotic signals to the interior of the cell. HGs are secreted in a highly methylated and acetylated form into the cell wall. In muro the degree of methylation/actetylation and polymerization is mediated by multigene families of pectin methylesterases (67 PMEs), PME inhibitors (76 PMEIs), pectin acetylesterases (12 PAEs), poly-galacturonases (66 PGs), and pectate lyases-like (27 PLLs)[1]. De-esterified carboxylic groups of HGs account for most cation exchange capacities of cell walls and bind Ca2+, Pb2+, Cu2+, Co2+, Ni2+, Zn2+, Cd2+ and Al3+ [2] . Recently, it has been shown that the overexpression of PME3 interferes with Zn2+ mediated growth processes[3] . To characterize in more detail growth effects and PME activity upon long term heavy metal and trace element exposure, a targeted genetic screen with mutants and overexpressors of pectin modifying genes has been initiated and the results will be presented and discussed.

1) Sénéchal et al., (2014) J Exp Bot 65, 5125-5160

2) Kartel et al (1999) Chemosphere 38:2591–2596

3) Weber et al. (2013) Plant J 76, 151-164

P487 - Trehalose metabolism plays an important role in flooding tolerance

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Enormous areas of land are flooded each year due to extreme weather events like cyclones, heavy rainfall and meltwater (or combinations of these events). These extreme weather events are increasing in frequency and severity due to climate change. Flooding restricts respiration and photosynthesis due to limited gas exchange in the affected organs. Not surprisingly flooded plants will rapidly encounter an energy crisis. In the last decade, the trehalose synthesis pathway and in particular the metabolite trehalose-6 phosphate (T6P) have been linked with energy sensing and the response to energy crisis. Here we show that trehalose metabolism is affected during flooding stress resulting in specific regulation of the levels of the signaling metabolite T6P, both during and after submergence. Accordingly, Arabidopsis mutants altered in endogenous T6P levels had altered flooding tolerance. The regulatory effect of T6P is presumably via its regulation of starch metabolism and SnRK1 activity and this was tested via measurement of starch and soluble sugar levels and SnRK1 activity. We hypothesize that specific dynamics of T6P both during and after flooding are critical to facilitate metabolic adaptation in response to energy supply and demand via regulation of SnRK1 activity. Our data strongly suggest that manipulation of the trehalose synthesis pathway will allow us modulate flooding tolerance through this pathway and could also be the basis of natural variation in flooding tolerance.

P488 - Rice Cyclophilin OsCYP18-2 is Translocated to the Nucleus by an Interaction with SKIP and Enhances Drought Tolerance in Rice and Arabidopsis

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Cyclophilin 18-2 (CYP18-2) genes, homologues of human *peptidyl-prolyl isomerase-like 1* (PPiL1), are conserved across multicellular organisms and *Schizosaccharomyces pombe.* Although PPiL1 is known to interact with ski-interacting protein (SKIP), a transcriptional co-regulator and spliceosomal component, there have been no functional analyses of PPiL1 homologues in plants. Rice cyclophilin 18-2 (OsCYP18-2) bound directly to amino acids 56-95 of OsSKIP and its binding was independent of cyclosporin A, a cyclophilin-binding drug. Moreover, OsCYP18-2 exhibited PPIase activity regardless of its interaction with OsSKIP. Therefore, the binding site for OsCYP18-2”s interaction with SKIP was distinct from the PPIase active site. OsCYP18-2”s interaction with SKIP full length protein enabled OsCYP18-2”s translocation from the cytoplasm into the nucleus, and AtSKIP interacted in planta with both AtCYP18-2 and OsCYP18-2. Drought and salt stress induced similar expression of *OsCYP18-2* and

OsSKIP. Over-expression of OsCYP18-2 in transgenic rice and *Arabidopsis thaliana* plants enhanced drought tolerance, and altered expression and pre-mRNA splicing patterns of stress-related genes in *Arabidopsis* under drought conditions. Furthermore, OsCYP18-2 caused transcriptional activation with/without OsSKIP in the GAL4 system of yeast; thus the OsSKIP-OsCYP18-2 interaction has an important role in the transcriptional and post-transcriptional regulation of stress-related genes, and increases tolerance to drought stress.

P489 - ROLE OF PLASMA MEMBRANE PROTON PUMP H+-ATPASES IN CESIUM TOLERANCE IN ARABIDOPSIS

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In 2011, the Fukushima nuclear power plant accident released a large amount of radioactive nuclides into the atmosphere of Japan, which then caused serious contamination of large ecosystems including agricultural farmlands. Among them, radiocesium (^{137}Cs) is one of the major radionuclides, which constitutes a considerable environmental threat because of its high relative mobility in the soil–plant system, long-term bioavailability, high radiotoxicity and relatively long half-life (30.17 yrs.). Finally, the ^{137}Cs uptake by plant roots is the main pathway for the migration of ^{137}Cs from soil to humans via plants. Cesium is a weakly hydrated alkaline metal with chemical similarities to potassium and several previous studies suggest that the uptake and the translocation of cesium is operated mainly by two transport pathways on plant root cell membranes, namely the K^+ transporter and the K^+ channel pathway. Since, the uptake of nutrients into and within the plant is energized and regulated by plasma membrane H^+-ATPases, we evaluate the role of several isoforms of plasma membrane H^+-ATPases in the uptake and the translocation of cesium in Arabidopsis plants. Altogether, our knowledge on plant uptake of radiocaesium will be important for devising effective strategies and developing techniques, such as agricultural countermeasures and phytoremediation, to minimize the transfer of radiocaesium from soil to humans.

P490 - Role of PI3K (PhosphatidylInositol 3-Kinase) and TOR (Target Of Rapamycin) kinases on proline metabolism upon salt stress in Arabidopsis thaliana

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The TOR kinase is a central node participating to the integration of information from the environment and their translation in developmental and metabolic responses. In animals TOR can be regulated by a class-III PI3K *via* PI3P (PhosphatidylInositol-3-Phosphate). In plants, these two kinases are known to be involved in the regulation of growth and the adaptation to various environments. In Arabidopsis, TOR forms a complex (TORC1) with two others proteins, LST8 and RAPTOR, and is activated under favorable conditions while nutrient limitations or stresses modify its activity. Accumulation of proline is a widespread plant adaptive response to environmental stresses such as salt constraint and is a consequence of both proline biosynthesis and catabolism regulation. LY294002, a PI3K and PI3K-related kinase inhibitor, affects PI3P levels *in vivo* and triggers a decrease in proline accumulation only in response to salt treatment in *A. thaliana* seedlings. This lower proline accumulation is correlated with a lower transcript level of Pyrroline-5-carboxylate synthetase 1 (*P5CS1*) and higher transcript and protein levels of Proline dehydrogenase 1 (*ProDH1*), encoding respectively the key-enzymes of proline biosynthesis and catabolism. *ProDH1* expression is also induced in both *pi3k*-hemizygous mutant and raptor mutants. Taken together, these data suggest that both PI3K and TORC1 are involved in the regulation of proline metabolism and that these signaling pathways could be interconnected.

P491 - MOLECULAR MECHANISMS UNDERLYING ARSENIC PERCEPTION IN PLANTS

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Arsenic has been a major challenge for organism survival since life inception. In plants, the mechanisms underlying arsenic response are mostly unknown. Arsenate is the most abundant chemical form of arsenic and based on its similarity with phosphate, it is easily incorporated into cells through the phosphate transporters. In plants, once arsenate is perceived, the phosphate transporters are rapidly downregulated. This response exerts a particular dilemma for plants; to suffer a phosphate starvation condition, or to be arsenic intoxicated. In order to withstand this particular situation, plants adapt the expression of arsenate / phosphate transporters to the detoxification capacity through the action of phytohormones. Here we will present our recent results to understand the molecular mechanisms underlying this coordinated response.

P492 - Using Quantitative Trait Locus Mapping to Better Understand Arsenic Tolerance Mechanism in Arabidopsis

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Arsenic is an environmental contaminant; either human activities or natural processes can cause arsenic pollution. Soil with a high arsenic content can inhibit normal crop growth and these crops will also have food safety issues. Oil crops like *Brassica juncea* grown in an arsenic environment can accumulate arsenic in its seeds and seed oil. Thus, arsenic pollution not only harms people"s health but also damages local agriculture, especially in developing countries. *Arabidopsis thaliana* is used as a model organism in this project to identify genes associated with arsenic tolerance, which can help to better understand arsenic tolerance and reduce the impact of arsenic contamination in agriculture. Hydroponic cultivation system was employed and chemical analysis was used to assess the presence of arsenic. After screening the arsenic tolerance of a recombinant inbred line population, quantitative trait locus mapping was used thereafter to find out locus or loci associated to arsenic tolerance and detoxification. We firstly confirmed arsenic accumulation in both seeds and seed oil of *Brassica juncea*. We then selected a recombinant inbred line population that was previously made for other research purpose, to screen arsenic tolerance. Finally, quantitative trait locus mapping and a further fine mapping were both processed to target arsenic tolerance related genes.

P493 - Two NAC transcription factors from Caragana intermedia altered salt tolerance of the transgenic Arabidopsis

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Plants are continuously challenged by different environment stresses, and they vary widely in their adjustability. NAC (NAM, ATAF and CUC) transcription factors are known to be crucial in plants tolerance response to abiotic stresses, such as drought and salinity. ANAC019, ANAC055, and ANAC072, belong to the stress-NAC TFs, confer the Arabidopsis abiotic stress tolerance. Here we isolated two stress-responsive NACs, CiNAC3 and CiNAC4, from Caragana intermedia, which were induced by ABA and various abiotic stresses. Localization assays revealed that CiNAC3 and CiNAC4 localized in the nuclei, consistent with their roles as transcription factors. Histochemistry assay using ProCiNAC4::GUS transgenic Arabidopsis showed that the expression of the GUS reporter was observed in many tissues of the transgenic plants, especially in the root vascular system. Overexpression of CiNAC3 and CiNAC4 reduced ABA sensitivity during seed germination, and enhanced salt tolerance of the transgenic Arabidopsis. The results indicate that CiNAC3 and CiNAC4 play essential roles not only in promoting lateral roots formation, but also in responding to salinity and ABA treatment of Arabidopsis.

P494 - Genome-wide Association Study for Shoot and Root Variations in Arabidopsis under different Growth Temperatures

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The analysis of shoot and root variations under different growth temperatures is of importance to reveal the mechanism of how plants interact with the environment. We selected 80 Arabidopsis ecotypes from the 1001 genome project for high throughput phenotyping 24 shoot traits and 14 root traits under contrasting growth temperatures during the light period (15ºC, 27ºC, and 20ºC as control). A web application for genome-wide association study (GWAS) (http://gwas.gmi.oeaw. ac.at) with Accelerated Mixed Model and the 250K SNPs was applied for GWAS. Based on Bonferroni threshold, 76 loci were significantly associated with 7 root traits, mainly with growth rate of total and lateral roots, length of lateral and tertiary roots at the growth temperature of 27ºC. At 15ºC, only 11 loci were significantly associated with length of lateral and primary roots. For the shoot traits, there were 4 loci significantly associated with dry weight, leaf area and its growth rate and stockiness at 15ºC, respectively, two of which also significantly associated at 20 ºC. The gene functions underlying the mapped SNPs include antioxidant/ oxidase protein, protein kinase, sodium/hydrogen exchanger, cellulose/ callus synthase, proteasome, DNA methyltransferase. Our results indicate that GWAS with a relative small number of ecotypes could be possible with high density SNPs and the genes relevant to stress reaction pay an important role for plants adapting to a range of growth temperatures.

P495 - Improvement of oxidative stress tolerance in Arabidopsis by over-expression of a heat shock factor A3

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Heat stress transcription factors (Hsfs) regulate plant responses to environmental stresses. In current study, transcription of *HsfA3*, a class A *Arabidopsis* Hsf, was induced by a reactive oxygen species, hydrogen peroxide (H_2O_2) and a herbicide, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) in seedlings. Transgenic plants overexpressing *HsfA3 (35S: HsfA3)* were tolerance to oxidative stress, as identified by lower levels of ion leakage and higher contents of chlorophyll fluorescence than wild-type *Arabidopsis* plants (wt) under light conditions. The transcriptional activity of galactinol synthase (*GolS*) genes, a key enzyme in synthesis of osmoprotective substances, especially raffinose family oligosaccharides (RFOs), was induced in *HsfA3* overexpressors. Among the RFOs, galactinol level was increased in *HsfA3* overexpressors compared with the wt under control growth conditions. Additionally, the transient transactivation assay and electrophoretic mobility shift assay showed that *GolS1* and *GolS2* were directly regulated by HsfA3. These data provide evidence that some *GolS* genes are directly regulated by HsfA3 and *HsfA3* plays an important role in improvement of oxidative stress tolerance by synthesis of galactinol in *Arabidopsis*.

P496 - Signaling components involved in the induction of acid phosphatases by phosphate starvation

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Induction and secretion of acid phosphatases (APases) is a hallmark response of plants to phosphate (Pi) starvation. These APases can enhance Pi utilization efficiency of plants under Pi starvation. We have used *Arabidopsis* to investigate the signaling mechanism that underlies this adaptive response. Using forward genetics, we first determined that three members of a purple acid phosphatase (PAP) family, AtPAP10, AtPAP12, and AtPAP26, are responsible for most of the Pi starvation-induced APase activity in *Arabidopsis*. We then identified sucrose and ethylene as two positive regulators of APase activity on the root surface. Using split-root experiments, we found that the root surface-associated AtPAP10 activity is differentially regulated by systemic and local signaling at multiple levels. Previously, a MYB-CC transcription factor, PHR1, had been shown to bind to the P1BS element on the *AtPAP10* gene promoter and serve as a transcriptional activator. In our work, we identified another cis-element, LPRE, on the *AtPAP10* promoter that is critical for the transcription of *AtPAP10*. Two PHR1-like proteins, PHL2 and PHL3, directly bind to LPRE and act as transcriptional activators. PHR1 can also bind to the LPRE. Interestingly, when PHR1 binds to the LPRE, it acts as a transcriptional repressor. Besides, a SPX domain-containing protein was found to be a negative regulator of AtPAP10 activity. A model for the transcriptional control of *AtPAP10* expression will be presented.

P497 - tasiRNA-ARF pathway moderates floral architecture in plants subjected to drought and high-salinity stress

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We studied the expression profiles of small RNAs in plants subjected to drought, cold, high-salinity stress, and ABA treatments, using 454 DNA sequencing technology. Expression of three groups of ta-siRNAs (TAS1, TAS2 and TAS3) and their precursors was downregulated in plants subjected to drought and high-salinity stress. Analysis of ta-siRNA synthesis mutants and mutated *ARF3*-overexpressing plants that escape the tasiRNA-ARF target indicated that self-pollination was hampered by short stamens in plants under drought and high-salinity stress. Microarray analysis revealed that expression of auxin biosynthesis-related genes, *YUCCAs* and auxin response-related genes was downregulated by drought stress and by the *RDR6* mutation. In addition, artificial auxin, 2,4-D treatment rescued short stamen phenotype of *rdr6*. The overall results of the present study indicated that tasiRNA-ARF is involved in maintaining the normal morphogenesis of flowers in plants under stress conditions through the fine-tuning expression changes of auxin-related genes.

P498 - Nitrate and phosphate signaling interactions

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Nitrogen and Phosphate are two of the most important elements sustaining plant growth and nutrition. However, Nitrate (NO_3^-) and inorganic phosphate (Pi) are also important signalling molecules whose interactions are not very well documented. Here, we identified a nitrate regulated GARP transcription factor, HRS1, which integrates NO_3^- and Pi signals at the root tip of Arabidopsis. HRS1 takes part in the regulation of the root shortening in Pi deficient conditions, only when NO_3^- is present. We propose an explanatory mechanism by which NO_3^- and Pi regulates the same transcription factor, at two different levels: transcriptionally and post transcriptionally[1]. We finally open perspectives showing interesting elements of the interaction between phosphate and nitrate signalling per se.

