15th International Conference on Arabidopsis Research

July 11 - 14, 2004 · Berlin · Germany

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Imprint

Abstract Book of the 15th International Conference on Arabidopsis Research
Published by: Max Planck Institute of Molecular Plant Physiology · Am Mühlenberg 1 · 14476 Potsdam

Coordination and Organisation: Isabell Witt
Database Publishing: MMDO Berlin
Design: Dirk Biermann
Printed by: sd:k Satz Druck GmbH Teltow

Potsdam/Golm, June 2004

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Max Planck Institute of Molecular Plant Physiology
Am Mühlenberg 1 · 14476 Potsdam · Germany

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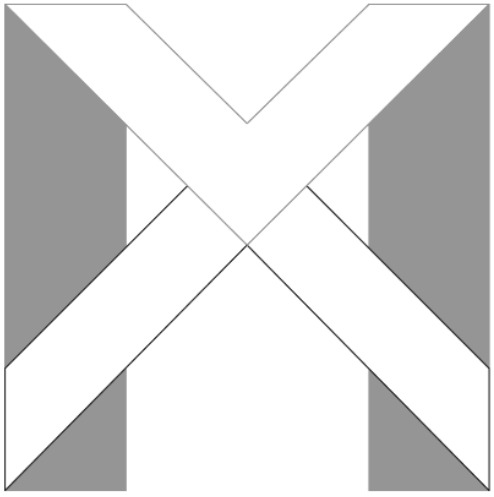

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The DBG promotes plant sciences by several activities:

- Editing the journal ***„Plant Biology***", published by the Georg Thieme Verlag, Stuttgart, Germany. "***Plant Biology***" is listed in the PubMed database and publishes preferentially short papers of general interest in plant sciences. Plant Biology online: www.thieme-connect.com.
- Organizing biennially a Scientific Congress (Botanikertagung)

  The 2004 Congress will be held in Braunschweig, Germany, September 5 - 10, 2004 (www.botanikertagung.de).
- Special Seminars, Workshops and Meetings are organized by the five Sections of the DBG:
  - ***Biodiversity and Evolutionary Biology***
  - ***Mycology and Lichenology***
  - ***Phycology***
  - ***Plant Physiology and Molecular Biology***
  - ***Secondary Plant Constituents and Natural Products***
- Awarding travel funds for young scientists to attend meetings of the DBG and its Sections.
- Awarding prizes for outstanding doctoral theses:

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The German Botanical Society has currently about 1,100 members from 17 countries. The annual membership fee is 75 € (Students: 35 €) and includes subscription to "***Plant Biology***". For further information see: www.deutsche-botanische-gesellschaft.de, contact the Executive Office (info@deutsche-botanische-gesellschaft.de), or the President of the DBG, Ulf-Ingo Flügge (ui.fluegge@uni-koeln.de).

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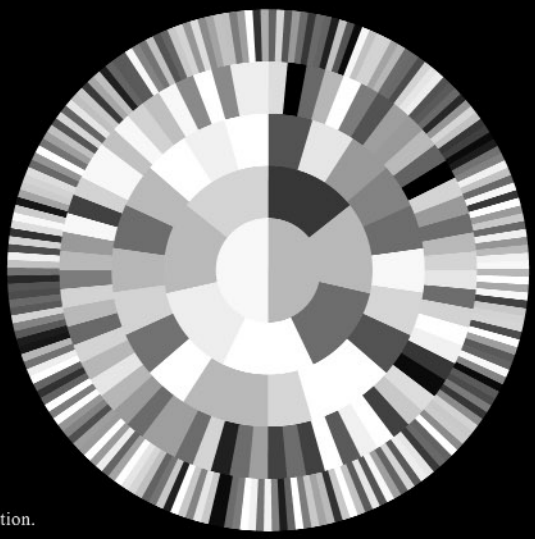

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15th International Conference on Arabidopsis Research

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Programme Overview

Sunday

ECCA

14:00 **Opening and Opening Lecture** by Enrico Coen

ECCA

15:00 - 16:30 **Overview lectures**
Development 1 (flower, fertilization, fruit, and seed) by Detlef Weigel
Metabolism (primary, secondary, cross-talk, and short distance metabolite transport) by Gloria Coruzzi

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia - parallel sessions**

ECCA
Development 1 - flower, fertilization, fruit, and seed
Session chair: Detlef Weigel

ECCR1
Metabolism - primary, secondary, cross-talk, and short distance metabolite transport
Session chair: Gloria Coruzzi

18:30 - 20:00 **Reception**

20:00 - 22:30 **Posters + beer/wine**
Workshops

Monday

ECCA

9:00 - 10:30 **Overview lectures**
Development 2 (shoot and root) by Jennifer Fletcher
Interaction with the environment 1 (abiotic) by Jian-Kang Zhu

10:30 – 11:00 **Coffee break**

11:00 - 12:30 **Symposia - parallel sessions**

ECCA
Development 2 – shoot and root
Session chair: Jennifer Fletcher

ECCR1
Interaction with the environment 1 - abiotic
Session chair: Jian-Khang Zhu

12:30 - 13:30 **Lunch**

13.30 - 15:00 **Poster session 1 - odd numbered posters**

ECCA

15:00 - 16:30 **Overview lectures**
Interaction with the environment 2 (biotic) by Jeff Dangl
Novel tools, techniques and resources by Josef Ecker

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia - parallel sessions**

ECCA
Interaction with the environment 2 - biotic
Session chair: Jeffrey Dangl

ECCR1
Novel tools, techniques and resources
Session chair: Josef Ecker

18:30 - 20:00 **Dinner**

20:00 - 22:30 **Posters + beer/wine**
Workshops

Programme Overview

Tuesday

ECCA

9:00 - 10:30 **Overview lectures**
Non-Arabidopsis (limitations of the Arabidopsis model) by Steven Tanksley
Long distance transport (signals including silencing and metabolites) by Ottoline Leyser

10:30 – 11:00 **Coffee break**

11:00 - 12:30 **Symposia - parallel sessions**

ECCA
Non-Arabidopsis - limitations of the Arabidopsis model
Session chair: Steven Tanksley

ECCR1
Long distance transport - signals including silencing and metabolites
Session chair: Ottoline Leyser

12:30 - 13:30 **Lunch**

13.30 - 15:00 **Poster session 2 - even numbered posters**

ECCA

15:00 - 16:30 **Overview lectures**
Cell biology by Gerd Jürgens
Natural variation and comparative genomics including genome evolution and adaptation by Thomas Mitchell Olds

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia - parallel sessions**

ECCA
Cell biology
Session chair: Gerd Jürgens

ECCR1
Natural variation and comparative genomics including genome evolution and adaptation
Session chair: Thomas Mitchell-Olds

19:30 - 23:00 **Conference dinner at the Diedersdorf Castle**

Wednesday

ECCA

9:00 - 10:30 **Overview lectures**
Modelling the virtual plant/ Bioinformatics by Przemyslaw Prusinkiewicz
Genetic mechanisms (transcriptional and chromatin regulation) by Marjori Matzke

10:30 -11:00 **Coffee break**

11:00 - 12:30 **Symposia - parallel sessions**

ECCA
Modelling the virtual plant/ Bioinformatics
Session chair: Przemyslaw Prusinkiewicz

ECCR1
Genetic mechanisms - transcriptional and chromatin regulation
Session chair: Marjori Matzke

ECCA

12:30 - 13:30 **Closing lecture** by Steven Briggs **and Concluding remarks**

13:30 - 14:30 **Lunch**

Detailed Programme

Sunday

ECCA

14:00 **Opening**
Opening lecture
From genes to morphogenesis by Enrico Coen (T11-029)

ECCA

15:00 – 16:30 **Overview lectures**
Development 1 – flower, fertilization, fruit, and seed
15:00 Recent advances in flower development by Detlef Weigel, Max Planck Institute for Developmental Biology (T01-003)

Metabolism - primary, secondary, cross-talk, and short distance metabolite transport
15:46 A systems approach to nitrogen networks by Gloria Coruzzi, New York University (T07-041)

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia - parallel sessions**

ECCA

Development 1 - flower, fertilization, fruit, and seed
Session chair: Detlef Weigel

17:00 Maria Costa, John Innes Centre
Molecular analysis of floral dorsoventral asymmetry (T01-009)

17:16 Sarah Liljegren, University of North Carolina at Chapel Hill
Cell separation in Arabidopsis flowers and fruit (T01-087)

17:37 Eva Sundberg, Swedish University of Agricultural Sciences
SHI family genes redundantly regulate gynoecium and leaf development in Arabidopsis (T01-093)

17:58 Michael Lenhard, University of Freiburg
Control of Arabidopsis petal size by a novel RING finger protein (T01-063)

18:14 Naoki Aono, National Institute for Basic Biology
Pollen specific MADS-box genes are involved in pollen germination (T01-021)

ECCR1

Metabolism – primary, secondary, cross-talk, and short distance metabolite transport
Session chair: Gloria Coruzzi

17:00 Mark Stitt, Max Plank Institute of Molecular Plant Physiology
Functional genomics of carbon-nitrogen interactions (T07-098)

17:20 Mary Lou Guerinot, Dartmouth College
Ionomics: Gene discovery in aid of plant nutrition, human health and environmental remediation (T07-097)

17:40 Marcus Fehr, Carnegie Institution
Genetically encoded sensors for metabolites (T07-029)

18:00 Anika Wiese, Utrecht University
A conserved uORF mediates sucrose-induced translational control on bZIP transcription factors (T07-003)

18:10 Hitoshi Sakakibara, RIKEN Plant Science Center
Nutritional regulation of cytokinin biosynthesis: A possible role for long-distance signaling molecule (T08-006)

18:20 Daniel Weicht, Max-Planck-Institute of Molecular Plant Physiology
PaVESy: Combining profiling data with pathway knowledge (T11-016)

18:30-20:00 **Reception**

20:00 - 22:30 **Posters + beer/wine**

20:00 - 21:10 **Workshops**
Introduction to The Arabidopsis Information Resource (TAIR)
Organisers: TAIR Curators

21:15 - 22:30 Advanced techniques in data mining using TAIR
Organisers: TAIR Curators

Monday

ECCA

9:00 – 10:30 **Overview lectures**
Development 2 – shoot and root
9:00 Regulatory mechanisms in shoot and root development by Jennifer Fletcher, USDA/UC Berkeley (T02-101)
Interaction with the environment 1 - abiotic
9:45 Abiotic stress signaling and tolerance by Jian-Kang Zhu, University of California-Riverside (T04-111)

10:30 – 11:00 **Coffee break**

11:00 - 12:30 **Symposia - parallel sessions**

ECCA

Development 2 – shoot and root
Session chair: Jennifer Fletcher

11:00 Keiko Torii, University of Washington
Synergistic interaction of ERECTA-family receptor-like kinases regulate cell proliferation, patterning, and organ growth (T02-012)

11:20 Robert Sablowski, John Innes Centre
Shoot stem cells: Not naive at all (T02-009)

11:40 Philip Benfey, Duke University
Radial patterning in Arabidopsis: Networks and movement (T02-110)

12:00 Gorou Horiguchi, National Institute for Basic Biology
ANGUSTIFOLIA3 encodes a homolog of synovial sarcoma translocation protein and mediates local cell proliferation for lateral expansion of leaf blade in Arabidopsis thaliana (T02-006)

12:10 Aaron Rashotte, University of North Carolina
Cytokinin regulated transcription factors (T02-063)

12:20 Ikram Blilou, Utrecht University
Redundant PIN gene activity as a major control mechanism in patterning and cell division in Arabidopsis root development (T02-120)

Detailed Programme

ECCR1

Interaction with the environment 1 – abiotic
Session chair: Jian-Khang Zhu

11:00 Markus Teige, University of Vienna
A MAP-kinase pathway for cold and salt signalling (T04-004)

11:15 Takashi Hirayama, RIKEN & Yokohama City University
Isolation of novel ABA-related mutants using ABA analogs (T04-020)

11:30 Reetta Ahlfors, University of Helsinki
Hormonal interactions in plant abiotic stress responses (T04-044)

11:45 Bassem Al-Sady, University of California-Berkeley
Functional requirements for PIF3 in the de-etiolation process (T04-024)

12:00 Sophie Filleur, Lancaster University
Nutritional regulation of root architecture by ANR1, a MADS-box transcription factor (T04-063)

12:15 Rhonda Meyer, Max Planck Institute of Molecular Plant Physiology
Enhanced heterosis for biomass production at elevated light intensities (T04-046)

12:30 - 13:30 **Lunch**

13.30 - 15:00 **Poster session 1 - odd numbered posters**

ECCA

15:00 - 16:30 **Overview lectures**

Interaction with the environment 2 – biotic

15:00 A synthesis for understanding disease and disease resistance by Jeff Dangl, University of North Carolina at Chapel Hill (T05-090)

Novel tools, techniques and resources

15:45 Genome-wide discovery of transcriptions units and functional elements in Arabidopsis by Josef Ecker, The Salk Institute (T10-055)

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia - parallel sessions**

ECCA

Interaction with the environment 2 – biotic
Session chair: Jeffrey Dangl

17:00 Catherine Golstein, Indiana University
Indirect activation of RPS5-mediated resistance by AvrPphB (T05-035)

17:15 Jane E Parker, Max Planck Instiute for Plant Breeding Research
Interaction dynamics of several immune response regulators in Arabidopsis (T05-089)

17:30 Volker Lipka, Center for Plant Molecular Biology – University of Tübingen
Genetic dissection of non-host disease resistance to fungal pathogens in Arabidopsis (T05-053)

17:45 Gernot Kunze, University of Basel
Elongation factor Tu – A novel PAMP involved in plant defence (T05-061)

18:00 Carole Asnaghi, CNRS
Systematic analysis of cytochromes P450 biotic stress signaling response in *A. thaliana* (T05-041)

18:15 Thierry Genoud, University of Fribourg
Identifying pathogen-induced changes in the plant defense signaling network (T05-058)

ECCR1

Novel tools, techniques and resources
Session chair: Josef Ecker

17:00 Samuel Hazen, The Scripps Research Institute
Mapping LUX ARRHYTHMO, a novel myb transcription factor essential for circadian rhythms, and other circadian clock mutants by oligonucleotide array genotyping (T10-052)

17:15 Markus Schmid, Max Planck Institute for Developmental Biology
AtGenExpress – Expression atlas of Arabidopsis development (T10-018)

17:30 Sinead Drea, John Innes Centre
A molecular atlas of transcription factor expression patterns in Arabidopsis (T10-014)

17:45 Ian Small, INRA
The AGRIKOLA project: Systematic RNAi in Arabidopsis (T10-041)

18:00 Martina Schad, Max Planck Institute of Molecular Plant Physiology
The essentials of tissue-specific protein and metabolite profiling - Laser Microdissection, LC/MS/MS and GCMS (T10-017)

18:15 Sacha Baginsky, ETH Zürich
Proteome analyses of different plastid types: A first step towards a "systems" analysis of plastid development and differentiation (T10-027)

18:30 - 20:00 **Dinner**

20:00 - 22:30 **Posters + beer/wine**

Workshops

AtGenExpress
Organiser: Lutz Nover, Goethe University Frankfurt

Proteomics in Arabidopsis
Organisers: Harvey Millar, the University of Western Australia, and Hans-Peter Braun, University of Hannover

Proteolytic enzymes and their role in plant biology
Organisers: Iwona Adamska, University of Konstanz, and Zach Adam, The Hebrew University

Detailed Programme

Tuesday

ECCA

9:00 – 10:30 **Overview lectures**

Non-Arabidopsis - limitations of the Arabidopsis model

9:00 Model systems, plant sciences, and the shift to horizontal biology by Steven Tanksley, Cornell University (T12-028)

Long distance transport - signals including silencing and metabolites

9:45 Long range signalling by Ottoline Leyser, University of York (T08-019)

10:30 – 11:00 **Coffee break**

11:00 - 12:30 **Symposia – parallel sessions**

ECCA

Non-Arabidopsis - limitations of the Arabidopsis model

Session chair: Steven Tanksley

11:00 Douglas Cook, University of California - Davis
Dissecting symbiotic nitrogen fixation in legumes (T12-025)

11:22 Dani Zamir, the Hebrew University of Jerusalem
Zooming-in on a tomato yield quantitative trait nucleotide (QTN) with wild species introgression lines (T12-027)

11:44 Katharina Schneider, GSF National Research Centre
Genome analysis in sugar beet (Beta vulgaris L.) (T12-002)

12:06 Ian Bancroft, John Innes Centre
Assessing the impact of polyploidy by comparative analysis of Brassica genome microstructure (T12-004)

ECCR1

Long distance transport - signals including silencing and metabolites

Session chair: Ottoline Leyser

11:00 Peter Doerner, University of Edingburgh
Phosphate signaling in Arabidopsis (T08-014)

11:22 Didier Reinhardt Bern, University of Michigan
Patterning of plants by auxin (T08-003)

11:44 Olivier Voinnet, CNRS
Genetic dissection of RNA silencing movement in Arabidopsis (T08-019)

12:06 Robin Cameron, McMaster University
Involvement of DIR1, a putative lipid transfer protein, in long distance signalling during Systemic Acquired Resistance (T08-001)

12:30 - 13:30 **Lunch**

13.30 - 15:00 **Poster session 2 – even numbered posters**

ECCA

15:00 - 16:30 **Overview lectures**

Cell biology

15:00 A journey through the plant cell by Gerd Jürgens, University of Tübingen (T03-077)

Natural variation and comparative genomics including genome evolution and adaptation

15:45 Natural genetic variation within and between species by Thomas Mitchell Olds, Max Planck Institute of Chemical Ecology (T06-010)

16:30 – 17:00 **Coffee break**

17:00 - 18:30 **Symposia – parallel sessions**

ECCA

Cell biology

Session chair: Gerd Jürgens

17:00 Jaideep Mathur, University of Toronto
Shaping plant cells using an actin mesh (T03-069)

17:20 Stéphanie Robert, INRA
Cellulose biosynthesis and cell elongation (T03-009)

17:40 Takashi Ueda, University of Tokyo
Function and differentiation of endocytic organelles in Arabidopsis cells (T03-065)

18:00 Mark Kwaaitaal, Wageningen University
Endocytosis of the receptor like kinases AtSERK1 and BRI1 in Arabidopsis (T03-056)

18.10 Michelle Speckhart, Louisiana State University
Isolation and characterization of SIAMESE, a putative cell cycle regulator involved in endoreplication (T03-075)

18:20 Edward Kraft, University of California – Davis
Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis by (T03-080)

ECCR1

Natural variation and comparative genomics including genome evolution and adaptation

Session chair: Thomas Mitchell-Olds

17:00 John Stinchcombe, Brown University
Ecological genomics of naturally occurring flowering time variation among Arabidopsis accessions (T06-020)

17:20 Günter Theißen, Friedrich Schiller University
A floral homeotic polymorphism in Capsella: Studying a hopeful monster (T06-025)

17:40 Brandon Gaut, University of California - Irvine
A population genomic search for maize domestication genes (T12-006)

18:00 Chris Toomajian, University of Southern California
The genomic pattern of polymorphism in Arabidopsis thaliana (T06-026)

18.15 Z. Jeffrey Chen, Texas A&M University
Progenitor-dependent gene expression and evolution of transcriptome in Arabidopsis allopolyploids (T06-023)

19:30 - 23:00 **Conference dinner at the Diedersdorf Castle**

Detailed Programme

Wednesday

ECCA

9:00 – 10:30 **Overview lectures**
Modelling the virtual plant/Bioinformatics

9:00 Modelling Arabidopsis thaliana from genes to phenotypes by Przemyslaw Prusinkiewicz, University of Calgary (T11-028)

Genetic mechanisms - transcriptional and chromatin regulation

9:45 Transcriptional and chromatin regulation: A dynamic affair by Marjori Matzke, Austrian Academy of Science (T09-059)

10:30 – 11:00 **Coffee break**

11:00 - 12:30 **Symposia - parallel sessions**

ECCA

Modelling the virtual plant/Bioinformatics
Session chair: Przemyslaw Prusinkiewicz

11:00 Jim Haseloff, University of Cambridge
New tools for computer visualisation and modelling of cell interactions (T11-010)

11:30 Henrik Jönsson, Lund University
Modelling and in vivo live imaging of the Arabidopsis shoot apical meristem (T11-026)

12:00 Dorota Kwiatkowska, Wroclaw University
Formation of flower primordia at the shoot apical meristem of Arabidopsis: A quantitative approach to the meristem surface growth (T11-001)

ECCR1

Genetic mechanisms - transcriptional and chromatin regulation
Session chair: Marjori Matzke

11:00 Claudia Köhler, University of Zürich
Epigenetic control of seed development (T09-044)

11:15 Heriberto Cerutti, University of Nebraska - Lincoln
Arabidopsis AtMut11, related to a subunit of trithorax-like complexes, is required for gene silencing and heterochromatin maintenance (T09-021)

11.30 Ortrun Mittelsten Scheid, Gregor Mendel Institute of Molecular Plant Biology
An inversion of dominance between epialleles in polyploid Arabidopsis (T09-008)

11:45 Tetsu Kinoshita, Japan National Institute of Genetics
Genomic imprinting of FWA gene in Arabidopsis endosperm (T09-025)

12:00 Daniel Schubert, University of Edinburgh
Control of Arabidopsis development by PG-dependent histone methylation (T09-028)

12:15 Diana Dugas, Rice University
miRNA regulation of lateral organ separation in Arabidopsis (T09-056)

ECCA

12:30 - 13:30 **Closing lecture**
What plant research will be like 10 years from now by Steven Briggs, Diversa Corporation (T12-026)

Concluding remarks

13:30 - 14:30 **Lunch**

Abstract Index

T01 Development 1 (Flower, Fertilization, Fruit, Seed)

T01-001 AtBRM, an ATPase of the SNF2 family, controls flowering in Arabidopsis

Sara Farrona, Lidia Hurtado, John L. Bowman, José Carlos Reyes

T01-002 FROM WHEAT TO ARABIDOPSIS: UTILIZING GENE EXPRESSION PROFILES FROM ISOLATED WHEAT EGG CELLS FOR DISCOVERY OF NOVEL AND EGG CELL SPECIFIC GENES IN ARABIDOPSIS

S. Sprunck, B. Bellmann, M. Gebert, U. Baumann, P. Langridge, T. Dresselhaus

T01-003 Recent advances in flower development

Detlef Weigel

T01-004 ANALYSING FUNCTIONAL DOMAINS OF THE CARPEL DEVELOPMENT GENE SPATULA OF ARABIDOPSIS THALIANA

Teodora Paicu, David R. Smyth

T01-005 PATTERN OF FUNCTIONAL EVOLUTION OF THE FLORAL MERISTEM IDENTITY PROTEIN LEAFY

Alexis Maizel, Detlef Weigel

T01-006 A genetic model for floral meristem development

Hao Yu, Toshiro Ito, Frank Wellmer, Elliot M Meyerowitz

T01-007 Floral homeotic genes are targets of gibberellin signaling in flower development

Hao Yu, Toshiro Ito, Yuanxiang Zhao, Jinrong Peng, Prakash Kumar, Elliot M Meyerowitz

T01-008 Isolation and characterizing of genes required for different aspects of petal and stamen differentiation

Moriyah Zik, Inbal Markovitz, Tamar Rozilio, Chloe C. Diamond, Vivian F. Irish

T01-009 Molecular analysis of floral dorsoventral asymmetry

M.M.R. Costa, S. Fox, C. Baxter, P. Cubas, E. Coen

T01-010 Using dominant mutants to identify natural modifiers of flowering time

Min Chul Kim, Janne Lempe, Anandita Singh, Detlef Weigel

T01-011 Misexpression of FLOWERING LOCUS C (FLC) results in varying degrees of repression of the floral transition depending on the promoter identity.

Melissa J. Hills, Barry Pogson, Jim Peacock, Elizabeth Dennis, Chris Helliwell

T01-012 The tetraspore kinesin stabilises the male meiotic cytokinetic apparatus in Arabidopsis thaliana

Valerie Bourdon, Janet Kenyon, Hugh G. Dickinson

T01-013 Roles of DELLA Proteins in Gibberellin-Regulated Seed Germination and Floral Development

Ludmila Tyler, Stephen G. Thomas, Jianhong Hu, Alyssa Dill, Jose M. Alonso, Joseph R. Ecker, Tai-ping Sun

T01-014 Protein- protein interactions between AGL24 and several MADS-box proteins involved in flowering

Miho Takemura, Rie Sawai, Takayuki Kohchi

T01-015 Regulating the Regulators: The plant specific BBR family of GAGA-repeat Binding proteins

Dierk Wanke, Kenneth Berendzen, Dora Szakonyi, Ingo Ciolkowski, Luca Santi, Guido Jach, Kurt Stüber, Kai Müller, Francesco Salamini

T01-016 Transcriptional regulation of the floral homeotic gene AGAMOUS

Sandra Stehling, Monika Demar, Detlef Weigel, Jan Lohmann

T01-017 Molecular variation of CONSTANS in natural accessions

Yasushi Kobayashi, Detlef Weigel

T01-018 Regulation of CONSTANS protein and its relationship to photoperiodic flowering in Arabidopsis

Wim Soppe, Federico Valverde, George Coupland

T01-019 A SUMO specific protease that regulates flowering of Arabidopsis

Yong-Fu Fu, Paul H. Reeves, Giovanni Murtas, George Coupland

T01-020 Genetic evidence for essential calcium transporters in pollen growth and fertilization.

Frietsch, S., Romanowsky, S.M., Schiøtt, M., Palmgren, M.G., Harper, J.F.

T01-021 Pollen specific MADS-box genes are involved in pollen germination

Naoki Aono, Saori Miyazaki, Naomi Sumikawa, Mitsuyasu Hasebe

T01-022 SLW1 Is Essential for the Female Gametophyte Development in Arabidopsis

Dong-Qiao Shi, De Ye, Wei-Cai Yang

T01-023 Nuclear Division during Gametogenesis in Arabidopsis Mutant dq1

Dong-Qiao Shi, De Ye, Wei-Cai Yang

T01-024 A molecular model for ACR4 function in the organisation of the L1 cell layer of developing organs

Miriam L. Gifford, Gwyneth C. Ingram

T01-025 'Integument-led' seed growth in the megaintegumenta (AUXIN RESPONSE FACTOR 2) mutant

Melissa Spielman, Marie C. Schruff, Rod J. Scott

T01-026 Genetic dissection of the AUXIN RESPONSE FACTOR2 mutant megaintegumenta

Marie C Schruff, Sushma Tiwari, Melissa Spielman, Rod J Scott

T01-027 EMBRYONIC FACTOR 1 (FAC1), encoding an AMP deaminase in Arabidopsis, is essential for activating zygotic embryogenesis

Jun Xu, Haiying Zhang, Conghua Xie, Paul Dijkhuis, Chun-ming Liu

T01-028 Gene regulatory network controlling seed maturation

Alexandra TO, Christiane VALON, Gil SAVINO, Jérôme GIRAUDAT, François PARCY

T01-029 Molecular mechanism of floral repression during vegetative development

Myriam Calonje, Lingjing Chen, Z. Renee Sung

T01-030 Overexpression of KNAT1 interferes with Arabidopsis ovule development

Elisabeth B. Truernit, James P. Haseloff

T01-031 Genetic analysis of circadian clock components in Arabidopsis

TSUYOSHI MIZOGUCHI, SUMIRE FUJIWARA, ATSUSHI ODA, MAYU NAKAGAWA, TAKEOMI TAJIMA, HIROSHI KAMADA, GEORGE COUPLAND

T01-032 LHY and CCA1, clock components in Arabidopsis, control photoperiodic flowering mainly through a transcriptional cascade, GI-CO-FT

SUMIRE FUJIWARA, GEORGE COUPLAND, HIROSHI KAMADA, TSUYOSHI MIZOGUCHI

T01-033 FLOWERING LOCUS T as a link between photoperiodic induction in leaves and evocation at shoot apex

Yasufumi Daimon, Sumiko Yamamoto, Mitsutomo Abe, Ayako Yamaguchi, Yoko Ikeda, Harutaka Ichinoki, Michitaka Notaguchi, Koji Goto, Takashi Araki

T01-034 Isolation and characterization of secreted proteins from the inflorescence of A.thaliana.

Martijn Fiers

T01-035 FIDGET (FIT), an APETALA2-like protein promotes flowering by direct activation of FT

Stephan Wenkel, Lionel Gissot, Jose Gentilhomme, George Coupland

T01-036 Inhibition of the V-ATPase leads to deformation of golgi stacks during male gametophyte development

Jan Dettmer, York D. Stierhof, Renate Schmidt, Karin Schumacher

T01-037 Identification and characterisation of three genes determining embryogenesis by means of T-DNA mutagenesis in Arabidopsis thaliana (L.)

Jana Repkova, Marketa Dudova, Tomas Kocabek

T01-038 Influence of methylation pathway genes on FLC expression in Arabidopsis thaliana.

Pavel Lízal, Simona Balková, Jirina Relichová

T01-039 Drawing a line in the Arabidopsis fruit: How the valve margin forms at the border between the valve and the replum

Adrienne Roeder, Sarah Liljegren, Cristina Ferrandiz, Martin Yanofsky

T01-040 GeBP and LEC genes, two putative pathways to regulated trichome formation.

Julien Curaba, Thomas Moritz, François Parcy, Vered Raz, Michel Herzog, Gilles Vachon

T01-041 Methods to identify in vivo target genes in Arabidopsis

Stefan de Folter, Lisette van Zuijlen, Gerco Angenent

T01-042 Loss-of-function mutation lhy-12 is caused by mis-splicing of the LHY gene and an intragenic suppressor mutation lhy-2 may partially restore the defect in Arabidopsis

ATSUSHI ODA, MAYU NAKAGAWA, GEORGE COUPLAND, TSUYOSHI MIZOGUCHI

T01-043 Identifying downstream targets of INDEHISCENT (IND), a bHLH transcription factor important for fruit dehiscence

Kristina Gremski, Pedro Robles, Martin F. Yanofsky

T01-044 Moleculer Basis of Late-Flowering Phenotype in Dominant fwa Mutants

Yoko Ikeda, Mitsutomo Abe, Takashi Araki

T01-045 Characterization of TSF, a homolog of floral pathway integrator FT

Ayako Yamaguchi, Yasushi Kobayashi, Sumiko Yamamoto, Mitsutomo Abe, Takashi Araki

T01-046 Epigenetics of seed development: what is the significance of imprinting?