1. Medici, A. et al. AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. *Nat Commun* 6: 6274 (2015).

P499 (Talk) - Light signaling controls nuclear architecture reorganization during seedling establishment

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Nuclear organization and genome expression are subject to extensive reprogramming during most developmental transitions. However, the mechanisms triggering them in response to environmental stimuli are poorly understood. Here, we determined that light perception triggers a switch between two different nuclear architectural schemes during Arabidopsis post-embryonic development. While progressive nucleus expansion and heterochromatin rearrangements in cotyledon cells are achieved similarly under light and dark conditions during germination, the later steps that lead to mature nuclear phenotypes are intimately associated with the photomorphogenic transition in an organ-specific manner. The light signaling integrators DET1 and COP1 maintain heterochromatin in a decondensed state in etiolated cotyledons. In contrast, under light conditions, cryptochrome-mediated photoperception releases nuclear expansion and heterochromatin compaction within conspicuous chromocenters. Finally, we report that global engagement of RNA Polymerase II in transcription is highly increased under light conditions, suggesting that cotyledon photomorphogenesis involves a transition from globally quiescent to more active transcriptional states. Given these findings, we propose that light-triggered changes in nuclear architecture underlie interplays between heterochromatin reorganization and transcriptional reprogramming associated with the establishment of photosynthesis.

P500 - The Arabidopsis nitrate transceptor NRT1.1/NPF6.3 that governs distinct signaling pathways is subjected to a complex post transcriptional regulation.

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Nitrate (NO_3^-) is both the main nitrogen source for higher plants, and a signal molecule regulating their metabolism and development. The roots sense the NO_3^- concentration in the soil solution, and trigger signalling pathways allowing plant adaptation to its availability. In Arabidopsis, this response depends on the NRT1.1/NPF6.3 transceptor. We recently reported that NRT1.1 acts not only as a NO_3^- transporter but also facilitates influx of the phytohormone auxin in a NO3 concentration dependent manner defining a new mechanism for sensing environmental stimuli, and for connecting nutrient and hormone signalling in the control of root development. NRT1.1 was also shown to regulate the expression of hundreds of genes in response to NO_3^- supply. Using point mutations in the NRT1-1 protein, we showed that NRT1.1 activates four separate signalling mechanisms, which have independent structural bases in the protein. We also show that these mutations have differential effects on genome-wide gene expression. Besides, our results also demonstrate the occurrence of at least two levels of posttranscriptional regulation of NRT1.1 affecting independently mRNA and protein accumulation depending of root tissues and NO3- concentration. Our findings add to the evidence that NRT1.1 is subjected to a complex regulation and triggers independent signalling pathways in Arabidopsis in response to different environmental conditions.

P501 - Functional analyses of CBL10 induced by ROS in salt and drought stresses

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Cellular levels of Ca^{2+} and Reactive Oxygen Species (ROS) that are tightly regulated to keep at low levels in cytosol are greatly fluctuated as second messengers to decode many environmental and developmental signals. Phytohormones are representative primary signals leading to various changes ROS or Ca^{2+} signaling with synergistic and antagonistic cross-talks. In addition to its diverse roles in plant development, brassinosteroids (BRs) are also involved in thermotolerance through heat-shock protein synthesis, dose-dependent cold resistance in Arabidopsis, and higher resistance to bacterial and fungal pathogens in tobacco and rice. However, how BR exerts their effects on stress tolerance are not understood yet. In this study, we found that the BR-induced ROS production was impaired in BR biosynthetic or signaling mutant. We also found that CBL10, one of the calcineurin B-like (CBLs) proteins that sense calcium, can be signaling component for BR-induced ROS by showing that ROS-induced CBL10 expression was nullified and the endogenous CBL10 expression in shoot was reduced in the BR signaling mutant. Moreover, we provided a possible explanation for positive role of CBL10 in salt tolerance by showing BR sensitivity in hypocotyl growth was reduced in cbl10 under salt condition. Additionally we found that CBL10 functions negatively in drought tolerance, opposite aspect for its positive function in salt resistance.

P502 (Talk) - Suppressor screening of autophagy-defective mutants based on early senescence

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Autophagy is a conserved mechanism for degradation of cellular contents to recycle nutrients, and it is coordinated by Autophagy related (ATG) proteins. Dysfunctional proteins or damaged organelles are recognized and enclosed in a double membrane vesicle called autophagosome,

and then delivered to the vacuole, where the contents are degraded by vacuolar hydrolases. In plants, autophagy is very important during development, senescence, and to deal with starvation and other adverse conditions[1]. Autophagy-defective mutants show an early senescence phenotype under normal growth condition and carbon- or nitrogen-starvation conditions (2, 3). This phenotype is particularly strong in *atg2,* in which the gene disrupted by a T-DNA insertion encodes ATG2, a protein involved in the formation/expansion of the double membrane of autophagosomes. *atg2* mutant plants grown without sugar and exposed to prolonged darkness cannot be recovered when they are exposed to light again, contrary to wild type plants. This condition was used to perform a suppressor screening on *atg2* after EMS mutagenesis, to identify genes connecting autophagy and senescence processes. A total of 6 suppressors were confirmed on M3 generation. The 6 candidates are currently being sequenced and characterized phenotypically.

P503 - Analysis of direct target genes of SOG1, a key transcription factor involved in DNA damage response

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Plants are constantly exposed to a risk of DNA damage induced by DNA replication error and environmental stresses such as UV, heavy metals and pathogen attacks. Therefore, DNA damage response is important for plants to survive under various environmental conditions. In *Arabidopsis*, it has been reported that SUPPRESSOR OF GAMMA RADIATION 1 (SOG1) transcription factor functions as a master regulator of DNA damage response. However, the signaling pathway downstream of SOG1 has been less understood. To uncover the mechanisms underlying DNA damage response controlled by SOG1, we performed microarray and ChIP-Seq analyses to identify direct target genes of SOG1. We identified about 120 genes as SOG1-direct target genes. Many genes related to DNA repair and cell cycle regulation were included in the target genes. Interestingly, some key genes in plant immune signaling were also identified as SOG1-target genes, suggesting that SOG1 is involved in defense response. Also, we could identify shared consensus sequence in the promoters of SOG1-direct target genes. One of the promoters with base substitutions in the consensus sequence did not respond to DNA damage *in planta*, indicating that the consensus sequence functions as cis-regulatory element recognized by SOG1. Our findings suggest that SOG1 directly induces a set of genes, which are involved in DNA repair, cell cycle and plant immunity system, through binding to the consensus sequence.

P504 - Plant Growth At High Densities: Shade Avoidance Responses To Neighbors

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One of the main problems in agriculture is the loss of crop yield due to weeds. The competition for resources, including light, between crops and weeds is particularly severe at high plant densities. In dense stands, the light environment is already changing before true shading occurs, due to selective wavelength reflection by neighboring plants. Previous studies have shown that the earliest neighbor responses in dense stands of Arabidopsis are induced through touching of leaves and lead to upward leaf movement (hyponasty). The resulting vertical canopy structure increases the horizontal reflection of far-red (FR) light by the elevated leaves towards neighboring plants. This FR enrichment in turn lowers the Red:Far-red light ratio (R:FR) perceived by neighbors, constituting a signal for upcoming competition for light. In response, plants follow a defensive strategy of ‘shade avoidance’ in order to survive as individuals. These responses lead to more open crop canopy, in which more light penetrates through the canopy facilitating weed growth. We will further elucidate the mechanism(s) involved in plant neighbor detection and study the consequence of plant neighbor detection for weed suppression by testing whether inhibition of shade avoidance responses through genetic approaches, leads to a better “closed” crop canopy that can effectively cooperatively suppress weeds.

P505 - Effects of a Variety of Environmental Factors on the Splicing of Chloroplast Introns

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Chloroplast gene expression is mainly regulated at the posttranscriptional levels, including intron splicing, rRNA processing, and editing. Although several recent studies have demonstrated that these posttranscriptional RNA metabolism in chloroplasts is affected by developmental and environmental cues, reports investigating the effects of environmental factors on chloroplast RNA metabolism are severely limited. To get insights into the importance of chloroplast RNA metabolism during stress responses, we carried out a comprehensive analysis of the intron splicing of chloroplast transcripts in *Arabidopsis thaliana* and cabbage (*Brassica rapa*) under different environmental conditions, such as application of photosynthesis electron transfer inhibitor, DCMU and DBMIB, and treatment of a variety of environmental stresses, including drought, salt, cold, UV, or heat stresses. The results showed that splicing patterns and transcript levels of the selected chloroplast genes are modulated by different environmental factors and photosynthesis electron transfer inhibitors. Taken together, our results demonstrate that splicing of chloroplast transcripts is affected differently by various environmental factors, which suggests that proper splicing of chloroplast genes is important for plant response to environmental cues. [Supported by grants from NRF and Next-Generation BioGreen21]

P506 - Nucleocytoplasmic NPR1 is Pivotal Signaling Component in Response to Salt Stress in tobacco roots

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Translocation of nonexpressor of pathogenesis-related (PR) gene 1 (NPR1) protein, a transcriptional coactivator for salicylic acid-mediated pathogen attack, from cytoplasm to the nucleus is involved in early regulatory pathway of salt stress in tobacco roots. NPR1-green fluorescence protein (GFP) fusion protein exhibited strong localization in the cytoplasm of the elongation zone (EZ) and differentiation zone (DZ) in control roots of NPR1-GFP transgenic plants (35S:: NPR1-GFP). But GFP fluorescence was not observed at all in the meristamatic zone (MZ), indicating that NPR1 is constitutively cleared by 26S proteasome, and then did not involve in cell division. We next analyzed subcellular localization of NPR1-GFP protein after salt stress. After 1h, translocation of cytosolic NPR1 fluorescence into the nucleus was clearly observed in the EZ, whereas NPR1-GFP protein was strongly localized in the plasma membrane of the DZ. Oligomeric NPR1 remained at a steady-state level in roots during salt stress. However, dimeric and monomeric NPR1, translocation forms from cytosol to nucleus, were significantly cleared by proteasome-mediated degradation in tobacco roots during salt stress. Tissue-specific distribution of NPR1 proteins was well in accordance with stress-induced pattern of reactive oxygen species (ROS) accumulation in roots during salt stress. These results indicated that the effects of NPR1 in response to salt stress are regulated in a ROS-mediated spatial subcellular manner.

P507 - ROS Homeostasis Under Anoxia is Mediated by the Universal Stress Protein HRU1

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The response of *Arabidopsis thaliana* to low oxygen is activated by the oxygen sensor RAP2.12, which controls a core set of hypoxia-responsive genes responsible for metabolic acclimation. Anoxia also induces genes that have been identified as related to reactive oxygen species (ROS). Here, we show that a low-oxygen responsive Universal Stress Protein (HRU1) is induced by RAP2.12 and modulates ROS production. Mutations in HRU1 result in misregulated ROS production and affect tolerance to both anoxia and submergence. We found that HRU1 affects ROS production by interacting with ROP2 small GTPase, RbohD NADPH oxidase, and thioredoxin-h, thus highlighting the existence of an anoxia-

specific mechanism for the modulation of ROS production. We suggest that the induction of HRU1 under anoxia integrates the anaerobic response with the production of ROS and consequent signaling.

P508 - Hydrotropic response deficiency in the ahr1 mutant affects the expression Upbeat1, High Ploidy2, and Cobra genes during water stress conditions

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Hydrotropism involves the perception of water and consequently the change of the growth direction of the root to the water source. This process is considered an adaptive strategy to resist drought. Interested in described if some root growth genes were altered in the *ahr1* mutant, we analyzed the expression of genes that participate in root development, like SUMO E3 Ligase (HIGH PLOIDY2, HPY2), a glycosylphosphatidylinositol (GPI)-anchored protein (COBRA), and a tonoplast intrinsic protein (GAMMA). We also analyzed the mRNA level of UPBEAT1 (*Upb1*) a negative regulator of peroxidases activity during root growth that directly regulates the expression of a set of peroxidases. Results showed that *Cobra* expression diminished in wild type roots grew on water stress system, however the mRNA levels did not change significantly in the *ahr1* roots, in the same conditions. Polar expansion of roots might be negatively regulated during water stress in Col-0 but did not affect *ahr1* root growth. Cobra function might be required for a longitudinal expansion allowing root growth during water stress. *Hyp2* expression diminished in wild type roots grew on water stress system, however the mRNA levels did not change significantly in the *ahr1* roots, in the same conditions. This down-regulation of *Hpy2* transcript levels might be related to an increase drought tolerance in *ahr1* mutant. Wild type roots and *ahr1* showed low levels of Upb1 expression during water stress.

P509 - Chloroplast signals in the control of plant growth, programmed cell death and defense mechanisms

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Plants are sessile organims and therefore have to adjust to a changing environment. Because they play a central role in various aspects of plant metabolism and their function is exquisitely sensitive to external conditions, chloroplasts are ideally suited to play a role as sensors of stresses, and a wealth of retrograde signaling pathways have been identified that modulate nuclear gene expression in response to external stimuli. Using various genetic approaches, we have shown that reduced photosynthesis impacts cell cycle progression, but also that chloroplast dysfunction causes constitutive activation of stress responses leading to the upregulation of specific cell cycle inhibitors and triggering premature exit of proliferation and cellular differentiation. In addition, chloroplast-derived signals also play a role in the control of programmed cell death: we have shown that photosynthetic activity is required for the onset of cell death, and that the production of 3'-phosphoadenosine 5'-phosphate (a metabolite involved in high-light response) in chloroplasts is a negative regulator of programmed cell death. Together, our results provide evidence for the central role of chloroplastderived signals in all aspects of plant development, biotic and abiotic stress response.

P510 (Talk) - Cross-species-functionality of a Salix caprea CELL WALL ASSOCIATED KINASE-LIKE protein in Arabidopsis

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Isolates of the fast growing willow tree Salix caprea are capable of tolerating and accumulating heavy metals from soil and thus are candidates for phytoremediation strategies. Previously, we have shown that upon long time exposure with cadmium (Cd) and zinc (Zn) a *CELL WALL ASSOCIATED KINASE-LIKE* gene (*ScWAKL*) was highly upregulated[1]. Phylogenetic analysis with all WAK and WAKL proteins of Arabidopsis, *Brachypodium distachyon, Populus trichocarpa* and *Salix* purpurea positioned the ScWAKL in a distinct subfamily specific to the Salicaceae. Here, we present the functional analysis of *ScWAKL* in a cross-species approach expressing a ScWAKL-GFP fusion protein in Arabidopsis. We show that ScWAKL-GFP is localized in the plasma membrane and does not affect growth as long as the transgenes are not exposed to heavy metal or trace elements. In root growth assays, ScWAKL-GFP transgenes exhibit the same responses as wild type on medium supplemented with manganese and nickel. While the transgenes are hypersensitive to cadmium, copper and zinc, they show a higher tolerance towards lead (Pb). To understand the molecular basis of this selective mechanism of recognition and response, expression of metal responsive genes was quantified and evaluated in the context of the ionome data of Cd and Pb treated ScWAKL-GFP and wildtype seedlings. A model will be presented describing how ScWAKL mediates selective responses towards Cd and Pb.

[1] Konlechner et al. (2013) Environ Pollut 178C, 121-127

P511 - Discovery and characterization of putative plant prions using an integrated approach

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Prions are self-replicating protein structures that can be transferred between cells or organisms by conversion of the native protein to the aggregated prion form. Prions have been associated with diseases in mammals, but in fungi and flies they govern diverse phenotypic switches, for example in metabolism and learning. Surprisingly, prions have not been identified in plants yet. Recently, we have taken advantage of a computationally study that predicted a set of prion candidates in *A. thaliana* to characterize potential plant prions. Using well established yeast assays we have shown that three of these, *NRPE1, PosF21* and *Med9*, form SDS-resistant aggregates, a property consistent with prions. Furthermore, the gold standard *S. cerevisiae* phenotype switching assay revealed that PosF21 behaves as a prion in yeast. Several *A. thaliana* mutants for these genes were selected and grown in several conditions affecting protein folding. These experiments uncovered a role for these genes in salt stress tolerance, with the line overexpressing NRPE1 prion domain being very sensitive to cold, heat and salt. Deleting or overexpressing these prion domains in *A. thaliana* transgenic lines will address their functional importance in plants. Changing chaperone levels in these plants will tell us whether the prion domain-dependent phenotypes we observe are chaperone dependent. This work could reveal the existence of prions involved in key biological processes in a new kingdom of life, plants.

P512 (Talk) - ABA and flg22 signaling pathways inducing stomatal closure: role of aquaporins in regulation of water and hydrogen peroxide transport

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Stomatal movements are crucial for controlling the plant water status under changing environmental conditions and for protecting plants against pathogens. Although stomatal movements involve marked water fluxes, the role of aquaporins (AQP) has remained hypothetical. Functional assays in epidermal peels of Arabidopsis leaves showed that *pip2;1* plants invalidated for plasma membrane AQP *PIP2;1* gene have a defect in stomatal closure in response to abscisic acid (ABA) and flagellin peptide flg22, but normal responses to dark, light or carbon dioxide. Guard cell (GC) protoplasts from wild-type plants showed an increase in osmotic water permeability (P_f) in response to ABA and flg22 which was fully abrogated in *pip2;1* GC protoplasts. Transgenic expression of hydrogen peroxide (H2O2) sensitive probe HyPer revealed that ABA and flg22 induce an accumulation of H_2O_2 in GCs of wild-type but not of *pip2;1* plants. OST1, a key kinase in ABA signaling was able to phosphorylate *in vitro* a cytosolic peptide of PIP2;1 at Ser121. OST1 also enhanced PIP2;1 water transport activity after co-expression in *Xenopus* oocytes. Furthermore, expression in *pip2;1* plants of phospho-mimetic or phospho-deficient forms of PIP2;1 indicated that the increase in GC protoplast P_f, intracellular accumulation of H_2O_2 and stomatal closure induced by ABA and flg22 require phosphorylation of Ser121. This work provides the first direct genetic evidence for aquaporin function in GCs. We propose that PIP2;1 plays a hydraulic and signaling role in GC pathways requiring H_2O_2, with a key regulatory role of Ser121 phosphorylation.

P513 - The Organization of Controller Motifs Leading to Robust Plant Iron Homeostasis

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Homeostatic mechanisms are essential to keep cells and organisms fit in a changing and challenging environment. An important task is to identify the principles, which contribute to the functionality and robustness of homeostatic mechanisms in the presence of environmental perturbations. Although key components of iron homeostasis have been identified in Arabidopsis and other plants, the ways these components integrate and interact are not well understood. We use a computational approach to describe robust iron homeostasis in Arabidopsis roots. We show that a functionally conserved hierarchical set-point structure of the key regulators needs to be established in order to obtain a concerted and integrated homeostatic behavior during high- and low-affinity iron uptake, assimilation, storage, as well as iron remobilization from the vacuolar store. If this hierarchical set-point structure is not met, several dysfunctional behaviors in iron regulation are observed. In agreement with experimental results the emerging model points towards a central regulatory function of the global transcription factor FIT during high-affinity uptake of iron. It further predicts a dramatic improvement in the iron uptake system's response time due to an experimentally identified stabilizing effect of FIT on the iron transporter IRT1. By including an additional inflow controller in the vacuolar membrane, the model suggests an iron biofortification approach, which allows an increased vacuolar iron storage capacity in roots even under iron limiting conditions.

P514 - RCI1A connects low temperature response with ET biosynthesis to regulate freezing tolerance in Arabidopsis

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For plants, as sessile organisms, extreme temperatures are among the environmental factors that most condition their development and distribution. Understanding how plants cope with extreme temperatures is interesting not only from a basic point of view but also from a more practical aspect to design molecular tools that can be used to improve crop tolerance to this adverse environmental condition. During the last years, an important effort has been devoted to unveil the molecular mechanisms underlying plant response to low temperature, and more specifically the process of cold acclimation, the adaptive process whereby many plants from temperate regions are able to increase their freezing tolerance after a period of exposure to low non-freezing temperature. In our lab, we previously identified the Arabidopsis *RARE COLD INDUCIBLE 1A (RCI1A)* gene, which encodes the 14-3-3 psi isoform. 14-3-3 proteins have the potential to regulate plant responses to abiotic stresses, however their role in such responses remains poorly understood. The functional characterization of RCI1A has revealed that this 14-3-3 isoform interacts with different key enzymes of the ethylene biosynthetic pathway to control the phytohormone levels which, in turn, regulate cold-induced gene expression and freezing tolerance. We propose that RCI1A negatively modulates ethylene biosynthesis and contributes to determine the precise levels of ethylene that are necessary for accurate development of constitutive freezing tolerance and cold acclimation in Arabidopsis. Work funded by the Ministerio de Economía y Competitividad, Grants BIO2010-17545 and BIO2013-47788-R. RC was supported by a JAE Doc contract from CSIC.

P515 (Talk) - Seed Longevities in Arabidopsis Natural Variations after Priming Treatments

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Seed longevity, the periods in which seeds remain viable, is more than 100 years in some cases. One of the determinants of seed longevity is dryness of the seeds, because seed longevity remarkably decreases as seed moisture content increases. On the other hand, priming is a kind of treatment given to seeds to achieve uniformed seed germination in crop cultivations. The treatment, which often includes temporal imbibition and subsequent drying of seeds, is required to release seed dormancy and activate metabolic events associated with germination, however, such a treatment reduces seed longevity. Thus, it is important to improve the seed longevity after priming for sustainable seed supply in agriculture. To clarify the molecular mechanisms involved in the regulation of seed longevity before and after priming treatments, we investigated the seed longevity in 235 accessions of *Arabidopsis* natural variations. After priming (imbibition for 3 days in the dark at 4°C and subsequent incubation for 12 hours in continuous light at 22°C) treatments, most of the accessions showed significant reduction in their seed longevity, whereas three accessions exhibited markedly longer longevity after the priming treatment compared with a standard accession Col-0. By using recombinant inbred lines from a cross between the accessions and Col-0, we are looking for quantitative trait locus determining the seed longevity after priming treatments.

P516 - Arabidosis DPB3-1 and NF-Y Subunits Forms a Transcriptional Complex and Enhances the Heat Stress-Specific Expression of DREB2A Target Genes

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Appropriate gene expression in response to changes of the environmental conditions is necessary for plants to survive. *Arabidopsis* DREB2A is an important transcription factor that regulates dehydration and heat stress-inducible genes. The *DREB2A* gene is induced by dehydration and heat stress conditions and the encoding protein is stabilized under those stress conditions. The DREB2A protein induces the expression of dehydration or heat stress-inducible target genes according the environmental conditions. However, detailed mechanisms in which DREB2A selects its target genes under stress conditions remain to be elucidated. In this study, we identified DPB3-1 as an interacting protein with DREB2A using yeast two-hybrid screening. *DPB3-1*-overexpressing *Arabidopsis* showed significantly increased heat stress tolerance, while the drought stress tolerance was not significantly changed. Moreover, the analysis of gene expression patterns revealed that the expression levels of heat stress-inducible DREB2A target genes were enhanced, while those of dehydration stress-inducible genes were not affected. These results suggest that DPB3-1 is a positive regulator of DREB2A specifically under heat stress conditions. Further analysis for protein interactions suggested that NF-Y subunits formed a heat stress-specific transcriptional complex with DPB3-1. Now, we are analyzing the detailed mechanisms in which the identified proteins regulate the stress-specific activity of DREB2A.

P517 (Talk) - HMA6 and HMA8 are two chloroplast Cu+-ATPases with different enzymatic properties

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Copper (Cu), a transition metal, is a key element in redox reactions but also a toxic compound when present in excess in the cell. Therefore, assimilation and distribution of Cu must be tightly regulated to fit cellular requirements. In Arabidopsis chloroplast, Cu plays a key role in the photosynthetic process as cofactor of the plastocyanin (PC). Since PC is an essential component of the chloroplast photosynthetic electron transfer chain, Cu delivery to the thylakoid lumen is a priority for plant cells. In Arabidopsis, Cu delivery into the thylakoids involves two transporters of the P_{IB-1} ATPases family, HMA6 located at the chloroplast envelope and HMA8 at the thylakoid membrane. To gain further insight into the way Cu is delivered to PC, we have analyzed the enzymatic properties of HMA8 and compared them to HMA6 ones. To achieve that goal, native and mutated forms of the transporters have been produced in *Lactoccoccus lactis*. Their enzymatic properties have been determined by *in vitro* phosphorylation assays, and confirmed by phenotypic analysis in yeast. These experiments reveal that HMA6 and HMA8 display different enzymatic properties: HMA8 has a higher apparent affinity for Cu^+ but a slower dephosphorylation kinetics than HMA6. Modeling experiments suggest that these differences could be explained by the electrostatic properties of the Cu releasing cavities of the two transporters and/or by the different nature of their cognate Cu acceptors (metallochaperone/PC).

P518 - Molecular and functional characterization of the mitochondrial proline dehydrogenase 1 in Arabidopsis thaliana

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Proline is accumulated in many plant species in response to environmental stresses. Upon relief from stress, proline is rapidly oxidized in mitochondria by the rate limiting proline dehydrogenase (ProDH) enzyme and then by the pyrroline-5-carboxylate dehydrogenase (P5CDH) enzyme. Although two *ProDH* genes have been identified in the Arabidopsis genome, little is known about the function of these isoforms. A viable double *prodh1prodh2* mutant was generated. To evaluate the contribution of each isoform to proline oxidation, mitochondria were either isolated from wild-type, *prodh1, prodh2, prodh1 prodh2* or *p5cdh* mutants. Oxygen consumption, ProDH activity and proteomic analysis were conducted. We show that root growth as well as root density are differentially affected in the different prodh mutants in response to proline. We also demonstrate that ProDH activity is linked to ProDH content. In addition we show that ProDH1 forms part of a low molecular weight complex in the mitochondrial membrane. Finally, protein separation by 2D Blue native / SDS PAGE in combination with immunoblotting and protein analysis by mass spectrometry allowed the identification of ProDH1 peptides in mitochondria. Molecular and biochemical analyses indicate a key role of ProDH1 in proline oxidation and electron transfer to the respiratory chain.

P519 - Regulation of Na+ transport, the role and control of AtHKT1;1 expression in Arabidopsis thaliana ecotypes Col-0 and C24

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Two Arabidopsis ecotypes, Col-0 and C24, have previously been identified as interesting candidates to study plant salinity tolerance. Notably, C24 accumulates significantly more Na^+ in the shoot than Col-0. Despite its superior Na^+ exclusion, the Col-0 ecotype is less salt tolerant than the C24 ecotype, based on its reduction in dry weight under stressed conditions.

We investigated the basis for the difference in shoot Na^+ accumulation between Col-0 and C24 using a genetic approach. Quantitative trait loci analysis of a Col-0 × C24 mapping population indicated that the difference in Na^+ accumulation mapped to a locus that included *AtHKT1;1. AtHKT1;1* encodes a protein likely to mediate the retrieval of Na^+ from the xylem, thereby reducing translocation of Na^+ to the shoot. C24 and Col-0 *HKTs* were compared at the protein and transcriptional levels. RT-PCR showed that the levels of mRNA of *AtHKT1;1* in roots were much lower in C24 than Col-0. To determine the mechanism of differences in expression levels between the two ecotypes, a series of *AtHKT1;1promoter:: GFP* constructs were tested in Arabidopsis. Results show that both the Col-0 and C24 *AtHKT1;1* promoters are able to drive *GFP* expression, suggesting that differences in the promoter region are not responsible for the low levels of *AtHKT1;1* mRNA in C24 roots. However, a transposable element identified in the second intron of the C24 *AtHKT1;1* genomic sequence seems likely to be determining differences between the two ecotypes.

P520 - Identification of Genetic Determinants of Root Hydraulics in Arabidopsis thaliana

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Roots play a crucial role in soil water uptake, and their hydraulics has been the object of intense research in many plant species. We are using a combination of forward and reverse genetics approaches to provide a more detailed overview of *Arabidopsis thaliana* root hydraulics. Study of natural variation among 13 *A. thaliana* accessions revealed the existence of a 2-fold variation for root hydraulic conductivity (Lp_r), and variable contribution of cell-to-cell and apoplastic water transport paths (Sutka *et al.*, 2011). The distinct suberization patterns observed among accessions did not correlate with their root hydraulic properties. In comparison, a positive overall correlation was found between transcripts abundance of certain plasma membrane intrinsic proteins aquaporins (PIPs) isoforms and Lp_r. Further, genetic characterization of two of the most highly expressed PIP aquaporins in roots i.e., *At*PIP2;1 and *At*PIP2;2 was performed (Péret *et al.*, 2012). Single mutants for these *PIPs* did not show any alteration of Lp_r, whereas Lp_r was found to be reduced by ~40% in the double mutants hinting to the functional redundancy for Lp_r between *PIP* paralogues. *A. thaliana* lines over-expressing AtPIP2;1 exhibited 47-63% increase in Lpr. The overall work provides clues on the main aquaporin isoforms that determine root hydraulics in *A. thaliana*. It also shows that a wide range of root hydraulic profiles, as previously reported in various species, can be observed in a single model species.

P521 (Talk) - Identification and characterization of low Ca sensitive mutants in Arabidopsis

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Calcium (Ca) deficiency symptoms such as blossom end rot in tomato or tip burn in foliar vegetables are one of agricultural problem which can be caused by not only low Ca concentration in soil but also other environmental stresses including drought and salinity. In spite of the economical importance of solving this problem, our understandings on molecular mechanisms of the adaptation to low Ca circumstances in plants is still limited. To identify the genes which are involved in low Ca adaptation and to clarify molecular mechanisms how plant face with low Ca, we characterized mutants sensitive to low Ca and identified several essential genes for low Ca adaptation in Arabidopsis. Some of these genes encode one of cell wall polymer synthase, and we also showed that these genes can complement a yeast mutant defective in homologue genes. Elemental analysis revealed that there was no significant difference between shoot Ca concentration of mutants and that of the wild type under the normal and the low Ca condition. The double mutant of these genes showed enhanced sensitivity to low Ca. It has been already reported that the activity of synthesis of this cell wall polymer is dependent on Ca concentration. It is conceivable that the defect in the genes promotes low synthesis activity under low Ca condition and causes the poor growth under low Ca.

P522 - Global Changes in Expression Pattern of Rice Non-Coding RNAs in Response to Nitrogen-Deficient Conditions

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Nitrogen is a key component of crop plants growth. To increase the crop yield, the enormous amount of nitrogen-containing fertilizer is used, which increases the total production cost and leads to environmental pollution caused by residual nitrogen source which crop doesn't take up. Small RNAs and long non-coding RNAs are non-coding RNAs, which regulates the expression of their target genes by their own specific molecular mechanisms. Recent studies showed that the expression pool of those non-coding RNAs could be modulated in response to nutrient-deficient conditions. In this study, we aim to investigate the transcriptome-wide responses of rice non-coding RNAs on nitrogen-deficient conditions. For this, we performed strand-specific RNA-Seq and small RNA-Seq for identifying nitrogen-responsive long non-coding RNAs and microRNAs, respectively. We observed the considerable expression changes of long non-coding RNA pool on nitrogen-deficient conditions, and some of those showed nitrogen starvation time-dependent expression patterns. Moreover, microRNAs, such as miR169 family, were also showed nitrogen starvation-dependent expression patterns. Our study suggests that long non-coding RNAs and microRNAs may have roles in regulating expression of rice genes in response to nitrogen starvation and supply state. This work is supported by a grant from the Next-Generation BioGreen 21 Program (PJ01101803), Rural Development Administration, Republic of Korea.