Abed Chaudhury, Ming Luo, Rachel Corvisy, Bjorg Sherman, WJ Peacock, ES Dennis

T01-047 Initiation of Seed Coat Development in Mutants Affecting Embryo and Endosperm Development

Allan Lohe, George Haughn, Abed Chaudhury

T01-048 CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis

Corbesier Laurent, Hailong An, Clotilde Roussot, Coral Vincent, Aidyn Mouradov, Paula Suarez-Lopez, George Coupland

T01-049 ENHANCERS OF LUMINIDEPENDENS DEFINE A ROLE FOR BRASSINOSTEROIDS IN FLORAL PROMOTION

Malgorzata Domagalska, Fritz M. Schomburg, Andrew J. Millar, Richard M. Amasino, Richard D. Vierstra, Ferenc Nagy, Seth J. Davis

T01-050 ECL1, which acts in parallel with CO, accelerates flowering by upregulating FT

Seung Kwan Yoo, Jong Seob Lee, Ji Hoon Ahn

T01-051 Partial complementation of the pollen defective apyrase mutation.

Carolin Wolf, Iris Steinebrunner

T01-052 Characterisation of AtNIC4, a member of the MATE family from Arabidopsis thaliana

Mandy Kursawe, Blazej Dolniak, Fabian Poree, Bernd Mueller-Roeber

T01-053 Intercellular protein trafficking of TFL1 and FT is essential for inflorescence meristem identity and floral transition in Arabidopsis.

Koji Goto, Akira Nakayama

T01-054 Characterization of Naturally Occuring Proteins Modified by the Phytohormone IAA from Bean and Arabidopsis seeds

Claudia Seidel, Alexander Walz, Seijin Park, Jerry Cohen, Jutta Ludwig-Müller

T01-055 Ectopic expression of the proline biosynthesis genes roID and AtP5CS affects axillary bud formation and flowering in Arabidopsis

Roberto Mattioli, Daniele Marchese, M.L.Mauro, S. D'Angeli, M.M Altamura, Paolo Costantino, Maurizio Trovato

T01-056 bHLHs transcription factors interacting with CO are potential regulators of flowering time

José Gentilhomme-Le Gourrierec, Lionel Gissot, Stephan Wenkel, George Coupland

T01-057 Exploiting the non-flowering fca-1 co-2 ga1-3 triple mutant and gene expression profiling to characterise the role of individual genes in the transition to flowering of Arabidopsis

Dean Ravenscroft, Federico Valverde, Seonghoe Jang, George Coupland

T01-058 Characterization of a late-flowering T-DNA tagged mutant of Arabidopsis thaliana

Maria Svensson, Sazzad Karim, Dan Lundh, Mikael Ejdebäck, Per Bergman, Abul Mandal

T01-059 Functional characterization of MAF2: an FLC Paralogue

Anandita Singh, Min Chul Kim, Janne Lempe, Sureshkumar Balasubramaniam, Detlef Weigel

T01-060 VRN5, a PHD/FNIII protein, is involved in vernalization by repressing FLC

Thomas Greb, Nuno Geraldo, Josh Mylne, Caroline Dean

T01-061 TRANSPARENT TESTA1 controls early and late steps of flavonoid biosynthesis in the endothelium of Arabidopsis thaliana seeds

Gui-Hua Lu, Martin Sagasser, Elmon Schmelzer, Klaus Hahlbrock, Bernd Weisshaar

T01-062 Comparative Protein Profiling and Expression Analyses of the Arabidopsis Subtilisin-like Serine Protease Family

Carsten Rautengarten, Berit Ebert, Patrick Giavalisco, Dirk Steinhauser, Thomas Altmann

T01-063 Control of Arabidopsis petal size by a novel RING finger protein

Sabine Disch, Jennifer C. Fletcher, Michael Lenhard

T01-064 Functional analysis of armadillo repeat-only (ARO) proteins in Arabidopsis thaliana

M. Gebert, T. Dresselhaus, S. Sprunck

T01-065 STY genes and Auxin in Arabidopsis development

Sohlberg JJ, Myrenås M, Eklund M, Sundberg E

T01-066 Floral induction by ambient growth temperature in Arabidopsis thaliana

Sureshkumar Balasubramanian, Janne Lempe, Sridevi Sureshkumar, Chris Schwartz, Joanne Chory, Detlef Weigel

T01-067 Using microarrays to identify genes implicated in pollen and anther development

Gema Vizcay-Barrena, Zoe Wilson

T01-068 Developmental regulation of transcription factor genes in Arabidopsis seeds

Anna Blacha, Armin Schlereth, Tomasz Czechowski, Yves Gibon, Mark Stitt, Wolf-Rüdiger Scheible, Michael Udvardi

T01-069 DAG1 and DAG2: two Arabidopsis transcription factors that play a maternal role in controlling seed germination

Julie Martone, Matteo Berretti, Stefano Gabriele, Gianluca Ragone, Paolo Costantino, Paola Vittorioso

T01-070 Antagonistic role of the bZIP transcription factors FD and FDP in controlling flowering and plant architecture

Philip A. Wigge, Min Chul Kim, Detlef Weigel

T01-071 Regulation of the circadian expression of GI

Cremer Frédéric, Coupland George

T01-072 Identification and analysis of genes mediating the vernalization response

Nuno Geraldo, Joshua S. Mylne, Thomas Greb, Clare Lister, Caroline Dean

T01-073 Characterization of The vanguard1 Mutant That Is Involved in Stabilization and Growth of Pollen Tube in Arabidopsis

Lixi Jiang, Shulan Yang, Li-Fen Xie, Ching San Puah, Wei-Cai Yang, Venkatesan Sundaresan, De Ye (Author of Correspondance))

T01-074 Analysis of flowering time in Arabidopsis and Lolium by micro-array analysis and heterologous overexpression

Stefano Ciannamea, Jacqueline Busscher-Lange, Richard Immink, Claus.H.Andersen, Gerco Angenent

T01-075 Cloning of ISE1 gene, which regulates plasmodesmatal function during embryogenesis in Arabidopsis

Insoon Kim, Michael Mindrionos, Marisa Otegui, Katrina Crawford, Euna Cho, Fred Hempel, Patricia Zambryski

T01-076 Regulation of egg cell identity in the female gametophyte

Rita Gross-Hardt, James M. Moore, Wendy B. Gagliano, Ueli Grossniklaus

T01-077 Functional analysis of SBP box gene SPL8

Yan Zhang, Peter Huijser

T01-078 Investigating the role of GABA in pollen tube growth and guidance in Arabidopsis

Emily Updegraff, Daphne Preuss

T01-079 Regulation of flowering time by gibberellins

Sven Eriksson, Henrik Böhlenius, Ove Nilsson

T01-080 Identification of enhancers of elf3-7 through activation tagging

Karen A. Hicks, Amy E. Aloe, Adam J. Booth

T01-081 Characterization and mapping of photoperiod-sensitive suppressors of elf3-1

Kathryn E. Lynd, Karen A. Hicks

T01-082 EMB Genes of Arabidopsis with Unknown Cellular Functions

Rosanna Pena-Muralla, Rebecca Rogers, David Meinke

T01-083 Identification of Genes Required for Embryo Development in Arabidopsis

Iris Tzafrir, Allan Dickerman, Colleen Sweeney, Steven Hutchens, Sandrine Casanova, Amy Fesler, Clay Holley, John McElver, George Aux, David Patton, David Meinke

T01-084 Disruption of abh1, the Arabidopsis mRNA cap binding protein, causes early flower development by affecting the transcript abundance of photoperiod and vernalization pathway regulators.

Josef M. Kuhn, Julian I. Schroeder

T01-085 Patterns of Gene Expression during Arabidopsis Flower Development

Frank Wellmer, Marcio Alves-Ferreira, Annick Dubois, Jose Luis Riechmann, Elliot M. Meyerowitz

T01-086 Novel developmental mutants of Arabidopsis thaliana

Mirza, J. I.

T01-087 Cell separation in Arabidopsis flowers and fruit

Sarah Liljegren, Adrienne Roeder, Lalitree Darnielle, Ji-Young Youn, Joseph Ecker, Martin Yanofsky

T01-088 A mutation in the TILTED1 locus uncovers the interplay of cell division and patterning during embryogenesis in Arabidopsis

Pablo D. Jenik, Rebecca E. Joy, M. Kathryn Barton

T01-089 LOV1 is a floral repressor that negatively regulates CO in Arabidopsis

So Yeon Yoo, Yunhee Kim, Jong Seob Lee, Ji Hoon Ahn

T01-090 Annual plant for a perennial problem

Eric Walton, Roger Hellens, Rong Mei Wu

T01-091 Functional analysis of a phosphatidic acid in ABA signaling during germination

Takeshi Katagiri, Masatomo Kabayashi, Kazuo Shinozaki

T01-092 Analysis of sepal and petal development using fl51 mutant of Arabidopsis

Noriyoshi Yagi, Seiji Takeda, Ryuji Tsugeki, Kiyotaka Okada

T01-093 SHI family genes redundantly regulate gynoecium and leaf development in Arabidopsis

Sandra Kuusk, Joel Sohlberg, Mattias Myrenås, Magnus Eklund, Eva Sundberg

T01-094 **Mutations in the Arabidopsis FLAKY POLLEN gene cause both sporophytic and gametophytic male sterility**
Sumie Ishiguro, Miho Yamada, Yuka Nishimori, Kiyotaka Okada, Kenzo Nakamura

T01-095 **Identification and Characterisation of Genes that Control Petal**
Higginson, T., Szecsi, J., Bordji, K., Vergne, P., Hugueney, P., Dumas, C., Bendahmane, M.

T01-096 **The transcript profile of cytoplasmic male sterile Brassica napus**
Jenny Carlsson, Matti Leino, Rita Teixeira, Ulf Lagercrantz, Kristina Glimelius

T01-097 **The Arabidopsis formin AtFH5 is a potential effector of Polycomb group activity in endosperm polarity**
Jonathan N. Fitz Gerald, Mathieu Ingouff, Christophe Guérin, Hélène Robert, Mikael Blom Sørensen, Laurent Blanchoin, Frédéric Berger

T01-098 **Establishment of fruit patterning in Arabidopsis**
Jose R. Dinneny, Detlef Weigel, Martin F. Yanofsky

T01-099 **Graft transmission of floral signalling in Arabidopsis is dependent on long-distance action of genes in the photoperiod pathway**
Colin Turnbull, Samuel Justin

T01-100 **The Genetic and Molecular Network of SOC1 for Flowering in Arabidopsis**
Horim Lee, Jihyun Moon, Ilha Lee

T01-101 **Interaction of Polycomb-group proteins controlling flowering in Arabidopsis**
Yindee Chanvivattana, Anthony Bishopp, Daniel Schubert, Christine Stock, Yong Hwan Moon, Renee Sung, Justin Goodrich

T01-102 **Isolation of novel mutants defective in pollen tube growth**
Ulrich Klahre, Benedikt Kost

T01-103 **Components of the Arabidopsis autonomous floral promotion pathway, FCA and FY, are conserved in grasses**
Somrutai Winichayakul, Nicola Beswick, Gregory Bryan and Richard Macknight

T02 Development 2 (Shoot, Root)

T02-001 **Root Hair Tip Growth Requires the Arabidopsis COW1 Gene which Encodes a Phosphatidyl Inositol Transfer Protein**
Karen Böhme, Yong Li, Florence Charlot, Claire Grierson, Katia Marrocco, Kyotaka Okada, Michel Laloue, Fabien Nogué

T02-002 **Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root**
Céline F. Mouchel, Georgette C. Briggs, Christian S. Hardtke

T02-003 **Arabidopsis auxin influx proteins AUX1 and LAX3: a tale of two carriers**
Ranjan Swarup, Ilda Casimiro, Kamal Swarup, Vanessa Calvo, Malcolm J. Bennett

T02-004 **Length and width: Both cell proliferation and cell elongation are controlled in a polar-dependent manner in a leaf, two-dimensional and determinate organ.**
Hirokazu Tsukaya

T02-005 **Leaf Vascular Patterning Mutants**
Jalean Petricka

T02-006 **ANGUSTIFOLIA3 encodes a homolog of synovial sarcoma translocation protein and mediates local cell proliferation for lateral expansion of leaf blade in Arabidopsis thaliana**
Gorou Horiguchi, Gyung-Tae Kim, Hirokazu Tsukaya

T02-007 **A novel class of Arabidopsis response regulator genes, the ectopic expression of which results in phenocopy of the wol cytokinin-receptor deficient mutant**
Takatoshi Kiba, Koh Aoki, Hitoshi Sakakibara, Takeshi Mizuno

T02-008 **Localization and activity of the embryo pattern regulator BODENLOS**
Alexandra Schlereth, Jasmin Ehrismann, Dolf Weijers, Gerd Jürgens

T02-009 **Shoot stem cells: not naive at all**
Jean-Luc Gallois, Fabiana Nora, Robert Sablowski

T02-010 **Rice Lectin-Receptor Kinase (OsLRK) senses galactose and plays a role in root development.**
Kolesnik Tatiana, Bhalla Ritu, Ramamoorthy Rengasamy, Ramachandran Srinivasan

T02-011 **A model of Arabidopsis leaf development**
Sarah Cookson, Christine Granier

T02-012 **Synergistic interaction of ERECTA-family receptor-like kinases regulate cell proliferation, patterning, and organ growth**
Keiko U. Torii, Chris T. Berthiaume, Emi J. Hill, Lynn J. Pillitteri, Elena D. Shpak

T02-013 **The NAC gene family in Zea mays: evidence for the conservation of NAM- and CUC-like functions during SAM development in monocots**
Zimmermann, Roman, Werr, Wolfgang

T02-014 **The DP-E2F-like DEL1 gene is a suppressor of the endocycle in Arabidopsis thaliana**
Kobe Vlieghe, Véronique boudolf, Gerrit Beemster, Sara Maes, Zoltan Magyar, Ana Atanassova, Janice de Almeida Engler, Dirk Inzé, Lieven De Veylder

T02-015 **Micro-RNA targeted TCP genes are regulated at several levels**
Carla Schommer, Javier F. Palatnik, Pilar Cubas, Detlef Weigel

T02-016 **The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis**
Claudia Hass, Jens Lohrmann, Florian Hummel, Sang Dong Yoo, Ildoo Hwang, Tong Zhu, Klaus Harter

T02-017 **Does the universally conserved eukaryotic release factor 1 have an additional function in Arabidopsis?**
Katherine Petsch, Dr Richard Moyle, Dr Jimmy Botella

T02-018 **Identification and Characterisation of an AHP1-interacting protein from Arabidopsis thaliana**
Grefen, Christopher, Bäurle, Isabel, Horak, Jakub, Harter, Klaus

T02-019 **Modulation of light (phytochrome B) signal transduction by the response regulator ARR4**
Gabi Fiene, Virtudes Mira-Rodado, Uta Sweere, Eberhard Schäfer, Klaus Harter

T02-020 **Growth control of the Arabidopsis root meristem by Cytokinin.**
Raffaele Dello Ioio, Alessandro Busetti, Paolo Costantino, Sabrina Sabatini

T02-021 **Plant organ growth involves the chromatin modifying complex Elongator**
Delphine Herve-Fleury, Hilde Nelissen, Leonardo Bruno, Dirk Inze, Mieke Van Lijsebettens

T02-022 **Comparitive protein profiling: Effects of ethylene and cytokinin on the proteome of Arabidopsis**
Naomi Etheridge, Scott Peck, G. Eric Schaller

T02-023 **FIC, a Factor Interacting with CPC, as a Putative Partner for Cell-to-Cell Movement**
Tetsuya Kurata, Masahiro Noguchi, Kiyotaka Okada, Takuji Wada

T02-024 **Role of sterols in the integration of shoot and root meristem function**
Keith Lindsey, Margaret Pullen, Jennifer Topping

T02-025 **ROOT HAIR PATTERNING AND THE REGULATION OF ROOT HAIR-SPECIFIC GENES**
Hyung-Taeg Cho, Sang Ho Lee, Dong Wook Kim

T02-026 **The AtMYB11 gene is a possible regulator of development in Arabidopsis thaliana**
K. Petroni, V. Calvenzani, D. Allegra, G. Falasca, MM. Altamura, C. Tonelli

T02-027 **HORMONAL MEDIATION OF KNOX ACTIVITY IN THE DISSECTED LEAF OF TOMATO**
Sophie Jasinski, Angela Hay, Hardip Kaur, Jean-Michel Davière, Andrew Phillips, Peter Hedden, Miltos Tsiantis

T02-028 **THE RKS FAMILY OF TRANSMEMBRANE RECEPTOR KINASES**
Ingrid Roxrud, Hilde-Gunn Opsahl Sorteberg, Ed D.L. Schmidt

T02-029 **Role of the UGF protein family during Arabidopsis thaliana development**
Vanessa Wahl, Tanja Weinand, Markus Schmid

T02-030 **CYTOKININ INDEPENDENT 2 (CKI2), a putative receptor histidine kinase of Arabidopsis**
Robert Meister, Shoba Sivasankar

T02-031 **A suppressor screening of jaw-D, a microRNA overexpressing mutant**
Heike Wollmann, Javier Palatnik, Detlef Weigel

T02-032 **CUC2 and CUC3 are involved in axillary meristem formation and post-embryonic organ separation.**
Ken-ichir Hibara, Masao Tasaka

T02-033 **A new GAL4-based activation tagging system for Arabidopsis root developmental study**
Keiji Nakajima, Takashi Hashimoto

T02-034 **Analysis of pale-green mutant apg6 using Ac/Ds transposon system in Arabidopsis.**
Fumiyoshi Myouga, Reiko Motohashi, Takashi Kuromori, Noriko Nagata, Kazuo Shinozaki

T02-035 **ead1, an orthologue of a human oncogene, is required for ethylene and auxin responses in Arabidopsis.**
Anna N. Stepanova, Jose M. Alonso

T02-036 **Understanding the Molecular Mechanism of TFL1**
LUCIO CONTI, YOSHIE HANZAWA, TRACY MONEY, OLIVER RATCLIFFE, DESMOND BRADLEY

T02-037 **Transcriptome analysis reveals an alternative mechanism for habituation**
Melissa S. Pischke, Edward L. Huttlin, Adrian D. Hegeman, Michael R. Sussman

T02-038 **Molecular genetic analysis of three bHLH genes involved in root hair and trichome differentiation**
Ryosuke Sano, Ryoko Nagasaka, Kayoko Inoue, Yumiko Shirano, Hiroaki Hayashi, Daisuke Shibata, Shusei Sato, Tomohiko Kato, Satoshi Tabata, Kiyotaka Okada, Takuji Wada

T02-039 **“Overexpression of CDK inhibitors at the SHOOTMERISTEMLESS domain causes precocious exit of cell cycle and affects morphogenesis in Arabidopsis”**
Carmem-Lara de O. Manes, Tom Beeckman, Juan Antonio Torres, Mirande Naudts, Jan Traas, Dirk Inzé, Lieven De Veylder

T02-040 **Identification and functional characterization of brassinosteroid-responsive genes**
Carsten Müssig, Danahe Coll-Garcia, Thomas Altmann

T02-041 **Functional analysis of the CLE40 signal in Arabidopsis root meristem development**
Yvonne Stahl, Rüdiger Simon

T02-042 **Genetic analysis of the SCABRA and RUGOSA genes**
Hricova, Andrea, Quesada, Victor, Micol, Jose Luis

T02-043 **Analysis of the distribution of Arabidopsis thaliana amidase1, an enzyme capable of forming indole-3-acetic acid from indole-3-acetamide.**
Tina Schäfer, Elmar W. Weiler, Stephan Pollmann

T02-044 **Altered Cytokinin Sensitivity 1 (AtACS1) encodes a cytokinin-binding protein involved in cytokinin perception**
Christopher G. Wilkins, David E. Hanke, Beverley J. Glover

T02-045 **Interactions between Lateral Organ Boundary gene family members (LBDs) and KNOX genes : new clues from the analysis of the lollo mutant**
Lorenzo Borghi, Silke Winters, Rüdiger Simon

T02-046 **Characterization of A and B-type cyclins in Arabidopsis**
J. Foreman, P. Doerner

T02-047 **At1g36390 is highly conserved, and may play a role in shoot development**
Horvath

T02-048 **Regulation of Lateral Root Formation by SLR/IAA14, ARFs, and Chromatin Remodeling Factor, SSL2/CHR6**
Hidehiro Fukaki, Yoko Okushima, Ryusuke Iida, Yoko Nakao, Naohide Taniguchi, Athanasios Theologis, Masao Tasaka

T02-049 **Effectors of SHOOTMERISTEMLESS function.**
Wei-Hsin Chiu, John Chandler, Wolfgang Werr

T02-050 **KNAT3 and KNAT4: two KNOX genes control aspects of plant development and are active in the shoot apical meristem.**
John Chandler, Wolfgang Werr

T02-051 **CYTOKININ RESPONSE GENES OF ARABIDOPSIS IDENTIFIED BY GENOME-WIDE TRANSCRIPTOME ANALYSIS REVEAL NOVEL CYTOKININ-SENSITIVE PROCESSES**
Wolfram Brenner, Georgy Romanov, Lukas Bürkle, Thomas Schmülling

T02-052 **DORNRÖSCHEN/ESR1 is putatively involved auxin-regulated embryonic development and interacts with PHAVOLUTA.**
John Chandler, Melanie Cole, Britta Grewe, Annegret Flier, Wolfgang Werr

T02-053 **Homeodomain Interactions at the Shoot Apex**
J. Peter Etchells, Anuj M. Bhatt, Joanne L. Dowding, Hugh G. Dickinson

T02-054 **A family of single MYB domain proteins redundantly inhibits trichome initiation on the epidermis of shoot organs**
Victor Kirik, Daniel Bouyer, Marissa Simon, John Schiefelbein, Martin Hülskamp

T02-055 **Vascular bundle differentiation in stems of the auxin mutants pin1, pinoid and monopteros**
Stieger Pia A

T02-056 **Phenotypical analysis of the cytokinin receptor mutants ahk2, ahk3 and ahk4 unveils partially redundant functions in shoot and root development**
Michael Riefler, Thomas Schmülling

T02-057 Genetic and biochemical evidence for the function of phospholipase A in auxin signal transduction

Scherer, Günther FE, Holk, André, Rietz, Steffen, Oppermann, Esther

T02-058 Mutations in the RETICULATA gene strongly modify internal architecture but not organ shape in vegetative leaves

Quesada, Victor, Kinsman, Elizabeth A., González-Bayón, Rebeca, Ponce, María Rosa, Pyke, Kevin A., Micol, José Luis

T02-059 ROT3 and ROT3 homolog, which fine-tune the biosynthesis of brassinosteroids in Arabidopsis, play critical roles in plant morphogenesis

Gyung-Tae Kim, Hoonsung Choi, Shozo Fujioka, Toshiaki Kozuka, Suguru Takatsuto, Frans E. Tax, Shigeo Yoshida, Hirokazu Tsukaya

T02-060 Identification of trichome specific promoter regions of GLABRA1 and TRIPTYCHON

Martina Pesch, Martin Hülskamp

T02-061 Non-cell autonomous action of TTG1 during trichome pattern formation

Daniel Bouyer, Arp Schnittger, Martin Hülskamp

T02-062 Cytokinin Regulated Transcription Factors

Aaron M. Rashotte, Joseph J. Kieber

T02-063 The cell-autonomous ANGUSTIFOLIA-gene regulates organ size and form in a non-cell-autonomous way

Stefanie Falk, Arp Schnittger, Elena Galiana Jaime, Martin Hülskamp

T02-064 Totipotency of pericycle cells in Arabidopsis thaliana root and hypocotyl explants for both root and shoot regeneration

R. Atta, A. Guivarc'h, L. Laurens, J. Traas, V. Giraudat-Pautot, D. Chriqui

T02-065 Role of CHAYOTE in root hair development and epidermal cell patterning

Olga Ortega-Martínez, Paul Linstead, Rachel Carol, Liam Dolan

T02-066 GONZO1 regulates leaf polarity in Arabidopsis

Michael R. Smith, Scott Poethig

T02-067 TYPE-B RESPONSE REGULATORS FUNCTIONALLY OVERLAP IN THE REGULATION OF CYTOKININ RESPONSES

Michael Mason, Dennis Matthews, Eric Schaller

T02-068 Analysis of TRANSPARENT TESTA GLABRA2 involved in trichome differentiation

Tetsuya Ishida, Sayoko Hattori, Kiyotaka Okada, Takuji Wada

T02-069 Arabidopsis BROS is involved in cell expansion-related organ development

Yuxin Hu, Huay Mei Poh, Nam-Hai Chua

T02-070 Quantitative Trait Loci for Root Architecture in Arabidopsis

Jennifer A Saleeba

T02-071 Diverse activities of Mei2-like RNA binding protein genes

Nena Alvarez, Garrett H. Anderson, Suzanne Lambie, Vernon Trainor, Maureen R. Hanson, Bruce Veit

T02-072 An Arabidopsis dwf8 mutant displays pleiotropic phenotypes that may or may not be associated with typical brassinosteroid dwarf mutants

Hyun-Kyung Lee, Ki-hong Song, Shozo Fujioka, Suguru Takatsuto, Shigeo Yoshida, Sunghwa Choe

T02-073 RHD6-like transcription factors involved in root hair development

Benoît Menand, Stéphane Jouannic, Eoin Ryan, Paul Linstead, Liam Dolan

T02-074 Isolation and characterization of gulliver mutants that are defective in the light and brassinosteroid signaling pathways

Mi Kwon, Su Youn Jang, Jun Ho Ko, Sungwha Choe

T02-075 Large-scale analysis of nuclear-encoded chloroplast proteins using Ac/Ds transposon system in Arabidopsis.

Reiko Motohashi, Fumiyoshi Myouga, Mieko Higuchi, Kinntake Sonoike, Noriko Nagata, Takuya Ito, Takashi Kuromori, Kazuo Shinozaki

T02-076 STRUBBELIG defines a novel receptor-mediated signaling pathway regulating meristem development in Arabidopsis

David Chevalier, Martine Batoux, Lynette Fulton, Ram Kishor Yadav, Kay Schneitz

T02-077 Axillary bud growth: one pathway or many?

Barbara Willett, Ottoline Leyser

T02-078 Discrete heterodimers direct nuclear import of the transcription factor SHOOT MERISTEMLESS in the shoot apical meristem of Arabidopsis

Melanie Cole, Wolfgang Werr

T02-079 Biological function studies of RHD6-like transcription factors involved in root hair development

Laurent Hoffmann, Benoît Menand, Paul Linstead, Liam Dolan

T02-080 Genetic analysis of regulators of axillary meristem initiation

Smita Raman, Silke Schulze, Oliver Clarenz, Thomas Greb, Klaus Theres

T02-081 FEZ and SMB encode two-plant specific transcription factors required for the orientation of cell division and cell specification in the Arabidopsis root cap.

Ana Campilho, Marion Bauch, Harald Wolkenfelt, Jim Haseloff, Ben Scheres

T02-082 VND7, a NAC-domain protein regulates xylem vessel formation in Arabidopsis

Minoru Kubo, Masatoshi Yamaguchi, Hiroo Fukuda, Taku Demura

T02-083 Isolation and characterization of the genes interacting with VND7 (Vascular-related NAC Domain Protein 7)

Masatoshi Yamaguchi, Minoru Kubo, Hiroo Fukuda, Taku Demura

T02-084 Identifying Targets of Inducible PLETHORA Root Identity Genes

Marijn Luijten, Ben Scheres, Renze Heidstra

T02-085 The HEMIVENATA gene encodes a TIP120 (CAND1) protein and is required for venation pattern formation

M. M. Alonso-Peral, H. Candela, J. C. del Pozo, M. R. Ponce, J. L. Micol

T02-086 Mutual antagonism between SHOOT MERISTEMLESS (STM) and (YABBY3)

Fabiana R. Nora, Robert Sablowski

T02-087 Regulation of LATERAL SUPPRESSOR ¯ a gene involved in the formation of axillary meristems

Andrea Eicker, Thomas Greb, Klaus Theres

T02-088 Genetic analysis of procambial development in the Arabidopsis root

Annelie Carlsbecker, Ove Lindgren, Martin Bonke, Siripong Thitamadee, Sari Tähtiharju, Ykä Helariutta

T02-089 Altered meristem patterning and hormone signaling in the cellulose deficient tsd1 (tumorous shoot development1) mutant, an allele of the KORRIGAN1 endo-1,4-β-glucanase

Eva Krupková, Markus Pauly, Thomas Schmülling

T02-090 Identification of permeable leaves mutants that exhibit surface defects in leaves using a new method

Hirokazu Tanaka, Toshihiro Tanaka, Chiyoko Machida, Masaru Watanabe, Yasunori Machida

T02-091 Isolation of two novel putative effectors of polar auxin transport in Arabidopsis thaliana

Nenad Malenica, Christian Luschnig

T02-092 Modulation of GA biosynthesis by other plant hormones in Arabidopsis thaliana

Jose Perez-Gomez, Ana M. Vidal-Rico, Nicholas Clark, Omar J. Ruiz-Rivero, Lindsey Woolley, Jeremy P. Coles, Andrew L. Phillips, Peter Hedden

T02-093 rol mutations suppress the root hair cell wall formation mutant Irx1

Anouck Diet, Nicolas Baumberger, Beat Keller, Christoph Ringli

T02-094 Investigating the roles of the Arabidopsis MAX genes in shoot branching control

Kath Bainbridge, Ottoline Leyser

T02-095 PLETHORA1 and PLETHORA2 are involved in the formation and maintenance of Arabidopsis root stem cells

Mitsuhiro Aida, Dimitris Beis, Renze Heidstra, Viola Willemsen, Ikram Blilou, Laurent Nussaume, Yoo-Sun Noh, Richard Amasino, Ben Scheres

T02-096 Dynamic growth maps modelling for Arabidopsis leaves

Bensmihen, S., Bangham, J.A., Coen, E.

T02-097 MGOUN3, AN ARABIDOPSIS GENE WITH PROTEIN INTERACTION MOTIFS IS ASSOCIATED WITH MERISTEM ORGANIZATION AND REGULATION OF FLOWERING TIME

GUYOMARC'H S., ZHOU D.-X., DELARUE M.