P523 - Lon protease domain-containing protein, LPCP1 regulates CBSX1, which is a key regulator of redox system in Arabidopsis chloroplast

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In our previous report, we identified proteins consisting of a single cystathionine b-synthase (CBS) domain pair stabilize cellular redox homeostasis via regulation of Trx (thioredoxin) systems. Based on Y2H, BiFC assay and immunoprecipitation, we found that LPCP1 (Lon protease domain-containing protein 1) interacts with CBSX1, one of CBS domain-containing protein in chloroplast. Knock-down lines constructed by the artificial microRNA showed severe sterility, due to their shorter siliques relative to the wild type and anther indehiscence. The sterility was severer with the higher *CBSX1* overexpression. Moreover, compared to the wild type, the expression levels of ROS-related genes were decreased in *LPCP1* knock-down lines but were increased in *LPCP1* overexpressed lines. These expression patterns were opposite to *CBSX1* knock-out and overexpressed lines. Taken together, we suggest that LPCP1 acts as an antagonistic regulator of CBSX1 which modulates Trx (thioredoxin) activity, so that maintains ROS homeostasis.

P524 - A device for imaging and asymmetric perfusion of Arabidopsis roots

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Plant roots are highly sensitive to changing environmental conditions such as water and nutrient availability, biotic and abiotic stresses. Response mechanisms involve local sensing, intracellular signaling and intercellular communication. We only begin to understand how environmental signals are integrated to modulate growth and development, how acclimation and immunity are triggered, and how responses are being coordinated between distal tissues in the plant. To understand how roots perceive and process information about their change in environment we need tools that allow live imaging of roots and provide precise control over the root microenvironment. Over the past years, a number of microfluidic devices have been developed that have substantially advanced experimental access to roots. A limitation of existing devices has been the inability to dynamically simulate heterogeneous environments, which is an important characteristic of natural growth conditions. Here we present a novel imaging and perfusion device for *Arabidopsis* roots that guides the growth direction and allows controlled symmetric or asymmetric perfusion of the root to apply treatments only to one side. This device allows us to distinguish cell-autonomous and coordinated responses that depend on intercellular communication. The possibility to switch the side of root treatment allows to study response kinetics to locally changing environmental conditions.

P525 (Talk) - A mutation in NAC103 suppresses ROS accumulation in root tips caused by excess boron stress

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Excess boron (B) is toxic to plants. The molecular mechanism of B toxicity is unclear. We have demonstrated that *rpt5a-6*, an *Arabidopsis thaliana* mutant of 26S proteasome, is sensitive to excess B. The mutant develops dead cells in its root meristem. We have also revealed that the sensitivity is suppressed by a mutation in a transcription factor *NAC103*, a substrate of proteasome, which functions in the unfolded protein response (UPR) pathway (Sun et al., 2013). Furthermore, the mutation in NAC103 alleviates DNA damage accumulation, which is caused by excess B stress (Sakamoto et al., 2011).

Based on the knowledge that one of the most common causes of DNA damage and unfolded protein production is oxidation by reactive oxygen species (ROS), we hypothesized that ROS accumulation is a primordial defect in excess B toxicity in root growth. DAB staining revealed that excess B causes H_2O_2 accumulation in root tips, especially in *rpt5a-6*, and the accumulation was reduced in *rpt5a-6 nac103-1* double mutant. We also identified some ROS productive genes which were upregulated by excess B stress, which supported our hypothesis. Considering the recent report that exogenous over-expression of *NAC103* resulted in ROS accumulation in canola leaves (Niu et al., 2014), we suggest that *NAC103* regulation through proteasome is essential for meristem maintenance, by preventing ROS accumulation under excess B stress.

P526 - Characterization of a MAPK signaling module involved in Arabidopsis response to wounding

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The sessile condition of plants exposes them continuously to any environmental stress. Injury, an important stress condition that plants must face, may cause harsh damages to the plant tissues and facilitate the entry of pathogens. Therefore, plants have evolved mechanisms to respond efficiently to wounding either by healing the damaged tissues or by isolating the wounded organs. Mitogen-Activated Protein Kinase (MAPK) modules, consisting of three kinases (MAP3K, MAP2K and MAPK) activated in cascade, play an important role in cellular signaling after stress perception by plants. In particular, several MAPKs have been reported to be activated by ABA, H2O2 or JA, that are well-known mediators of plant responses to environmental stresses, and produced upon wounding. The aim of this project is to identify a wounding-induced MAPK module and to characterize its functions in the wounding response by identifying the upstream activating signals as well as the downstream cellular responses triggered by this pathway (gene expression, resistance to pathogens, etc.). Our first biochemical and genetic results led us to identify some MAPKs activated in response to wounding and JA in a MAP2K-dependent manner. The role of this pathway in the wound-induced cellular responses has also been tested.

P527 - Analysis of Splicing, Processing, and Expression of Chloroplast Genes in Arabidopsis thaliana and Coffea arabica under Abiotic Stress Conditions

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Although many recent studies have demonstrated that chloroplast gene expression is regulated mainly at post-transcriptional levels, the splicing and processing of chloroplast RNA transcripts and their effects on plant growth and stress responses are largely unknown. To understand how splicing and processing of chloroplast transcripts are related to plant growth and stress responses, we analyzed the intron splicing, gene expression, and rRNA processing of chloroplast transcripts in *Arabidopsis thaliana* and Coffee tree (*Coffea arabica*) under normal and stress conditions. The results showed that intron splicing and expression of many chloroplast genes were differently affected in Coffee plants under drought, cold (6oC), or combined drought and heat (38oC) stress conditions. In addition, splicing of many chloroplast RNAs and processing of rRNAs were impaired in Arabidopsis mutants lacking chloroplast-localized DEAD-box RNA helicase or S1-domian RNA-binding protein, which results in abnormal growth and sensitivity to environmental stresses. Taken together, these results demonstrate that correct splicing and processing of chloroplast RNAs are important for the growth of the plants under normal conditions as well as for the adaptation of the plants to abiotic stresses. [Supported by grants from NRF and Next-Generation BioGreen21]

P528 - The Ethylene Response Factor-VII genes RAP2.12, RAP2.2 and RAP2.3 regulate low oxygen, oxidative and osmotic stress responses.

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The ERF-VII transcription factor RELATED TO APETALA2.12 (RAP2.12) was previously identified as an activator of the *ADH1-LUC* reporter gene (Papdi et al., 2008, Plant Physiol, 147: 528-542). RAP2.12 protein functions in oxygen sensing via the N-end rule protein degradation pathway (NERP, Gibbs, D.J. et al., 2011, Nature 479, 415-418, Licausi, F. et al., 2011, Nature 479, 419-422). Inducible overexpression of RAP2.12 and its homologs RAP2.2 and RAP2.3 conferred tolerance to anoxia, oxidative and osmotic stresses, and enhanced the sensitivity to ABA, while the *rap2.12-2 rap2.3-1* double mutant was hypersensitive to submergence and osmotic stress. All three RAP2 factors sustained ABA-mediated activation of *ADH1* and activated hypoxia marker genes. RAP2.12 protein level is not only controlled by NERP, but is also regulated by the RING-domain containing SEVEN IN ABSENTIA of Arabidopsis thaliana 2 (SINAT2). Silencing of SINAT1/2 genes lead to enhanced RAP2.12 abundance independently of the presence or absence of its N-terminal degron. These results suggest, that RAP2.12 and its homologs act redundantly in multiple stress responses that sequentially occur during and after submergence in Arabidopsis. Besides NERP, alternative protein degradation pathways may regulate the abundance of RAP2 transcription factors during distinct stresses (Papdi et al., 2015, Plant J. doi: 10.1111/tpj.12848). Research was suported by the OTKA grants K-81765, NN-110962, IPA project HUSRB/1002/214/036.

P529 - Sequence comparison of four hyperaccumulator Noccaea caerulescens accessions and related species including Arabidopsis thaliana

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Noccaea caerulescens is a metal hyperaccumulator plant which has a great potential to serve as a model in plant evolutionary genomics. Various accessions show remarkable differences in their abilities to accumulate and tolerate metals. All accessions are hyperaccumulators for zinc. Calaminous accessions show a great difference in cadmium tolerance and accumulation. Ganges (GA) is a hyperaccumulator, La Calamine (LC) is hypertolerant and Lellingen (LE) is sensitive to cadmium. Serpentine accession Monte Prinzera (MP) is a Ni hyperaccumulator. In this study we compare the sequences from the accessions of metal hyperaccumulator to related species, including a non-metal hyperaccumulator species *Arabidopsis thaliana*, to examine the evolution of metal hyperaccumulation and –tolerance. We have used Illumina system to sequence and de novo assemble the whole transcriptomes including both shoots and roots of four contrasting accessions of *N. caerulescens*. The transcriptomes were assembled in search of sequence differences that might affect gene function. Assembly was conducted using the Trinity assembly program and proteins were predicted using Transdecoder and Augustus. The transcriptome assembly produced ca. 65,000 nucleotide

contigs for each accession, of which ca. 45,000 yielded a predicted protein sequence. Protein sequences from each species and accession were grouped into orthologous sequences and a multiple alignment produced. The significance of the results will be discussed.

P530 - Root cap cells play a key role in phosphate uptake and homeostasis

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Mineral ion uptake by plants represents one of the initial food chain inputs, and a crucial factor that controls yield. Cellular and molecular mechanisms driving ion absorption remain poorly characterised and often involve broad multigenic families and overlapping functions. In the model plant Arabidopsis, the expression of several high affinity phosphate (Pi) transporters (PHT1 family) is highly regulated depending on the phosphate status of the plant and restricted to specific cell layers of the root. By specific complementation of *phf1-1* mutant (strongly altered in phosphate uptake regulation), using GAL4-enhancer trap strategy, we investigated the role of several root cell layers. Coupling this technique with high-resolution real time 33P-imaging, we were able to differenciate Pi uptake and Pi translocation in the plant and more specifically in the root tip. We showed that the root cap accounts for a significant amount of the whole plant phosphate uptake (around 20%). Such Pi absorption is efficient for shoot biomass production and repression of Pi-starvation-induced genes. This work reveals that the root cap surrounding and protecting the root stem cell niche (meristem) at the root tip harbors an unexpected crucial role for Pi nutrition, extending the role of this tissue from that previously described in environmental perception (gravity, light, moisture).

P531 - Functional characterization of salicylic acid-inducible genes coding for GSTs and GRXs in the defense response to stress in Arabidopsis thaliana

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Plants are constantly exposed to several biotic and abiotic stress conditions that increase the production of reactive oxygen species (ROS). The survival of plants depends on a complex balance between the production and detoxification of ROS. Salicylic acid (SA) is a key hormone in the establishment of the defense response to stress, being essential for the production and also for the contention of the oxidative burst needed to establish the defense responses. SA induces the expression of genes coding for proteins with antioxidant and detoxifying function, among them *GLUTATHIONE S-TRANSFERASES (GSTs)* and *GLUTAREDOXINS (GRXs)*. In this work, we performed an extensive microarray analysis of available databases to determine the expression patterns of *GSTs* and *GRXs* under different stress conditions where SA is involved as a signal. We selected 2 *GSTs* and 2 *GRXs* genes and confirmed their expression patterns under stress conditions using real-time PCR. We used mutant or silenced plants for the selected *GSTs* and *GRXs* genes and evaluated their relevance to overcome different stress conditions such as treatments with methyl viologen, and UV-B radiation. Our results indicate that SA induces the expression of a set of *GSTs* and *GRXs* genes in a temporal specific manner and that these genes are important for the contention of oxidative damage produced by different types of abiotic stress, suggesting a particular role for them in controlling ROS accumulation in the plant defense responses.

P532 - X-ray induced Arabidopsis long non-coding RNAs derived from transposons/repeats are regulated by ATM

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Double-strand breaks (DSBs) are a severe threat to genome integrity and invoke a rapid turn on of DNA repair processes. Concomitantly, transcriptional and transpositional reactivation of individual transposons (TEs) has been observed. However, the genome-wide scale and mechanism of DNA damage-induced TE reactivation are not well understood. In this study, two weeks old wild type and ATM (ataxia telangiectasia mutated)-deficient Arabidopsis plants were treated with 80 Gy X-ray irradiation. Three hours after the treatment RNA was isolated from the plantlets for transcriptome analysis by RNA sequencing. Mapping the reads against the Arabidopsis genome sequence and a comprehensive repetitive DNA element library revealed that TEs and repeats are transcriptionally up- and downregulated after DNA damage and this transcriptional response is largely ATM-dependent. Interestingly, many ATM-dependently regulated TEs/repeats are part of long non-coding RNAs (lncRNAs). We discuss factors downstream of ATM that might play a role in regulating TEs/lncRNAs in response to DNA damage.

533 - Intrinsically disordered proteins as modulators of plant plasticity: How disorder leads to orderly plant growth

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The DRM (dormancy-related) protein family - is an example of a terrestrial-plant-specific Intrinsically disordered protein (IDP) family whose expression is strongly associated with multiple abiotic stresses. During stress, other unrelated IDPs, have been shown to stabilize both the cytoskeleton and mitochondrial membranes and act as a “molecular shields” to protect enzymes. In contrast the mode of action of the highly conserved plant-specific DRM protein family remains to be determined. Multiple lines of bioinformatic-based evidence support the prediction of protein disorder and the presence of multiple conserved molecular recognition features (MoRFs), which are short disordered regions that are postulated to become fully ordered upon binding to a partner in this physically small (12-15 kDa) protein family. It has also been demonstrated that the two closely related family members AtDRM1 and AtDRM2 overexpression lines are hypersensitive to various abiotic stimuli and display growth retardation under standard growth conditions; the converse being true in down-regulated lines. The lack of recovery of true DRM1 knock out plants to date suggests loss-of-gene-induced-lethality and a fundamental role for DRM in a plant”s growth cycle.

534 - Phytochrome Control of Resource Allocation and Growth in Arabidopsis

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Plants use light as a source of energy to drive photosynthetic carbon (C) fixation, and as a signal to provide information about the environment. Light is sensed by a suite of receptors, including members of the phytochrome family. Phytochrome function has been intensively researched during seedling establishment, yet phytochromes are principal environmental regulators of adult plant architecture, resource allocation and growth. Currently, we understand almost nothing of how light signalling is integrated with photosynthesis to optimise autotrophic growth. Our recent work has provided some of the first intriguing insights into how phytochrome regulates C metabolism. Phytochrome ko mutants are reported to have less chlorophyll, yet they accumulate more sucrose and starch than wild type plants during the day time. However, growth, biomass and protein production are severely compromised in these mutants. Our metabolome data show that phytochrome mutants have elevated levels of amino acids and tricarboxylic acid cycle (TCA cycle) intermediates: a profile is that is associated with physiological stress. Indeed, preliminary experiments suggest that light mutants have a constitutive-stress phenotype that results in increased tolerance to dark induced starvation and senescence. Our data therefore indicate that phytochromes play a vital role in switching the metabolic state to favor

growth or a stress-resistance depending on the availability of light for photosynthesis.

P535 - Identification of genome-wide binding sites of a bZIP transcription factor ZW1 under normal and drought conditions in rice

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Transcription factors such as many members in basic leucine Zipper (bZIP) family have been shown to play important roles in regulating drought responses in plants, but their molecular mechanisms is largely unclear mainly because a complete profile of all the putative targeting genes or binding sites for a given transcription factor was seldom reported. Here, we performed a genome-wide binding site (or target gene) identification of a bZIP transcription factor ZW1 by using chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-Seq methods. By ChIP-seq, 681, 2128, and 7194 genes associated with ZW1 binding sites were identified in wild type rice Zhonghua 11, under normal growth condition, wild type rice under moderate drought stress, and *ZW1*-overexpression rice under normal condition, respectively. The ABA responsive element (ABRE) was highly enriched in the ZW1-binding peaks. Further analysis showed that ABRE was required for ZW1 binding to the promoters of target genes. Genome-wide expression profiling by RNA-Seq revealed ZW1 plays important roles in many biological processes. Integration analyze the results of ChIP-Seq and RNA-Seq, 131 direct target genes of ZW1 were identified. Furthermore, we found the biotic stress and abiotic stress processes were mainly affected by ZW1. Interestingly, a type 2C protein phosphatase (PP2C) gene, which is a homolog of the negative regulator ABI1 in ABA signaling, was directly regulated by ZW1. These results provided valuable clues to completely unveil the molecular mechanism of the bZIP transcription factor in the regulation of drought tolerance in rice.

P536 - Genetic analysis of mitochondrial functions and stress responses

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Plant mitochondria have essential role in adaptive responses to unfavorable environmental conditions. Reactive oxygen species (ROS) can be produced in the mitochondrial electron transport chain (mETC) under stress, where Complex I and III are the major sites of ROS synthesis. Stabilization of the electron flow in the mETC can protect plants by reduction of oxidative damage, control of redox balance and support photosynthesis during stress. To reveal the function of genes encoding subunits of Complex I and III of the mitochondrial electron transport in stress responses, we are characterizing *Arabidopsis thaliana* mutants carrying mutations in genes encoding such proteins. When compared to wild type several mutants showed morphological and physiological changes under stress conditions. Phenotypic differences in tolerance to drought and salinity were revealed through in vitro germination and growth tests, as well as by complex phenotyping of soil-grown plants. Several mutants showed altered tolerance to osmotic or salt stress. Two mutant alleles were characterized in detail in which the mutations disrupted the NDUSF8a gene. Both *ndusf8a* mutants were hypersensitive to salt stress, although they had lower H_2O_2 content and lipid peroxidation rates during osmotic stress. Changes in chlorophyll fluorescence under stress showed that these mutations influence photosynthesis and the NDUSF8a gene is important in regulation of stress responses. Supported by OTKA NN-110962 and EPPN program.

Systems biology and new approaches

Posters 537 to 555

P537 - A multi-scale model capturing the fractal nature of cauliflowers

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Fractals are mathematical objects forming mesmerizing shapes due to a high degree shape irregularity and self-similarity. Many biological objects display fractal features. The curd morphology of *Brassica Oleracea*, (i.e., broccolis, cauliflowers and Romanesco) are among the most striking example. However, how such biological curiosities can develop from rules governing meristem growth and genetic regulation largely remains elusive. Using the genetic knowledge established in Arabidopsis, where a mutant (namely the double mutant *ap1 cal*) generates cauliflower-like structures, we have built a gene regulatory network whose properties recapitulate the recursive behavior of a fractal development. We have then integrated this genetic network within a 3D model of Arabidopsis development. This integration revealed missing links between the GRN and the growth parameters. We have explored the biological nature of these links and were able to produce a multiscale model of plant growth capturing the various curd shapes found in brassicacae genera.

P538 - Genome-Wide Profiling of Translatome Changes During Seed Germination

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Seed germination is a crucial stage during the plant life cycle. Seeds are unique structures that contain reserves used by the growing seedling until photosynthesis is established. Seed stored mRNAs are produced during seed development, retain their function during dry storage, and are translated during the early phases of imbibition. The importance of these mRNAs is demonstrated by the absolute requirement of translation for germination. Here we present the genome-wide profiling of the total mRNA and polysome associated mRNA pool (translatome) from fully-after-ripened dry seeds to greening seedlings. For these analyses ribosomes are size separated on a sucrose gradient, which allows the separation of polysomes (mRNAs that contain more than one ribosome and therefor represent mRNAs that are actively translated). These analyses provided insight in the translation dynamics during seed germination, and identified two phases during germination where translational control of gene regulation is especially evident. Different sequence motives were enriched in the mRNAs regulated specifically in the two phases indicating specific regulatory mechanisms being active during germination.

P539 - From Bench to Bountiful Harvests - Past, Present and Future Research Under the Umbrella of the Multinational Arabidopsis Steering Committee (MASC)

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The Multinational Arabidopsis Steering Committee (MASC) has its origin in the 1990s when scientists from the US, Europe, Japan and Australia formed an *ad hoc* committee to promote large-scale studies in *Arabidopsis thaliana*. During the last two decades the idea of a combined and coordinated effort accompanied by the policy of open data sharing has proven successful and led to the establishment of *Arabidopsis thaliana* as the reference plant. The MASC is led by chair and co-chair with support of the only funded member the MASC coordinator (DFG). Today eight MASC subcommittees monitor and coordinate research in major Arabidopsis research areas: Bioinformatics, Epigenetics and Epigenomics, ORFeomics, Metabolomics, Natural Variation and Comparative Genomics, Phenomics, Proteomics

as well as Systems and Synthetic Biology. Two additional key groups of MASC represent major Arabidopsis projects and resources as well as representatives from 26 countries. MASC publishes an annual report that outlines progress and activities of the Arabidopsis community as well as analysis and recommendations for the next year according to the road map (2012-2021) "From Bench to Bountiful Harvests" (Lavagi, Plant Cell, 2012). The MASC report 2014/2015 will be published at the 26th International Conference on Arabidopsis Research (ICAR) in Paris, France. The 27th ICAR will be in Gyeongju, South Korea, June 29th- July 3rd 2016. If you would like to know more about MASC, please visit: www.arabidopsisresearch.org

P540 - Systems genetics identifies regulatory gene modules that quantitatively regulate root growth

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Plants have evolved a high level of phenotypic plasticity in traits related to growth and development. While this modulation of growth and development occurs in response to environmental conditions, both its onset and extent are genetically determined. However, very little is known about the genes and genetic mechanisms that govern the plasticity of growth and development. There is growing evidence that rather than single genes, networks of epistatically interacting genes are key for this genetic regulation. We use an approach combining custom phenotyping pipelines that enable us to capture quantitative root phenotypes of a very large number of genetically distinct individuals, genome wide association studies to identify the associated loci in the genome, and systems-biology driven approaches to identify the gene networks and pathways that quantitatively regulate root growth. Using these approaches, we have recently identified and experimentally verified multiple novel regulators. Importantly, we identified a regulatory module of leucine-rich repeat receptor-like kinase (LRR-RLK) genes that regulate root growth in an epistatic manner. We are currently investigating the interaction of these genes at the molecular level and have found that at least two of these LRR-RLKs act in the same protein complex. Overall, our results demonstrate that, using a systems-genetics approach, it is possible to identify genetic networks that quantitatively regulate plant growth and development.

P541 - Emergence of novel phenotypes in co-evolving biological systems:allelic diversification and dominance at the selfincompatibility locus in Arabidopsis

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System biology approaches are currently changing the way we think about evolutionary processes by revealing the high level of integration of biological systems, whereby individual interacting components evolve under a strong co-evolutionary constraint, rather than as independent entities. This raises the question of how novel phenotypes can emerge in such highly integrated co-evolving biological systems. To shed light on this fundamental biological process, we focus on the sporophytic self-incompatibility (SI) system in outcrossing *Arabidopsis* species. SI is a reproductive system by which hermaphrodite flowering plants recognize and specifically reject self-pollen. In the Brassicaceae, it is based on a molecular lock-and-key mechanism involving two genes (a male and a female determinant). Both display large allelic series (at least 50 alleles within outcrossing *Arabidopsis* species) and are tightly linked in a small non-recombining region which ensures strict haplotypic association between co-adapted alleles. This reproductive model system allows the study of two co-evolutionary processes: i) between the male and the female reproductive proteins allowing self-pollen rejection and ii) between small non-coding RNAs and their target sites controlling the dominance/recessivity interactions between SI alleles. By using a multidisciplinary approach combining functional and genetic approaches, we will aim to decipher mechanisms of emergence of functional and regulatory novelty.

P542 - Information extraction from articles for the elaboration of the regulatory networks involved in Arabidopsis seed development

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Seed is the main vector for breeding and production of annual field crops, and the accumulation of seed storage compounds (sugars, lipids, proteins) is of primary importance for food, feed and industrial uses. Seed development requires the coordinated growth of different tissues and involves complex genetics and environmental regulations. A comprehensive understanding of the molecular network underlying these regulations remains a major scientific challenge with important potential impact for agriculture and industry. Knowledge on these regulations is spread in a high number of scientific articles (*e.g.* Pubmed query "*Arabidopsis seed*" yields more than 6000 references) and is difficult to analyze. The molecular and genetic mechanisms are described by complex expressions that involve biological entities linked by various specific semantic relations. The aim of this work is to automatically extract the information (*i.e.* entities and relations between entities) by developing generic Natural Language Processing and Machine Learning methods. The approach consists in 1) the formal annotation of examples in a set of documents with respect to an annotation model, 2) training methods on the examples and, 3) the application of the methods to new texts to extract knowledge. Last we plan to integrate the extracted knowledge in a comprehensive regulatory model, with database and graphical representation tools. We expect these tools to be useful for analyzing other gene regulatory networks.

P543 - SmartLeaf: An automated system for tracking circadian leaf movement rhythms

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Leaf movement in plants is an example of a rhythmic output under control of the circadian clock. Circadian research groups use leaf movement rhythms in *Arabidopsis thaliana* as a high-throughput assay for circadian clock function. However, current systems possess serious limitations. Firstly, the cost of the camera system is prohibitively expensive for all but the best-funded laboratories. Secondly, current methods involve multiple time-consuming manual steps for the researcher. Here we present SmartLeaf, a high-throughput automated system for measuring leaf movement rhythms in *Arabidopsis*. The system consists of two components: a time-lapse image capture program based on web cameras and an automated leaf-tracking algorithm. SmartLeaf successfully images and tracks wild type and several mutant lines at the seedling stage, validating our system as a method. SmartLeaf is a system for image capture and tracking of leaf movement in *Arabidopsis*. Here it is applied for period estimation of several mutant genotypes demonstrating its effectiveness. SmartLeaf is automated, affordable and accessible to the non-specialist.

P544 - Dynamic chromatin changes in response to nitrate in Arabidopsis root

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Our understanding of plant gene regulation is constrained by our limited knowledge of plant cis-regulatory DNA and its dynamics in response to environmental cues. Nitrate is a potent signal that control global gene expression, shaping plant growth and development. We integrated transcriptomics, Pol II ChIP-seq and mapped DNase I hypersensitive sites (DHSs) in *Arabidopsis* root in response to nitrate to uncover regulatory landscape dynamics, disclosing hundreds of nitrate sensitive elements and enabling mapping of key TF regulatory circuits underlying this fundamental response. This information will facilitate developing strategies to improve N-use efficiency and enhance plant production.

P545 (Talk) - Imaging analysis of phosphate absorption and distribution of Plants

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Phosphorus is one of the crucial minerals for plant growth and therefore (Perret *at al.*, 2014), Plants have evolved various physiological mechanisms to improve the utilization of phosphorus. To understand how plants sense phosphate availability from the environment remains a challenging question. Understanding phosphate acquisition (Nussaume *et al.*, 2011) and its usage by plants is also essential to optimize fertilizer usage to sustain crop yield. Orthophosphate ion, the main source of phosphorus available for plants, seems to act as a signal element itself for signaling processes. In order to analyze the spatiotemporal dynamics of phosphate in plants we have developed real-time radioisotope imaging systems (RRIS) both at whole plants level and microscopic level (Kanno *et al.*, 2007; 2012)). It provides unique opportunity to follow movement of phosphate radiotracer (32-P, 33-P) in living plants. Both systems allow quantitative analysis and possess wide dynamic range of detection. A system which combined a microscope was able to image tracer in the plant tissue enlarged 40 times. It also offers opportunity to access chemiluminescent and fluorescence signals in the same plants. It offers opportunity to analyze plants where phosphate transport has been manipulated to investigate the role of different cell layers to phosphate transport. Kanno *et al.* J. of Radio analytical Nuclear Chemistry, Vol.272, No.3, pp.565-570, 2007 Nussaume *et al.* Frontiers in Plant Science, Vol.2, No.83, pp.1-12, 2011 Kanno*et al.* Royal Society, Philosophical Transaction B, Vol.367, pp1501-1508, 2012 Perret *at al.* Plant Physiol., 166(4), pp1713-23, 2014

P546 - Prometheus: Omics Portal for Comparative Genomics

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Since NGS (Next Generation Sequencing) technology were developed in mid 2000s, the huge amount of genome information has been generated and analyzed. Studies of reference genomes were contributed to unveiling biological responses, cloning of interested genes, molecular marker development, and development of new medicine. As number of sequenced genomes were increased, a lot of tools or pipelines were developed to identify the gene function or gene families such as InterPro, Pfam, SignalP, PSortII, ChloroP, TMHMM2, and NetPhos. Furthermore, rapidly increasing -genome information have been a major reason for spurring - genome wide functional studies of genes of interest or gene families through comparative genomics. Although many web-based platforms for comparative genomics were developed, application of those platforms were limited as they allow only small numbers of genome or tools. The web-based comparative genomics platform for large amount of genome information and various tools is desirable for comprehensive genome wide gene family studies or functional studies for interested genes as well as their evolutionary studies. Here we present Prometheus, the omics portal for comparative genomics. The Prometheus is a web-based and cloud computing-based comparative genomics platform and contains more than 30,000 of genome information from prokaryotic to eukaryotic genome with 3 primary and 20 secondary database generated from various analysis such as InterPro, TargetP, and OrthoMCL. In addition, the system of My Genes in the Prometheus gives chance to analyze interested genes with various tools or Chlosha, cloud-based analysis pipeline in the Prometheus as well as Chlosha II, a version for advanced users to allow customized analysis pipeline. Furthermore, assembly and annotation pipeline for genome or transcriptome will be added in the Prometheus in the near future.

P547 - TAIR: A Sustainable Community Database for International Arabidopsis Research

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Started as a NSF-funded project in 1999, TAIR has served as the primary source and the main portal of curated Arabidopsis data, with worldwide usage of 66,000 visitors per month and over 2 million visits per year. Since the end of our NSF funding in 2013, TAIR has transitioned to a new funding model supported by institutional and individual subscriptions. We are now in the second year of user-supported funding. Subscription results indicate that this model is capable of sustaining TAIR for the long term and will enable TAIR to continue to grow and develop at a rate that will keep pace with the evolving needs of the research community.

P548 - Identification of chemical regulating cryptochrome-mediated blue light signaling in Arabidopsis by forward chemical genetics approach

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Chemical genetics involves the use of diverse small active or synthetic molecules to evaluate the biological processes. Classical forward genetics screening focus on identification of target genes and pathways through the study of phenotype induced by random mutagenesis. This study aims at using conditional chemical forward genetic approach to first identify specific small molecule that promote hypocotyl growth of *Arabidopsis* seedlings under blue light and further identify the key protein modulators that correspond to the phenotype. A total of 3,700 active small molecules were used for the screening of potential chemicals that promote hypocotyl elongation of wild type seedlings under continuous blue light. Of these, a specific small molecule was identified to only induced hypocotyl growth under blue and not under darkness, red or far-red light. Further evaluation using blue light related mutants confirmed the chemical induced physiological change was related to the cryptochrome-mediated blue light signaling. Microarray analysis showed that approximately 3% of the total genes were differentially regulated for at least 2-fold and of these about 300 genes were regulated at the similar manner in wild type (Col-0) and mutant (*hy5*) seedlings. Further evaluation of possible protein targets will provide a better understanding of the etiolation state of *Arabidopsis* under blue light and its roles and involvement under cryptochromes signaling.