T02-098 Analysis of the transcriptional regulation of cell specialisation during leaf development in Arabidopsis thaliana

Dajana Lobbes, Cathie Martin, Jonathan Clarke

T02-099 Regulatory Mechanisms in Shoot and Root Development

Jennifer C. Fletcher, Leor Williams, Stephen P. Grigg, Mingtang Xie, Sioux Christensen

T02-100 Comparative chloroplast proteomics of a cpSRP54 deletion mutant in Arabidopsis thaliana

Heidi Rutschow, Jimmy Ytterberg, Robert Nilsson, Klaas J. van Wijk

T02-101 Identification and characterization of mutations suppressing the wol mutation in the CRE1/WOL cytokinin receptor gene

Ari Pekka Mähönen, Ykä Helariutta

T02-102 A genetic interaction analysis of incurvata mutants identifies microRNA targets and microRNA machinery elements

P. Robles, S. Jover-Gil, H. Candela, J. M. Barrero, J. L. Micol, M. R. Ponce

T02-103 A mutational analysis of the ABA1 gene of Arabidopsis thaliana highlights the involvement of ABA in vegetative development

J. M. Barrero, P. Piqueras, M. González-Guzmán, R. Serrano, P. L. Rodríguez, M. R. Ponce, J. L. Micol

T02-104 Regeneration of shoots through the action of ESR genes

Yoshihisa Ikeda, Stephen H. Howell, Nam-Hai Chua

T02-105 The INCURVATA4 gene encodes the ATHB-15 transcription factor and is probably regulated by a microRNA

I. Ochando, S. Jover-Gil, J. J. Ripoll, H. Candela, A. Vera, M. R. Ponce, A. Martínez-Laborda, J. L. Micol

T02-106 SCHIZORIZA is required for root patterning in Arabidopsis

Monica Pernas, Eoin Ryan, Panaglyota Mylona, Paul Linstead, Liam Dolan

T02-107 Characterization of cell type specific transcription factors and their regulatory network in Arabidopsis roots

Ji-Young Lee, Juliette Colinas, Kenneth D. Birnbaum, Philip N. Benfey

T02-108 Radial Patterning in Arabidopsis: Networks and movement

Philip N. Benfey, Ken Birnbaum, Kim Gallagher, JiYoung Lee, HongChang Cui, Alice Paquette, Teva Vernoux, Mitch Levesque

T02-109 Root hair development in adaptation to Fe and P deficiency

Margarete Mueller, Wolfgang Schmidt, Bettina Linke, Thomas J. Buckhout

T02-110 Expression of the bHLH genes GL3 and EGL3 during cell fate specification in the Arabidopsis root epidermis

Christine Bernhardt, Myeong Min Lee, Antonio Gonzalez, Fan Zhang, Alan Lloyd, John Schiefelbein

T02-111 The cyclophilin AtCyp95 regulates root system development

Karen Deak, Jocelyn Malamy

T02-112 TCP transcription factors control cell division and differentiation in patterning of organ development.

Tomotsugu Koyama, Keiichiro Hiratsu, Masaru Ohme-Takagi

T02-113 In planta functions of the Arabidopsis cytokinin receptor family

Masayuki Higuchi*, Melissa S. Pischke*, Ari Pekka Mähönen, Kaori Miyawaki, Yukari Hashimoto, Motoaki Seki, Masatomo Kobayashi, Kazuo Shinozaki, Tomohiko Kato, Satoshi Tabata, Ykä Helariutta, Michael R. Sussman, Tatsuo Kakimoto

T02-114 Isolation and analyses of a thick-leaved mutant N692

Noriyuki N. Narita, Gorou Horiguchi, Hirokazu Tsukaya

T02-115 Genetic Analysis of Vascular Development in Arabidopsis

Ryuji Tsugeki, Yoshinori Sumi, Nozomi Maruyama, Kiyotaka Okada

T02-116 Transcription profiling with the Complete Arabidopsis Trancriptome Microarray (CATMA): analysis of cell elongation in the hypocotyl.

Renou Jean Pierre, Pelletier Sandra, Lemonnier Gaëtan, Martin-Magniette Marie-Laure, Taconnat Ludivine, Bitton Frédérique, Vernhettes Samantha, Caboche Michel, Höfte Herman

T02-117 Study of miRNA targeting

Enrique Cortes-Valle, David Brice, David Baulcombe

T02-118 Redundant PIN gene activity as a major control mechanism in patterning and cell division in Arabidopsis root development.

Ikram Blilou, Marjolein Wildwater, Viola Willemsen, Ivan Papanov, Jiri Friml, Renze Heidstra, klaus Palme, Ben Scheres

T02-119 AtRaptor and meristem activity

Garrett H. Anderson, Maureen R. Hanson

T02-120 Profiling Primary Auxin Responses and Transcriptional Regulation Mediated by AXR1 and SCFTIR1 Functions

Keithanne Mockaitis, Sunethra Dharmasiri, Nihal Dharmasiri, Mark Estelle

T02-121 GRAS Proteins involved in a variety of developmental processes

Petra Ziemer, Cordelia Bolle

T03 Cell Biology

T03-001 Mitochondrial Biogenesis in Arabidopsis

Ryan Lister, May-Nee Lee, Monika Murcha, Orinda Chew, Rachel Clifton, Joshua Heazlewood, A. Harvey Millar, James Whelan

T03-002 An Arabidopsis Mitochondrial Proteome

Joshua L. Heazlewood, Jim Whelan, A. Harvey Millar

T03-003 Regulation of signaling and membrane dynamics by RAC GTPase in Arabidopsis

Shaul Yalovsky, Daria Bloch, Meirav Lavy, Limor Poraty, Keren Shichrur, Keren Bracha-Drori, Nadav Sorek, Achi Krauz, Hasana Strenberg, Irena Potnov, Einat Sadot

T03-004 A role for ubiquitin in plant cell death

Marcus Garzon, Peter Schlögelhofer, Claudia Kerzendorfer, Andreas Bachmair

T03-005 The N-end rule pathway for protein degradation

Xiao-jun Yin, Marcus Garzon, Andrea Faust, Alexander Yephremov, Andreas Bachmair

T03-006 Comparative and functional genome analysis corroborate the existence of ESCRT (endosomal protein sorting complexes required for transport) in Arabidopsis thaliana

Verena Winter, Sabine Müller, Marie-Theres Hauser

T03-007 Molecular characteristics of REP (Rab Escort Protein) subunit of Rab prenyltransferase from Arabidopsis thaliana

Magdalena Wojtas, Ewa Swiezewska

T03-008 From genomics to cellular dynamics: Dissection of guard cell ABA signal transduction mechanisms

June M. Kwak, Nathalie Leonhardt, Izumi Mori, Miguel A. Torres, Jeff Dangl, Jonathan Jones, Zhen-Ming Pei, Julian I. Schroeder

T03-009 Cellulose biosynthesis and cell elongation

Samantha Vernhettes, Thierry Desprez, Martine Gonneau, Herman Höfte, Michel Juraniec, Stéphanie Robert

T03-010 Uncovering COP9 signalosome-dependent processes in plants through the isolation of new CSN interacting factors

Silvia Iafrate, Paolo Costantino, Xing-Wang Deng, Giovanna Serino

T03-011 The family of conserved glycoproteases from higher plants and bacteria

Kirsten Haußühl, Christian Weiss, Pitter Huesgen, Patrick Dessi, Alexander Böhm, Elisabeth Glaser, Winfried Boos, Iwona Adamska

T03-012 Analysis of RNase Z proteins from Arabidopsis thaliana

Edyta Bocian, Maria Ptak, Anita Marchfelder, Stefan Binder

T03-013 Has the Arabidopsis NAC domain protein ATAF1 a regulatory function within stress and glucose signaling?

Sarah Himbert, Klaus Salchert, László Ökrész, Csaba Koncz, Tatjana Kleinow

T03-014 Changes in local auxin concentrations control valve margin formation in the Arabidopsis fruit

Lars Østergaard, Sarah J. Liljegren, Martin F. Yanofsky

T03-015 Knock-out of the Mg-protoporphyrin IX methyltransferase in Arabidopsis : effects on chloroplast development and chloroplast-to-nucleus signalling.

Dominique Pontier, Catherine Albrieux, Jacques Joyard, Thierry Lagrange, Maryse A. Block

T03-016 Identification and characterization of proteins that interact with an Arabidopsis kinesin

Irene S. Day, Vaka S. Reddy, Tyler Thomas, A.S.N. Reddy

T03-017 PAS1 immunophilin targets a NAC¯like transcription factor to the nucleus during the cell cycle

Smyczynski Cybelle, Vaillant Emilie, Grandjean Olivier1, Masson Thimoté, Bellec Yannick, Jean-Denis Faure

T03-018 Molecular and Functional Characterization of Metacaspases in Arabidopsis

Naohide Watanabe, Eric Lam

T03-019 Characterization of CULLIN3 in Arabidopsis thaliana

Monika Dieterle, Alexis Thomann, Yves Parmentier, Wen-Hui Shen, Thomas Kretsch, Pascal Genschik

T03-020 Mechanisms generating specificity within the Arabidopsis CBL-type calcium sensor protein / CBL interacting protein kinases signaling network

Oliver Batistic, Stefan Weinl, Dragica Blazevic, Cecilia D'Angelo, Jörg Kudla

T03-021 Localization of an ascorbate-reducible cytochrome in the plant tonoplast. Possible involvement in iron metabolism

Dan Griesen, Alajos Berczi, Amy Vargas, Han Asard

T03-022 “Light regulation of cell cycle progression in living plants”

Carmem-Lara de O. Manes, François-Yves Bouget

T03-023 Systematic determination of protein localisation in Arabidopsis cells

Matthew Tomlinson, Olga Koroleva, Peter Shaw, John Doonan

T03-024 Chloroplast division site placement requires dimerisation of the ARC11/ AtMinD1 protein in Arabidopsis

Makoto Fujiwara, Ayako Nakamura, Ryuuichi Itoh, Yukihisa Shimada, Shigeo Yoshida, Simon Geir Møller

T03-025 Genetic dissection of mucilage secretory cell differentiation in Arabidopsis

Andrej Arsovski, Phoenix Bouchard-Kerr, Theodore M. Popma, George W. Haughn, Tamara L. Western

T03-026 GIANT CHLOROPLAST 1 is essential for correct plastid division in Arabidopsis

Jodi Maple, Makoto T. Fujiwara, Nobutaka Kitahata, Tracy Lawson, Neil R. baker, Shigeo Yoshida, Simon Geir Møller

T03-027 AtNAP7 is a plastidic SufC-like ABC/ATPase essential for Arabidopsis embryogenesis

Xiang Ming Xu, Simon Geir Møller

T03-028 Analysis of a putative monoubiquitination-mediated protein degradation pathway in Arabidopsis

Christoph Spitzer, Swen Schellmann, Martin Hülskamp

T03-029 Comprehensive oligo-microarray analysis of gene expression profiles during cell elongation

Shigeru Sato, Nanae Yamada, Shiho Nakamoto, Takashi Hibino

T03-030 A novel CDK-phosphorylation cascade in Arabidopsis thaliana

Akie Shimotohno, Hirofumi Uchimiya, Masaaki Umeda

T03-031 Functional analysis F-box protein family using antisense lines

Hirofumi Kuroda, Akie Ishikawa, Motoaki Seki, Kazuo Shinozaki, Minami Matsui

T03-032 ACTIVATION TAGGING AS A TOOL TO IDENTIFY AND DISSECT A NEW GENE FAMILY MEMBERS AND THEIR FUNCTIONS IN A.THALIANA

Gigolashvili Tamara, Mock Hans-Peter, Fluegge Ulf-Ingo

T03-033 The Arabidopsis co-chaperone ROF2 encodes a new heat-shock induced FKBP immunophilin, which is developmentally regulated.

Keren Aviezer-Hagai, Julia Skovorodnikova, Odelia Farchi-Pisanty, Adina Breiman

T03-034 Regulation of signal transduction by nucleo-cytoplasmic partitioning of proteins in Arabidopsis thaliana

Katja Schmied, Miriam Dewald, Dorothea Haasen, Bernd Weisshaar, Thomas Merkle

T03-035 Identification of SNARE molecules involved in the post-Golgi network pathways in Arabidopsis

Tomohiro Uemura, Takashi Ueda, Akihiko Nakano, Kunio Takeyasu, Masa H. Sato

T03-036 In vitro evolution of telomerase-deficient tissue cultures of Arabidopsis thaliana

Petra Bulankova, Matthew J. Watson, Karel Riha, Dorothy E. Shippen, Boris Vyskot

T03-037 The chloroplast SRP-pathway: Molecular analysis of protein-protein interactions

Silke Funke, Jan C. Pasch, Danja Schünemann

T03-038 DOES AtWNK8 REGULATE THE V-ATPase BY PHOSPHORYLATING VHA-C?

Anne Hermesdorf, Angela Brüx, Karin Schumacher

T03-039 The Arabidopsis KLUNKER gene encodes a putative regulator of the Arp2/3 complex

Moola Mutondo, Ilona Zimmermann, Rainer Saedler, Martin Hülskamp

T03-040 Exocyst complex in plants

Zarsky V, Synek L, Elias M, Moore I, Drdova E, Quentin M, Kakesova H, Ziak D, Hala M, Cvrckova F, Soukupova H

T03-041 Stability of Microtubules Containing Modified Alpha-Tubulin

Tatsuya Abe, Kuniko Naoi, Takashi Hashimoto

T03-042 DISTORTED2 encodes for the Arabidopsis ARPC2 subunit of the ARP2/3 complex

Rainer Saedler, Neeta Mathur, Bhylahalli P. Srinivas, Birgit Kernebeck, Martin Hülskamp, Jaideep Mathur

T03-043 Cellular localization of AtFH8, an Arabidopsis Class I formin

Veronika Mikitova, Viktor Zarsky, Fatima Cvrckova

T03-044 A novel function of cyclin-dependent kinase inhibitors - KRP1/ICK1 can block mitosis and trigger endoreduplication

Christina Weinl, Suzanne Kuijt, Arp Schnittger

T03-045 Identification and Characterisation of the Arabidopsis thaliana DISTORTED Mutant GNARLED

Ilona Zimmermann, Moola Mutondo, Rainer Saedler, Martin Hülskamp

T03-046 Screen for plasmodesmal associated proteins

Marcella B. Pott, Mark Kearley, David Ehrhardt

T03-047 Progression through meiosis I and meiosis II in Arabidopsis anthers is regulated by an A-type cyclin predominately expressed in prophase I

Yixing Wang, Jean-Louis Magnard, Sheila McCormick, Ming Yang

T03-048 Carbon Dioxide-Induced Modulation of Cytosolic Calcium Pattern During CO2 Signal Transduction in Guard Cells.

Julian Schroeder, Erwin Grill, Jared Young

T03-049 Isolation and characterization of Arabidopsis thaliana spiral3 mutant

Masayoshi Nakamura, Yugo Komiya, Takashi Hashimoto

T03-050 Proteomic analysis of glutathione S-transferases of Arabidopsis thaliana reveals differential salicylic acid-induced expression of the plant-specific phi and tau classes

Pia G Sappl, Luis Oñate-Sánchez, Karam B Singh, A Harvey Millar

T03-051 DNA replication licensing affects cell proliferation or endoreplication in a cell type-specific manner

M. Mar Castellano, M. Beatrice Boniotti, Elena Caro, Arp Schnittger, Crisanto Gutierrez

T03-052 A Transcriptomic and Proteomic Characterisation of the Arabidopsis Mitochondrial Protein Import Apparatus and its Response to Mitochondrial Dysfunction

Ryan Lister, Orinda Chew, May-Nee Lee, Joshua L. Heazlewood, Rachel Clifton, Pia Sappl, Karen L. Parker, A. Harvey Millar, James Whelan

T03-053 Global transcription analysis of Arabidopsis core cell cycle regulators in suspension-cultured cells and plants reveals multiple and highly specific profiles of gene expression

Margit Menges, James A.H. Murray

T03-054 **AraPerox: A Database of plant peroxisomal proteins**
Sigrun Reumann, Changle Ma, Steffen Lemke, Lavanya Babujee

T03-055 **Subcellular and functional analyses of regulatory proteins from plant peroxisomes**
Changle Ma, Sigrun Reumann

T03-056 **Endocytosis of the receptor like kinases AtSERK1 and BRI1 in Arabidopsis.**
Mark Kwaaitaal M.Sc., Dr. Eugenia Russinova, Prof. Dr. Sacco C. de Vries

T03-057 **A Proteomic Analysis of Leaf Peroxisomes**
Lavanya Babujee, Franziska Lüder, Virginie Wurtz, Hartmut Kratzin, Sigrun Reumann

T03-058 **Genetic analysis of the AtRabGDI family**
Hana Soukupova, Michal Hala, Lukas Synek, Viktor Zarsky

T03-059 **Regulation and compartmentation of glutathione biosynthetic enzymes**
Andreas Wachter, Thomas Rausch

T03-060 **det3: Life with 50% V-ATPase activity**
Angela Brüx, Matthias Grauer, Karin Schumacher

T03-061 **Isolation of mutants affecting endoreduplication by an enhancer/ suppressor screen of multicellular Arabidopsis trichomes**
Farshad Roodbarkelari, Arp Schnittger

T03-062 **An Arabidopsis mutant that has a defect in organization of endomembranes**
Kentaro Tamura, Tomoo Shimada, Maki Kondo, Mikio Nishimura, Ikuko Hara-Nishimura

T03-063 **CDKA;1 is essential for Arabidopsis embryo and gametophyte development**
Moritz Nowack, Paul Grini, Marcel Lafos, Csaba Koncz, Arp Schnittger

T03-064 **Study of the Arabidopsis ORC subunits during the cell cycle and plant development**
Sara Diaz-Triviño, Mar Castellano, Mari-Paz Sanchez, Crisanto Gutierrez

T03-065 **Function and differentiation of endocytic organelles in Arabidopsis cells**
Takashi Ueda, Tomohiro Uemura, Masa H. Sato, Akihiko Nakano

T03-066 **Genetic analysis of peroxisomal biogenesis and function in Arabidopsis**
Bethany K. Zolman, Melanie Monroe-Augustus, Illeana Silva, Bonnie Bartel

T03-067 **Study of root hair tip growth of Arabidopsis by video-enhanced light microscopy**
Miroslav Ovečka, František Baluška, Irene Lichtscheidl

T03-068 **Role of Heat Stress Granules for mRNP Storage and Decay**
Christian Weber, Markus Fauth

T03-069 **Shaping plant cells using an actin mesh**
JAIDEEP MATHUR

T03-070 **Towards a transcript profiling of Arabidopsis trichomes**
Marc Jakoby, Doris Falkenhahn, Arp Schnittger

T03-071 **Characterization of Arabidopsis mutants defective in the Peroxisomal Targeting Signal receptors PEX7 and PEX5**
Andrew W. Woodward, Bonnie Bartel

T03-072 **Cyclic AMP signaling during the plant cell cycle: Isolation of a putative cyclic nucleotide dependent protein kinase from Arabidopsis thaliana and Nicotiana tabacum BY-2.**
Luc Roef, Carl Van Ingelgem, Lieven De Veylder, Dirk Inzé, Harry Van Onckelen

T03-073 **Mitosis-specific accumulation of PORCINO reveals requirement for de novo synthesis of alpha/beta-tubulin heterodimers in elongating cells**
Katharina Steinborn, Gerd Jürgens, Ulrike Mayer

T03-074 **In vivo role of GNOM dimerisation**
Nadine Anders, Gerd Jürgens

T03-075 **Isolation and characterization of SIAMESE, a putative cell cycle regulator involved in endoreplication**
Michelle Speckhart, Matt Brown, Viktor Kirik, Martin Hülskamp, Dirk Inzé, Lieven De Veylder, John C. Larkin

T03-076 **Developmentally regulated nuclear-envelope targeting in plants.**
Shalaka Patel, Annkatrin Rose, Tea Meulia, Iris Meier

T03-077 **A journey through the plant cell**
Gerd Juergens

T03-078 **Expression of a fungal cellulose-binding domain in Arabidopsis thaliana**
Michaël QUENTIN, Jan DERKSEN, Henry van der VALK

T03-079 **Characterization of the evolutionary conserved F-Box protein FBP7 in Arabidopsis**
Luz Irina A. Calderón V., Carola Kuhnle, Claus Schwechheimer

T03-080 **Functional Analysis of the RING-type Ubiquitin Ligase Family of Arabidopsis**
Edward Kraft, Sophia Stone, Herborg Hauksdottir, Andy Troy, Jill Herschleb, Luis Williams, Judy Callis

T03-081 **Regulated degradation of AUX/IAA proteins through a family of SCF F-box proteins**
Sunethra Dharmasiri, Nihal Dharmasiri, Sutton Mooney, Mark Estelle

T03-082 **AtNAP AND AtPIR ENCODE SUBUNITS OF A PUTATIVE WAVE**
Brembu Tore, Winge Per, Seem Martin, Bones Atle M.

T03-083 **GENETICALLY INDUCED CELL ABLATION PRODUCE MINELESS PLANTS ¯ PLANTS WHERE MYROSIN CELLS HAVE BEEN REMOVED.**
Borgen Birgit H., Thangstad Ole P., Grønseth L, Rossiter John T, Bones Atle M.

T03-084 **The apoplastic alpha-fucosidase of Arabidopsis thaliana (AtFXG1): Phenotypic characterization of a null mutant and of transgenic plants over-expressing AtFXG1**
José Antonio Abelenda, Gloria Revilla, Ignacio Zarra

T03-085 **Trithorax protein ATX1 is essential for actin-based plant tip growth: do actin-driven endosomes provide the link?**
A. Hlavacka, B. Voigt, D. Menzel, D. Volkmann, Z. Avramova, F. Baluska

T03-086 **GFP-FABD2 construct allows in vivo visualization of the actin cytoskeleton in all cell types of Arabidopsis thaliana**
B. Voigt, J. Samaj, F. Baluska, D. Menzel

T04 Interaction with the Environment 1 (Abiotic)

T04-001 **Towards Understanding Changes to Arabidopsis Mitochondrial Function During Abiotic Stress**
A. Harvey Millar, Joshua L. Heazlewood, Orinda Chew, Lee J Sweetlove, Jim Whelan

T04-002 **Functional analysis of Arabidopsis thaliana diacylglycerol kinase 2**
Fernando C. Gómez-Merino, Charles A. Brearley, María Inés Zanor, Bernd Mueller-Roeber

T04-003 **Cellular model for chilling tolerance activated by glycine betaine**
John Einset

T04-004 **A MAP-kinase pathway for cold and salt signalling**
Markus Teige, Elisabeth Scheikl, Thomas Eulgem, Robert Doczi, Kazuya Ichimura, Kazuo Shinozaki, Jeffery L. Dangl, Heribert Hirt

T04-005 **The Basic Helix-Loop-Helix genes involved in iron deficiency responses in Arabidopsis**
Hong-yu Wang, Marc Jakoby, Wim Reidt, Bernd Weisshaar, Helmut Bäumlein, Petra Bauer

T04-006 **Yellow Stripe-Like Family members may be involved in metals homeostasis.**
Adam Schikora, Marie Le Jean, Catherine Cuire, Jean-François briat

T04-007 **Genome Wide RNA Expression and Metabolic Analysis of high light adaptation in wild type, tocopherol minus (vte1), and complemented (pvte-vte1) mutants of Arabidopsis thaliana.**
Sean J Coughlan, Eveline Bergmuller, Marion Kanwischer, Joachim Kopka, Peter Doerman, Edgar B Cahoon

T04-008 **Functional analysis of heat shock induced heat shock factor genes in Arabidopsis thaliana**
Mukesh Kumar, Christian Lohmann, Friedrich Schöffl

T04-009 Transcriptional Regulation of ABA-responsive Genes in Seeds, Germination Stage Plants, and Vegetative Growth Stage Plants.

Kazuo Nakashima, Yasunari Fujita, Koji Katsura, Kyonoshin Maruyama, Motoaki Seki, Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki

T04-010 Genetic and molecular characterisation of a novel Major Facilitator Superfamily protein implicated in zinc homeostasis in Arabidopsis

Michael J. Haydon, Christopher S. Cobbett

T04-011 Signals and signal transduction in the control of nuclear expression of chloroplast antioxidants

Isabelle Heiber, Andrea Pena, Jehad Shaikh Ali, Elke Ströher, Bodo Raatz, Karl-Josef Dietz, Margarete Baier

T04-012 Expression in Multi-Gene Families: Analysis of Chloroplast and Mitochondrial Proteases

Galit Sinvany, Olga Davydov, Giora Ben -Ari, Alexander Raskind, Zach Adam

T04-013 Plant Modulates Its Genome Stability in Response to Stress

Youli Yao, Igor Kovalchuk

T04-014 Role of the zinc finger homeodomain (ZFHD) and NAC transcription factors in drought-inducible expression of the erd1 gene

Lam-Son Phan Tran, Kazuo Nakashima, Yoh Sakuma, Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki

T04-015 Xanthine deyhdrogenase from Arabidopsis thaliana: An old fellow in purine catabolism and a new player in reactive oxygen species metabolism.

Christine Hesberg, Ralf R. Mendel, Florian Bittner

T04-016 RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phot1

Sayaka Inada, Maki Ohgishi, Tomoko Mayama, Kiyotaka Okada, Tatsuya Sakai

T04-017 The Arabidopsis AtMYB60 transcription factor is specifically expressed in guard cells and is involved in the regulation of stomatal movements

Cominelli E, Galbiati M, Conti L, Sala T, Leonhardt N, Vavasseur A, Vuylsteke M, Dellaporta S, Tonelli C

T04-018 The impact of elevated boron on the development of Arabidopsis thaliana

Tomas Kocabek, Stephen Rolfe, Ali Al-Zwi

T04-019 The interacting roles of light regulation and ethylene biosynthesis in modulating hypocotyl gravitropism

Marcia A. Harrison, Justin D. Hogan, John E. Porter

T04-020 Isolation of novel ABA-related mutants using ABA analogs

Takashi Hirayama, Noriyuki Nishimura, Tomo Yoshida, Maki Murayama, Shinpei Hayashi, Takashi Kuromori, Tadao Asami, Kazuo Shinozaki

T04-021 Identification of a new ABA biosynthesis locus, AtABA4, in Arabidopsis thaliana

Helen North, Aurélie De Almeida, Annie Marion-Poll

T04-022 Ethylene responses in Arabidopsis seedlings' roots require a local boost in auxin production.

Anna N. Stepanova, Joyce M. Hoyt, Alexandra A. Hamilton, Jose M. Alonso

T04-023 The calcium sensor CBL1 and its interacting protein kinase CIPK1 mediate osmotic stress responses in Arabidopsis

Cecilia D´Angelo, Stefan Weinl, Joachim Kilian, Oliver Batistic, Jörg Kudla

T04-024 Functional requirements for PIF3 in the de-etiolation process

Bassem Al-Sady, Elena Monte, Rajnish Khanna, James Tepperman, Enamul Huq, Peter H Quail

T04-025 DegP1 Protease in Arabidopsis - Possible Role in Degradation of Oxidatively Damaged Membrane Proteins

Kapri-Pardes, E., Adam, Z.

T04-026 Characteristics of GABI-Kat mutant AnnAt1 annexin (line 327B12)

Gorecka Karolina M., Konopka Dorota, Buszewska Malgorzata E., Hennig Jacek, Pikula Slawomir

T04-027 The ABC of guard cell regulation

Markus Klein, Su Jeoung Suh, Annie Frelet, Enrico Martinoia

T04-028 Expression analysis, characterization of mutants and biochemical studies of selected osmotic stress-responsive members of the aldehyde dehydrogenase (ALDH) gene superfamily in Arabidopsis

Hans-Hubert Kirch, Andrea Ditzer, Simone Schlingensiepen, Simeon Kotchoni, Dorothea Bartels

T04-029 Functional characterization of RCI2A and RCI2B

Ballesteros, Maria L., Medina, J., Salinas, J.

T04-030 Functional Characterisation of Arabidopsis thaliana group I GSK-3/Shaggy-like Kinases

Wilfried Rozhon, Elena Petutschnig, Claudia Jonak

T04-031 Functional Analysis of Arabidopsis Group III and IV GSK-3/shaggy-like Kinases

Elena Petutschnig, Wilfried Rozhon, Claudia Jonak

T04-032 Genetic analysis of zig suppressor 3 suppressing AtVti11 deficiency

Tetsuya Takahashi, Mitsuru Niihama, Miyo Terao Morita, Masao Tasaka

T04-033 The location of QTL for nutrient stress and heavy metal tolerance using Stepped Aligned Inbred Recombinant Strains (STAIRS) in Arabidopsis thaliana.

Ankush Prashar, T. M. Wilkes, J. Pritchard, M. J. Kearsey

T04-034 Characterisation of Integrators of Light Perception to the Circadian Clock

Elsebeth Kolmos, Mark R. Doyle, Andras Viczian, Joachim Uhrig, Richard M. Amasino, Ferenc Nagy, Seth J. Davis

T04-035 Identification of sugar-regulated genes in Arabidopsis thaliana using high-throughput RT-PCR and Affymetrix gene chips.

Daniel Osuna, Rosa Morcuende, Wolf-Rüdiger Scheible, Mark Stitt

T04-036 Arabidopsis thaliana dehydrins ERD 14, LTI 29 and COR 47 protect thylakoid membranes during freezing

Vladan Bozovic, Janne Svensson, Jürgen M. Schmitt, Carsten A. Köhn

T04-037 Characterization of environmentally-controlled protein phosphorylation in photosynthetic membranes of plants by mass spectrometry

Alexander V. Vener, Maria Hansson, Inger Carlberg

T04-038 PP2C type phosphatase regulates stress-activated MAP kinase

Alois Schweighofer, Heribert Hirt, Irute Meskiene

T04-039 Interaction of phosphate- and sugar-sensing in Arabidopsis thaliana

Renate Müller, Lena Nilsson, Tom Hamborg Nielsen

T04-040 Salt stress in Arabidopsis: Characterisation of nhx1 ion transporter mutants

Moez Hanin, Faiçal Brini, Khaled Masmoudi

T04-041 Expression pattern and physiological functions of the Early light-induced proteins (Elips) in Arabidosis thaliana

Marc Christian Rojas Stütz, Iwona Adamska

T04-042 Can differences in carbon distribution within the plant explain responses of root elongation to water deficit : an analysis in Arabidopsis thaliana

S Freixes, M-C Thibaud, M Seguela, B Muller

T04-043 Genetic variability of leaf expansion responses to water deficit in Arabidopsis thaliana.

C. Granier, L. Aguirrezabal, K. Chenu, G. Rolland, S. Bouchier, T. Simonneau, F. Tardieu

T04-044 Hormonal interactions in plant abiotic stress responses

Reetta Ahlfors, Enric Belles-Boix, Mikael Brosche, Dirk Inze, Hannes Kollist, Saara Lång, Kirk Overmyer, Tapio Palva, Pinja Pulkkinen, Airi Tauriainen, Hannele Tuominen, Jaakko Kangasjärvi

T04-045 Locating Sodium Chloride Associated QTL within Arabidopsis Using STAIRS

B.Ranavaya, T.Wilkes, J.Pritchard, M.J.Kearsey

T04-046 Enhanced Heterosis for Biomass Production at Elevated Light Intensities

Rhonda C. Meyer, Martina Becher, Hanna Witucka-Wall, Marianne Popp, Thomas Altmann

T04-047 Analysis of cold-induced gene expression in two Arabidopsis accessions of contrasting freezing tolerance.