P549 - Form transcription to translation: Post-Transcriptional Coordination of the Iron Deficiency Response in Arabidopsis

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Acclimation to changing environmental conditions is mediated by proteins, the abundance of which is carefully tuned by an elaborate interplay of DNA-templated and post-transcriptional processes. To dissect the mechanisms that control and mediate cellular iron homeostasis, we conducted quantitative high-resolution iTRAQ proteomics and microarray-based transcriptomic profiling of iron-deficient *Arabidopsis thaliana* plants. A total of 13,706 and 12,124 proteins were identified with a quadrupole-orbitrap hybrid mass spectrometer in roots and leaves, respectively. This deep proteomic coverage allowed accurate estimates of post-transcriptional regulation in response to iron deficiency. Similarly regulated transcripts were detected in only 13% (roots) and 11% (leaves) of the 886 proteins that differentially accumulated between iron-sufficient and iron-deficient plants, indicating that the majority

of the iron-responsive proteins was post-transcriptionally regulated. Mutants harboring defects in the in the *RING DOMAIN LIGASE1 (RGLG1)* and *RING DOMAIN LIGASE2 (RGLG2)* showed a pleiotropic phenotype that resembled iron-deficient plants with reduced trichome density and the formation of branched root hairs. Proteomic and transcriptomic profiling of *rglg1 rglg2* double mutants revealed that functional RGLG protein is required for the regulation of a large set of iron-responsive proteins including the coordinated expression of ribosomal proteins. This integrative analysis provides a detailed catalog of post-transcriptionally regulated proteins and allows the concept of a chiefly transcriptionally regulated iron deficiency response to be revisited.

P550 (Talk) - ATHB5 mediates a hypocotyl-specific gene regulatory network driving both expansin gene expression and the final step of Arabidopsis seed germination

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Plant growth is controlled by co-ordinated gene expression programmes in 3D space over time. The GA-DELLA signalling module controls plant cell growth through the proteolytic degradation of DELLA repressor proteins and transcriptional regulation of cell wall modifying gene expression. While the molecular basis of this growth control switch is well characterised, less is known about the pathways linking DELLA proteins to cell wall modifications, and how these events unfold within the context of dynamic organ growth. The embryo-to-seedling transition represents a simplified growth system which is inhibited by DELLA and occurs exclusively through cell shape changes. Quantitative 3D analysis of organwide cell shape changes driving this transition identified a sub-domain of cells in the upper hypocotyl responsible for completing the final step of germination. We bioinformatically inferred a DELLA-repressed gene regulatory network (GRN) between the transcription factor ATHB5 and the growth-promoting gene *EXPA3*. The spatial relationship between ATHB5 and *EXPA3* was quantified organ-wide at single cell resolution using custom-made image analysis software. These network components are specifically induced in the upper hypocotyl prior to the completion of seed germination and spatiotemporally co-ordinated across individual cell types. This ATHB5-mediated GRN provides a mechanistic link between GA signalling and cell type specific growth driving the seed to seedling transition.

P551 - Construction of an Arabidopsis electron microscopy atlas

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Transmission electron microscopy (TEM) techniques are superior for acquiring images of limited regions under high magnification but not for wide areas such as those of plant tissues and large cells. To capture such digital images, we developed an auto-acquisition TEM system, in which a remote PC is used to adjust a X-Y stage, electron beam, and CCD camera. We successfully acquired gigapixel-class high-resolution TEM images, which were merged using an image-tiling program. To take gigapixel-class TEM pictures of *Arabidopsis*, organs such as roots and leave were fixed using a highpressure freezing method or general chemical method. After embedding in resin, large ultrathin sections were cut and placed on a hole grid. We then acquired several thousand overlapping TEM micrographs using the auto-acquisition TEM system and merged. The gigapixel TEM images obtained included transverse and/or radial longitudinal sections of root tip (columella, meristematic zone, elongation zone), cotyledon, anther, and shoot apical meristem. Using these gigapixel TEM images, we are now constructing a zoomable website, the "Arabidopsis Electron Microscopy Atlas". The gigapixel TEM images can be converted to pyramidal tiled multi-resolution images and mounted on a web server. Such websites are accessible from any PC, tablet, or smartphone. This atlas is useful to for observing the ultrastructure of organelles in different developmental stages and under various environmental conditions.

P552 - Absolute units at the transcriptional level for cracking our understanding of circadian regulation in Arabidopsis

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A combination of experimental and theoretical work has been fundamental for understanding how circadian rhythms emerge from the complex interaction of genes. Moreover, current clock models have lacked absolute units on the concentration scale, potentially hiding areas of ignorance behind unrealistic mechanisms. Therefore, we converted the parameters to absolute units, within biologically relevant ranges. Exploring the literature showed us that some parameters follow a log-normal distribution. Nonetheless, we found an important lack of quantitative information for plant-relevant parameters for example transcription rates and K_ds of protein-protein interaction. Which is a problem that needs to be solved by the community. We observe by using P2011 model and mRNA data with absolute units, that transcription rates do not violate maximum estimates from other eukaryotes apart from *GIGANTEA (GI)*. Furthermore, using a translation model and the TiMet Project mRNA datasets showed that *PSEUDO RESPONSE REGULATORs (PRRs)* have higher translation rates than expected. Surprisingly, P2011 and the translation model give similar predictions for the relative levels between the *PRRs* apart from *TIMING OF CAB EXPRESSION1 (TOC1)*. Finally, we tested how experimental error constrains our understanding, by propagating this uncertainty into model predictions.

P553 - Modelling plant heterosis using genome-scale metabolic networks

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Applications of genome-scale metabolic reconstructions have continued to expand over the last decade. Herein, we introduce a novel method to simulate and predict plant heterosis using a manually curated genome-scale model of Arabidopsis thaliana. The approach provides evidences of the presence of metabolic bottlenecks explaining the superior performance of heterozygous hybrid plants over their parental inbred lines. The presented results bring new clues on the genetic and molecular basis of heterosis, a phenomenon that has remained enigmatic for decades, despite its paramount agronomic importance.

P554 - The use of bulk segregant analysis to identify single nucleotide polymorphism related to Arabidopsis root morphological traits

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The root morphology is highly responsive to nitrate, which acts at multiple stages of root development. To elucidate the natural genetic variations related to root morphology, two F2 populations were generated from two crosses between contrasting natural accessions of *Arabidopsis thaliana*, Pyl-1 (Le Pyla, France) crossing Bor-1 (Borky, Czech Republic) and C24 (Coimbra, Portugal) crossing Ost-0 (Osthammar, Sweden). Upon *in vitro* culture at 10 mM NO_3^-, Pyl-1 had almost no visible lateral root (LR), while Bor-1 had about a dozen of long LRs. C24 had much shorter primary root length (LPR) compared with Ost-0. For each outcross, around 450 F2 individuals were phenotyped and pools of 40 plants with extreme traits were identified. Bulked DNAs were sequenced by Illumina NextSeq platform. Raw reads were then filtered and mapped to Col-0 genome followed by consensus-calling programs. The SHOREmap pipeline (*Nature Methods* 8:550-551) was used for estimating allele frequencies (AFs). By comparing the four bulks of recombinant genomes, we were able to detect a common peak of AF on the long arm of chromosome 4. Further GO enrichment analysis revealed genes with high AF values across all the chromosomes involved in signal transduction. Phenotypes of knockout lines of candidate genes will be challenged in order to confirm the roles of these genes in *Arabidopsis* root development.

P555 - CRISPR Primer Designer: Design primers for knockout & chromosome imaging CRISPR-Cas system

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Clustered regularly interspaced short palindromic repeats – CRISPR associated system (CRISPR-Cas) enables biologists to edit genome precisely and provides a powerful tool for perturbing endogenous gene regulation, modulation of epigenetic markers and genome architecture. However, there are concerns rose about the specificity of the system, especially the usages of knocking out a gene. Previous designing tools either were mostly built-in websites or ran as command-line program, and none of them ran locally and acquired a user-friendly interface. In addition, with the development of CRISPR derived-systems, such as chromosome imaging, there were still no tools helping users to generate specific end-user spacers. We here presented CRISPR Primer Designer for researchers to design primers for CRISPR applications. The program has a user-friendly interface, and can analyze the BLAST results by using multiple parameters and score for each candidate spacers, generate the primers when using a certain plasmid. In addition, CRISPR Primer Designer runs locally and can be used to search spacer clusters, and exports primers for CRISPR-Cas system based chromosome imaging system. CRISPR Primer Designer can be downloaded at: http://www.plantsignal. cn/CRISPR/crispr_primer_designer.html.

Epigenetics

Posters 556 to 573

P556 - Discovering the enigmatic DNMT2 in Arabidopsis thaliana: more than a tRNA cytosine methyltransferase

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DNA methyltransferase 2 (DNMT2) is highly conserved among different kingdoms and does not have a biological function well defined so far; however, it has been shown that DNMT2 can methylate RNA in animal and plant cells, specifically the cytosine 38 (C38) of transfer RNAs (tRNAs). It is also responsive to stress conditions in animal cells and it seems to somehow play a role in early development and male gametogenesis in animals. This work aims to investigate the possible role(s) of *DNMT2* in *Arabidopsis thaliana*. *dnmt2* mutant displays no obvious phenotypes in embryo or plant development. Using sodium bisulfite treated-RNA high throughput sequencing, we found two new target candidates for AtDNMT2: tRNA Gly^{GCC} and tRNA Gly^{CCC}. To get a better view of the possible molecular roles of AtDNMT2, we analyzed its cellular localization. Based on transient expression assays, we showed that AtDNMT2 is mostly localized in the nucleus and it might interact with cytoskeleton proteins. To evaluate possible molecular and cellular pathways that DNMT2 could influence, we constructed Col-0 and *dnmt2* RNA-seq libraries and found that it may be involved in several cellular processes: metabolic, developmental, stress response, cell organization, signal transduction, among others. Our preliminary data suggests that *AtDNMT2* is playing various roles in Arabidopsis cells, probably acting as co-factor of the epigenetic machinery and indeed participating in tRNA C38 methylation of specific tRNAs.

P557 - Imprinting and redundancy of a cluster of Mcm1/SRF Type-I (γ) MADS-Box Factors in Plant Reproduction

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MADS box transcription factors are evolutionary conserved proteins found in yeast, animals and plants. The MADS box transcription factors can be divided into two groups, Type I and Type II. Type I have been shown to be regulated by an epigenetic mechanism called imprinting where only one parental copy is active. More specifically, the maternally expressed MADS box gene AGL36 has been shown to be regulated by a dual switch comprising of the METHYLTRANSFERASE1 (MET1), which maintains CG methylation and DEMETER (DME), which removes methylation by glycosylation. Moreover, the FERTILIZATIONINDEPENDENT-SEED Polycomb repressive complex 2 (FIS-PRC2) is required for repression of AGL36. *AGL36* clusters with *AGL34, AGL35*, and *AGL90*, sharing a high degree of sequence similarity and many of the AGLs have been found to interact and may form dimers or tetramers. AGL36 interacts with AGL28 and AGL62 where the *agl62* mutant has an endosperm phenotype, but preliminary studies show that none of the other single mutants have an apparent phenotype. Double and triple mutants are therefore necessary to investigate redundant functions. In addition, *AGL28* and *AGL90* do not appear to be regulated by a dual MET1/DME switch. The Type I transcription factors are predicted to have been duplicated through local and more recent duplication events and we will report on the characterization of one such cluster and the nature of the surrounding region (e.g. transposon presence) in the genus Arabidopsis.

P558 - Histone deacetylase HD2C interacts with BRM-containing SWI/SNF chromatin remodeling complex and coregulates response to heat stress in Arabidopsis.

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Histone acetylation is one of the key mechanisms regulating chromatin structure and transcriptional activity of genes. The addition of acetyl group to lysine side chain, by loosening chromatin structure, makes DNA more accessible to RNA polymerase and other regulatory factors and promotes transcription. Histone acetylation is a reversible process that depends on the interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs). Both HATs and HDACs act as components of multiprotein complexes and are recruited to target loci by specific transcriptional regulators. Arabidopsis HD2C is the most thoroughly studied histone deacetylase of the HD2-type. It functions as a transcriptional repressor and was shown to be involved in responses to ABA and abiotic stresses. In this work, we show evidence for the interaction between Arabidopsis HD2C histone deacetylase and BRM-containing SWI/SNF chromatin remodeling complex. Moreover, we reveal a novel function of HD2C and BRM ATPase as regulators of heat stress response and show that they co-regulate a subset of genes necessary for proper response and adaptation of Arabidopsis to high temperature.

P559 - Genome-wide Transcriptome Analysis of Brassica napus Epilines Selected for Energy Use Efficiency and High Yield

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Plants utilize sophisticated epigenetic regulatory mechanisms to coordinate changes in gene expression and thus, rapidly to respond to the environment. Moreover, one of the most complex quantitative traits in plants, yield, has been demonstrated to possess an epigenetic component that correlates with energy homeostasis (De Block M, Van Lijsebettens M, 2011). The energy use efficiency (EUE) rate was used as a selection marker for artificial selection in isogenic *Brassica napus* doubled haploid populations. Selected lines with high EUE had an increased seed production. All selected lines were found to be genetically identical but epigenetically different. Epigenetic states as well as the agronomic characteristics of the lines were stably transmitted for over eight generations (Hauben *et al.*, 2009). To investigate regulatory and metabolic pathways associated with transcriptional states, we performed

RNA-seq analysis on EUE1 epiline. For functional analyses of Brassica differentially expressed genes Arabidopsis orthologous were used. The functional categorization of EUE1 DEGs showed an enrichment of GO terms related to drought stress response. A more detailed analysis revealed an over-representation of stress responsive genes. A hierarchical clustering of EUE1 RNAseq data with Arabidopsis water stress-related transcriptome data sets showed a clear correlation with drought stress treatments. These data were confirmed by drought stress experiments on EUE1 plants.

P560 - Silencing of developmentally regulated genes by the MUT9 kinases in Arabidopsis

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MUT9 is a small family of plant-specific kinases first described in Chlamydomonas reinhardtii. In this alga, MUT9 is responsible for the phosphorylation of histone H3 threonine 3 (H3T3ph), a modification associated to the chromatin of transcriptionally silent transgenes and transposons. We present here the initial characterization of four putative MUT9 orthologs in Arabidopsis, At2g25760, At3g13670, At5g18190 and At3g03940. RT-PCR and promoter fusion with GUS indicate that the four genes are expressed in the majority of plant organs. Analysis of translation fusions with the fluorescent protein GFP showed that MUT9 proteins localize into the nuclei, sometimes distributed as a discrete foci. We also isolated homozygous T-DNA mutants in the four *MUT9* genes. Analysis of these mutants did not reveal any obvious phenotypes or defects in gene silencing at the loci tested. However, crossing the T-DNA lines in all possible combinations revealed a double mutant combination displaying a drastic phenotype. Plants of this double mutant were dwarf in appearance, often produced floral buds with narrower sepals resulting in exposed floral organs. RNA-seq and a candidate gene approach indicated that this mutant is defective in the silencing of developmentally regulated genes such as *PISTILLATA, APETALA1* and *MEDEA*. Thus, the MUT9 family appears to play essential role(s) in developmental programs and gene silencing in Arabidopsis. Funding: this work was supported by a FAPESP Jovem Pesquisador grant (N° 11/50483-2) to JAC-M

P561 - Primer-dependent and primer-independent initiation of double stranded RNA synthesis by Arabidopsis RNAdependent RNA polymerases RDR2 and RDR6

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In plants and many other eukaryotes, cellular RNA-dependent RNA polymerases (RDRs) are fundamental components of RNA silencing, a conserved RNA-based mechanism involved in stability, protection, inheritance and expression of eukaryotic genomes at transcriptional or post-transcriptional levels. In *Arabidopsis thaliana* genetic studies have demonstrated that RDR2 and RDR6 are involved in the synthesis of double stranded RNA (dsRNA) from single stranded RNA (ssRNA) targeted by RNA silencing. The dsRNA is subsequently cleaved by the ribonuclease DICER-like into secondary small interfering RNAs (siRNAs) that reinforce and/or maintain the silenced state of the target RNA. Models of RNA silencing propose that RDRs could use primer-independent and primer-dependent initiation to generate dsRNA from a transcript targeted by primary siRNA or microRNA (miRNA). However, the biochemical activities of RDR proteins are still partly understood. Here, we obtained active recombinant RDR2 and RDR6 in a purified form. We demonstrate that RDR2 and RDR6 have primer-independent and primer-dependent RNA polymerase activities with different efficiencies. We further show that RDR2 and RDR6 can initiate dsRNA synthesis either by elongation of 21- to 24- nucleotides RNAs hybridized to complementary RNA template or by elongation of self-primed RNA template. These findings provide new insights into our understanding of the molecular mechanisms of RNA silencing in plants.

P562 - Molecular Basis Of Transgenerational Progressivity Of DNA Methylation Restoration

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Genetic dissection of DNA methylation of repeats elements in Arabidopsis has revealed a complex interplay between de novo RNA-directed DNA methylation and maintenance methylation, which is orchestrated by the ATP-dependent chromatin remodeler DECREASE OF DNA METHYLATION 1 (DDM1; Law & Jacobsen, Nat Rev Genet 2010). Plants mutant for *DDM1* show much-reduced DNA methylation over transposable elements and other repeats. Although at some loci this hypomethylation is stably inherited upon restoration of DDM1 function, this is not the case for a large class of repeat sequences, which reacquire wild-type DNA methylation instead. This restoration of DNA methylation is however not immediate but rather proceeds in an incremental manner over several successive generations and during the sexual phase of the plant cycle (Teixeira et al., Science 2009; Teixeira & Colot, Heredity 2010). Here, we show that progressive DNA methylation almost exclusively affects CG sites that are located within nucleosomal DNA, thus revealing a key role for chromatin organization in determining how DNA methylation patterns are established.

P563 - SHOOT GROWTH1 Maintains Arabidopsis Epigenomes by Regulating IBM1

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Maintaining correct DNA and histone methylation patterns is essential for the development of all eukaryotes. In Arabidopsis, we identified SHOOT GROWTH1 (SG1)/INCREASE IN BONSAI METHYLATION2 (IBM2), a novel protein involved in the control of gene methylation. SG1 contains both a Bromo-Adjacent Homology (BAH) domain found in several chromatin regulators and an RNA-Recognition Motif (RRM). The sg1 mutations are associated with drastic pleiotropic phenotypes. The mutants degenerate after few generations and are similar to mutants of the histone demethylase INCREASE IN BONSAI METHYLATION1 (IBM1). A methylome analysis of sg1 mutants revealed a large number of gene bodies hypermethylated in the cytosine CHG context, associated with an increase in di-methylation of lysine 9 on histone H3 tail (H3K9me2), an epigenetic mark normally found in silenced transposons. The sg1 phenotype is suppressed by mutations in genes encoding the DNA methyltransferase CHROMOMETHYLASE3 (CMT3) or the histone methyltransferase KRYPTONITE (KYP), indicating that SG1 functions antagonistically to CMT3 or KYP. We further show that the IBM1 transcript is not correctly processed in sg1, and that the functional IBM1 transcript complements sg1. Altogether, our results suggest a function for SG1 in the maintenance of genome integrity by regulating IBM1.

P564 - Role of RdDM in regulation of a cluster of Type-I (γ) MADS-box transcription factor genes

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The RNA Directed DNA methylation (RdDM) pathway has been shown to be involved in several different processes during plant development and reproduction. RdDM produce and utilize small interfering RNA to direct methylation of DNA and thus can regulate expression of genes through epigenetic mechanisms. RdDM has been linked to the regulation of a cluster of Type-I (γ) MADS-box transcription factor genes called AGAMOUS LIKE (AGL). In mutants lacking key components of the RdDM machinery, several of the AGLs show altered expression patterns compared to Wildtype. It has been reported that several AGLs are upregulated in developing seeds when the maternal contributor lacks a functional RNA polymerase IV of the RdDM pathway. In this study, we have investigated the parental origin of the differential expression of

Type-I (γ) MADS-box transcription when RdDM is not functional in either of the parents. Several AGLs have also been shown to be imprinted and we investigate the hypothesis that RdDM is involved in parent- of- origin dependent gene expression.

P565 - INCURVATA11 is involved in chromatin remodeling

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We performed several screens for leaf mutants and found that mutations classified together based on morphological phenotype actually affect genes involved in a single pathway or molecular mechanism. Gene–morphology relationships among our mutants were reproducible and in not few cases predictable. One of the most represented phenotypic classes that we found was that of mutants with incurved leaves, some of which had defects in chromatin remodeling, an essential process for all eukaryotes that impacts growth and development. The *incurvata11-1 (icu11-1)* mutant exhibits curly leaves, a phenotype that we already observed in mutants carrying alleles of *CURLY LEAF (CLF)* and *ICU2*, both of which are involved in chromatin remodeling. *ICU11* belongs to the CP family, which also seems to participate in chromatin remodeling, as indicated by the synergistic phenotypes of double mutant combinations of cp alleles and alleles of *CLF* and *TERMINAL FLOWER 2 (TFL2)*. The CP family includes redundant and essential genes in Arabidopsis: the *icu11 cp2* and *cp3 cp4* double mutants are lethal. In addition, we found the ICU11 and CP2 proteins solely localized at the cell nucleus. Many genes were found derepressed in a global expression analysis of *icu11-1* leaves. Taken together, our results indicate that *ICU11* and other *CP* genes are new players on the chromatin remodeling scene.

P566 - Long noncoding RNAs in root developmental plasticity

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In recent years, noncoding RNAs have emerged as major components of the eukaryotic transcriptome. The production of 24nt siRNAs depends on noncoding transcription mediated by the plant-specific RNA polymerases Pol IV and Pol V, leading to DNA methylation and transcriptional gene silencing. Besides, Pol II is also capable of transcribing functional long noncoding RNAs (lncRNAs). Genome wide analyses have revealed the existence of thousands of lncRNAs in several plant species. In the last years, several Pol II lncRNAs have been characterized in a wide range of regulatory mechanisms in plants, including the recruitment of chromatin remodelers, the modulation of alternative splicing factors, the fine-tune of miRNA activity or the control of mRNA translation or accumulation. Recently, dual noncoding transcription by alternative polymerase complexes was implicated in epigenetic and chromatin conformation dynamics, impacting gene expression. In our lab, we study the role of lncRNAs in the control of gene expression, leading to the modulation of far-reaching developmental outputs

P567 - Phosphate starvation in rice induces mitotically heritable modulation of DNA methylation at starvation responsive genes

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Cytosine DNA methylation (mC) is a genome modification that can regulate the expression of coding and non-coding genetic elements, however little is known about the involvement of mC in response to environmental cues. Using whole-genome bisulfite sequencing to assess the spatio-temporal dynamics of mC in rice grown under phosphate (Pi) starvation and recovery conditions, we identified widespread Pi starvation-induced changes in mC in rice roots and shoots, preferentially localized in TEs and proximal to highly induced genes. These changes in mC occurred after changes in proximal gene transcription and were mostly *DCL3a*-independent. Furthermore, these changes in mC could be mitotically but not meotically propagated. Similar analyses performed in Arabidopsis revealed a limited effect of Pi starvation on mC, suggesting a species-specific mechanism. Overall, this study provides insights into a cellular activity that potentially repress the activity of specific TEs in genomic regions that must be transcriptionally activated to respond to environmental perturbation.

P568 - DNA methylation and small RNAs cooperatively repress Athila retrotransposon to prevent developmental defects

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Small RNAs and DNA methylation have pivotal roles in regulating gene expression. In Arabidopsis thaliana, RDR6, an RNA dependent RNA polymerase, has been shown to generate 21nt siRNAs required for post-transcriptional gene silencing. Because *RDR6*-dependent epigenetically activated 21nt easiRNAs are also generated from transposable elements, which are silenced by DNA methylation, crosstalk between 21nt siRNA and DNA methylation is suggested. Consistently, we observed the strong genetic interaction between *rdr6* and mutants defective in DNA methylation. The *rdr1 rdr2 rdr6 ddm1* quadruple mutant, losing both 21nt and 24nt siRNAs and DNA methylation, has dramatic floral defects, growth defect and loss of fertility. These defects are not observed in *rdr1 rdr2 ddm1* or *rdr1 rdr2 rdr6*. Segregation analyses in a *rdr1 rdr2 rdr6* background indicates that *RDR6* cooperates with DNA methylation to repress a single causative locus. Using EMS mutagenesis, we isolated suppressors that recovered these pleiotropic phenotypes, all of which gained hyper-methylation at a specific centromeric *Athila* retrotransposon. In addition, replacing easiRNA with *RDR6*-independent hairpin small RNAs from this *Athila* element suppressed multiple phenotypes of the quadruple mutant, suggesting that *Athila* is the causative locus. Our data provide evidence that *RDR6*-dependent 21nt easiRNA compensates for the loss of DNA methylation, preventing developmental defects caused by a specific perturbed transposon.

P569 - Small molecules with big impact: functional characterization of embryonic miRNAs

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Plant microRNAs (miRNAs) are short non-coding RNAs (20-24nt) that post-transcriptionally regulate gene expression throughout plant life, and have diverse regulatory roles throughout development. Consistent with their importance during development, key developmental regulators, such as transcription factors and F-box proteins are downstream targets of plant miRNAs. We recently demonstrated that miRNAs are required for both body plan formation, as well as proper developmental timing, during embryogenesis (Nodine and Bartel, 2010). However, the functions of individual miRNAs during embryogenesis are largely unknown. This is largely due to the inability to profile small RNA populations from early embryos. We modified next generation sequencing protocols to enable highly accurate and reproducible small RNA and transcriptome profiling from very low amounts of starting RNA. We used these approaches to profile the small RNAs and mRNAs in wild type and miRNA-deficient (*dcl1-5*) embryos. By comparing the RNA populations of Col-0 and *dcl1-5* embryos, we have obtained a genome-wide view of miRNA/target interactions in developing embryos. Furthermore, fluorescence-based miRNA sensors together with RNA in situ hybridizations for selected miRNAs gave cellular resolution of miRNA activity and expression

domains during embryogenesis. We also systematically inhibited specific miRNA families in wild-type embryos, and identified a set of miRNAs that are required for embryo development. Together, our results demonstrate that miRNAs likely have a large impact on the gene regulatory networks at the beginning of plant life.

Keywords: small RNA, miRNAs, embryo, development

Reference: Nodine, M. D. and Bartel, D. P. (2010) MicroRNAs prevent precocious gene expression and enable pattern formation during plant embryogenesis. Genes & development 24, 2678-2692.

P570 - Binding of ubiquitin by the WIYLD domain of the histone H3K9 methyltransferase SUVR4 changes its activity and product specificity

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In eukaryotes, different chromatin states facilitate or repress gene expression and restrict the activity of transposable elements. Post-translational modifications of amino acid residues on the N-terminal tails of histones are suggested to define such states. The histone lysine methyltransferase SUVAR3-9 RELATED4 (SUVR4) of *Arabidopsis thaliana* functions as a repressor of transposon activity. Binding of ubiquitin by the WIYLD domain facilitates the addition of two methyl groups to monomethylated lysine 9 of histone H3. By using NMR spectroscopy we identified SUVR4 WIYLD (S4WIYLD) as a domain with a four-helix bundle structure, in contrast to three-helix bundles of other ubiquitin binding domains. A model of the complex suggests that the N-terminal and C-terminal parts of S4WIYLD constitute a surface that interacts with charged residues close to the hydrophobic patch of ubiquitin. The WIYLD domains of the closely related SUVR1 and SUVR2 *Arabidopsis* proteins also bind ubiquitin, indicating that this is a general feature of this domain. SUVR4 localizes to the entire nucleoplasm and is associated with euchromatic genes in ChIP experiments, suggesting that SUVR4 may have euchromatic targets in addition to transposons. Ongoing ChIP-seq and RNA-seq experiments will clarify whether SUVR4 has a role outside of transposon repression. We propose that SUVR4 is involved in the epigenetic defense mechanism by trimethylating H3K9 to suppress potentially harmful transposon activity together with a possible activity on euchromatic genes.

P571 - Deciphering Dynamic Chromatin 3D Organization by chromatin remodeler subunit BAF60.

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Spatial organisation of genomes are dynamically regulated by chromatin interactions, which can be deduced using chromosome conformation capture (3C)-based techniques. This global chromatin interaction patterns regulate the cellular and genetic process in Arabidopsis thaliana. SWI/SNF are multi-protein complexes which mediate ATP-dependent chromatin remodeling to regulate gene expression. This regulation is mediated by changes in the topology of the DNA. BAF60 is a SWI/SNF subunit targets floral repressor FLC involving the photoperiod flowering pathway in Arabidopsis. BAF60 accumulates in the nucleus and controls the formation of the FLC gene loop by modulating histone density, composition, and post-translational modification. To decipher the role of BAF60 at the genome wide level and to uncover looping interactions, we performed and integrated ChIP-seq, RNA-seq and Hi-C data. We generated interaction maps in BAF60 RNAi and found that BAF60 is a key regulator of chromatin architecture which controls the formation of loop structure to modulate gene expression. Analysis of other Histone modification data with Hi-C data shows that BAF60 specific chromatin interaction patterns were correlated with other epigenetic marks. Altogether our data open new avenue in understanding the mechanism of action of SWI/SNF complexes.

572 - Coordination of transcriptional initiation and elongation via an antisense transcript-mediated chromatin mechanism

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The mechanistic basis of how chromatin influences transcription remains unresolved. Here, through a combination of experiments and mathematical modeling we find the mechanism underlying transcriptional repression of *Arabidopsis FLOWERING LOCUS C (FLC)* involves tight coordination of transcriptional initiation and elongation. This is demonstrated through quantitative model predictions followed by detailed measurements of total and chromatin-bound *FLC* intronic RNA, a new methodology for analyzing elongation rate changes. Such coordination of initiation and elongation is a key feature of an integrated mechanism involving both a changed chromatin state and the FLC antisense RNA, *COOLAIR*. We propose coordinated initiation and elongation rate changes via the chromatin state could be a general feature of robust transcriptional regulation.

P573 - Enzymatic and Structural Assays of Class II AtHDA5

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Arabidopsis thaliana histone deacetylase 5 (*At*HDA5), a homolog of yeast RPD3 class II, contains the histone deacetylase

(HD) conserved domain and shares the classII *At*HDA18 HD domain with 88% protein sequence identity. The N terminus of *At*HDA5 from residues 10th -26th was predicted as nuclear localization signal (NLS) and the C terminus is a highly variable region. Since the plant histone deacetylases remained undiscovered in molecular levels, it worths to study the molecular function and protein structures. In this study, the recombinant *At*HDA5 was constructed with a Maltose Binding Protein fusion tag for protein expression and purification. Interestingly, *At*HDA5 showed two different sizes of polymer and monomer forms after the analyses of size-exclusion chromatography and dynamic light scattering. To confirm the enzymatic function, Boc-Lys(Ac)-AMC substrate was used to check the activity of polymer and monomer forms. To identify the phosphorylation sites, the mutants of *At*HDA5 ND26, *At*HDA5 S7/8A and *At*HDA5 S7A were prepared and showed the enzymatic activity decreased with 26%-33% compared to wildtype. The mutant with mimic phosphorylation S7/8D could rescue the 15% activity from S7/8A. The results showed that phosphorylation in AtHDA5 would regulate the enzyme activity. Truncated C-terminal (residues 385-661) caused the loss of enzyme activity of histone deacetylase. Subcelluar localization confirmed AtHDA5 located at cytoplasm. Transmission electron microscopy was used to reconstruct a low resolution of 3D model of *At*HDA5. The preliminary structure of MBP-*At*HDA5 shows a monomeric form (~18 Å). A homology modeling of *At*HDA5 was generated with the template of human HDAC4 (pdbid: 2VOW). Furthermore, the AtHDA5 model was used to reveal the substrate Boc-Lys(Ac)-AMC in the binding pocket of AtHDA5. The enzymatic and structural analyses would provide the insight into the molecular mechanisms for regulating gene expression of plants.