Matthew A Hannah, Dirk K Hincha, Arnd G Heyer

T04-048 Characterization of NAC genes that are modulated by hormones that mediate stress response

Giovanna Frugis, Elisabetta Di Giacomo, Adelaide Iannelli, Domenico Mariotti, Nam-Hai Chua

T04-049 The tup5 mutant shows blue light-dependent root growth inhibition and decreased far red light inhibition of seed germination

Nathalie Frémont, Michael Riefler, Thomas Schmülling

T04-050 Phytohormones maintain the circadian clock in Arabidopsis thaliana

Shigeru Hanano, Malgorzata Domagalska, Claudia Birkemeyer, Joachim Kopka, Seth J. Davis

T04-051 PHYTOCHROME-INTERACTING FACTOR 1, a Basic Helix-Loop-Helix Transcription Factor, is a Critical Regulator of the Chlorophyll Biosynthetic Pathway

Enamul Huq, Bassem Al-Sady, Matthew Hudson, Matthew Hudson, Klaus Apel, Peter H. Quail

T04-052 Expression profiling and T-DNA knockout analysis of the Arabidopsis thaliana annexin multigene family

Greg Clark, Sharmistha Barthakur, Araceli Cantero¯Garcia, Stanley J Roux

T04-053 Transcriptional regulation of mitochondrial alternative respiratory pathway genes in response to stress

Rachel Clifton, Ryan Lister, Karen Parker, Dina Elhafez, David Day, James Whelan

T04-054 Characterization of Different Sensitive Mutants to UV-B Radiation from Activation Tagging Lines

Youichi Kondou, Miki Nakazawa, Takanari Ichikawa, Mika Kawashima, Akie Ishikawa, Kumiko Suzuki, Shu Muto, Minami Matsui

T04-055 Arabidopsis MYC(bHLH) and MYB proteins function as transcriptional activators in abscisic acid signaling

Hiroshi ABE, Takeshi URAO, Motoaki SEKI, Takuya ITO, Masatomo KOBAYASHI, Kazuo SHINOZAKI, Kazuko YAMAGUCHI-SHINOZAKI

T04-056 Dynamics of root to shoot signaling of ABA revealed by in vivo imaging of water-stressed Arabidopsis

Christmann, Alexander, Grill, Erwin, Müller, Axel

T04-057 The SPA1 family: WD-repeat proteins with a central role in suppression of photomorphogenesis

Sascha Laubinger, Kirsten Fittinghoff, Ute Hoecker

T04-058 Overexpression of AtMYB90 gene confers the enhancement of salt tolerance

Domenico Allegra, Barbara Marongiu, Chiara Tonelli

T04-059 Localisation of light stress proteins in photosynthetic complexes of Arabidopsis thaliana

Reiser Verena, Norén Hanna, Heddad Mounia, Adamska Iwona

T04-060 Functional analysis of two members of the CHX family of putative sodium transporters in Arabidopsis

H.J. Newbury, D. Hall, J. Pritchard

T04-061 LOW-LIGHT- AND ETHYLENE-INDUCED HYPONASTIC GROWTH IN ARABIDOPSIS THALIANA

F.F Millenaar, M. Cox, L.A.C.J Voesenek, A.J.M. Peeters

T04-062 Functional analysis and subcellular localization of STO in planta

Martin Indorf, Ralf Markus, Gunther Neuhaus, Marta Rodriguez-Franco

T04-063 Nutritional regulation of root architecture by ANR1, a MADS-box transcription factor

S Filleur, BG Forde

T04-064 Differential effect of modifications on polyamine metabolism in salt stress responses.

Enrique Busó, Francisco Marco, María Teresa Collado, Rubén Alcázar, Teresa Altabella, Antonio F. Tiburcio, Pedro Carrasco

T04-065 Genetical genomics of petiole movement

Basten Snoek, Laurentius Voesenek, Anton Peeters

T04-066 Analysis of PTEN-like gene homologues from Arabidopsis thaliana.

Anne PRIBAT, Christophe ROTHAN, Veronique GERMAIN

T04-067 The UV-B response in Arabidopsis involves the bZIP transcription factor HY5

Roman Ulm, Alexander Baumann, Attila Oravecz, Zoltan Mate, Edward Oakeley, Eberhard Schäfer, Ferenc Nagy

T04-068 VITAMIN C IS IMORTANT FOR ACCLIMATION TO AND GROWTH IN HIGH LIGHT

Müller-Moule, Patricia, Golan, Talila, Niyogi, Krishna K.

T04-069 Comparative micro-array analysis of zinc deficiency and zinc excess response of Arabidopsis thaliana and the zinc hyper-accumulator Thlaspi caerulescens

Judith E. van de Mortel, Wilbert van Workum, Henk Schat, Mark G.M. Aarts

T04-070 Genetic analysis of suppressor mutants of shoot gravitropism 2.

Kiyoko Kuramasu, Takehide Kato, Miyo Terao Morita, Masao Tasaka

T04-071 Hormonal regulation on molecular level in Arabidopsis thaliana seedlings under sulphur starvation

C. Birkemeyer, A. Luedemann, V. Nikiforova

T04-072 Raffinose is dispensable in cold acclimation of Arabidopsis thaliana

E Zuther, K Büchel, M Hundertmark, M Stitt, DK Hincha, AG Heyer

T04-073 CHARACTERIZATION OF COL3, A POSITIVE REGULATOR OF LIGHT SIGNALING.

Sourav Datta, Xing-Wang Deng, Magnus Holm

T04-074 Characterization of a novel, RCD1-related gene family

Tiina Kuusela, Jaakko Kangasjärvi

T04-075 Salicylic acid accumulation interferes with excess light acclimation.

Dietmar Funck, Alfonso MAteo, Phil M. Mullineaux, Stanislaw Karpinski

T04-076 Phytochelatin synthase catalyzes key step in turnover of glutathione conjugates

Andreas Beck, Klaus Lendzian, Matjaz Oven, Alexander Christmann, Erwin Grill

T04-077 P-regulated transcription factors in Arabidopsis revealed by comprehensive real-time RT-PCR

Wenming Zheng, Rajendra Bari, Georg Leggewie, Katrin Piepenburg, Michael Udvardi, Wolf-Ruediger Scheible

T04-078 LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy

Alfonso Mateo, Per Mühlenbock, Christine Rustérucci, Chang Chi-Chen, Zbigniew Miszalski, Barbara Karpinska, Jane E. Parker, Philip M. Mullineaux, Stanislaw Karpinski

T04-079 CHARACTERIZATION OF THE PHOSPHATE SIGNAL TRANSDUCTION PATHWAY IN ARABIDOPSIS THALIANA

THIBAUD, misson, nussaume

T04-080 Poly(ADP-ribose) Polymerases (PARPs) in Arabidopsis

Charlene Calvert, Sue Butcher, Mark Coleman

T04-081 AN ARABIDOPSIS THALIANA T-DNA TAGGED MUTANT DEFECTIVE FOR A PEPTIDE TRANSPORTER GENE INDUCED BY WOUNDING AND NaCl

Sazzad Karim, Maria Svensson, Mikael Ejdebäck, Abul Mandal, Dan Lundh, Minna Pirhonen, Kjell-Ove Holmström

T04-082 Roles of the Pseudo Response Regulator genes in the Arabidopsis circadian clock

Patrice A Salomé, C. Robertson McClung

T04-083 Identification of a new root-specific ethylene-insensitive mutant potentially involved in auxin biosynthesis

Joyce M. Hoyt, Anna N. Stepanova, Alexandra A. Hamilton, Jose M Alonso

T04-084 SGR6, a novel protein, is involved in a signaling process of the shoot gravitropism

Daisuke Yano, Miyo Terao Morita, Masao Tasaka

T04-085 Functional Genomics of Abscisic Acid-Insensitive-1-, -3- and -5-Like Gene Families

Srinivas S.L. Gampala, Vijaykumar Veerappan, Mi-Young Kang, Christopher D. Rock

T04-086 Analyses of knockout mutants for the cell wall associated receptor like kinase genes reveal their important roles in Arabidopsis heavy metal responses.

Angela Jackson, Xuewen Hou, Hongyun Tong, Joseph Verica, Lee Chae, Zheng-Hui He

T04-087 A rice calcium binding protein OsCBL1 activates two reversely-regulated protein kinases and affects stress-responsive gene expression in transgenic Arabidopsis

Hee Han, Min-Ju Chae, Ji-Yeon Hong, In-Sun Hwang, Seok-Cheol Suh, In-Sun Yoon

T04-088 Arabidopsis pdr2 Reveals a Phosphate-sensitive Checkpoint in Root Development

Carla Ticconi, Carla Delatorre, Steffen Abel

T04-089 Molecular genetic characterization of SGR5 encoding a zinc-finger protein required for gravitropism of Arabidopsis.

Miyo T. Morita, Shinichiro Kiyose, Takehide Kato, Masao Tasaka

T04-090 Identification and Molecular Characterization of the Arabidopsis Mutants Showing Low Acid Phosphatase Activities under Phosphate-Deficient and Phosphate-Sufficient Conditions

Yongmei Jin, Kunfeng Li, Soyun Won, Minkyun Kim

T04-091 Comparative analysis of ten new alleles of the circadian clock gene ZEITLUPE

Laszlo Kozma-Bognar, Eva Kevei, Peter Gyula, Reka Toth, Balazs Feher, Anthony Hall, Ruth M. Bastow, Megan M. Southern, Victoria Hibberd, Maria M. Eriksson, Seth J. Davis, Shigeru Hanano, Woe-Yeon Kim, David E. Somers, Ferenc Nagy, Andrew J. Millar

T04-092 May Na+ and Cl- accumulation in rosette leaves be compatible with normal growth of Arabidopsis thaliana (accession COL) ?

Hounaïda Attia, Mokhtar Lachaâl, Mokhtar Hajji

T04-093 The model system Arabidopsis halleri: towards an understanding of plant metal homeostasis and metal accumulation

Michael Weber, Aleksandra Trampczynska, Annegret Bährecke, Stephan Clemens

T04-094 salt-induced expression of peroxisoem-associated genes requires components of the ethylene, jasmonate and ABA signalling pathways

W.L.Charlton, K. Matsui, B. Johnson, I.A.Graham, M. Ohme-Takagi, A. Baker

T04-095 Molecular Analysis of Phytochelatin Synthesis: AtPCS2 from Arabidopsis thaliana and the metallophyte Arabidopsis halleri

Pierre Tennstedt, Stephan Clemens

T04-096 Genetic complementation of phytochrome chromophore-deficient hy2 mutant by expression of phycocyanobilin:ferredoxin oxdoreductase in Arabidopsis

Chitose Kami, Keiko Mukougawa, Takuya Muramoto, Naoko Iwata, Akiho Yokota, Tomoko Shinomura, J. Clark Lagarias, Takayuki Kohchi

T04-097 Using Arabidopsis thaliana to progress in modelling plant transpiration under fluctuating environments.

Simonneau T, Lebaudy A, Hosy E, Granier C, Aguirrezabal L, Dauzat M, Rolland G, Sentenac H, Tardieu F

T04-098 **Genetic interaction of growth in leaves regulated by light environment -Light signals oppositely control growth between leaf blade and petiole-**
Toshiaki Kozuka, Gyung-Tae Kim, Gorou Horiguchi, Hirokazu Tsukaya

T04-099 **Effect of NaCl on photosynthesis of two accessions of Arabidopsis thaliana**
Dhouha Saadaoui, Zeineb Ouerghi, Mokhtar Hajji, Mokhtar Lachaâl

T04-100 **Glycine betaine accumulating Arabidopsis thaliana survives strong salt treatment ¯ a cDNA microarray study**
Peter Olsson, Leif Bülow

T04-101 **Characterization of QTL underlying whole-plant physiology in Arabidopsis: delta C13, stomatal conductance, and transpiration efficiency**
Thomas E. Juenger, John McKay, Joost Keurentjes, Jim Richards

T04-102 **A SEMIDOMINANT MUTATION IN A PHOSPHATE TRANSPORTER INCREASED ARSENIC ACCUMULATION IN ARABIDOPSIS THALIANA.**
Pablo Catarecha, María Dolores Segura, Joaquín Iglesias, María Jesús Benito, Javier Paz-Ares, Antonio Leyva

T04-103 **Characterization of sas1: a novel salt, ABA and sugar hypersensitive Arabidopsis mutant.**
Laura Zsigmond, Csaba Koncz, László Szabados

T04-104 **Identification of Potential Substrates of AtCPK11, a Calcium-Dependent Protein Kinase Induced by Drought and Salt Stress in Arabidopsis thaliana**
Miguel A. Rodriguez Milla, Yuichi Uno, Jared Townsend, Eileen Maher, John C. Cushman

T04-105 **Crosstalk and differential response to abiotic and biotic stressors reflected at the transcriptional level of effector genes from secondary metabolism**
Sabine Glombitza, Pierre-Henri Dubuis, Oliver Thulke, Gerhard Welzl, Lucien Bovet, Michael Götz, Matthias Affenzeller, Dieter Ernst, Harald K. Seidlitz, Daniele Werck-Reichhart, Felix Mauch, Tony R. Schaeffner

T04-106 **new insights in the ascorbate glutathione cycle from studies of the dehydroascorbate reductase in Arabidopsis thaliana**
Stefan Kempa, Dirk Steinhauser, Viktoria Nikiforova, Holger Hesse, Joachim Kopka, Rainer Hoefgen

T04-107 **Positional cloning and characterization of the Arabidopsis pho2 mutant**
Rajendra P. Bari, Mark Stitt, Joachim Uhrig, Wolf-Rüdiger Scheible

T04-108 **Potential role of a member of the PHO1 gene family in Pi re-distribution in Arabidopsis**
Aleksandra Stefanovic, C cile Ribot, Yong Wang, Lassaad Belbarhi, Julie Chong, Yves Poirier

T04-109 **SRR1, a gene involved in phyB signalling and circadian clock function.**
Vincent Fiechter, Christian Fankhauser

T04-110 **The Role of GRAS proteins in Phytochrome Signal Transduction**
Patricia Torres-Galea, Cordelia Bolle

T04-111 **Abiotic stress signaling and tolerance**
Jian-Kang Zhu

T04-112 **Role of membrane fluidity in cold perception in Arabidopsis thaliana suspension cells.**
VAULTIER Marie-Noëlle, ZACHOWSKI Alain, RUELLAND Eric

T05 Interaction with the Environment 2 (Biotic)

T05-001 **Immunolocalization of a Fusarium-induced stress associated protein in wheat (Triticum aestivum) root.**
Bhabatosh Mittra, Jibanananda Mishra, Mohmmad Asif, Taspos K. Das, Prasanna Mohanty

T05-002 **Analysis of PMR6: linking an altered cell wall composition with powdery mildew resistance**
Sonja Vorwerk, Shauna Somerville, Chris Somerville

T05-003 **Understanding the molecular mechanisms of the glucosinolate-myrosinase system in plant-aphid interactions**
Carina Barth, Georg Jander

T05-004 **DETERMINATION IN ARABIDOPSIS THALIANA OF PGPR EFFECT, ISR ACTIVITY AND THE POSSIBLE ISR-RESPONDING-WAY IN HIZOBACTERIAS ISOLATED FROM THE ROOTS OF NICOTIANA GLAUCA.**
Domenech, J.

T05-005 **Genomewide transcriptional analysis specifies the Fusarium toxin Zearalenone to interfere with stress responses and cell wall modification in Arabidopsis thaliana**
Ulrike Werner, Gerhard Adam, Marie-Theres Hauser

T05-006 **Identification of membrane-associated and infection-related transcripts of Arabidopsis by microarray analysis of polysomal fractions**
Mark de Jong, Guido Van den Ackerveken

T05-007 **Observations of new colonies on the root surface of Arabidopsis thaliana by Azorhizobium caulinodans**
Taichiro Iki, Hiroshi Oyaizu

T05-008 **Identification of genetic suppressors and enhancers of rar1 in Arabidopsis**
Paul Muskett, Jane Parker

T05-009 **Is Annexin 1 involved in cellular defense against oxidative stress in Arabidopsis?**
Konopka Dorota, Witek Kamil, Bandorowicz-Pikula Joanna, Pikula Slawomir, Hennig Jacek

T05-010 Testing the infectivity and RNA recombination of brome mosaic bromovirus on Arabidopsis gene-knockout lines related to RNA interference/PTGS.

Aleksandra Dzianot, Jozef J. Bujarski

T05-011 Transcriptome analysis of Arabidopsis clubroots and disease resistance of CKX gene overexpressing plants indicate a key role for cytokinin in disease development

Siemens, Johannes, Keller, Ingo, Sarx, Johannes, Kunz, Sabine, Schuller, Astrid, Nagel, Wolfgang, Schmülling, Thomas, Parniske, Martin, Ludwig-Müller, Jutta

T05-012 The jasmonate-insensitive mutant jin1 shows increased resistance to biotrophic as well as necrotrophic pathogens

Susanne Berger, Anja Nickstadt, Bart Thomma, Juergen Zeier, Christiane Loeffler, Ivo Feussner, Jaakko Kangasjaervi, Dierk Scheel

T05-013 Virulent bacterial pathogens induce a pathogen-mediated hypersensitive cell death in pflp-trangenic Arabidopsis

Feng, Teng-Yung, Huang Hsian-En, Ger Mang-Jye

T05-014 The Arabidopsis gene CAD1 controls programmed cell death in the plant innate immune system and encodes a protein containing a MACPF domain.

Chizuko Morita-Yamamuro, Tomokazu Tsutsui, Masanao Sato, Masanori Tamaoki, Daisuke Ogawa, Hideyuki Matsuura, Teruhiko Yoshihara, Yutaka Sonoda, Akira Ikeda, Ichiro Uyeda, Junji Yamaguchi

T05-015 The interaction between AtbZIP10 and LSD1 ¯ a new mechanism for the regulation of pathogen response?

Katia Schütze, Christina Chaban, Hironori Kaminaka, Christian Näke, Jan Dittgen, Jeff Dangl, Klaus Harter

T05-016 Da(1)-12 x Ei-2 Recombinant Inbred Lines: A Tool for Mapping Genes that Control Resistance to Specialist Insect Herbivores

Marina Pfalz, Heiko Vogel, Tom Mitchell-Olds, Juergen Kroymann

T05-017 Regulation of Cell Death in Arabidopsis by the LSD1-Gene Family

Petra Epple, Charles C. Clover, Ben F. Holt III, Hironori Kaminaka, Jeffery L. Dangl

T05-018 Structure / Function Analyses of Pseudomonas syringae Type III Effectors

Darrell Desveaux, Alex U. Singer, Laurie Betts, Jeffrey H. Chang, Zachary Nimchuk, Sarah R. Grant, John Sondek, Jeffery L. Dangl

T05-019 Characterization of AvrPpiB, a P. Syringae type III effector protein that enhances bacterial virulence on Arabidopsis.

Ajay Kumar Goel, Ryan A. Matthews, Sarah R. Grant, Jeffery L. Dangl

T05-020 Dissecting the role of WRKY transcription factors by comparative protein profiling

Janna Brümmer, Bekir Ülker, Lucia Jorda, Hikaru Seki, Imre Somssich

T05-021 Mutations in Arabidopsis RIN4 that affect the virulence of AvrRpm1, AvrB, and AvrRpt2 and R-gene mediated HR.

Han Suk Kim, Darrell Desveaux, Alex Singer, John Sondek, Jeff Dangl

T05-022 Searching For Novel Components Involved In Plant Nonhost Disease Resistance In Arabidopsis

Landtag, Jörn, Westphal, Lore, Lipka, Volker, Dittgen, Jan, Schulze-Lefert, Paul, Scheel, Dierk, Rosahl, Sabine

T05-023 An Arabidopsis mutant with decreased non-host penetration resistance has increased resistance to a host pathogen.

Monica Stein, Bi-Huei Hou, Shauna C Somerville

T05-024 Putative plant molecular target molecules of the pathogenicity protein effector POPP1 secreted by Ralstonia solanacearum.

Laurent Sauviac, Nigel H. Grimsley

T05-025 Reactive Oxygen species (ROS) mediate the IAA-induced ethylene production

Yoon Jung Song, Jung Hee Joo, Yun Soo Bae, June Seung Lee, Kyoung Hee Nam

T05-026 Characterization of two Arabidopsis-Erwinia pathosystems

Mathilde Fagard, Camille Roux, Marie-Anne Barny, Dominique Expert

T05-027 SYNTAXIN REQUIRED FOR PENETRATION RESISTANCE

Hans Thordal-Christensen, Jin-long Qiu, Helge Tippmann, Karen L. Olesen, Farhah Assaad, David Ehrhardt

T05-028 Isolation and Identification of Phosphatidic Acid Targets from Plants

Christa Testerink, Henk L. Dekker, Ze-Yi Lim, Melloney K. Johns, Andrew B. Holmes, Chris G. de Koster, Nicholas T. Ktistakis, Teun Munnik

T05-029 An Arabidopsis Mlo knock-out mutant phenocopies the barley mlo broad spectrum powdery mildew resistance phenotype

Chiara Consonni, H. Andreas Hartmann, Paul Schulze-Lefert, Ralph Panstruga

T05-030 Genetic variation of powdery mildew resistance in Arabidopsis thaliana as a resource for the identification of novel host “compatibility factors”

Katharina Goellner, Ralph Panstruga

T05-031 Arabidopsis, a model plant to study the molecular bases of biological control

Feng Dong Xin, Olivier Jocelyne, Deslandes Laurent, Hu Jian, Trigalet Danièle, Trigalet André, Marco Yves

T05-032 Sulfate-based performance of Arabidopsis thaliana in response to fungal infection

Cordula Kruse, Ricarda Jost, Rüdiger Hell

T05-033 RepA protein from geminivirus alters cell proliferation in Arabidopsis thaliana.

Bénédicte Desvoyes, Elena Ramirez-Parra, Crisanto Gutierrez

T05-034 The Arabidopsis csb3 mutant shows enhanced resistance to biotrophic pathogens

Gil-Morrió, Mª José, Jordá, Lucía, Mauch-Mani, Brigitte, Vera, Pablo

T05-035 Indirect activation of RPS5-mediated resistance by AvrPphB

Catherine Golstein, Jules Ade, Mark Stoutemyer, Roger Innes

T05-036 Analysis of mechanism underlying the inhibition of AvrRpm1/RPM1 functions by AvrRpt2 in the Arabidopsis thaliana

Tack-Min Kwon, Soon-Jae Jeong, Young-Byung Yi, Doh-Hoon Kim, Jaesung Nam

T05-037 Aquaporin genes regulated by dark adaptation and far-red light illumination in roots of Arabidopsis thaliana

Toshifumi Nagata, Kumi Sato-Nara, Atsushi Nagasaka, Hizuru Yamasita, Qiang Sun, Motoaki Seki, Kazuo Shinozaki, Hitoshi Suzuki

T05-038 UTA1 locus encoding AtVDAC1 regulates the competency of Arabidopsis to Agrobacterium-mediated transformation

Yoojin Choi, Soo-Jae Jeong, Young-Byung Yi, Doh-Hoon Kim, Kyung-Hoan Im, Jaesung Nam

T05-039 Expression profiling of the constitutive allene oxide synthase mutant cas1

Schwandt S., Reymond P., Müssig C., Weiler E.W., Kubigsteltig I.

T05-040 TRANSCRIPTION FACTOR Dof15 AS A CANDIDATE GENE REGULATING AUXIN/ INDOLE GLUCOSINOLATE HOMEOSTASIS IN ARABIDOPSIS

Aleksandra Skirycz, Michael Reichelt, Claudia Birkemeyer, Joachim Kopka, Maria Ines Zanor, Jonathan Gershenzon, Jan Szopa, Bernd Mueller-Roeber, Isabell Witt

T05-041 Systematic analysis of cytochromes P450 biotic stress signaling response in A. thaliana

Carole Asnaghi, Frédérique Hilliou, Luc Sofer, Alain Hehn, Simon Goepfer, René Feyereisen, Daniele Werck-Reichhart

T05-042 Characterization of antifungal compounds purified from transgenic Arabidopsis plants expressing a bacterial non-heme haloperoxidase gene

Miguel F.C. De Bolle, Jan Sels, Inge E. Velghe, Geert J.A. Schoofs, Wendy Van Hemelrijck, István Nagy, René De Mot, Bruno P.A.Cammue

T05-043 Gene Expression Profiling to elucidate EDS1 and PAD4 functions in Arabidopsis

Michael Bartsch, Lucía Jordá, Jane Parker

T05-044 Characterization of two novel Arabidopsis mutants demonstrates the diversity of defence pathways involved in BABA induced resistance.

Víctor Flors, Gábor Jakab, Jurriaan Ton, Brigitte Mauch-Mani

T05-045 Investigating the basis for differential functions between Arabidopsis SGT1a and SGT1b

Shigeyuki Betsuyaku, Laurent D. Noël, Paul R. Muskett, Jane E. Parker

T05-046 Cloning of Arabidopsis homologues of IAA- amidohydrolases from Chinese cabbage and expression during the development of clubroot disease

Astrid Schuller, Jutta Ludwig-Müller

T05-047 Structure-function studies of Arabidopsis thaliana TGG4 Myrosinase

Romit Chakrabarty, Derong Ding, Yongsheng Wang, Derek Andersson, Jesper Danielsson, Johan Meijer

T05-048 **Bacillus as beneficial bacteria for plant protection**
Jesper Danielsson, Christina Dixelius, Oleg Reva, Johan Meijer

T05-049 **FERMENTING OVER A COMPLEX MATTER: Heterologous expression of Brassica napus Myrosinase Binding Proteins in Pichia pastoris.**
Frédéric D. DUVAL, Johan MEIJER, Lars RASK

T05-050 **SAG101, a novel EDS1 interactor, is involved in plant disease resistance**
Marcel Wiermer, Bart J. Feys, Riyaz A. Bhat, Lisa J. Moisan, Nieves Medina-Escobar, Jane E. Parker

T05-051 **Analysis of Atapy1 promoter activity in clubroot disease caused by P. brassicae**
Francis Jacob, Jutta Ludwig-Müller, Iris Steinebrunner

T05-052 **Characterization of the transcriptional changes that result from infection with Pseudomonas syringae**
Natalie Weaver, Dong Wang, Jun Lu, Thomas B Kepler, Xinnian Dong

T05-053 **Genetic dissection of non-host disease resistance to fungal pathogens in Arabidopsis**
Volker Lipka, Jan Dittgen, Paul Schulze-Lefert

T05-054 **A new Myrosinase gene family in Arabidopsis thaliana**
Derek Andersson, Romit Chakrabarty, Jiaming Zhang, Johan Meijer

T05-055 **The role of RIN13 (RPM1 Interacting protein 13) in RPM1 mediated disease resistance in Arabidopsis**
Jong-Hyun Ko, Antionious Al-Daoude, Marta de Torres Zabala, Murray Grant

T05-056 **Host Factors Controlling Potato Virus X Movement in Arabidopsis thaliana**
Osman Mewett, Aidong Yang, Dave Edge, Alan Williams, Sue Angell

T05-057 **ARF-GTPases in plant pathogen interactions**
Ulrike Unte, Joachim Uhrig, Paul Schulze-Lefert, Volker Lipka

T05-058 **Identifying pathogen-induced changes in the plant defense signaling network**
Thierry Genoud, John Pufky, Patrick Bozo, Patrick Hurban, Jean-Pierre Métraux

T05-059 **Identification and Characterisation of a Novel Pathogen Regulated Gene by Enhancer Trapping**
Katherine Coutts, Ingela Fridborg, Alan Williams, Aidong Yang, Stuart MacFarlane, Sue Angell

T05-060 **Functional characterisation of LRR-type receptor-like kinases implicated in pathogen defense**
Birgit Kemmerling, Anne Schwedt, Ullrich Dubiella, Thorsten Nürnberger

T05-061 **Elongation factor Tu ¯ a novel PAMP involved in plant defence**
Gernot Kunze, Pascal Bittel, Anne Caniard, Delphine Chinchilla, Silke Robatzek, Cyril Zipfel, Thomas Boller, Jürg Felix

T05-062 Functional Genomics of Arabidopsis Heat Stress Transcription Factors (Hsfs)

Sachin Kotak, Markus Port, Arnab Ganguli, Frank Bicker, Pascal von Koskull-Döring

T05-063 Dissecting the oxylipin signature using a Gene Specific Tag (GST) microarray.

Robin Liechti, Aurélie Gfeller, Edward E. Farmer

T05-064 Identification of CPR5-interacting factors using split-ubiquitin system

Jinyoung Yang, Lisa k Anderson, Xinnian Dong

T05-065 Physiological plasticity of inducible defence responses in Arabidopsis

Tatiana Mishina, Jürgen Zeier

T05-066 Disease Resistance Signalling in snc1, a constitutively active TIR-NB-LRR Resistance-gene

Sandra Goritschnig, Yuelin Zhang, Xin Li

T05-067 NPR1 modulates salicylate- and jasmonate-dependent defense responses in plants

Steven H. Spoel, Gerold J.M. Beckers, Corné M.J. Pieterse, Xinnian Dong

T05-068 EDM2: a novel regulator of disease resistance in Arabidopsis thaliana

Thomas Eulgem, Hyeong Cheol Park, Xiao-Jun Wang, Greg Frank, Alayne Cuzick, John M. McDowell, Eric B. Holub, Jeffery L. Dangl

T05-069 Cell-specific Gene Activation by Salicylic Acid

Kate Wilson, John Carr

T05-070 dsRNAi ¯ A reverse genetic tool to discover gene function in plant nonhost resistance

Christina Neu, Bekir Ülker, Paul Schulze-Lefert

T05-071 OsEREBP BINDS TO TATA-BINDING PROTEIN, THEREBY REPRESSING THE BASAL TRANSCRIPTION OF GENES

Kim, Yun Ju, Kim, Jee Eun, Jung, Eui-Hwan, Kim, Sang Hee, Hwang, Seon Hee, Lee, Jung-Sook, Suh, Seok-Cheol, Hwang Duk-Ju

T05-072 Analysis of CIR1-mediated disease resistance in Arabidopsis

Shane Murray, Maryke Carstens, Sally-Ann Walford, Katherine Denby

T05-073 The PEN1 syntaxin defines a novel compartment upon fungal attack and is required for the timely assembly of papilla

Farhah Assaad, Jin-Long Qiu, Heather Youngs, David Ehrhardt, Laurent Zimmerli, Monika Kalde, Gehard Wanner, Scott Peck, Katrina Ramonell, Herb Edwards, Chris Somerville, Hans Thordal-Christensen

T05-074 Enhanced resistance to Cucumber mosaic virus in the Arabidopsis thaliana ssi2 mutant is mediated via an SA-independent mechanism

Ken-Taro Sekine, Ashis Nandi, Takeaki Ishihara, Shu Hase, Masato Ikegami, Jyoti Shah, Hideki Takahashi

T05-075 Identification of General and Isolate-Specific Botrytis cinerea resistance mechanisms in Arabidopsis

Katherine Denby, Nicolette Adams, Shane Murray, Heather Rowe, Dan Kliebenstein

T05-076 Cell death induced by AtMEK5 activation is different with pathogen induced HR-cell death

Hongxia Liu, Ying Wang, Tianhong Zhou, Yujing Sun, Guoqin Liu, Dongtao Ren*

T05-077 RPM1-interacting protein RIN12 is a positive regulator of defense responses

Andrew Plume, Antonious Al-Daoude, Marta de Torres Zabala, Monaz Mehta, Murray Grant

T05-078 IDENTIFICATION OF KEY ARABIDOPSIS GENES REQUIRED FOR RESISTANCE AGAINST BOTRYTIS CINEREA

Pedro L Nurmberg, Gary J Loake

T05-079 Antagonistic interactions between the SA- and JA- signaling pathways in Arabidopsis modulate expression of defense genes and gene-for-gene resistance to Cucumber mosaic virus

Hideki Takahashi, Yoshinori Kanayama, Ming Shu Zheng, Tomonobu Kusano, Shu Hase, Masato Ikegami, Jyoti Shah

T05-080 Arabidopsis basal immunity to the food-borne human pathogen Escherichia coli O157:H7

William Underwood, Roger Thilmony, Thomas Whittam, Sheng Yang He

T05-081 The BIK1 gene of Arabidopsis encodes a protein kinase required for resistance to Botrytis cinerea.

Paola Veronese, Fatma Ouaked, Heribert Hirt, Tesfaye Mengiste

T05-082 The Hyaloperonospora parasitica avirulence gene ATR13 reveals an intense “arms race” in progress with the Arabidopsis resistance gene RPP13.

Rebecca Allen, Peter Bittner-Eddy, Laura Grenville, Sharon Hall, Julia Meitz, Anne Rehmany, Jim Beynon

T05-083 scv1 is a suppressor of cpr5-mediated disease resistance

Lisa K. Anderson, Lena X. Gong, Xinnian Dong

T05-084 Global transcription profile changes controlled by the Arabidopsis gene Botrytis susceptible 1 (AtMYB108).

Paola Veronese, Qingqiu Gong, Pinghua Li, Hans Bohnert, Tesfaye Mengiste1

T05-085 Prediction of multiple Arabidopsis targets for the Pseudomonas effector protease AvrRpt2

Stephen Chisholm, Douglas Dahlbeck, Nandini Krishnamurthy, Kimmen Sjolander, Brian Staskawicz

T05-086 Functional analysis and expression studies of the flagellin receptor FLS2

Silke Robatzek, Delphine Chinchilla, Zsuzsa Bauer, Cyril Zipfel, Gernot Kunze, Pascal Bittel, Anne Caniard, Georg Felix, Thomas Boller

T05-087 The Dead Zone: Exploration of the Host Pathogen Interface of the Alternaria brassicicola-Brassicaceae Pathosystem

C.B. Lawrence, T.K. Mitchell, J. Glazebrook, R.A. Cramer, K.D. Craven, J.L. Pilon, J.W. Davis

T05-088 HPH, a Repressor of Phototropic Responsiveness in Arabidopsis

Brandon Celaya

T05-089 Interaction Dynamics of Several Immune Response Regulators in Arabidopsis

J. Parker, B. Feys, L. Moisan, N. Medina-Escobar, M. Wiermer, M. Bartsch

T05-090 A Synthesis for Understanding Disease and Disease Resistance

Jeff Dangl

T06 Natural Variation and Comparative Genomics Including Genome

T06-001 Transposon Activation in Arabidopsis Neopolyploids

Andreas Madlung, Brian Watson, Hongmei Jiang, Trevor Kagochi, R.W. Doerge, Luca Comai, Robert Martienssen

T06-002 An inducible targeted tagging system for localized saturation mutagenesis in Arabidopsis thaliana.

Bindu Nishal, Titima Tantikanjana, Venkatesan Sundaresan

T06-003 Diversity and redundancy within the Arabidopsis formin family

Denisa Pickova, Fatima Cvrckova, Marian Novotny, Martina Horackova, Viktor Zarsky

T06-004 Comparative Biochemical Genomics of Glucosinolate Chain Elongation in Arabidopsis & Friends

Markus Benderoth, Susanne Textor, Juergen Kroymann

T06-005 Quantitative trait locus analysis of the phase of the Arabidopsis circadian clock

Chiarina Darrah, Dr Anthony Hall, Dr Harriet McWatters

T06-006 Genomics of the Arabidopsis and rice CBL-CIPK signaling networks

Stefan Weinl, Oliver Batistic, Cecilia D`Angelo, Jörg Kudla

T06-007 Comparative transciptomics analyses of abiotic stress responses in Arabidopsis

Joachim Kilian, Stefan Weinl, Cecilia D'Angelo, Oliver Batistic, Jörg Kudla

T06-008 Geographical variation in Arabidopsis thaliana arises from recombination

Heike Schmuths, Konrad Bachmann

T06-009 Natural Variation of Flowering Time of Arabidopsis thaliana Wild Strains with Focus on FRIGIDA and FLOWERING LOCUS C

Janne Lempe, Sureshkumar Balasubramanian, Sridevi Sureshkumar, Detlef Weigel

T06-010 Natural Genetic Variation Within and Between Species

Thomas Mitchell-Olds

T06-011 Mapping Quantitative Trait Loci governing flowering time in Arabidopsis using Stepped Aligned Recombinant Inbred Strains(STAIRS)

S A C N Perera, T M Wilkes, M J Kearsey

T06-012 Evolutionary potential of the chalcone synthase cis-regulatory region in Arabidopsis thaliana

de Meaux Juliette, Goebel Ulrike, Pop Ana, Mitchell-Olds Tom

T06-013 Trichome evolution in tetraploid Arabidopsis

Kentar Shimizu, Michael D Purugganan, Kiyotaka Okada

T06-014 Elucidating the Molecular Basis of Heterosis in Arabidopsis thaliana

Martina Becher, Rhonda Meyer, Otto Törjek, Hanna Witucka-Wall, Oliver Fiehn, Joachim Fisahn, Achim Walter, Thomas Altmann

T06-015 QTLs for an important growth trait (Specific Leaf Area).

Hendrik Poorter, Yvonne de Jong, Stefan Bosmans, Ton Peeters

T06-016 Genetic analysis of natural variation for mineral composition and phytate levels in Arabidopsis thaliana seeds

Artak Ghandilyan, Henk Schat, Mohamed El-Lithy, W.H.O. Ernst, Maarten Koornneef, Dick Vreugdenhil, Mark G.M. Aarts

T06-017 Investigation of the molecular basis of heterosis using a combined genomic and metabolomic approach

Hanna Witucka-Wall, Rhond C. Meyer, Otto Törjek, Eugenia Maximova, Oliver Fiehn, Martina Becher, Anna Blacha, Michael Udvardi, Wolf-Rüdiger Scheible, Thomas Altmann

T06-018 Comparative analysis of the FRIGIDA genomic region reveals a recent transposition event in Arabidopsis thaliana

Sandip Das, Christa Lanz, Stephan Schuster, Detlef Weigel

T06-019 High diversity genes in the Arabidopsis genome

Jennifer M. Cork, Michael D. Purugganan

T06-020 Ecological genomics of naturally occurring flowering time variation among Arabidopsis accessions

John R. Stinchcombe, Cynthia Weinig, Charlotte Mays, Michael D. Purugganan, Johanna Schmitt

T06-021 Gene Subfunctionalization and Neofunctionalization in Arabidopsis Gene Families studied using Gene Expression and Sequence Data

Kiana Toufighi, David Guttman, Nicholas J. Provart

T06-022 A START: Putative lipid/sterol binding proteins in plant genomes

Kathrin Schrick, Diana Nguyen, Wojciech M. Karlowski, Klaus F.X. Mayer

T06-023 Progenitor-dependent gene expression and evolution of transcriptome in Arabidopsis allopolyploids

Jianlin Wang, Lu Tian, Hyeon-Se Lee, Meng Chen, Jinsuk J. Lee, Jiyuan J. Wang, Ning E. Wei, Sheetal Rao, Hongmei Jiang, Brian Watson, Andreas Madlung, Thomas C. Osborn, R. W. Doerge, Luca Comai, Z. Jeffrey Chen

T06-024 Natural variation in root system architecture

Jonathan Fitz Gerald, Melissa Lehti-Shiu, Paul Ingram, Jocelyn Malamy

T06-025 A floral homeotic polymorphism in Capsella: studying a hopeful monster

Guenter Theissen, Pia Nutt, Barbara Neuffer

T06-026 The genomic pattern of polymorphism in Arabidopsis thaliana

Chris Toomajian, Mattias Jakobsson, Badri Padhukasahasram, Vincent Plagnol, Keyan Zhao, Joy Bergelson, Martin Kreitman, Magnus Nordborg

T06-027 Mining publicly available sequence information to detect SNP markers

Norman Warthmann, Joffrey Fitz, Detlef Weigel

T06-028 The genetic architecture of trichome density in A. thaliana: results from multiple mapping populations

Vaughan Symonds, Alan Lloyd

T06-029 Adaptive trait genes in Arabidopsis thaliana: Discerning the role of selection and demography in patterns of genetic variation.

Brad Rauh, Karl Schmid

T06-030 FRI haplotype structure: implications for detecting selection and for genome-wide association mapping

Maria Jose Aranzana, Sung Kim, Keyan Zhao, Rana Goyal, John Molitor, Chikako Shindo, Clare Lister, Chunlao Tang, Honggang Zheng, Paul Marjoram, Caroline Dean, Magnus Nordborg

T06-031 Two Arabidopsis halleri MTP1 genes co-segregate with naturally selected zinc tolerance and account for high MTP1 transcript levels

Ute Krämer, Dörthe B. Dräger, Martina Becher, Anne-Garlonn Desbrosses-Fonrouge, Christian Krach, Rhonda C. Meyer, Katrin Voigt, Pierre Saumitou-Laprade, Ina N. Talke

T06-032 Natural variation, genomic imprinting and modifiers of mea seed abortion in the Arabidopsis species genepool.

Spillane C, Escobar JM, Baroux C, Hu H, Page D, Juenger T, Tessadori F, Gheyselinck J, Fransz P, Grossniklaus U

T06-033 Heterosis and transcriptome remodelling in Arabidopsis thaliana

David Stokes, Colin Morgan, Carmel O’Neill, Ian Bancroft

T07 Metabolism (Primary, Secondary, Cross-Talk and Short Distance Metabolite Transport)

T07-001 The At4g12720 gene encoding a homologue of the human GFG protein is active on ADP-ribose and flavine adenine dinucleotide (FAD).
Olejnik Kamil, Kraszewska Elzbieta

T07-002 Characterisation of the transparent testa 10 mutant affected in Arabidopsis seed coat flavonoid metabolism
Lucille Pourcel, Jean-Marc Routaboul, Michel Caboche, Loïc Lepiniec, Isabelle Debeaujon

T07-003 A conserved uORF mediates sucrose-induced translational control on bZIP transcription factors
Wiese, A, Elzinga, N, Wobbes, B, Rahmani, F, Smeekens, S

T07-004 Different roles of branched-chain aminotransferases (BCAT) in the metabolism of aliphatic amino acid in plants
Joachim Schuster, Oliver Schmidt, Tanja Knill, Stefan Binder

T07-005 Roles of BOR1 homologs in B transport
Toru Fujiwara, Kyoko Miwa, Yuko Nakagawa, Masaharu Kobayashi, Akira Nozawa, Junpei Takano

T07-006 The regulatory role of the acetohydroxyacid synthase small subunit in Arabidopsis
Robert Ascenzi, Gregory J. Budziszewski, Charles L. Ortlip, Bijay K. Singh

T07-007 Functional analysis of cell wall components by expressing a xyloglucanase in Arabidopsis thaliana
Carsten H. Hansen, Ulrike Hänsel, Kirk M. Schnorr, Markus Pauly

T07-008 A second starch phosphorylating enzyme is required for normal starch degradation in Arabidopsis leaves: the phosphoglucan, water dikinase (PWD)
Oliver Kötting, Axel Tiessen, Peter Geigenberger, Martin Steup, Gerhard Ritte

T07-009 Arabidopsis thaliana P450 transcript profiling and functional genomics
Hui Duan, Shahjahan Ali, Sanjeewa Rupasinghe, Natanya Civjan, Jyothi Thimmapuram, Lei Liu, Mark Band, Stephen Sligar, Daniele Werck-Reichhart, Mary A. Schuler

T07-010 P, A and L boxes do it together: A statistical approach for the investigation on stress inducible cis-elements in Arabidopsis
Kenneth Berendzen, Dierk Wanke, Kurt Stüber, Björn Hamberger

T07-011 Regulation and Localization of the Beta-cyano-L-alanine Converting Enzyme Nitrilase 4 in Arabidopsis thaliana
Julia J. Volmer, Markus Piotrowski

T07-012 Analysis of the involvement of AtNIT1 homologous enzymes in the metabolism of glucosinolate-derived nitriles

Tim Janowitz, Markus Piotrowski

T07-013 Expression profiling and functional definition of Arabidopsis CYP86A and CYP94B proteins as fatty acid hydroxylases

Hui Duan, Natanya Civjan, Stephen G. Sligar, Mary A. Schuler

T07-014 Proanthocyanidin metabolism in Arabidopsis: dissecting biosynthetic and regulatory functions in developing seed coat

Isabelle Debeaujon, Nathalie Nesi, Lucille Pourcel, Jean-Marc Routaboul, Pascual Perez, Martine Devic, Olivier Grandjean, Michel Caboche, Loïc Lepiniec

T07-015 Chemical Analysis of Arabidopsis Mutants in the Phenylpropanoid Pathway

Abdelali Hannoufa, Ulrike Schäfer, Gordon Gropp, Delwin Epp

T07-016 Genetic dissection of the regulation of cell wall matrix polymer biosynthesis by UDP-D-glucose 4-epimerase isoforms in Arabidopsis thaliana

Georg J. Seifert, Keith Roberts

T07-017 A biotechnological approach to reduce photorespiratory losses. Effects of the expression of Glycolate oxidase in plastids of Arabidopsis thaliana.

Verónica Maurino, Holger Fahnenstich, Ulf-Ingo Flügge

T07-018 A gene family in Arabidopsis with cystine-lyase and tyrosine aminotransferase-activities

Heike Hollaender-Czytko, Janine Grabowski, Iris Sandorf, Katrin Weckermann, Elmar W. Weiler

T07-019 Functional Analysis of Plant Nucleotide Metabolism:The Purine and Pyrimidine Phosphoribosyltransferase Gene Family in A. thaliana

Sandra Messutat, Steffen Müller, Peter Lange, Rita Zrenner, Ralf Boldt

T07-020 Oxylipin-signaling system of chloroplasts from Arabidopsis thaliana: Searching for the initial lipase(s)

Melanie Jünger, Stephan Pollmann, Christine Böttcher, Elmar W. Weiler

T07-021 Equilibrative nucleoside transporters (ENT) influence physiology and development in Arabidopsis

Michaela Traub, Martin Flörchinger, H. Ekkehard Neuhaus, Torsten Möhlmann

T07-022 Identification of new glucosinolate-biosynthesis genes induced by coronatine

Markus Piotrowski, Andreas Schemenwitz, Anna Lopukhina, Tim Janowitz, Elmar W. Weiler, Claudia Oecking

T07-023 Translational/post-translational regulation of a boron transporter AtBOR1

Junpei Takano, Kyoko Miwa, Masaharu Kobayashi, Hiroaki Hayashi, Tadakatsu Yoneyama, Nicolaus von Wirén, Toru Fujiwara

T07-024 Respiratory supercomplexes of plant mitochondria

Holger Eubel, Jesco Heinemeyer, Stefanie Sunderhaus, Mariano Perales, Hans-Peter Braun

T07-025 gamma-aminobutyric acid (GABA) metabolism in plants

Anke Hüser, Rainer Waadt, Ulf-Ingo Flügge, Frank Ludewig

T07-026 ISI1, a phloem located regulator of carbohydrate allocation in Arabidopsis

Fred Rook, Fiona Corke, Rachel Holman, Alexander G. May, Michael W. Bevan

T07-027 Expression Analysis of Nucleotide Metabolism Genes in Arabidopsis

Peter R. Lange, Rita Zrenner

T07-028 Expression patterns of five Arabidopsis thaliana UDP-D-glucose-4-epimerase genes in leaves .

Olga V. Voitsekhovskaja, Christine Barber, Georg J. Seifert

T07-029 Genetically encoded sensors for metabolites

Marcus Fehr, Karen Deuschle, Melanie Hilpert, Sylvie Lalonde, Wolf B. Frommer

T07-030 The Arabidopsis plastidic Glucose 6-Phosphate/Phosphate translocator GPT1 is essential for pollen maturation and female gametogenesis

Anja Schneider, Patrycja Niewiadomski, Silke Knappe, Karsten Fischer, Ulf-Ingo Flügge

T07-031 Analysis of a putative plastidic transporter affecting photosynthesis in Arabidopsis thaliana

Daniel Marquardt, Anja Schneider, Ulf-Ingo Flügge

T07-032 Functional analysis of the nucleotide sugar conversion pathway in Arabidopsis

Björn Usadel, François Guerineau, Markus Pauly

T07-033 The role of the oxPPP during the development of Arabidopsis thaliana.

Patrycja Niewiadomski, Eric van der Graaff, Karsten Fischer, Ulf-Ingo Flügge, Anja Schneider

T07-034 A novel MYB factor involved in the regulation of phenylpropanoid biosynthesis as pathogen response?

Bettina Berger, Tamara Gigolashvili, Ulf-Ingo Flügge

T07-035 Threonine aldolase, a previously uninvestigated component of plant amino acid metabolism

Vijay Joshi, Georg Jander

T07-036 Spatial and temporal expression of sucrose synthase gene family members

Zuzanna Bieniawska, Paul Barratt, Vera Thole, Alison M. Smith, Rita Zrenner

T07-037 a mutation in the sucrose transporter gene SUC2 leads to changes in metabolic gene expression

Julie C Lloyd, Oksana V Zakhleniuk

T07-038 Contribution of the myo-inositol oxygenase (miox) gene family of Arabidopsis thaliana to ascorbate biosynthesis

Argelia Lorence, Jon Robinson, Boris I. Chevone, Pedro Mendes, Craig L. Nessler

T07-039 Identification and characterization of a putative glucuronic acid reductase in Arabidopsis thaliana

Argelia Lorence, Amber M. Rogers, Pedro Mendes, Wenyan Zhang, Boris I. Chevone, Craig L. Nessler

T07-040 Reduction of cytokinin biosynthesis genes

Jennifer Tomscha, Joe Kieber

T07-041 A Systems Approach to Nitrogen Networks

Coruzzi, G., Gutierrez, R., Lejay, L., Shasha, D., Palenchar, P., Cruikshank, A.

T07-042 Elevated plastid-derived isoprenoid synthesis in the prl1 mutant of Arabidopsis thaliana

Doris Albinsky, Hiroyuki Kasahara, Juan M. Estevez, Kazumi Nakabayashi, Yuji Kamiya, Shinjiro Yamaguchi

T07-043 The three desulfo-glucosinolate sulfotransferase proteins in Arabidopsis have different substrate specificities

Marion Klein, Jim Tokuhisa, Michael Reichelt, Jonathan Gershenzon, Jutta Papenbrock

T07-044 High Sugar Response Mutant 5 encodes a F-box protein: A link between regulation of carbohydrate resource allocation and SCF ubiquitin ligase mediated protein degradation?

Georg Hemmann, Rachel Holman, Fiona Corke, Michael W. Bevan

T07-045 Overexpression of a specific Sucrose-Phosphat-Synthase (SPS) isoform from Arabidopsis thaliana sensitive to phosphorylation

Lehmann, Ute, Glinski, Mirko, Baessler, Olivia, Wienkoop, Stefanie, Weckwerth, Wolfram

T07-046 Phosphorylation studies on sucrose-phosphate synthase based on mass

Mirko Glinski, Ute Lehmann, Anne-Claire Cazale, Tina Romeis, Wolfram Weckwerth

T07-047 Characterization of two splicing variants of AtUPS 5

Anja Schmidt, Nadine Baumann, Michael Fitz, Wolf B. Frommer, Marcelo Desimone

T07-048 Oxygen sensing and adaptive metabolic responses to low internal oxygen in plants

Joost T van Dongen, Helene Vigeolas, Anke Langer, Anja Froehlich, Peter Geigenberger

T07-049 Expression profile and functional characterization of the Nucleobase-Ascorbate Transporter multigene family in Arabidopsis thaliana

Esther Grube, Verónica Maurino, Karsten Fischer, Markus Gierth, Ulf-Ingo Flügge

T07-050 ANALYSIS OF PROTEINS HOMOLOGOUS TO PLASTIDIC PHOSPHATE TRANSLOCATORS IN ARABIDOPSIS THALIANA

Marcella Santaella-Tenorio, Silke Knappe, Ulf-Ingo Flügge, Karsten Fischer

T07-051 Nutrient-dependent and hormonal regulation of sulfate transporters in Arabidopsis

Akiko Maruyama-Nakashita, Yumiko Nakamura, Tomoyuki Yamaya, Hideki Takahashi

T07-052 Systematic in-depth analysis of nitrogen signalling in Arabidopsis thaliana (L.)

Jens-Holger Dieterich, Tomasz Czechowski, Rosa Morcuende, Mark Stitt, Wolf-Rüdiger Scheible, Michael K. Udvardi

T07-053 Soluble Cytosolic Heteroglycans Acts as Substrate for the Cytosolic (Pho 2) Phosphorylase

Fettke, Joerg, Tiessen, Axel, Eckermann, Nora, Steup, Martin

T07-054 Two interacting high-affinity sulfate transporters regulate the uptake of sulfate in response to sulfur conditions.

Naoko Yoshimoto, Kazuki Saito, Tomoyuki Yamaya, Hideki Takahashi

T07-055 The PMEI-RP family: Inhibitors of one single protein family interact with apparently unrelated classes of target enzymes

Sebastian Wolf, Manuela Link, Christina Hofmann, Michael Hothorn, Klaus Scheffzek, Thomas Rausch, Steffen Greiner

T07-056 Functional Characterisation of the ERD6 Sugar Transporter Family

Barbara Hannich, Michael Buettner

T07-057 A FUNCTIONAL GENOMICS APPROACH TO PLANT SOLUBLE PYROPHOSPHATASES

Neslihan Ergen, Steffen Greiner, Thomas Rausch

T07-058 Functional analysis of the CYP76 family of P450 genes in A. thaliana

Sebastien Grec, Elisabeth Mueller, Danièle Werck-Reichhart

T07-059 In Arabidopsis thaliana, the invertase inhibitor isoforms AtC/VIF1 and 2 show distinct target enzyme specificities and developmental expression profiles

Manuela Link, Thomas Rausch, Steffen Greiner

T07-060 The role of CP12 in the co-ordination of chloroplast metabolism

Raines CA, Kaloudas D, Singh P, Lloyd JC, Howard T, Zahkleniuk O

T07-061 Structure-Function Relationship within the Invertase/Pectinmethylesterase Inhibitor Family of Arabidopsis thaliana

Michael Hothorn, Sebastian Wolf, Steffen Greiner, Klaus Scheffzek

T07-062 QTL analysis of carbohydrates and growth-related traits in a new recombinant inbred population derived from the Ler x Kond cross

Mohamed E. El-Lithy, Leónie Bentsink, José Broekhof, Hein van der Poel, Michiel van Eijk, Maarten Koornneef, Dick Vreugdenhil

T07-063 Photorespiration and glycolate cycle: Old subject, new insights

Richter A., Bauwe U., Boldt R., Hartwig T., Michl K., Bauwe H., Kolukisaoglu Ü.

T07-064 ENZYME REGULATION THROUGH PROTEIN-PROTEIN INTERACTIONS: GAPDH-CP12-PHOSPHORIBULOKINASE SUPRAMOLECULAR COMPLEX IN Arabidopsis thaliana

Marri Lucia, Sparla Francesca, Zaffagnini Mirko, Pupillo Paolo, Trost Paolo

T07-065 Loss of the hydroxypyruvate reductase, AtHPR1, does not lead to lethality under ambient CO2 conditions

Hartwig T., Michl K., Boldt R., Kolukisaoglu Ü., Bauwe H.

T07-066 Investigating the active sites of Arabidopsis thaliana cytochrome P450 monooxygenases hydroxylating aromatic rings

Sanjeewa Rupasinghe, Mary A. Schuler

T07-067 Small molecular weight Phospholipase A2 proteins in Arabidopsis.

Gert-Jan de Boer, Michel Haring

T07-068 Sugar signals interact with thioredoxin-mediated light activation of ADPglucose pyrophosphorylase in Arabidopsis leaves

Anna Kolbe, Axel Tiessen, Janneke H.M. Hendriks, Jeannette Kley, Peter Geigenberger

T07-069 Structure-Based Design of 4-Coumarate:CoA Ligase Variants with New Catalytic Properties

Katja Schneider, Klaus Hövel, Dietmar Schomburg, Hans-Peter Stuible, Erich Kombrink

T07-070 Biochemical links between growth, nitrogen, and carbon utilisation in Arabidopsis thaliana ecotypes

Joanna Cross, Oliver Blaesing, Yves Gibon, Linda Bartetzko, Melanie Hoene, Manuela Guenther, Sonja Koehler, Mark Stitt

T07-071 Mutants in medium long and long chain acyl-CoA oxidase activity demonstrate that long chain acyl-CoA activity is important in seed viability and essential for seedling establishment.

Elizabeth L. Rylott, Helen Pinfield-Wells, Alison D. Gilday, Ian A. Graham

T07-072 Reserve Mobilisation in the Arabidopsis Endosperm Fuels Hypocotyl Elongation in the Dark, is Independent of Abscisic Acid and Requires the PHOSPHOENOLPYRUVATE CARBOXYKINASE1 Gene

Steven Penfield, Elizabeth R. Rylott, Alison D. Gilday, Stuart Graham, Tony R. Larson, Ian A. Graham

T07-073 Role of chloroplast lipids in photosynthesis and oxidative stress

Amelie Kelly, Marion Kanwischer, Svetlana Porfirova, Peter Dörmann

T07-074 The Arabidopsis seed mucilage mutant mum5 encodes a putative pectin methylesterase

Facette, Michelle R, Somerville, Chris R

T07-075 Genome wide analysis of Arabidopsis gene expression under sulfur starvation reveals the involvement of key transcription factors controlling sulfur assimilation metabolism

Bertrand Gakière, Tilbert Kosmehl, Monika Adamik, Stefan Kempa, Holger Hesse, Rainer Hoefgen

T07-076 Multiple regulations on the antagonistic cross-talks between jasmonate- and salicylate-signaling pathways

Hwang Bae Sohn, Song Yion Yeu, Yeon Jong Koo, Myeong Ae Kim, Eun Hye Kim, Sang Ik Song, Ju-Kon Kim, Jong Seob Lee, Jong-Joo Cheong, Yang Do Choi

T07-077 Obtusifoliol 14alpha-demethylase mutants reveal that sterols regulate plant growth and development via brassinosteroids-dependent and independent pathways

Ho Bang Kim, Hubert Schaller, Chang-Hyo Goh, Hyoungseok Lee, Sunghwa Choe, Chung Sun An, Kenneth A. Feldmann, Rene Feyereisen

T07-078 Analysis of Putative Signal Termination Mutants from Arabidopsis Thaliana

Bhadra Gunesekera, Glenda Gillaspy

T07-079 Biochemical and Molecular Analysis of Constitutive and Inducible Terpene Volatile Emission from Arabidopsis thaliana

Dorothea Tholl, Feng Chen, Christian Abel, Jana Petri, Eran Pichersky, Jonathan Gershenzon

T07-080 Uniform stable isotope labeling of Arabidopsis thaliana opens hetero-nuclear multi-dimensional NMR-based metabolomics

Jun Kikuchi, Kazuo Shinozaki, Takashi Hirayama

T07-081 Identification and functional analysis of cis-prenyltransferases in Arabidopsis thaliana

Seiji Takahashi, Daiju Terauchi, Yugesh Kharel, Tanetoshi Koyama

T07-082 Using microarray data to examine co-regulation in the amino acid metabolic pathways of A. thaliana

Peter M. Palenchar, Daniel Tranchina, Dennis E. Shasha, Rodrigo A. Gutierrez, Laurence V. Lejay, Gloria M. Coruzzi

T07-083 Functional Analysis of Plant Nucleotide Metabolism: The Nucleoside Mono- and Diphosphate Kinase Gene Families in Arabidopsis thaliana

Claudia Kopka, Peter R. Lange, Ralf Boldt, Rita Zrenner

T07-084 Biosynthesis and distribution of glutathione in developing Arabidopsis embryos

Andreas J Meyer, Narelle G Cairns, Christopher S Cobbett

T07-085 Amino acid modifications as a principal basis for the diversity of aliphatic glucosinolates

Jim Tokuhisa, Jan-Willem de Kraker, Susanne Textor, Jonathan Gershenzon

T07-086 A Novel Nuclear Calmodulin-binding Protein Modulates Glucosinolate Accumulation in Arabidopsis

Marganit Levy, Qiaomei Wang, Steffen Abel

T07-087 Flavonol glycosyltransferases in Arabidopsis thaliana

Burkhard Messner, Patrik Jones, Birgit Geist, Susanna Holzinger, Kazuki Saito, Tony R. Schaeffner

T07-088 MOLECULAR AND GENOMIC ANALYSIS OF NITROGEN REGULATION OF AMINO ACID PERMEASE I (AAP1) IN ARABIDOPSIS

Mengjuan Guo, Daniel R. Bush

T07-089 **Accelerated senescence and changes in primary and secondary metabolism due to the overexpression of a novel transcription factor**
Zanor, Maria Ines, Palacios-Rojas, Natalia, Witt, Isabel, Müller-Röber, Bernd

T07-090 **Potential role of a member of the PHO1 gene family in Pi**
Aleksandra Stefanovic, Cécile Ribot, Yong Wang, Lassaad Belbarhi, Julie Chong, Yves Poirier

T07-091 **TRANSCRIPTOME AND METABOLIC PROFILE ANALYSIS TO CHARACTERIZE PLANT RESPONSE TO METHANOL TREATMENTS**
Hugo Peña-Cortes, Jorge Valdes, Valeria Espinoza, Fernando Dorta, Elizabeth Sanchez, Joachim Kopka, Lothar Willmitzer, Ingrid Ramirez

T07-092 **Identification of Arabidopsis Seed Color Mutants with Altered Oil Content**
Diane Ruezinsky, Georg Jander, Charlotte Weigel, Crystal Hewitt, Rob Last, Kristen Bennett

T07-093 **Regulation of the Anthocyanin Pathway by bHLH, Myb, and TTG1 proteins in Arabidopsis**
Tony Gonzalez, Alan Lloyd

T07-094 **Functional analysis of sterol-C24-methyltransferase in arabidopsis**
Pierrette Bouvier-Navé, Félix Muller, Aurélie Schaeffer, Vincent Compagnon, Pierre Benveniste, Hubert Schaller

T07-095 **Comparative analysis of the regulatory events that modulate the plastidic MEP isoprenoid pathway in Arabidopsis.**
Arturo Guevara, Carolina San Roman, Ma. Analilia Arroyo, Elena Cortés, María de la Luz Gutierrez-Nava, Patricia León

T07-096 **Genomewide diurnal and circadian changes in transcript levels of A. thaliana revealed by microarray analysis**
Oliver Bläsing, Yves Gibon, Oliver Thimm, Svenja Meyer, Axel Nagel, Mark Stitt

T07-097 **Ionomics: Gene Discovery in Aid of Plant Nutrition, Human Health and Environmental Remediation**
Guerinot, M.L., Eide, D.J., Harper, J.F., Salt, D.E., Schroeder, J.I. and Ward, J.M.