AUTHOR INDEX

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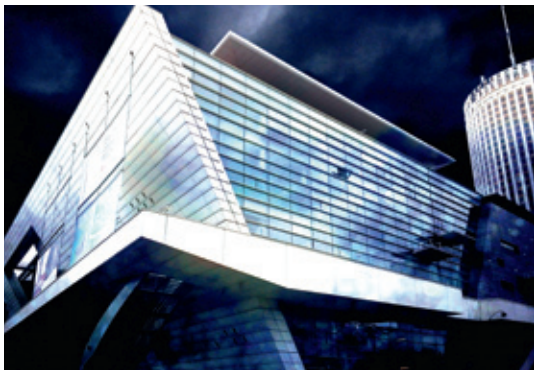
SOCIAL PROGRAM

WELCOME RECEPTION



The **Welcome reception** will take place at the Congress Center, in the exhibition/poster area, after the opening ceremony and the first keynote lecture.

Sunday July 5, 2015 at 6:00 pm

Palais des Congrès de Paris – Level 2

Reservation was required. To access the cocktail, please wear your badge (you can pick it up at the welcome desk if you have not printed it at home).

GALA DINNER & SEINE RIVER CRUISE

The ICAR Gala dinner will be held on the **Alizé boat** on **Thursday, July 9, 2015 from 7:00 pm**

The cruise will start at 8:15 pm sharp and end at 10:30 pm. Be on time!

The party will last till midnight or so!

Address:

Boarding **Port de la Rapée**, at the foot of the "Charles de Gaulle" Bridge (Paris 12th district)

Access:

Metro 1 Gare de Lyon station + 6 min walk

RER A D Gare de Lyon station + 6 min walk

From the conference venue: Metro 1 from Porte Maillot to Gare de Lyon (Direction Château de Vincennes) - 30 minutes

Free car park on the quay

Reservation was required. To access the Gala dinner, pick up your ticket from the welcome desk at the conference venue and present it upon your arrival at the Alizé boat.

If you have not booked your ticket, please note that you are welcome to join us from 10:30 pm on the boat to party with us (access upon presentation of your badge).

2013 Impact Factor: 6.605

ISSN: 1674-2052 (Print)
EISSN: 1752-9867 (Online)
CN 31-2013/Q

About the Journal

Molecular Plant (MP) is dedicated to serving the plant science community by publishing novel and exciting findings with high significance in plant biology. The journal focuses broadly on cellular biology, physiology, biochemistry, molecular biology, genetics, development, plant-microbe interaction, genomics, bioinformatics, and molecular evolution.

The 2013 MP's Impact Factor is 6.605, ranking into the Top 4 primary research journals in Plant Science category.

Publisher and Sponsor

Molecular Plant is published by the MP Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., sponsored by the Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and the Chinese Society of Plant Biology.

Why Publish with *Molecular Plant*?

Provide a high profile venue for your outstanding work
Fair review and rapid publication
Renowned international editorial board
Optional open access for all papers

GENERAL INFORMATION

HOW TO REACH THE CONGRESS VENUE?

Palais des Congrès de Paris
2 Place de la Porte Maillot
75017 Paris
ICAR exhibition and conference rooms: **Level 2, Paris Side**

By car:
From A1, A4, A6, A10, A15: Take Paris direction, access via western section of the ring road, exit Porte des Ternes
From A3, A13, A14: Take Paris direction, access via southern section of the ring road, exit Porte Maillot

By Public Transportation:
- Metro Line 1 (direction La Défense) Stop at Porte Maillot - Palais des Congrès
- Bus : Line PC1, PC 3, 82, 73, 43, 244
- RER Line C - Stop at Neuilly - Porte Maillot - Palais des Congrès

Download the free App for your smartphone

Visit Paris by Metro is the official application of the RATP. Fully translated into English, German, Dutch, Italian, Spanish and Japanese, it is intended for use by anyone who wishes to effortlessly explore Paris by Metro.
We invite you to upload it to help you getting around in Paris and visiting the best place of the Capital. The name of the App is: **Visiter Paris en métro - RATP**

PARKING

A VINCI PARK car park with almost 1,700 spaces is open 24/7 for your convenience. Access via "Place de la Porte Maillot". Telephone: 0033 1 40 68 00 11.

REGISTRATION AND WELCOME DESK

If you provided a correct email address when you registered, your personal e-badge has been sent by email the week before the Conference. If you have printed your e-badge, please go directly to the welcome desk upon your arrival to get your delegate bag.

Opening hours of the Welcome Desk - Maillot Hall - Level 2

• Sunday, July, 5	3:00 pm - 7:00 pm
• Monday/Tuesday/Wednesday/Thursday	08:30 am - 6:00 pm

Please note that there will be no admittance to the congress without the appropriate identification badge

INTERNET ACCESS

A free wifi will be provided by the organisers on the venue. It is limited to 4 hours per day by connected device and disconnects every 15 minutes if not used. It only works on the frequency band 2.4 Ghz.
Select the VIPARIS network
Password : 12345678

LIST OF PARTICIPANTS

You will find the list of participants on your personal ICAR account.

ABSTRACT BOOK

The abstract book is uploaded on the USB stick provided in your delegate bag, together with the MASC report.

SPEAKER PREVIEW ROOM

All speakers must go to the Preview Room in order to download their presentation and associated files, preferably the day before and at least 4 hours before the start of the session.
Speakers of the morning sessions should download their presentation the day before.

The Preview room is located in **room 204**. Opening hours of preview room:

• Sunday, July 5	3:00 pm - 6:00 pm
• Monday/Tuesday/Wednesday	8:30 am - 6:00 pm
• Thursday, July 9	8:30 am - 11:30 am

Presentations must be provided in their final version. Speakers will be able to check their presentation in the preview room with some assistance if required.

POSTER SESSIONS

Posters will be displayed at different spots on Level 2 of the Palais des Congres of Paris for the whole duration of ICAR2015. Posters will be grouped according to thematic sessions. A detailed map is provided page ... of the conference booklet.

There are **3 dedicated poster sessions:**

- Monday July 6 at 6:00 pm (2 hours) - Even numbers (002, 004, 006 etc.)
- Tuesday July 7 at 6:00 pm (2 hours) - Odd numbers (001, 003, 005 etc.)
- Wednesday July 8 at 6:00 pm (2 hours) - Even and odd numbers, at the convenience of the poster authors

The presenting author of each poster is required to be present at the dedicated poster sessions according to even or odd numbers.
Drinks will be provided during the poster sessions.

CERTIFICATE OF ATTENDANCE

Your certificate of attendance will be accessible online on your personnal ICAR account the night after you came to the congress. Do not forget to get scanned everyday!

BREAKS & LUNCHES

Refreshments breaks will be provided every morning at 10:30 am and every afternoon at 4:00 pm in the Maillot Hall, next to the exhibitors and posters.

Lunch buffets will be available in the Maillot Hall from 12:30 to 2:00 pm.

LIABILITY

The Organizing Committee will not be liable for personal accidents or loss/damage of private properties of congress participants either during the congress or during the gala dinner. It is therefore recommended that participants arrange their own personal health, accident and travel insurance.

TAXI

Taxis G7: 39 85 or +33 1 41 27 66 99
Taxi bleus: 36 09 or +33 1 49 63 10 10
Alpha Taxis: +33 1 45 85 85

OTHER INFORMATION

Pharmacy opens daily from 9.00 am to 8.00 pm.
Cash dispensers are available on levels -1 and 0.
Smoking is forbidden throughout the facility.
Most of the public areas are accessible to persons with reduced mobility. Special parking spaces are reserved for the disabled in the public car park. All levels are accessible by elevator.

PLANNING AT A GLANCE

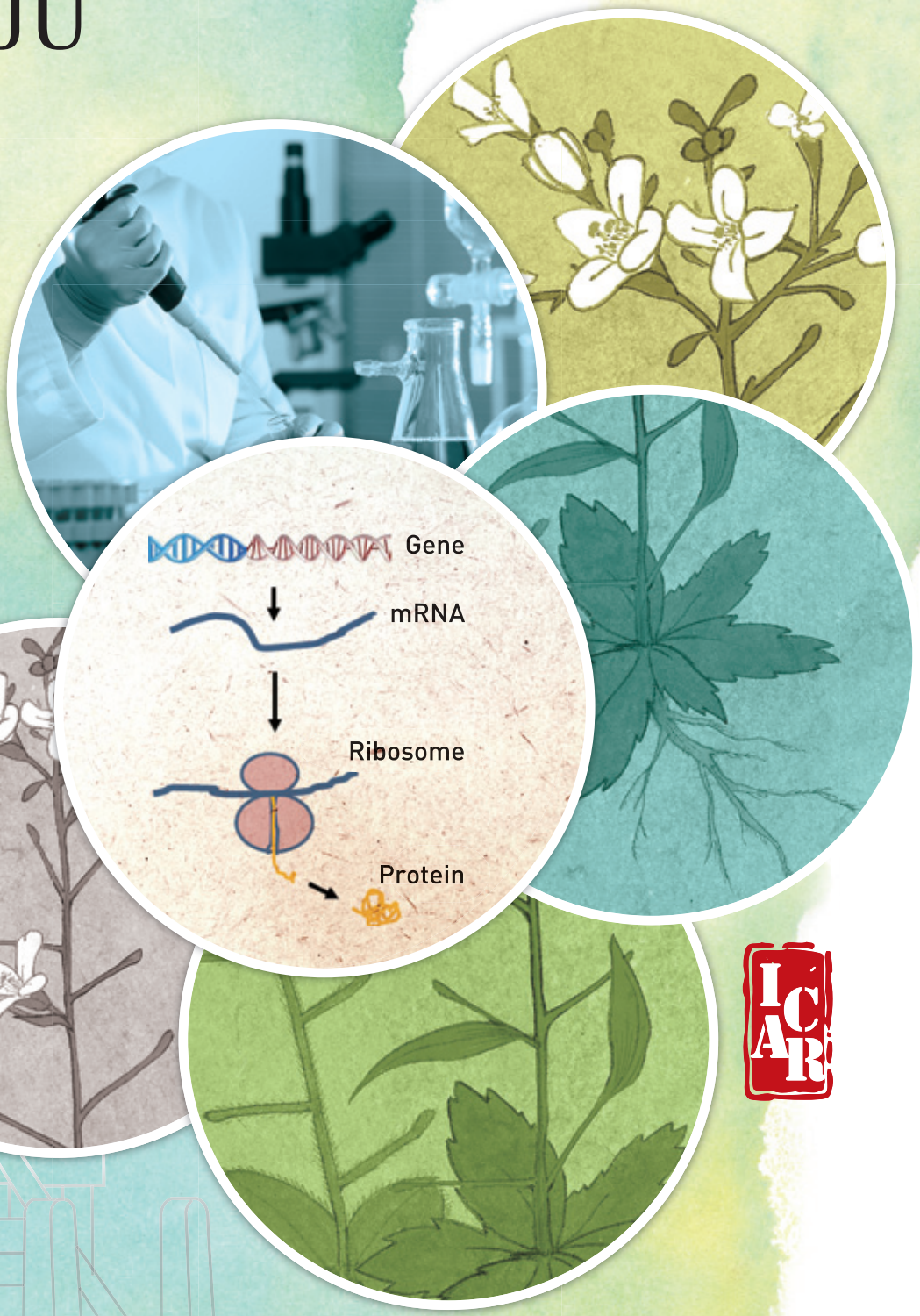
	SUNDAY JULY 5	MONDAY JULY 6	TUESDAY JULY 7	WEDNESDAY JULY 8	THURSDAY JULY 9
8:30 AM	ICAR 2015 PARIS - FRANCE	Registration/Poster setup	Registration/Poster setup	Registration/Poster setup	Registration
9:00 AM		**Plenary session** **Nutrition & metabolism I** 9:00 - 10:30 am *Blue Amphi*	**Plenary session** **Response to environment I** 9:00 - 10:30 am *Blue Amphi*	**Plenary session** **Nutrition & metabolism II** 9:00 - 10:30 am *Blue Amphi*	**Plenary session** **Response to environment II** 9:00 - 10:30 am *Blue Amphi*
10:30 AM		**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*
11:00 AM		**Plenary session** **Plant growth & development I** 11:00 - 12:30 am *Blue Amphi*	**Plenary session** **Reproduction: from flowering to seeds I** 11:00 - 12:30 am *Blue Amphi*	**Plenary session** **Plant growth & development II** 11:00 - 12:30 am *Blue Amphi*	**Plenary session** **Reproduction: from flowering to seeds II** 11:00 - 12:30 am *Blue Amphi*
12:30 PM		**Lunch** - *Maillot Hall* 12:30 - 2:00 pm	**Lunch** - *Maillot Hall* 12:30 - 2:00 pm	**Lunch** - *Maillot Hall* 12:30 - 2:00 pm	**Lunch** - *Maillot Hall* 12:30 - 2:00 pm
02:00 PM		Registration/Poster setup Maillot Hall **Thematic concurrent sessions** 2:00 - 4:00 pm **Primary metabolism, photosynthesis, biomass** *Room 242 AB* **Cell biology** *Blue Amphi* **Transcriptional regulations** *Maillot room*	Registration/Poster setup Maillot Hall **Thematic concurrent sessions** 2:00 - 4:00 pm **Biotic interactions** *Blue Amphi* **Development** *Maillot room* **Genome and chromatin dynamics** *Room 242 AB*	Registration/Poster setup Maillot Hall **Thematic concurrent sessions** 2:00 - 4:00 pm **Secondary metabolism** *Room 242 AB* **Reproduction** *Maillot room* **Post-transcriptional and post-translational regulations** *Blue Amphi*	Registration Maillot Hall **Thematic concurrent sessions** 2:00 - 4:00 pm **Abiotic stress** *Blue Amphi* **System biology and new approaches** *Room 242 AB* **Epigenetics** *Maillot room*
03:00 PM	**Registration** **Poster Set-up** 3:00 - 7:00 pm *Maillot Hall*				
04:00 PM		**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*	**Coffee break** - *Maillot Hall*
04:30 PM		**Workshops** 4:30 - 6:00 pm **W1** *Maillot room* **W2** *Room 242 AB* **W3** *Blue Amphi* **W4** *Room 243* **W5** *Room 241*	**Workshops** 4:30 - 6:00 pm **W6** *Room 241* **W7** *Blue Amphi* **W8** *Maillot room* **W9** *Room 243* **W10** *Room 242 AB*	**Thematic concurrent sessions** 4:30 - 6:30 pm **Translational biology and biotechnologies** *Room 242 AB* **Hormone signaling** *Blue Amphi* **Natural variation and evolution** *Maillot room*	**Closing Keynote Lecture** 4:30 - 5:45 pm *Blue Amphi*
05:00 PM	**Opening ceremony** **Keynote Lecture** 4:45 - 6:00 pm *Blue Amphi*				
06:00 PM	**Welcome reception** 6:00 - 7:30 pm *Maillot Hall*	**Poster session** **Odd numbers** With drinks 6:00 - 8:00 pm *Maillot Hall & Foyers*	**Poster session** **Even numbers** With drinks 6:00 - 8:00 pm *Maillot Hall & Foyers*	**Poster session** **Even and odd numbers** With drinks 6:30 - 8:00 pm *Maillot Hall & Foyers*	Free time
07:00 PM					**Seine River Cruise - Gala Dinner** 7:00 - 00:30 pm
08:00 PM					

ICAR 2016 KOREA GyeongJu

International Conference on Arabidopsis Research

ICAR 2016 KOREA, GYEONGJU

From 6. 29 To 7. 3



5 Keynote Speakers | 28 parallel sessions covering almost all areas in Arabidopsis research | 4 Workshops | 6 Postertalk sessions for junior scientists | A variety of cultural and sightseeing programs

Lead Organizer: Inhwan Hwang, Professor(POSTECH, KOREA)