T07-098 **Functional Genomics of Carbon-Nitrogen Interactions**
M.Stitt

T08 Long Distance Transport (Signals Including Silencing and Metabolites)

T08-001 **Involvement of DIR1, a putative lipid transfer protein, in long distance signaling during Systemic Acquired Resistance**
Robin K Cameron, Melody Neumann, Zhiying Zhao, Asif Mohammed, Karen Haines

T08-002 **How are signalling pathways involving Jasmonate and Calcium linked to the wound response in Arabidopsis.**
Valerie Hawkes, John Turner

T08-003 Patterning of plants by auxin

Didier Reinhardt, Eva-Rachele Pesce, Pia Stieger, Therese Mandel, Kurt Baltensperger, Malcolm Bennett, Jan Traas, Jirí Friml, Cris Kuhlemeier

T08-004 Expression Pattern of an Arabidopsis Dehydrin, Homologous to an Iron Transport Protein from Ricinus

Niklas Piening, Ruth Stadler, Alexandra Graf, Dirk Becker, Norbert Sauer, Horst Lörz, Manfred Gahrtz

T08-005 Expression profiling of membrane transporters in Arabidopsis

Eric van der Graaff, Anja Schneider, Rainer Schwacke, Patrycja Niewiadomski, Ulf-Ingo Flügge, Reinhard Kunze

T08-006 Nutritional regulation of cytokinin biosynthesis: a possible role for long-distance signaling molecule

Hitoshi Sakakibara, Kentaro Takei, Mikiko Kojima, Nanae Ueda, Tomoyuki Yamaya

T08-007 Novel proteins in the phloem of Brassicaceae.

Anna Kolasa, Patric Giavalisco, Kristin Kapitza, Julia Kehr

T08-008 Functional analysis of the transcription factor TF55

Janina Lisso, Yvonne Schmiele, Ursula Uwer, Thomas Altmann

T08-009 C8 GIPK, a GAI-interacting protein kinase that controls hypocotyl elongation in Arabidopsis

Hanbing Li, Melina Zourelidou, Carola Kuhnle, Claus Schwechheimer

T08-010 Essential role of riboflavin pathway in jasmonate signaling

Shi Xiao, Liangying Dai, Fuquan Liu, Zhilong Wang, Wen Peng, Daoxin Xie

T08-011 Molecular analysis of plasmodesmata

Stefan Meyer, Norbert Sauer

T08-012 Expression of AtMHX, a transporter involved in long distance metal transport, is governed at both the transcriptional and translational levels

Ora Assael-David, Helen Saul, Irina Berezin, Benayahu Elbaz, Vered Saul, Talya Mizrachy-Dagri, Jianxin Chen, Emil Brook, Orit Shaul

T08-013 Phosphate signaling in Arabidopsis.

Peter Doerner, Fan Lai, Jennifer Whyte

T08-014 Determinants of polar localization of PIN proteins in Arabidopsis

Justyna Wiśniewska, Daniela Seifertová, Eva Benková, Anne Vieten, Jozef Mravec, Jiři Friml

T08-015 Non-Genomic Effect Of Auxin On Protein Trafficking

Tomasz Paciorek, Juergen Kleine-Vehn, Eva Zazimalova, Jan Petrasek, David Morris, Neil Emans, Nadia Ruthardt, Gerd Juergens, Niko Geldner, Jiri Friml

T08-016 Novel Feedback Regulations in Efflux-Dependent Auxin Distribution

Jiri Friml, Anne Vieten, Michael. Sauer, Marta Michniewicz, Tomasz Paciorek, Justyna Wisniewska, Gerd Juergens

T08-017 Role Of Protein Phosphorylation In Polar Auxin Transport In Arabidopsis

Marta Michniewicz, Yang Xiong, Dolf Weijers, Remko Offringa, Jiri Friml

T08-018 Genetic dissection of RNA silencing movement in Arabidopsis

Patrice Dunoyer, Olivier Voinnet

T08-019 Long Range Signalling

Ottoline Leyser

T08-020 Functional analysis of the CHoR protein

Marc-André Lohse, Stefanie Hartje, Sabine Zimmermann, Antje Schneider, Gunnar Plesch, Bernd Mueller-Roeber

T09 Genetic Mechanisms (Transcriptional and Chromatin Regulation)

T09-001 Brca2 is essential to meiosis in Arabidopsis

Dray E, Siaud N, Richaud A, Doutriaux MP

T09-002 Arabidopsis Mutants Enhanced in RNA Silencing

Konstantina Boutsika, Francesco Di Serio, Eugene Glazov, Ueli Klahre, Frederick Meins

T09-003 Nuclear transcriptional control of chloroplast functions: analysis of 101 nuclear transcriptomes reveals distinct regulons and their relationship to metabolism and chromosomal gene distribut

Erik Richly, Alexander Biehl, Angela Dietzmann, Christos Noutsos, Dario Leister

T09-004 Effects of mutations causing reduced DNA methylation on interhomologue chromosome association in Arabidopsis thaliana.

Koichi Watanabe, Naohiro Kato, Eric Lam

T09-005 FUNCTIONAL CHARACTERIZATION OF ARABIDOPSIS AtSWI3 GENES ENCODING HOMOLOGS OF A CORE SUBUNIT OF YEAST SWI/SNF CHROMATIN REMODELING COMPLEX

Sarnowski T.J., Swiezewski S., Rios G., Pawlikowska K., Kwiatkowska A., Kozbial M., Kozbial P., Kuras M., Koncz C., Jerzmanowski A.

T09-006 Genetic dissection of early meiotic prophase events in maize and Arabidopsis.

Wojtek P. Pawlowski, Inna N. Golubovskaya, Liang Shi, Jingqiu Li, Waiking Kwan, Xun Wang, Robert B. Meeley, William F. Sheridan, W. Zacheus Cande

T09-007 Two BRCA2-like genes are needed for homologous recombination repair in Arabidopsis

Yuichi Ishikawa, Kiyomi Abe, Keishi Osakabe, Masaki Endo, Yuji ito, Takashi Kuromori, Kazuo Shinozaki, Hiroaki Ichikawa, Toshiaki Kameya, Seiichi Toki

T09-008 An inversion of dominance between epialleles in polyploid Arabidopsis

Mittelsten Scheid, O., Afsar, K., Paszkowski, J.

T09-009 Exploring early signaling pathways in phytochrome B-regulated seedling de-etiolation

Rajnish Khanna, Christina Lanzatella, Peter H. Quail

T09-010 Functional identification of microRNA targets in Arabidopsis

Rebecca Schwab, Javier Palatnik, Markus Riester, Carla Schommer, Markus Schmid, Detlef Weigel

T09-011 TT2, TT8, and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in Arabidopsis thaliana.

Baudry A, Heim MA, Dubreucq B, Caboche M, Weisshaar B, Lepiniec L

T09-012 Identification and characterisation of Aurora-like kinases in Arabidopsis thaliana.

Dmitri Demidov, Andreas Houben

T09-013 Developmental silencing and gene knockout analysis of the Ser/Arg-rich splicing factor SR45

Dheepa Balasubramanian, John Kronforst, Mary A. Schuler

T09-014 Palindromic ACGT-core motifs, the designated bZIP transcription factor binding sites, gather near putative transcriptional initiation sites in front of the ATG

Dierk Wanke, Katia Schütze, Kenneth Berendzen, Ingo Ciolkowski, Christina Chaban, Klaus Harter

T09-015 Expression and Functional Connections of Arabidopsis Two-Component Signaling Elements

Jakub Horak, Christopher Grefen, Klaus Harter

T09-016 Towards a complete transcript map in Arabiodopsis thaliana mitochondria

Joachim Forner, Bärbel Weber, Caterina Wiethoelter, Stefan Binder

T09-017 Interaction between pRb and FIE polycomb protein, point at a possible mechanism regulating endosperm development

Assaf Mosquna, Aviva Katz, Susana Shochat, Gideon Grafi, Nir Ohad

T09-018 A ROS Repressor-Mediated Binary Regulation System for Control of Gene Expression in Transgenic Plants

Ulrike Schäfer, Dwayne Hegedus, Nicholas Bate, Abdelali Hannoufa

T09-019 SCL14 ACTIVATES ACTIVATION SEQUENCE-1 (AS-1) THROUGH INTERACTION WITH TGA FACTORS

Tanja Siemsen, Ralf Weigel, Christiane Gatz

T09-020 The Rad17 homologue of Arabidopsis is involved in the regulation of DNA damage repair and homologous recombination

Fabian Heitzeberg, I-Peng Chen, Frank Hartung, Nadiya Orel, Karel J. Angelis, Holger Puchta

T09-021 Arabidopsis AtMut11, related to a subunit of trithorax-like complexes, is required for gene silencing and heterochromatin maintenance

Jianping Xu, Karin van Dijk, Shirley Sato, Thomas Clemente, Heriberto Cerutti

T09-022 Molecular basis of vernalization requirement and response

Caroline Dean, Josh Mylne, Thomas Greb, Nuno Geraldo, Gyorgy Szittya, Catherine Baxter, Fuquan Liu, Chikako Shindo, Lynne Barratt, Clare Lister

T09-023 Role of E2F transcription factor in the control of Arabidopsis cell growth and differentiation

Elena Ramírez-Parra, Angeles López-Matas, Corinne Fründt, Crisanto Gutiérrez

T09-024 Sub-nuclear localization of chromatin remodeling factor DDM1

Katarzyna Olczak, John Gittins, Andrzej Jerzmanowski, Jan Brzeski

T09-025 In vivo investigation of the transcription, processing, endonucleolytic activity and functional relevance of the spatial distribution of a plant miRNA

Eneida Abreu Parizotto, Patrice Dunoyer, Nadia Rahm, Christophe Himber, Olivier Voinnet

T09-026 Genomic imprinting of the FWA gene in Arabidopsis endosperm

Tetsu Kinoshita, Asuka Miura, Yeonhee Choi, Yuki Kinoshita, Xiaofeng Cao, Steven E. Jacobsen, Robert L. Fischer, Tetsuji Kakutani

T09-027 Histone methylation and heterochromatin assembly in Arabidopsis thaliana

Jörg Fuchs, Zuzana Jasencakova, Armin Meister, Steve Jacobsen, Ingo Schubert

T09-028 Contribution of target transgene position and structure to RNA-directed promoter methylation and TGS

Ute Fischer, Renate Schmidt, M. Florian Mette

T09-029 Control of Arabidopsis development by Polycomb-group dependent histone methylation

Daniel Schubert, Justin Goodrich

T09-030 Control of plant development by the miR-JAW and miR-159 microRNAs

Javier Palatnik, Ed Allen, Carla Schommer, Rebecca Schwab, Norman Warthmann, Xuelin Wu, Jim Carrington, Detlef Weigel

T09-031 Alternating partnerships of FIE with SET domain PcG members, mediate different developmental programs in Arabidopsis

Moran Oliva, Ofir Chakim, Aviva Katz, Nir Ohad

T09-032 Arabidopsis thaliana AtPOLK encodes a DinB-like DNA polymerase that extends mispaired primer termini and is highly expressed in a variety of tissues

Maria Victoria García-Ortiz, Rafael R. Ariza, Peter D. Hoffman, John B. Hays, Teresa Roldán-Arjona

T09-033 Arabidopsis Cellular Responses to DNA Damage

Lu Liang, Jean Molinier, Barbara Hohn

T09-034 The PCF-like subfamily of TCP proteins in Arabidopsis thaliana : molecular and genetic studies.

O. Navaud, P. Dabos, C. Bardet, C.Hervé, D.Trémousaygue

T09-035 **AtERF2 is a Positive Regulator of Defence Gene Expression in Arabidopsis**
McGrath, Ken, Kazan, Kemal, Schenk, Peer, Manners, John, Maclean, Don

T09-036 **FLC the epicentre of an expression domain**
Finnegan E. Jean, Sheldon Candice C., Peacock W. James, Dennis Elizabeth S.

T09-037 **Efficient gene targeting in plants by transient inhibition of non-homologous recombination**
Sylvia de Pater, Paul Bundock, Vanessa Costa, Teresa Samson, Paul Hooykaas

T09-038 **DNA-binding function and physiological function of the Dof transcription factors conserved only in higher plants**
Yoshimi Umemura, Kyoko Matsubara, Muneharu Esaka

T09-039 **Plant specific GAGA-binding proteins regulate MADS-box gene expression through DNA remodelling**
Maarten Kooiker, Chiara A. Airoldi, Prescilla S. Manzotti, Bilitis Colombo, Laura Finzi, Martin M. Kater, Lucia Colombo

T09-040 **The influence of the light period on redox regulation and stress responses**
Beril Becker, Simone Holtgrefe, Sabrina Jung, Regina Brockmann, Andrea Kandlbinder, Karl-Josef Dietz, Jan E. Backhausen, Renate Scheibe

T09-041 **Effector of Transcription (ET): A novel plant protein family repressing gibberellin mediated processes**
Rumen Ivanov, Mats Ellerström, Wim Reidt, Jens Tiedemann, Helmut Bäumlein

T09-042 **The chromatin-remodelling complex FACT associates with actively transcribed regions of the Arabidopsis genome**
Meg Duroux, Andreas Houben, Jiří Friml, Klaus D. Grasser

T09-043 **Signal pathway of the endoplasmic stress response**
Koizumi Nozomu, Iwata Yuji

T09-044 **Post-translational modifications of histones in Arabidopsis thaliana ¯ a proteomics approach towards understanding the histone code**
Eveline Bergmüller, Wilhelm Gruissem

T09-045 **Epigenetic control of seed development**
Claudia Köhler, Lars Hennig, Wilhelm Gruissem, Ueli Grossniklaus

T09-046 **Mitochondria and plastids: Complex machineries transcribe simple genomes**
Kristina Kühn, Karsten Liere, Daniela Kaden, Birte Kuhla, Monika Swiatecka, Uwe Richter, Andreas Weihe, Thomas Börner

T09-047 **Expression of nuclear genes for organellar RNA polymerases in Arabidopsis**
Carola Emanuel, Andreas Weihe, Thomas Börner

T09-048 **Analysis of a suppressor mutant of the immunophilin-like twisted dwarf1 (twd1) gene mutation**
Claudia Moeller, Dierk Wanke, Burkhard Schulz

T09-049 Arabidopsis HAF2 encoding transcription coactivator TAFII250 is required for leaf greening and genetically interacts with photomorphogenic regulators

BERTRAND C., BENHAMED M., DELARUE M., ZHOU D.-X.

T09-050 Specific heterodimerization of group C and group S Arabidopsis thaliana bZIP transcription factors

Fridtjof Weltmeier, Andrea Ehlert, Xuan Wang, Jesús Vicente-Carbajosa, Pilar Carbonero, Wolfgang Dröge-Laser

T09-051 The INCURVATA2 gene is involved in chromatin-mediated cellular memory

J. M. Barrero, M. R. Ponce, J. L. Micol

T09-052 A molecular and structural analysis of introns that reside in non-coding sequence.

Roger P. Hellens, Cas Simons

T09-053 Specific Methylation-Mediated Silencing of 4CL::GUS Transgene - Expression

B. Soltani, J. Ehlting, C. J. Douglas

T09-054 Helper component proteinase (HC-Pro) as a tool to dissect the mechanisms of RNA silencing and microRNA (miRNA) mediated RNA degradation

Lewis Bowman, Mathew Endres, Braden Roth, Ge Xin, Xuemei Chen, Vicki Vance

T09-055 Histone H1 is required for maintaining the pattern of DNA methylation in Arabidopsis

Andrzej T. Wierzbicki, Andrzej Jerzmanowski

T09-056 Chromatin assembly and gene silencing during development involve MSI1-like proteins

Lars Hennig, Romaric Bouveret, Vivien Exner, Wilhelm Gruissem, Claudia Köhler, Nicole Schönrock

T09-057 MicroRNA regulation of lateral organ separation in Arabidopsis

Diana Dugas, Allison Mallory, David Bartel, Bonnie Bartel

T09-058 Developmental defects triggered by ectopic expression of microRNAs in Arabidopsis

Heather A. Fitzgerald, Kristin D. Kasschau, Taiowa Montgomery, James C. Carrington

T09-059 Inheritance of methylation patterns of transgenes displaying post-transcriptional gene silencing in Arabidopsis thaliana

Matthias Arlt, Daniel Schubert, Renate Schmidt

T09-060 Transcriptional and chromatin regulation: a dynamic affair

Marjori Matzke

T09-061 A gene-specific RNA sensing mechanism, not position effects, triggers silencing in T-DNA transformants

Renate Schmidt, Daniel Schubert, Berthold Lechtenberg, Alexandra Forsbach, Mario Gils

T09-062 Functional characterization of the Polycomb group protein MEDEA from Arabidopsis thaliana

U. Akinci, C. Köhler ,U. Grossniklaus

T10 Novel Tools, Techniques and Resources

T10-001 A global view of cellular identity in the Arabidopsis root

Kenneth D. Birnbaum, Dennis E. Shasha, Jean Y. Wang, Jee W. Jung, Georgina M. Lambert, David W. Galbraith, Philip N. Benfey

T10-002 TILLING - high throughput functional genomics

Heike Wohlgemuth, Jeff Harford

T10-003 Quantitative Immunodetection using Infrared Technology

Heike Wohlgemuth, Jim Wiley

T10-004 K+ channel interactions detected by a system optimized for systematic studies of membrane protein interactions

Petr Obrdlik, Mohamed El-Bakkoury, Tanja Hamacher, Corinna Cappellaro, Cristina Vilarino, Carola Fleischer, Jose L. Revuelta, Eckhard Boles, Bruno André, Wolf B. Frommer

T10-005 Functional Genomics using RIKEN Arabidopsis Full-length cDNAs

Seki, M., Ishida, J., Nakajima, M., Enju, A., Sakurai, T., Iida, K., Satou, M., Akiyama, K., Oono, Y., Fujita, M., Kamei, A., Yamaguchi-Shinozaki, K., Ecker, J.R., Davis, R.W., Theologis, A., Shinozaki, K.

T10-006 Professor

Bernd Markus Lange

T10-007 A Comparison of Global gene Expression and MPSS profiling in Arabidopsis thaliana.

Sean J Coughlan, Blake Meyers, Vikas Agrawal, Hassan Ghazal, Pam Green

T10-008 Proteomic analysis of nuclear components from Arabidopsis suspension cells and Arabidopsis plants

Maciej Kotlinski, Tomasz Calikowski, Andrzej Jerzmanowski

T10-009 Bimolecular fluorescence complementation - a novel tool for in planta protein interaction studies

Christina Chaban, Michael Walter, Katia Schütze, Oliver Batistic, Claudia Oecking, Wolfgang Werr, Jörg Kudla, Klaus Harter

T10-010 Identification of apoplastic plant proteins by Transposon Assisted Signal Trapping (TAST)

Anja M. Kuschinsky, Carsten H. Hansen, Kirk M. Schnorr, Markus Pauly

T10-011 Plant resources database at the MPI-MP

Karin I. Köhl, Alexander Lüdemann, Joachim Kopka, Arnd G. Heyer

T10-012 Development of a quality-controlled cDNA micro-array method for expression profiling

Thomas Degenkolbe, Matthew Hannah, Susanne Freund, Dirk K. Hincha, Arnd G. Heyer, Karin I. Köhl

T10-013 Report of Resource Project in RIKEN BRC

Masatomo Kobayashi, Hiroshi Abe, Satoshi Iuchi, Toshihiro Kobayashi

T10-014 A molecular atlas of transcription factor expression patterns in Arabidopsis

Derbyshire, P., Drea, S., Crawford, B., Corsar, J., Shaw, P., Doonan, J., Dolan, L.

T10-015 A method to isolate chloroplasts from specific cell types of Arabidopsis thaliana

Elisabeth B. Truernit, Julian M. Hibberd

T10-016 Plant Bimolecular Fluorescence Complementation (PBFC) ¯ a system for detecting protein-protein interactions in plants

Keren Shichrur, Keren Bracha-Drori, Moran Oliva, Aviva Katz, Ruthie Angelovici, Nir Ohad, Shaul Yalovsky

T10-017 The essentials of tissue-specific protein and metabolite profiling - Laser Microdissection, LC/MS/MS and GCMS

Martina Schad, Richard D. Smith, Patrick Giavalisco, Oliver Fiehn, Stefanie Wienkoop, Wolfram Weckwerth, Julia Kehr

T10-018 AtGenExpress ¯ Expression atlas of Arabidopsis Development

Markus Schmid, Stefan Henz, Timothy Davison, Utz Pape, Martin Vingron, Bernhard Schölkopf, Detlef Weigel, Jan U. Lohmann

T10-019 Novel Gene Discovery in Arabidopsis thaliana

Beverly Underwood, Yongli Xiao, William Moskal, Udana Torian, Julia Redman, Hank Wu, Christopher Town

T10-020 RNAi for Plant Functional Genomics

Chris Helliwell, Varsha Wesley, Anna Wielopolska, Louisa Matthew, Neil Smith, Ming-bo Wang, David Bagnall, Ian Small, Ian Moore, Peter Waterhouse

T10-021 NARC - Norwegian Arabidopsis Research Centre - University of Oslo

Barbro E. Saether, Reidunn B. Aalen

T10-022 Imposing rigorously identical water deficits to different Arabidopsis thaliana accessions. An automated system for high throughput analyses of plant responses to soil water deficit.

C. Granier, P. Hamard, M. Dauzat, K. Chenu, L. Aguirrezabal, J.J. Thioux, B. Muller, F. Tardieu, T. Simonneau

T10-023 Real-Time RT-PCR profiling of over 1,400 Arabidopsis transcription factors: Unprecedented sensitivity reveals novel root- and shoot-specific genes

CZECHOWSKI T, BARI R, STITT M, SCHEIBLE WR, UDVARDI MK

T10-024 Search for Tissue-specific Promoters in Arabidopsis

Lee Theresa, Ahn Il-pyung, Kang Sang-ho, Park Yong-hwan, Suh Seok-cheol, Kim Young-mi

T10-025 Fox Hunting: A novel gain-of-function gene-hunting technique.

Takanari Ichikawa, Miki Nakazawa, Mika Kawashima, Haruko Iizumi, Hirofumi Kuroda, Youichi Kondow, Yumi Tsuhara, Kumiko Suzuki, Akie Ishikawa, Motoaki Seki, Miki Fujita, Reiko Motohashi, Noriko Nagata, Kazuo Shinozaki, Minami Matsui

T10-026 Laser microdissection: A tool to perform tissue-specific transcript profiling using microarray analysis

Regine Kleber, Julia Kehr

T10-027 Proteome analyses of different plastid types: A first step towards a "systems" analysis of plastid development and differentiation

Torsten Kleffmann, Anne von Zychlinski, Asim Siddique, Doris Russenberger, Wayne Christopher, Kimmen Sjolander, Wilhelm Gruissem, Sacha Baginsky

T10-028 Stable high-level transgene expression in Arabidopsis thaliana using gene silencing mutants and matrix attachment regions

Katleen M.J. Butaye, Ilse Geudens, Inge J.W.M. Goderis, Piet F.J. Wouters, Stijn L. Delauré, Bruno P.A. Cammue, Miguel F.C. De Bolle

T10-029 Cellular and subcellular protein profiling: Comprehensive Shotgun LC/MS/MS analysis in Arabidopsis thaliana

Stefanie Wienkoop, Ute Lehmann, Daniela Zoeller, Berit Ebert, Joachim Fisahn, Wolfram Weckwerth

T10-030 Development of IRES-mediated gene expression system in plants

Naoko Matsuo, Kazuyuki Hiratsuka

T10-031 A novel approach to dissect the abscission process in Arabidopsis.

Gonzalez-Carranza, Zinnia H., Roberts, Jeremy A.

T10-032 Enrichment of phosphorylated proteins from A. thaliana

Florian Wolschin, Wolfram Weckwerth

T10-033 Analysis of gene expression within single cells from Arabidopsis thaliana

Schliep, Martin, Zoeller, Daniela, Simon-Rosin, Ulrike, Fisahn, Joachim

T10-034 Gene Identification by Transcript Based Cloning

James Hadfield, Giles Oldroyd

T10-035 Integrative metabolic networks in plants: What do we get from it?

Morgenthal, Glinski, Wienkoop, Steuer, Weckwerth

T10-036 Protein profiling at the single cell level in Arabidopsis thaliana

Berit Ebert, Stefanie Wienkoop, Christian Melle, Ferdinand von Eggeling, Wolfram Weckwerth, Joachim Fisahn

T10-037 Genome-wide Analysis of Recombination Frequency in Arabidopsis thaliana

Janny L. Peters, Filip Cnudde, Paul Wijnhoven, Nigel Grimsley, Barbara Hohn, Tom Gerats

T10-038 Exploiting Laser Capture Microdissection to elucidate spatial gene expression during plant embryogenesis.

Matthew Spencer, Stuart Casson, Keith Lindsey

T10-039 The Genome Laboratory- Genomic Services for Arabidopsis Research

James Hadfield, Leah Clissold

T10-040 An alternative tandem affinity purification strategy applied to Arabidopsis protein complex isolation

Vicente Rubio, Yunping Shen, Yusuke Saijo, Yule Liu, Giuliana Gusmaroli, Savithramma P. Dinesh-Kumar, Xing Wang Deng

T10-041 The AGRIKOLA project: systematic RNAi in Arabidopsis

Thomas Altmann, Javier Paz-Ares, Jim Beynon, Murray Grant, Pierre Hilson, Ian Small

T10-042 Functional Analysis of EMB Genes Using Epitope-Tagged Proteins

Michael Berg, Rebecca Rogers, David Meinke

T10-043 Toward the high throughput identification of the binding sites of TGA transcription factors using a whole-genome promoter array

Françoise Thibaud-Nissen, Julia Redman, Christopher Johnson, Todd Richmond, Roland Green, Jonathan Arias, Christopher Town

T10-044 Transcriptome and proteome analysis of the light induced greening of an Arabidopsis cell culture

Yasuo Niwa, Anne von Zychlinski, Torsten Kleffmann, Philip Zimmermann, Wilhelm Gruissem, Sacha Baginsky

T10-045 CSB.DB - A Comprehensive Systems-Biology Database

Dirk Steinhauser, Bjoern Usadel, Alexander Luedemann, Oliver Thimm, Joachim Kopka

T10-046 Subcellular-Targeting for Efficient Expression of Foreign Gene in Transgenic Plant

Kim Young-mi, Theresa Lee, Ahn Il-pyoung, Kang Sang-ho, Park Yong-hwan, Suh Seok-cheol

T10-047 Insertional mutagenesis by Ac/Ds transposon system and a phenome analysis of transposon-tagged lines in Arabidopsis

Takashi Kuromori, Takuji Wada, Masahiro Yuguchi, Takuro Yokouchi, Kiyotaka Okada, Asako Kamiya, Yuko Imura, Takashi Hirayama, Kazuo Shinozaki

T10-048 AGRIKOLA: a systematic approach for hpRNA induced gene silencing

The Agrikola consortium, Magdalena Weingartner, Karin Köhl, Thomas Altmann

T10-049 The use of STAIRS for mapping genes controlling reporter gene transfer and integration into Arabidopsis thaliana using Agrobacterium-mediated transformation.

Angela Oldacres, Fadhilah Zainudin, Joanne Billington, Tim Wilkes, Mike Kearsey, Ian Puddephat, H. John Newbury

T10-050 T-DNA insertion mutagenesis: identification of tagged Arabidopsis genes by insert mapping and promoter trapping.

László Szabados, Edit Ábrahám, Isabella Kovács, Attila Oberschall, Martha Alvarado, Laura Zsigmond, Irén Kerekes, Gábor Rigó, Réka Nagy, Inga Krasovskaja, Csaba Koncz

T10-051 Development of a novel reporter to monitor homoeologous recombination events in Arabidopsis.

Liang Liang Li, Martine Jean, Samuel Santerre-Ayotte, Francois Belzile

T10-052 Mapping LUX ARRHYTHMO, a novel myb transcription factor essential for circadian rhythms, and other circadian clock mutants by oligonucleotide array genotyping

Samuel P Hazen, Justin O Borevitz, Thomas F Schultz, Frank G Harmon, Jose L Pruneda-Paz, Joseph R Ecker, Steve A Kay

T10-053 Identification of genetic regions controlling Agrobacterium-mediated transformation of Arabidopsis thaliana

Joanne Billington, Fadhilah Zainudin, Angela M Oldacres, Dr Ian Puddephat, Dr H. John Newbury

T10-054 Functional Annotation of the Arabidopsis Genome Using Controlled Vocabularies

Suparna Mundodi, Tanya Berardini, Leonore Reiser, Mary Montoya, Dany Yoo, Iris Xu, Sue Rhee

T10-055 GENOME-WIDE DISCOVERY OF TRANSCRIPTION UNITS AND FUNCTIONAL ELEMENTS IN ARABIDOPSIS

Joseph R. Ecker et al.

T11 Modeling the Virtual Plant / Bioinformatics

T11-001 Formation of flower primordia at the shoot apical meristem of Arabidopsis ¯ a quantitative approach to the meristem surface growth

Dorota Kwiatkowska

T11-002 Bicistronic and fused monocistronic transcripts are derived from adjacent Arabidopsis loci

Jyothi Thimmapuram, Hui Duan, Lei Liu, Mary A. Schuler

T11-003 MotifMapper: A modular based collection of Visual Basic routines for the analysis of correlative sequence data

Kenneth Berendzen, Dierk Wanke, Csaba Koncz, Imre E. Somssich, Kurt Stüber

T11-004 UniProt and the Swiss-Prot Plant Proteome Annotation Project (PPAP)

Michel Schneider, Michael Tognolli, Amos Bairoch

T11-005 AthaMap, an online resource for in silico transcription factor binding sites in the Arabidopsis thaliana genome

Nils Ole Steffens, Claudia Galuschka, Lorenz Bülow, Martin Schindler, Reinhard Hehl

T11-006 Metabolite fingerprinting: an ICA approach

M. Scholz, S. Gatzek, A. Sterling, O. Fiehn, J. Selbig

T11-007 Computational comparison of eukaryotic SNF1 and plant-specific SnRK1 protein kinase phosphorylation motifs on the basis of mutual information (MI).

Jan Hummel, Nima Keshvari, Wolfram Weckwerth, Joachim Selbig

T11-008 DIAGNOSIS OF PLANT METABOLISM

Yves Gibon, Jan Hannemann, Oliver Bläsing, Joachim Selbig, Oliver Thimm, Melanie Höhne, Mark Stitt

T11-009 Non-Random Distribution of Transcription Factor Binding Sites in the Arabidopsis thaliana Genome

Claudia Galuschka, Nils Ole Steffens, Lorenz Bülow, Reinhard Hehl

T11-010 New tools for computer visualisation and modeling of cell interactions.

Tim Rudge, Sarah Hodge, Smita Kurup, Jean-Maurice Assie, Lilian Ricaud, Jennifer Clark, Jim Haseloff

T11-011 Arabidopsis Microarray Resource at TAIR

Margarita Garcia-Hernandez, Nick Moseyko, Suparna Mundodi, Neil Miller, Mary Montoya, Jessie Cui Zhang, Iris Xu, Dan Weems, Seung Yoon Rhee

T11-012 Quantitative modelling of Arabidopsis thaliana development

Yvette Erasmus, Enrico Coen, Lars Muendermann, Przemyslaw Prusinkiewicz

T11-013 DegP/HtrA proteases in plants: A proposal for a new classification and nomenclature

Pitter Huesgen, Holger Schuhmann, Sadok Legroune, Jaime Garcia-Moreno, Iwona Adamska

T11-014 Model of rosette development and expansion in Arabidopsis thaliana subjected to various temperature and incident radiation conditions

Christophe A., Chenu K., Lecoeur J.

T11-015 Leaf development in response to light in Arabidopsis thaliana: a quantitative approach using 3D virtual plants to compare genotypes

Chenu K., Franck N., Lecoeur J.

T11-016 PaVESy: Combining profiling data with pathway knowledge

Alexander Luedemann, Claudia Birkemeyer, Daniel Weicht, Joachim Selbig, Joachim Kopka

T11-017 GABI-Primary Database: A Comprehensive Database for Plant Genome Data

Svenja Meyer, Axel Nagel

T11-018 MapManXT a generic software tool to functionally assign plant's genome and metabolome enabling integration and display of complementary high-throughput data onto biochemical pathway maps

Oliver Thimm, Juliane Fluck, Axel Nagel, Svenja Meyer, Daniel Weicht, Yves Gibon, Henning Redestig, Oliver Bläsing, Joachim Selbig, Mark Stitt

T11-019 The SYSTERS Protein Family Web Server: Shortcut from large-scale sequence information to phylogenetic information

Thomas Meinel, Eike Staub, Antje Krause, Hannes Luz, Stefanie Hartmann, Ute Krämer, Joachim Selbig, Martin Vingron

T11-020 ARAMEMNON 2: a database and data mining tool for Arabidopsis and rice membrane proteins

Rainer Schwacke, Eric van der Graaff, Anja Schneider, Ulf-Ingo Flügge, Reinhard Kunze

T11-021 ARABI-COIL - an Arabidopsis Coiled-coil Protein Database

Annkatrin Rose, Sankaraganesh Manikantan, Shannon J. Schraegle, Michael A. Maloy, Eric A. Stahlberg, Iris Meier

T11-022 TAIR (The Arabidopsis Information Resource): New Tools and Data

Eva Huala, Margarita Garcia-Hernández, Suparna Mundodi, Tanya Berardini, Katica Ilic, Nick Moseyko, Leonore Reiser, Peifen Zhang, Julie Tacklind, Brandon Zoeckler, Douglas Becker, Neil Miller, Mary Montoya, Dan Weems, Iris Xu, Thomas Yan, Daniel Yoo, Jessie Zhang, Seung Yon Rhee

T11-023 Genomic and systems biology approaches to understand CN signal interactions in Arabidopsis.

Gutierrez, R.A., Lejay, L., Shasha, D., Coruzzi, G.

T11-024 Detecting Chromosome Features using an Unsupervised Probabilistic Multigram Model: a Case Study of the Arabidopsis thaliana Genome

Terry Clark, John Goldsmith, Daphne Preuss

T11-025 The Botany Affymetrix Database: e-Northerns and Expression Angling

Kiana Toufighi, Eugene Ly, Nicholas J. Provart

T11-026 Modeling and in vivo live imaging of the Arabidopsis shoot apical meristem

Henrik Jönsson, Marcus Heisler, Bruce E. Shapiro, Victoria Gor, G. Venugopala Reddy, Elliot M. Meyerowitz, Eric Mjolsness

T11-027 GENEVESTIGATOR: Arabidopsis thaliana microarray database and analysis toolbox.

Philip Zimmermann, Matthias Hirsch-Hoffmann, Wilhelm Gruissem, Lars Hennig

T11-028 Modeling Arabidopsis thaliana from genes to phenotypes

Przemyslaw Prusinkiewicz

T11-029 From Genes to Morphogenesis

Enrico Coen

T12 Non-Arabidopsis (Limitations of the Arabidopsis Model)

T12-001 Arabidopsis cannot survive long periods of anoxia: analysis of gene expression during mitochondrial biogenesis using rice germination as a model system

Katharine A. Howell, Linne E. Jenkin, A. Harvey Millar, James Whelan

T12-002 Genome analysis in sugar beet (Beta vulgaris L.)

Katharina Schneider, Diana Bellin, Sandra Hunger, Silke Möhring, Elena Pestsova, Francesco Salamini, Britta Schulz

T12-003 CYCLOIDEA and Floral Symmetry in Aster family

Minsung Kim, Pilar Cubas, Amanda Gillies, Richard Abbott, Enrico Coen

T12-004 Assessing the impact of polyploidy by comparative analysis of Brassica genome microstructure

Dr. Ian Bancroft

T12-005 Development of three different cell types is associated with the activity of a specific MYB transcription factor in the ventral petal of Antirrhinum majus flowers

Glover, Beverley J, Perez-Rodriguez, Maria, Jaffe, Felix, Butelli, Eugenio, Martin, Cathie

T12-006 A population genomic search for maize domestication genes

Stephen Wright, Irie Bi Vroh, Masanori Yamasaki, Steven Schroeder, John Doebley, Michael McMullen, Brandon S. Gaut

T12-007 Wox gene phylogeny and expression in Maize and Arabidopsis: A comparison of embryonic pattern formation

Judith Nardmann, Wolfgang Werr

T12-008 Tobacco functional genomics: Uncovering the importance of "-like" and "unknown" genes for plant fitness

Wolfgang Lein, Mark Stitt, Frederik Börnke, Uwe Sonnewald, Thomas Ehrhardt, Andreas Reindl

T12-009 Cold-induced pollen sterility in rice is associated with a disruption in sugar metabolism and increase in ABA levels

Sandra N. Oliver, Joost Van Dongen, Peter Geigenberger, Hargurdeep S. Saini, Chris L. Blanchard, Paul E. Roffey, Elizabeth S. Dennis, Rudy Dolferus

T12-010 Molecular and Genetic Analysis of rough endosperm Mutants in Maize

Diego Fajardo, Susan Latshaw, Donald R. McCarty, A. Mark Settles

T12-011 Drought Stress Tolerance: from gene discovery in Arabidopsis to an application in Crops

Jacqueline Heard, Don Nelson, Tom Adams, Karen Gabbert, Jingrui Wu, Oliver Ratcliffe, Bob Creelman, Brendan Hinchey, Emily Reisenbigler, Paolo Castiglioni, Meghan Galligan, Bob Bensen, Kris Hardeman, Neal Gutterson, Stan Dotson

T12-012 Subcellular analysis of carbon partitioning and regulation of ADP-Glucose-pyrophosphorylase in rice endosperm

Sonja Reiland, Anna Kolbe, Axel Tiessen, Joost T. van Dongen, Peter Geigenberger

T12-013 Two Zn-responsive metallothionein genes from Thlaspi caerulescens

Viivi Hassinen, Pauliina Halimaa, Arja Tervahauta, Kristina Servomaa, Sirpa Kärenlampi

T12-014 Ecological Genomics of Glucosinolates in Boechera (Brassicaceae)

Aaron J. Windsor, Alice M. Shumate, Nataˇa Formanová, Thomas Mitchell-Olds

T12-015 PROTEIN-PROTEIN INTERACTIONS BETWEEN MADS BOX TRANSCRIPTION FACTORS DIRECTING FLOWER DEVELOPMENT IN SUNFLOWER AND CHRYSANTHEMUM

Shulga Olga, Shchennikova Anna, Angenent Gerco, Skryabin Konstantin

T12-016 Proteomic profiling of Thlaspi caerulescens populations

Marjo Tuomainen, Naoise Nunan, Arja Tervahauta, Viivi Hassinen, Satu Lehesranta, Sirpa Kärenlampi

T12-017 Cytokinin signaling in secondary vascular development

Kaisa M. Nieminen, Leila Kauppinen, Marjukka Laxell, Sari Tähtiharju, Juha Immanen, Ykä Helariutta

T12-018 **Altered apyrase activity influences potato (Solanum tuberosum) plant development and tuber yield**
David Riewe, Jeremy Clark, Peter Geigenberger

T12-019 **Linkage Disequilibrium and Potential Demographic Factors Shaping Genetic Variation in Arabidopsis lyrata subsp. petraea**
Lawton-Rauh, Amy, Mitchell-Olds, Tom

T12-020 **Changes in the modifications of core histone H3 after salt stress in the BY-2 tobacco cell line and Arabidopsis thaliana cells**
Sokol A., Prymakowska-Bosak M., Jerzmanowski A.

T12-021 **Population biology of the other Arabidopsis: life history, ecology and population dynamics of A. lyrata, a close relative of A. thaliana.**
Clauss, MJ, Mitchell-Olds, T

T12-022 **Use of halophytic Arabidopsis relative model systems (ARMS) to reveal unique genetic components of salt tolerance**
Gunsu Inan, Qingqiu Gong, Shisong Ma, Mark Fredricksen, Huazhong Shi, Paul M. Hasegawa, Hans J. Bohnert, Robert J. Joly, Jian-Kang Zhu, Ray A. Bressan

T12-023 **Modification of Flower Color in Dianthus caryophyllus by Genetic Transformation**
Sung-Jin Kim, Ji-Sun Baek, Youn-Hee Choi, Kwang-Woong Lee

T12-024 **Analysis of reproductive mode, ploidy, and flowering time in Boechera (Brassicaceae) populations**
M. Eric Schranz, Thomas Mitchell-Olds

T12-025 **Dissecting symbiotic nitrogen fixation in legumes**
Cook, Douglas, Ané, Jean-Michel, Penmesta, R. Varma, Riely, Brendan

T12-026 **What plant research will be like 10 years from now.**
Steve Briggs

T12-027 **Zooming-in on a Tomato Yield Quantitative Trait Nucleotide (QTN) with Wild Species Introgression Lines**
Eyal Fridman, Fernando Carrari, Yong-Sheng Liu, Alisdair Fernie, Dani Zamir

T12-028 **Model Systems, Plant Sciences, and the Shift to Horizontal Biology**
Steven D. Tanksley, Andre Kessler

T13 Others

T13-001 **Expression Profiles of Arabidopsis thaliana During Natural Leaf Senescence**
Chen-Kuen Wang, Kin-Ying To

T13-002 Exploring a new detergent inducible promoter active in higher plants and its potential biotechnological application

Gretel M. Hunzicker, Elmar W. Weiler, I. Kubigsteltig

T13-003 SH2 proteins in plants : The story is just beginning

Latha Kadalayil

T13-004 Autophosphorylation Activity of the Arabidopsis Ethylene Receptor Multigene Family

Patricia Moussatche, Harry J. Klee

T13-005 Protein-protein interactions in the cytokinin signal transduction pathways of Arabidopsis thaliana

Heyl, A., Dortay, H., Bürkle, L., Schmülling, T.

T13-006 CHARACTERIZATION OF LOSS-OF-FUNCTION MUTANTS OF CYTOKININ OXIDASE/DEHYDROGENASE GENES AND SUPPRESSOR-MUTAGENESIS OF TRANSGENIC PLANTS WITH REDUCED CYTOKININ CONTENT

Isabel Bartrina, Tomá˘ Werner, Michael Riefler, Thomas Schmülling

T13-007 Characterization of the Cullins AtCUL3a and AtCUL3b in Arabidopsis thaliana

Perdita Hano, Aysegül Mutlu, Hanjo Hellmann

T13-008 The Arabidopsis Biological Resource Center ¯ 2003-2004 Activities; Resource Acquisitions and Stock Distribution

Randy Scholl, Emma Knee, Luz Rivero, Deborah Crist, Natalie Case, Rebecca Klasen, James Mann, Julie Miller, Garret Posey, Pamela Vivian, Zhen Zhang, Ling Zhou

T13-009 Genomics-Related Stocks Distributed by ABRC

Randy Scholl, Emma Knee, Deborah Crist, Luz Rivero, Natalie Case, Rebecca Klasen, James Mann, Julie Miller, Garret Posey, Pamela Vivian, Zhen Zhang, Ling Zhou

T13-010 Cytokinin oxidases/dehydrogenases (CKX) of Arabidopsis as a tool to study cytokinin functions in shoot and root development

Tomas Werner, Ireen Köllmer, Thomas Schmülling

T13-011 Cell wall polysaccharide analysis of tomato fruits and quantitative trait loci (QTL) mapping of responsible regions in the tomato genome

Antje Bauke, Dani Zamir, Markus Pauly

T13-012 Genetic mapping and characterization of the Arabidopsis trichome birefringence (tbr) mutant.

Ana-Silvia Nita, Ravit Eshed, Deborah P. Delmer, Wolf-Rüdiger Scheible

T13-013 Genetic and molecular dissection of the role of CPD steroid hydroxylase in regulation of brassinosteroid hormone biosynthesis and signalling in Arabidopsis

Marcel Lafos, Zsuzsanna Koncz, Csaba Koncz

T13-014 Functional Analysis of Arabidopsis At4g23740 GeneF

X. Zhang, J. Heinz, J. Choi, C. Chetty

T13-015 Dynamics of protein complexes through the cell cycle: a proteome technical approach

Noor Remmerie, Peter Deckers, Kris Laukens, Harry Van Onckelen, Erwin Witters

T13-016 Localisation of the Agrobacterium oncoprotein A4-Orf8 and its constituent parts.

Umber M., Clément B., Gang S., Voll L., Weber A., Michler P., Helfer A., Otten L.

T13-017 Molecular analysis of xyloglucan-specific galactosyltransferases in Arabidopsis

Xuemei Li, Israel Cordero, Nicholas Carpita, Wolf-Dieter Reiter

T13-018 Autophosphorylation sites of AtCPK5

Camille N. Strachan, Adrian D. Hegeman, Aaron Argyros, Estelle M. Hrabak, Jeffrey F. Harper, Nancy D. Denslow, Alice C. Harmon

T13-019 DIURNAL CHANGES IN CYTOKININ LEVELS IN ARABIDOPSIS PLANTS

Hoyerová K, Kamínek M.

T01 Development 1 (Flower, Fertilization, Fruit, Seed)

T01-001

AtBRM, an ATPase of the SNF2 family, controls flowering in Arabidopsis

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In the cellular nucleus the DNA is associated with proteins to form the chromatin. Regulation of the chromatin structure is essential to control gene expression during developmental processes. SNF2 family proteins are implicated in DNA metabolism through chromatin remodeling. We have characterized one protein of this family in Arabidopsis thaliana, ARABIDOPSIS THALIANA BRAHMA. AtBRM is a close homolog of the ATPase of Drosophila SWI/SNF complex. AtBRM is expressed in all the organs of the plants, mostly in meristem and fast dividing tissues. Plants with low levels of AtBRM obtained by RNAi (atbrm plants) showed a pleitropic phenotype. We have focused our analysis in the study of changes in the reproductive development in these plants. The flowers of atbrm plants had small petals and stamens, immature anthers, homeotic transformations and reduced fertility. These plants were able to flower earlier than wild type plants under inductive and non-inductive photoperiods. The expression of several genes of the autonomous pathway, such as CO, FT and SOC1, is upregulated in silenced plants grown under non-inductived conditions. These results indicated that AtBRM plays an important role in repression of photoperiod-dependent flowering

T01-002

FROM WHEAT TO ARABIDOPSIS: UTILIZING GENE EXPRESSION PROFILES FROM ISOLATED WHEAT EGG CELLS FOR DISCOVERY OF NOVEL AND EGG CELL SPECIFIC GENES IN ARABIDOPSIS

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Little is known about the complex transcript composition of the female gametes (egg cell and central cell) from seed plants. Moreover, transcriptional changes occurring after fertilization and during very early embryogenesis are not well characterized. In many angiosperm plants including Arabidopsis, the limiting factors hindering the isolation of female gametes are their small size and inaccessibility, since the cells are deeply embedded in maternal tissues. Using a microdissection technique for wheat ovaries, we are isolating egg cells and central cells from unpollinated wheat ovules, as well as defined stages of zygotes and early embryos. cDNA populations generated from few reproductive cells were subsequently used to study gene expression profiles after bioinformatical analysis of some thousand ESTs. The expression of selected candidates was further studied by RT-PCR. Numerous cDNAs represent novel genes, specifically expressed in the egg cell and/or the 2-celled proembryo. In addition, many wheat ESTs exhibit significant similarity to "hypothetical" genes from Arabidopsis and/or rice. We will report about our approach to discover novel/hypothetical genes in Arabidopsis whose expression is not known until now. Three "hypothetical" gene families of Arabidopsis are analyzed as an example in more detail on both, expressional and functional level, all representing potential key genes involved e.g. in cell identity, signalling and fertilization.

T01-003

Recent advances in flower development

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I will review recent advances in flower development, focusing on progress in understanding mechanisms of floral induction and early floral patterning, including the identification of direct target genes and the role of microRNAs in flowering.

T01-004

ANALYSING FUNCTIONAL DOMAINS OF THE CARPEL DEVELOPMENT GENE SPATULA OF ARABIDOPSIS THALIANA

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The gynoecium developmental gene SPATULA controls the internal growth of carpel margins and the pollen tract tissue derived from them. It encodes a basic Helix-Loop-Helix (bHLH) transcription factor that is expressed in these tissues, and in other tissues unaffected in spatula mutants where it may have redundant partners. By dissecting and analysing different domains of the SPATULA protein, we aim to understand how it functions.

The most significant functional domain is the bHLH region, known in other bHLH proteins to play a role in sequence specific DNA binding (the basic region) and in dimerization (the helix-loop-helix region). Overlapping the basic region is a bipartite nuclear localization region (NLS). When a p35S:SPT: GFP reporter gene is biolistically transfected to onion epidermal cells, the gene product is localised to the nucleus. A short 27 amino acid sequence (including the NLS) alone is sufficient to direct nuclear import. Conversely, upon deleting the bipartite NLS, the fusion protein is now found in both the cytoplasm and the nucleus (similar to the control p35S:GFP). However, simultaneous introduction of p35S:SPT-NLS:GFP and p35S:SPT into onion cells results in localisation of the fluorescent protein only to nuclei. This indicates that the SPT protein is able to homo-dimerise, and that only one member of the dimer needs to have a NLS for it to be directed into the nucleus. Similar experiments involving p35S:ALC (ALCATRAZ) and p35S:IND (INDEHISCENT), two other bHLH genes expressed in the gynoecium, also showed evidence for some preferential movement of SPT-NLS:GFP to the nucleus, as expected if these proteins could hetero-dimerise with SPT. Further experiments using the yeast two hybrid system are under way.

Other putative functional secondary structures, localized in the N-terminal region, are two helical regions, one charged and one amphipathic. The protein also includes also two acidic and several serine rich domains found to act as activation domains in other transcription factors. By removing different putative functional domains we are testing which are absolutely required for complementation of the spatula mutant.

Heisler et al . 2001 Development 128, 1089-1098.

T01-005

PATTERN OF FUNCTIONAL EVOLUTION OF THE FLORAL MERISTEM IDENTITY PROTEIN LEAFY

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Two genes, LEAFY (LFY) and APETALA1 (AP1), are required to specify floral identity in Arabidopsis. Expression of LFY in otherwise non-reproductive meristems can cause their conversion into floral meristems in a variety of species, demonstrating that it is a master regulator of floral development. AP1 is a direct target of LFY, and both act in a partially redundant manner to specify floral meristem identity. At least some of this function is due to activation and regulation of various homeotic genes, which control the identity of different floral organs. Biochemically, LFY is a sequence specific DNA-binding protein with no similarity to any other plant or animal protein.

Homologs of LFY have been cloned from many seed plants (angiosperms and gymnosperms), as well as from the more distant non-flowering Pteridophytes and Bryophytes (ferns and mosses). All share two highly conserved domains. Despite their similarity to their seed plant counterparts, the function of the LFY homologs in species that arose prior to evolution of floral structure is unclear.

We have decided to take advantage of the molecular diversity generated during evolution to obtain insights into the structure-function relationships of LFY. We sampled a set of homologs derived from all major clades of extant plants and tested the functionality in several ways. By combining classical phenotypic analysis with genome-wide molecular profiling, we assayed the potency of the different homologs to rescue a strong null allele of LFY. We also assayed in yeast models their transcriptional activity and DNA binding activity. Several conclusions arose from this study. First, flowers and activity of LFY homologs in Arabidopsis date to the same evolutionary time point. Almost all angiosperms homologs are functionally interchangeable. However, homologs from the non-flowering clades can only partially complement a lfy null allele, their potency being inversely proportional to their evolutionary distance. Second, among the molecular targets of LFY, AP1 is the main output. AP1 is the only target upregulated by the homologs from non-flowering plants. Third, the different homologs vary in their DNA binding affinity. By building chimera between reference homologs, we have established that the conserved domains contribute more than the non-conserved domains to diversification of LFY activity. Finally the role of criticals amino-acids for evolution of LFY function is examined.

T01-006

A genetic model for floral meristem development

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The transition from vegetative to reproductive growth is a dramatic developmental change in the life of plants, in which newly formed meristems acquire floral rather than inflorescence identity. Floral meristem identity genes LEAFY (LFY) and APETALA1 (AP1) promote establishment and maintenance of floral identity in newly formed floral primordia. Without their activity, the floral primordia develop with inflorescence characteristics. The underlying molecular-genetic mechanism remains unknown. Our studies show that these phenotypes are due in large part to the ectopic expression of AGAMOUS-LIKE 24 (AGL24), a central regulator of floral meristem identity. We present evidence that AGL24 is an early target of transcriptional repression by LFY and AP1. Without such repression, continued AGL24 expression in floral meristems is sufficient to cause floral reversion regardless of the activation of floral organ identity genes. This reveals that LFY and AP1 promote floral development not only by positively regulating genes activated in flower development, but also by repressing AGL24, a promoter of inflorescence fate.

Yu, H., Ito, T., Wellmer, F., Meyerowitz, E.M. (2004) Nature Genetics 36, 157-161.

T01-007

Floral homeotic genes are targets of gibberellin signaling in flower development

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Gibberellins (GAs) are one class of phytohormones involved in the regulation of flower development in Arabidopsis. The GA-deficient ga1-3 mutant shows retarded growth of all floral organs, especially the abortive stamen development that results in complete male sterility. Until now it has not been clear how GA regulates the late-stage development of floral organs after the establishment of their identities within floral meristems. We have identified that gradual rescue of floral defects in ga1-3 can be achieved by various combinations of null mutations of DELLA proteins. In particular, the synergistic effect of rga-t2 and rgl2-1 could substantially restore flower development in ga1-3. These genetic data suggest that GA promotes flower development by suppressing the effects of nuclear DELLA regulatory proteins in its signaling pathway. We further found that the transcript levels of floral homeotic genes APETALA3 (AP3), (PISTILLATA) PI, and AG (AGAMOUS) are immediately upregulated in young flowers of ga1-3 upon GA treatment. Using a steroid-inducible activation of RGA, we demonstrated that these floral homeotic genes are transcriptionally repressed by RGA activity in young flowers, while the expression of (LEAFY) LFY and APETALA1 (AP1) is not significantly affected. In addition, we observed the partial rescue of floral defects in ga1-3 by overexpression of AG. Our results indicate that GA promotes the expression of floral homeotic genes by antagonizing the effects of DELLA proteins, which secures continued flower development.

Yu, H., Ito, T., Zhao, Y., Peng, J., Kumar, P., Meyerowitz, E.M. (2004) Proc. Natl. Acad. Sci. USA (in press)

T01-008

Isolation and characterizing of genes required for different aspects of petal and stamen differentiation

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We are studying the molecular mechanisms underlying petal and stamen formation by identifying and characterizing genes required for different aspects of these processes. In Arabidopsis, the identity of petals and stamens is established by the activity of two transcription factors, APETALA3 (AP3) and PISTILLATA (PI) that function as an obligatory heterodimer. In spite of extensive forward mutagenesis screens, not many genes have been identified that function downstream of AP3/PI and are required for normal petal and stamen development. These genes have not been identified, probably due to redundancy in gene function or lethality caused by mutations in these genes that prevent their recovery based on visible phenotypes. To circumvent these problems we are taking an approach aimed at first identifying genes that are specifically expressed in petal and/or stamens and then studying their function in the formation of these organs. To that end, we have previously conducted a cDNA based microarray screen comparing gene expression profiles of different mutant or transgenic lines with altered AP3/PI function, resulting in lack of, or production of, ectopic petals and/or stamens (1). We are currently supplementing this screen by conducting an additional microarray study intended to isolate petal and stamen expressed genes that are direct downstream targets of AP3/PI. Using the Affymetrix full genome Arabidopsis oligo-array, we are performing time course experiments with an inducible AP3 line and identifying genes that display changes in expression early after the induction of AP3 activity. Genes that are expressed immediately following AP3 induction are likely to be directly affected by AP3/PI rather than through intermediary regulators. Further, we are focusing on a subset of genes identified in these microarray screens as petal and/or stamen specific genes, and studying their function in petal and/or stamen organogenesis. This is done by combining detailed expression studies, application of reverse genetics tools and biochemical characterization of the encoded proteins. Preliminary results demonstrating the detailed expression of several of these genes in petals and/or stamens and their possible function will be presented.

(1) Zik, M., and Irish, V.F. 2003. Plant Cell, 15: 207-222.

T01-009

Molecular analysis of floral dorsoventral asymmetry

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Dorsoventral asymmetry in flowers is thought to have evolved independently multiple times in different species
from radially symmetric ancestors. In Antirrhinum, dorsoventral asymmetry of the flower and its component organs
requires the combined activity of four key genes: CYCLOIDEA (CYC), DICHOTOMA (DICH), RADIALIS (RAD) and
DIVARICATA (DIV). We are currently analysing how these genes, all coding for transcription factors, interact to establish
a basic asymmetric pre-pattern in the Antirrhinum flower meristem and exploring the extent to which these processes
are conserved in Arabidopsis, a species with radially symmetric flowers.
CYC, DICH and RAD are expressed dorsally in floral primordia and promote dorsal petal and stamen identity. Genetic studies have revealed that RAD is downstream of CYC and DICH. We have obtained the DNA-binding site consensus for CYC protein by random binding-site selection and shown that CYC can bind directly to the RAD promoter and intron. To confirm that CYC interacts with RAD in vivo, transgenic Arabidopsis plants overexpressing inducible CYC fused to the rat glucocorticoid receptor (35S:CYC::GR) were generated and crossed to RAD:RAD plants. The 35S:CYC::GR heterozygous plants grown in the presence of DEX were dwarfed with smaller leaves but bigger flowers than WT. In addition, we overexpressed RAD in Arabidopsis and the transgenic plants obtained were also dwarfed with smaller epidermal cells.

T01-010

Using dominant mutants to identify natural modifiers of flowering time

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To identify new naturally occurring genetic variants affecting flowering time, we have crossed dominant mutants to a panel of wild Arabidopsis thaliana accessions. We started with dominant alleles of FWA, which encodes a homeodomain transcription factor. Its epigenetic allele, fwa, causes late flowering phenotype. We crossed fwa-2 mutants with 22 relatively early flowering natural accessions and monitored flowering time of the F1 plants. Most F1 plants showed an increment of 50 to 100% in total leaf number compared to that of parental accessions. However, the F1 derived from a cross between fwa-2 and LI-2 accession produced an extremely late flowering phenotype. A control cross showed that the late flowering phenotype was not fwa-2 dependent, since the F1 between two the early flowering accessions Ler, the parent of fwa-2, and LI-2 also flowered late.
Crossing LI-2 with different FRI FLC combinations (e.g., fri flc-3, FRI flc-3, or fri FLC), as well as sequencing the FRI gene from LI-2, suggested that a functional FRI allele from LI-2 causes late flowering in the F1 by activating the weak FLC allele of Ler. In addition to delayed flowering, we observed aerial rosettes in the latest plant derived from a backcross of the F1 into Ler. Such a phenotype has previously been shown by Grbic and colleagues to be conferred by synergistic activation of FLC by ART1 and FRI loci in the Sy-0 ecotype. Furthermore, the FLG locus, which has been mapped as a QTL between the Cvi and Ler accessions by Koornneef, Alonso-Blanco and colleagues, also enhances FLC action. We have started to examine possible candidate genes with known effects on flower development in this region, including the HUA2 locus identified by Chen and coworkers. We observed that the late flowering phenotype co-segregates with the HUA2 allele from LI-2. Sequencing revealed changes at amino acid level in the HUA2 allele of LI-2 compared to Columbia. We are now testing whether the dominant HUA2 allele of LI-2 accession modulates flowering phenotype and aerial rosette formation by acting through the FRI/FLC pathway.
This work is supported by Max-Planck Society, KOSEF, and EMBO.

T01-011

Misexpression of FLOWERING LOCUS C (FLC) results in varying degrees of repression of the floral transition depending on the promoter identity.

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Flowering in Arabidopsis is regulated by a number of different environmental and endogenous signals including an extended period of low temperature or vernalization. FLOWERING LOCUS C (FLC) encodes a MADs domain transcription factor and is known to play a critical role in the vernalization response (1;3). The expression of FLC is down-regulated in response to vernalization resulting in the promotion of flowering.
FLC is expressed throughout development at different levels in a range of plant tissues including the vegetative apex, root tissue, leaves and stems according to mRNA gel blot analysis (1;3). In which cells and at what time during development FLC expression is sufficient to repress flowering is currently unknown.
I have used a two component system to manipulate FLC expression in a tissue specific manner. This system has been described by Moore et al. and involves a novel transcription factor, LhG4, that activates the expression of both a GUS reporter gene and an additional cloned gene (2). Using this system, a number of Columbia lines were established by John Bowman and Yuval Eshed with a variety of tissue-specific promoters driving the expression of LhG4. I have used these lines to activate the tissue-specific expression of FLC.
A number of different lines resulted in flowering significantly later than control plants. These results suggest that FLC may have the ability to repress flowering in more than one tissue and/or at more than one point in development. Further experiments are underway to characterize these lines.

1. Michaels & Amasino (1999)
2. Moore et al. (1998)
3. Sheldon et al. (1999)

T01-012

The tetraspore kinesin stabilises the male meiotic cytokinetic apparatus in Arabidopsis thaliana

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Male meiotic cytokinesis in angiosperms is organised by a specialised cytoskeleton. Instead of the preprophase band and phragmoplast microtubules of somatic cells, the nascent microspore nuclei generate arrays of radiating microtubules over their surfaces. These radial microtubular systems (RMS) serve both to define the cytoplasmic domain of the young microspore and, where they intersect with their neighbours, determine the position of the callose internal walls of the tetrad.
Mutation of the TETRASPORE (TES) locus causes dramatic instability of the RMS with the result that no internal tetrad walls are formed, the young microspore nuclei aggregate and a single large spore (tetraspore) develops containing all four meiotic products. Further, the microspore nuclei may fuse at this stage resulting in 'spores' containing between one and four nuclei. Surprisingly, pollen mitosis 1 and 2 proceed normally in a common cytoplasm with the result that the TES pollen tube delivers sperm pairs of varying ploidy. TES encodes a kinesin with an N-terminal motor domain belonging to a plant-specific sub-family. A description of the tes mutant phenotype is presented, including changes to the RMS cytoskeleton during male germline development, as are details of TES expression patterns in somatic and reproductive tissues. Despite the specificity of the mutant phenotype, the TES transcript is found in all actively dividing tissues. We show that TES binds to ANP1 - a MAPKKK - to itself and to HIK, its closest Arabidopsis homologue.

T01-013

Roles of DELLA Proteins in Gibberellin-Regulated Seed Germination and Floral Development

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Bioactive gibberellins (GAs) are phytohormones that regulate developmental processes ranging from seed germination to vegetative growth and floral organ formation. RGA and GAI, two well-characterized negative components of the GA signaling pathway, control such growth processes as stem elongation, but had not been shown to play a major role in regulating either seed germination or flower development. GAI and RGA are members of the GRAS family of putative transcriptional regulators. Unlike other GRAS family members, RGA and GAI contain a DELLA motif, which appears to be necessary for the GA-responsiveness of these proteins. Three RGL (RGA-like) genes had been identified through their sequence similarity to RGA and GAI. RGL1, RGL2, and RGL3 all contain DELLA motifs. To further investigate the functions of RGA, GAI, and especially the RGL genes, we used quantitative PCR (qPCR) to determine the transcript levels for these five genes in various tissues. The qPCR data supported the idea of functional redundancy: RGA and, to a lesser extent, GAI were expressed at relatively high levels across tissues, while the RGL genes showed peaks in expression in germinating seeds and/or flowers. We also identified T-DNA insertion alleles of each of the RGL genes. Under normal growth conditions, rgl single, double, and triple mutants were morphologically similar to wild-type controls. Experiments employing GA-deficient conditions confirmed that RGL2 negatively regulates seed germination. Flower development, in contrast, clearly involves the action of multiple DELLA proteins: Mutant combinations in the ga1-3 GA-deficient background indicated that RGA, RGL1, and RGL2 function together to regulate floral development. At the protein level, RGL2 $^{-}$ like RGA and GAI $^{-}$ appears to be degraded in response to GA, through a pathway which requires the F-box protein SLY.

T01-014

Protein- protein interactions between AGL24 and several MADS-box proteins involved in flowering

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An Arabidopsis MADS-box gene, AGL24 functions in the promotion of flowering. Interestingly, SVP which represses flowering, is phylogenetically very close to AGL24. Recent works suggest that MADS-box proteins form complexes with other MADS-box proteins. To reveal whether AGL24 protein form complexes with other MADS-box proteins involved in flowering, proteins interacting with AGL24 were screened by yeast two-hybrid method. As a result, several MADS-box genes such as SOC1, AP1 and FUL were isolated. Studies using with yeast experiments also indicated that AGL24 interacted with CAL and FLC, but not with SVP and AGL24 itself. Furthermore, it was found that SVP interacted with SOC1, AP1, FUL, CAL and FLC. Then, the interactions among these proteins were confirmed by in vitro binding assays. Based on the expression patterns of these genes, AGL24 may interact with SOC1 in shoot apical meristems during vegetative development and with AP1 CAL, and FUL in early floral meristems. These results suggest that AGL24 and SVP may function antagonistically through the interactions with same MADS-box proteins. Now, we are analyzing the interactions between AGL24 and these MADS-box proteins in planta.

T01-015

Regulating the Regulators: The plant specific BBR family of GAGA-repeat Binding proteins

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BBR proteins comprise a novel class of transcription factors that are confined to the plant kingdom. They have recently been identified as essential key-regulators of homeobox gene expression in barley. BBR-proteins have been identified due to their specific binding to a conserved element with its simple sequence repeat consensus of (GA/TC)7 or higher. BBR proteins have properties of animal GAGA-binding factors, but they exhibit no sequence homologies to Trl and Psq of Drosophila, which encode functionally analogous proteins.
In the dominant mutant Hooded (K), the barley ortholog of KNOTTED was overexpressed as a result of a duplication of 305 bp in Intron IVcontaining an (GA/TC)8 element. Surprisingly, gemonic screening reveals functional elements are in both promoter and intron sequences.
So far three distinct regions could be identified common to most BBR proteins: An N-terminal putative activation domain, a NLS and a highly conserved domain at its C-terminus, which mediates DNA-binding.
By structural means, the protein family can be subdivided into two groups based upon their N-terminal domain. Similarly, phylogenetic analysis based solely on the DNA-binding domain sequence strongly supports the division into two distinct groups.
The basic DNA-binding domain, a 90 amino acid region, is structured as a typical zink-finger-like motif putatively comprising two ß-sheets followed by an a-helix. A full genome analysis of (GA/TC)n-elements conducted with the use of the MotifMapper program package (www.motifmapper.de) strongly supports the BBR function in regulating transcriptional regulators.

T01-016

Transcriptional regulation of the floral homeotic gene AGAMOUS

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The homeotic gene AGAMOUS (AG) plays a central role during Arabidopsis thaliana flower development, since it not only specifies the reproductive organs, but also terminates stem cell proliferation in the center of the flower. Multiple transcriptional inputs contribute to region specific AG activation via regulatory elements located in the second intron. We have shown previously that two transcription factors LEAFY (LFY) and WUSCHEL (WUS) are important direct activators, however their activity is not sufficient for correct AG expression. Detailed knowledge of the regulators involved in AG activation is a prerequisite for the understanding of patterning and stem cell control in emerging flowers.
In an attempt to identify additional direct AG regulators, we performed yeast one-hybrid screens using cis-regulatory elements that had been shown to be evolutionary conserved within the family of Brasicacae. One of these highly conserved motifs was a 33 bp element termed AAGAAT-box that we could show to be important for activation of a AG::GUS reporter in Arabidopsis. In yeast we were able to identify nine putative transcription factors that interacted with this element. Five of them belong to the group of Myb-like transcription factors with a single MYB domain, the other four are closely related bZIP transcription factors. Close inspection of the AAGAAT-box sequence revealed consensus-binding sites both for Myb-like and bZIP transcription factors. Analysis of the nine transcription factors in a yeast transactivation assay revealed that all members of the MYB-like group were able to activate transcription from the AAGAAT-box trimer, whereas only one bZIP was a potent activator. Interestingly, we could observe cooperative effects between specific members of the two groups.
Expression analysis by interrogating the AtGenExpress database indicated that all candidates were present in floral tissue, with most of them being transcribed also in other parts of the plant. In situ hybridization confirmed expression in floral tissue for five of the nine candidate genes.
To asses the biological function of the newly identified candidate regulators, we currently examine the phenotype of knock out lines and plants carrying over- expression alleles. To get an insight into their role in AG regulation, we are analyzing in detail the response of various AG::GUS reporter lines, which will include binding site deletion lines, in loss and gain of function experiments.

T01-017

Molecular variation of CONSTANS in natural accessions

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The timing of the floral transition is one of an important quantitative trait in the natural field. Extensive molecular genetic studies have revealed pivotal regulatory factors controlling flowering-time, and distinct or integrated pathways for flowering. However, little is known about how such a regulatory network has been maintained and adapted under the natural conditions.

Naturally occurring variation refers to phenotypic and/or genetic differences in natural populations. Since such a variation is assumed to be a trait maintained in the natural habitats, it has been focused on as a case for evolutionary genetic studies.

We have taken a sequence-based approach, focusing on the flowering-time gene CONSTANS (CO), to identify potentially interesting natural variants. CO encodes a putative transcription factor, carrying three functionally separated domains: B-box domain (zinc finger-like), CCT domain (containing NLS region), and putative transcriptional activation domain.

So far, we have screened the entire transcribed region of CO (5'UTR, cds, intron, and 3'UTR) from more than 150 natural accessions, and found several candidates for functional variants. These studies were guided by the identification of residues that are conserved in CO homologues in both Arabidopsis and other species. Characterization of these candidate variants will be presented.

T01-018

Regulation of CONSTANS protein and its relationship to photoperiodic flowering in Arabidopsis

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Arabidopsis flowers earlier under long-day (LD) than under short-day (SD) conditions. This flowering time behaviour depends on the presence of functional CONSTANS (CO) protein. Transcription of CO is circadian regulated and peaks at the end of a LD and during the night, both under LD and SD conditions. Despite the similar expression pattern, CO protein only accumulates under LD conditions, at the end of the afternoon and in the evening in the presence of light. This accumulation of CO is regulated antagonistically by photoreceptors, with phytochrome B negatively, and phytochrome A and the cryptochromes positively influencing CO stability. We have shown that CO is present in a phosphorylated and in an unphosphorylated form. Furthermore, CO is degraded through the proteasome at the beginning of the day and during the night. Presently, we are studying the significance of CO phosphorylation and the mechanisms which underlie changes in CO stability during the day and its degradation by the proteasome.

T01-019

A SUMO specific protease that regulates flowering of Arabidopsis

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Post-translational modification is important in regulation of protein function. The covalent attachment of the ubiquitin-like modifier (SUMO) to its targets (sumoylation) represents, after ubiquitylation, the best-studied example of a protein modification by attachment of a peptide to target proteins. Accumulating evidence shows that sumoylation is involved in a wide range of biological processes, including transcription, the cell cycle, apoptosis, chromatin integrity and dynamics, and nucleocytoplasmic transport. Protein sumoylation may regulate plant development since the Arabidopsis genome contains genes predicted to encode all the components of the SUMO system defined in other organisms. The early in short days 4 (esd4) mutant showed extremely early flowering in both long days and short days and alterations in other developmental events. We have identified ESD4 and shown that it encodes a protease with strong similarity to yeast and animal proteases specific to SUMO. ESD4 has the activity in vitro of a SUMO protease, and is responsible in vivo for recycling SUMO from SUMO-conjugates, because esd4 mutants accumulated higher levels of SUMO-conjugates compared to wild type plants and over-expression of SUMO enhanced the biochemical and morphological phenotype of esd4. ESD4 is localized at the periphery of the nucleus. We are identifying SUMO targets with affinity purification following by 2-DE and MALDI-TOF and try to explain how the SUMO system is involved in flowering control.

T01-020

Genetic evidence for essential calcium transporters in pollen growth and fertilization.

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Calcium dynamics are thought to play a central role in pollen development, as evidenced by pharmaco-chemical approaches and visualization of calcium gradients and oscillations. Using Arabidopsis as a model system, we provide the first genetic evidence to support a model in which calcium signals are natural regulators of pollen tube growth and fertilization. Calcium signals are largely controlled by influx (through channels) and efflux (through pumps and antiporters). We have identified T-DNA gene disruptions in all 14 calcium pumps, and 18 of 20 cyclic nucleotide gated channels (CNGCs). From this set of mutants, distinct pollen specific phenotypes have been found for disruptions of ACA9 (a calmodulin activated plasma membrane calcium pump) and CNGC18 (a cyclic nucleotide and calmodulin regulated putative calcium channel).

Calcium Pump Mutation: Three independent gene disruptions in ACA9 resulted in partial male sterility. Homozygous aca9 mutants showed an 80% reduction in seed set. Mutant aca9 pollen displayed a reduced growth potential and a high frequency of aborted fertilization.

Channel Mutation: Two independent disruptions in CNGC18 resulted in complete sterility. We were unable to identify a homozygous mutant in more than 400 F1 progeny. In vitro germination of heterozygous cngc18 pollen in the quartet background showed that mutant pollen tubes germinate, but only grow a short distance, with a “kinky-like” non-directional growth, often prematurely terminating with a bursting event.

Although the aca9 and cncg18 mutants show defects in different stages of pollen tube development, they both identify pollen specific ion transporters that are regulated by calmodulin. A hypothesis to be tested is that both ACA9 and CNGC18 are essential for calmodulin-regulated calcium oscillations required for plasma membrane signaling during pollen growth and fertilization.

Schiøtt et al., (2004) A plant plasma membrane Ca2+ pump is required for pollen tube growth+fertilization. PNAS in press

T01-021

Pollen specific MADS-box genes are involved in pollen germination

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Sexual reproduction of land plants depends on gametophytic tissues, sperm cells and egg cells. Sperm cells and egg cells are borne in pollen grain and in embryo sac, respectively, multicellular gametophytes of seed plants. For fertilization, sperm cells must travel through pollen tube to an embryo sac, which lies within a pistil and cannot contact directly with pollen grains. To carry out this process, pollen grains germinate and the pollen tube elongates. The pollen tube is guided in ovule. Little is known what genes do regulate these events. We found that several MADS-box genes are involved in the first step of these succeeding actions, pollen germination. We had found that eight MADS-box genes are expressed specifically in pollen of Arabidopsis thaliana (Kofuji et al, 2003). We focused on three MADS-box genes classified into MIKC*-type class, which is considered to be diverged from well-known MIKC-type (MIKCc-type) class before the divergence of bryophytes. Promoter-GUS analyses showed that these genes were expressed in vegetative cells of pollen and one of three genes was expressed already in the anthers of young flower buds. Single and double mutants of each gene displayed normal phenotype, suggesting that they have redundant functions. We found that the rate of in vitro germination of triple mutant pollen was much reduced compared to that of wild type pollen, although the mutant pollen can germinate normally on a stigma. Results on microarray analysis revealed that the expression level of several cell wall related genes such as glycosyl hydrolase family genes were reduced in triple mutant pollen. Results on morphological analyses will be presented.

Kofuji et al, (2003) Mol. Biol. Evol. 20, 1963-1977.

T01-022

SLW1 Is Essential for the Female Gametophyte Development in Arabidopsis

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During female gametophyte development, the functional megaspore undergoes three subsequent nuclear divisions, giving rise to an 8-nucleate embryo sac. Cellularization of the coenocytic embryo sac results in the formation of functional multicellular female gametophyte. Gametophytic mutants are often manifested by non-Mendelian segregation of KanR marker gene in the Ac/Ds gene trap system. A gametophytic mutant, slw1, was isolated because of its distorted KanR segregation ratio. The ratio of KanR to KanS of slw1 is about 1:1, instead of typical Mendelian 3:1 segregation. Reciprocal cross with wild type plants indicated that Ds could not be transmitted through megagametophytes. Confocal analysis of mutant ovules showed that nuclear division of mitosis in embryo sac was affected. And mature 7-cell embryo sac can not be formed before pollination. Molecular analysis demonstrated that a single Ds element was inserted into the ORF of SLW1 gene. Complementation test indicated that the phenotype was generated because of the disruption of the target gene. Protein localization investigation indicated that the SLW1 protein localized in the nuclei of the cells. As suggested that the protein be possibly involved in cell division. The result of GUS staining and in situ hybridization showed that the expression of SLW1 gene confines in cell division active part of plant, embryo sac and pollen grain.

T01-023

Nuclear Division during Gametogenesis in Arabidopsis Mutant dq1

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During female gametophyte development, the functional megaspore undergoes three subsequent nuclear divisions, giving rise to an 8-nucleate embryo sac. Cellularization of the coenocytic embryo sac results in the formation of functional multicellular female gametophyte. Gametophytic mutants are often manifested by non-Mendelian segregation of KanR marker gene in the Ac/Ds gene trap system. A gametophytic mutant, dq1, was isolated because of its distorted KanR segregation ratio. The ratio of KanR to KanS of dq1 is about 1:1, instead of typical Mendelian 3:1 segregation. Reciprocal cross with wild type plants indicated that Ds could not be transmitted through megagametophytes. Confocal analysis of mutant ovules showed that nuclear division of mitosis in embryo sac was affected. And mature 7-cell embryo sac can not be formed before pollination. Molecular analysis demonstrated that a single Ds element was inserted into the ORF of DQ1 gene. Complementation test indicated that the phenotype was generated because of the disruption of the target gene. Protein localization investigation indicated that the DQ1 protein localized in the nuclei of the cells. As suggested that the protein be possibly involved in cell division. The result of GUS staining and in situ hybridization showed that the expression of DQ1 gene confines in cell division active part of plant, embryo sac and pollen grain.

T01-024

A molecular model for ACR4 function in the organisation of the L1 cell layer of developing organs

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Mechanisms regulating cell layer organisation in developing plant organs are fundamental to plant growth. In order to understand the signalling pathways potentially involved in this process, we have studied the receptor kinase-encoding ARABIDOPSIS CRINKLY4 (ACR4) gene, and shown that its expression is restricted to the L1-layer of most meristems and organ primordia. Our functional analysis has revealed a role for ACR4 in regulating cellular organisation during the outgrowth of developing sepal margins and ovule integuments. We show that ACR4 encodes a functional kinase and that ACR4 protein is abundant in the anticlinal and inner periclinal membranes of L1 cells (Gifford et al., 2003). In order to further investigate the mechanism of ACR4 function we have carried out a comprehensive functional dissection of the ACR4 protein, based on the ability of deletion derivatives to complement the mutant phenotype. This has permitted identification of functionally important domains of ACR4 and the formulation of a functional model for ACR4 as a partially redundant component of a developmentally crucial signalling pathway involved in the maintenance of L1-layer integrity throughout Arabidopsis development. In particular our model proposes roles for other members of the CR4-Like protein family of Arabidopsis, which are in the process of being confirmed.

Gifford, M.L., Dean, S. and Ingram, G.C. (2003). Development 130, 4249-4258.

T01-025

'Integument-led' seed growth in the megaintegumenta (AUXIN RESPONSE FACTOR 2) mutant

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Arabidopsis thaliana seeds consist of three main components: the maternally derived seed coat, which develops from the integuments surrounding the embryo sac; and the two fertilization products, embryo and endosperm, which have maternal and paternal genetic contributions. We have previously shown that endosperm growth is an important factor in the final size of the seed (1, 2). Here we describe a different phenomenon, 'integument-led' seed growth, exemplified by the megaintegumenta (mnt) mutant phenotype. The mnt mutation has a maternal effect on seed size, and increased seed size is associated with extra cell division in the integuments. mnt mutants have a variety of other phenotypes, including thick stems and impaired opening of flowers, which also involve abnormalities in cell division and/or expansion. mnt is a mutant allele of the AUXIN RESPONSE FACTOR 2 (ARF2) gene (see our related poster, Schruff et al.).

(1) Scott et al. (1998), Development 125, 3329.
(2) Adams et al. (2000), Development 127, 2493.

T01-026

Genetic dissection of the AUXIN RESPONSE FACTOR2 mutant megaintegumenta

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Auxin signalling has important effects on cell division, elongation, differentiation and patterning by evoking complex changes in the transcription of various gene families.
Auxin Response Factors (ARFs) are a family of transcription factors that have been shown to respond indirectly to auxin signalling by regulating early auxin response genes. We have found that the mnt (megaintegumenta) mutant has a disruptive lesion in ARF2 which has previously been shown to act as a transcriptional repressor (1). The mutation has pleiotropic effects on the phenotype, some of which are presented in detail in our related poster (see Spielman et al.).
The identity of the mutated gene was confirmed via genetic mapping, mutant rescue and an allelism test with another arf2 mutant. Sequencing confirmed that a single base pair change is responsible for the disruption of gene function in mnt by interfering with an intron splice site. We have cloned ARF2 in Brassica napus and sequence alignments with other plant species will be presented. Various expression analyses are currently being undertaken.

(1) Tiwari et al. (2003) Plant Cell, 15, 533

T01-027

EMBRYONIC FACTOR 1 (FAC1), encoding an AMP deaminase in Arabidopsis, is essential for activating zygotic embryogenesis

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Fusion of the egg and sperm cells produces a zygote, a totipotent cell that develops into an embryo and then an individual plant. Screening of EMS mutagenized population leads to the identification of a genetic locus, EMBRYONIC FACTOR 1 (FAC1), which is essential for early zygotic embryogenesis. Mutation of the FAC1 genes leads to embryos being arrested at the zygote or the first cell division stage. Heterozygous plants carrying these mutations showed typical mendelian segregations, in which 25% progeny seeds were aborted. The FAC1, as identified by positional cloning, is a single-copy gene encoding adenosine monophosphate deaminase (AMPD). A knockout line with a T-DNA insertion in the same gene showed identical phenotypes and failed to complement the fac1 mutation, confirming that the cloning result. During embryogenesis FAC1 is expressed in the zygote, embryo proper and endosperm, but not in the suspensor. Genetic analysis in combination with expression studies revealed that paternal-derived FAC1 gene are expressed at the zygote stage, which counts as the earliest expressed paternal genes known so far in plants. FAC1 expression is also associated with somatic embryo induction, suggesting a critical role in embryogenesis. The FAC1 gene can complement the yeast AMPD mutation only when its N-terminal hydrophobic domain was removed. As being the critical enzyme in converting AMP to IMP, FAC1 may generate a high energy potential during zygotic and somatic embryogenesis through irreversibly degrading AMP.

Liu, C.M., and Meinke, D.W. (1998). Plant J 16, 21-31.
Lukowitz, W., et al., (2004). Cell 116, 109-119.

T01-028

Gene regulatory network controlling seed maturation

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The conquest of most terrestrial niches by land plants has been greatly facilitated by the appearance of seeds. Seeds offer plants a unique opportunity to interrupt their life cycle, withstand adverse environmental conditions in a desiccated state and then resume growth using endogenous storage compounds. These seed specific traits (desiccation tolerance, storage accumulation and entry into quiescence) are acquired during a developmental phase called seed maturation, which is genetically controlled in Arabidopsis by four genes named ABI3, FUS3, LEC1 and LEC2 and encoding transcription factors. How these four genes interact to control together the various facets of seed maturation is unknown. By analyzing the expression of reporter constructs for ABI3, FUS3 and LEC2 in various mutant and transgenic backgrounds, we show that the four genes belong to a complex regulatory network that enables them to regulate each other's expression locally and redundantly. This network contains features reminiscent of the mechanisms governing animal development, such as positive feedback loops used here to stabilize ABI3 and FUS3 expression. Our results also suggest that this network might have arisen in evolution via the duplication of a single autoregulatory gene, a likely general mechanism used in the evolution of developmental processes.

T01-029

Molecular mechanism of floral repression during vegetative development

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Flowering time in higher plants is finely regulated by floral inducers and floral repressors. Recent investigations of early flowering mutants in Arabidopsis reveal a variety of mechanisms by which flowering can be delayed and vegetative growth extended (Sung et al., 2003). While some early flowering genes interfere with the signaling process or inhibit the expression of floral inducer, other genes such as CURLY LEAF (CLF) and EMBRYONIC FLOWER (EMF) genes probably delay flowering by repressing the flower organ identity genes. CLF and EMF2 encode Polycomb Group (PcG) proteins. In animals, PcG proteins repress their target genes by modifying histone tails through deacetylation and methylation, generating a PcG-specific histone code that recruits other chromatin remodeling proteins to establish a stable, heritable mechanism of epigenetic expression control. Owing to their conserved structural and functional relationship to animal PcG proteins, plant PcG proteins might function through a similar biochemical mechanism. PcG proteins control multiple aspects of Arabidopsis development. The PcG target genes that have been identified so far encode MADS box proteins. The deregulated expression of ten MADS Box genes, such as, AG, AP3 and PI in emf mutants suggests that these genes might be directly regulated by EMF2 PcG complex (Moon et al., 2003). To investigate this possibility, we are performing Chromatin Immunoprecipitation (ChIP) analysis on nuclear extracts from Arabidopsis WT and emf2 mutant seedling using anti-EMF2 antiserum. Different regions of the AP3 and PI promoters and of the AG second intron were analyzed by PCR following the immunoprecipitations. The results are consistent with hypothesis that EMF2 protein complex maintain the repression of the MADS box genes in Arabidopsis vegetative development.

Moon et al., 2003. Plant Cell, 15(3):681-93.
Sung et al., 2003. Current Opinion in Plant Biology 6:29-35.

T01-030

Overexpression of KNAT1 interferes with Arabidopsis ovule development

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The ovule is the female reproductive organ of higher plants. During Arabidopsis thaliana ovule development two integuments grow out from the chalaza, enclose the embryo and eventually develop into the seed coat (1). Cell division and elongation in the ovule integuments need to be precisely and locally regulated to achieve full enclosure of the plant embryo.
With the ultimate goal of identifying the factors that regulate integument morphogenesis, we started to analyse aspects of outer ovule integument development. Cell divisions in the outer integument were mapped and the cellular events that lead to the development of highly specialized seed coat cells (2) were monitored using fluorescent intracellular markers. Moreover, Arabidopsis enhancer-trap lines were used to map domains of gene expression during integument development and will be used to identify genes that are expressed in this tissue.
The Arabidopsis outer ovule integument initially consists of two cell layers. Overexpression of the homeodomain protein KNAT1 increases the number of cell divisions specifically in the outer layer of the outer integument. Enhancer-trap lines showing expression in specific domains of the outer integument were crossed into KNAT1 overexpressing plants and were used as markers to further characterize the effect of KNAT1 expression on integument development. The down-regulation of a marker that was specifically expressed in the outer layer of the outer integument suggests that KNAT1 interferes with outer layer cell identity.

(1) Schneitz, Curr. Op. Plant Biol. (1999), 2:13 ¯ 17
(2) Windsor et al., Plant Journal (2000): 22:483-493

T01-031

Genetic analysis of circadian clock components in Arabidopsis

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Circadian rhythms are driven by endogenous biological clocks that regulate many processes in a wide variety of organisms. At least 4 genes, LHY (LATE ELONGATED HYPOCOTYL), CCA1 (CIRCADIAN CLOCK ASSOCIATED 1), TOC1 (TIMING OF CAB EXPRESSION 1) and GI (GIGANTEA), have been shown to be closely associated with clock function in Arabidopsis. Loss-of-function of either LHY or CCA1 shortens the period of the rhythm in the expression of clock-controlled genes under continuous light, and also accelerates flowering under short days (SD). By constructing lhy cca1 double mutants, we have shown that LHY and CCA1 are partially redundant and essential for the maintenance of circadian rhythms in constant light (1).

To identify mutations which affect circadian rhythms, we have screened EMS-mutagenised the single loss-of-function mutant of lhy (lhy-12) seeds for plants which flowered earlier than the lhy-12 in SD. We found that at least two mutations, 38elf-1 and 70elf-1, in a similar way to the cca1, enhanced 1) early flowering phenotype in SD and 2) shift in the phase of expression of GI, of the lhy-12 mutant. We have identified both of the 38ELF and 70ELF genes by map-based cloning. Characterization of these enhancer mutations is underway. They may define further cca1 loss-of-function alleles or genes required for CCA1 regulation.

(1) Mizoguchi et al., Developmental Cell 2, 629-641, 2002

T01-032

LHY and CCA1, clock components in Arabidopsis, control photoperiodic flowering mainly through a transcriptional cascade, GI-CO-FT

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Circadian clock components, LATE ELONGATED HYPOCOTYL (LHY), CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), TIMING OF CAB EXPRESSION 1 (TOC1) and GIGANTEA (GI) are thought to compose a negative feed-back loop to control their expression reciprocally in Arabidopsis (1). In photoperiodic floral induction pathway of Arabidopsis, CONSTANS (CO), a downstream factor of GI, promotes flowering through a floral activator, FLOWERING LOCUS T (FT). Loss-of-function of both LHY and CCA1 causes acceleration of flowering under short days (2).

Here we show that Arabidopsis lacking both LHY and CCA1 proteins (lhy cca1) alters expression profiles of GIGANTEA (GI), a gene that accelerates flowering in response to light/dark cycles, under long days (LD) and short days (SD). Early flowering phenotype of the lhy cca1 is completely suppressed by loss-of-function alleles of gi (gi-3 and gi-6). Loss-of-function alleles of CONSTANS (CO), a gene which functions downstream of GI in LD pathway, also partially suppress the flowering phenotype of the lhy cca1. Late flowering phenotype of lhy-1 (LHY-ox) is largely overcome by overexpression of GI (35S::GI) under LD. Early flowering phenotype of 35S::GI is partially suppressed by co-2, fha-1 and fca-1, in a similar way to the lhy cca1. In wild-type, almost no FT transcript is detected in SD. However, higher levels of FT expression is observed in two early flowering plants, the lhy cca1 and 35S::GI even under SD condition. The higher levels of FT expression in the lhy cca1 and 35S::GI line are reduced by co-2. Loss-of-function of ft delays flowering time of the lhy cca1 and 35S::GI line.
These results suggest that clock components, LHY and CCA1, regulate Arabidopsis flowering mainly though a transcriptional cascade of floral activator genes, GI-CO-FT (GCF transcriptional cascade).

1) Mizoguchi and Coupland, Trends Plant Sci 5, 409-411, 2000
2) Mizoguchi et al., Developmental Cell 2, 629-641, 2002

T01-033

FLOWERING LOCUS T as a link between photoperiodic induction in leaves and evocation at shoot apex

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Flowering in Arabidopsis is regulated by several pathways which converge on the transcriptional regulation of floral pathway integrators including FT. FT is a direct target of CO and encodes a protein with similarity to mammalian proteins (PEBP/RKIP) involved in cellular signaling. FT transcription is immediately induced in cotyledon and leaf vascular tissues upon transfer from short-day to inductive long-day photoperiods. Promotion of flowering by FT requires the activity of another flowering-time gene FD which encodes a bZIP transcription factor preferentially expressed in the shoot apex. FD is involved in transcriptional activation of the floral meristem identity genes AP1 and CAL redundantly with LFY. ft; lfy and fd; lfy double mutants are very similar in severe reduction of AP1 mRNA levels and strong defects in floral specification. Loss of FT function suppresses ectopic up-regulation of AP1 in seedlings by FD overexpression. Mutant forms of FD which lack a C-terminal potential phosphorylation site cannot interact with FT in yeast cells and fail to complement fd late-flowering phenotype even by over-expression. These and other evidences suggest that FT and FD are inter-dependent in promotion of floral transition and activation of AP1 expression. Since the activity of FD, which is preferentially expressed in shoot apex, seems to require protein/protein interaction with FT, shoot apex is likely the site of action of FT protein. Consistent with this, restoration of FT function in shoot apex through expression by FD regulatory sequences or only in L1 of shoot apex by PDF1 promoter can rescue ft late-flowering phenotype in FD-dependent manner. These raise an interesting possibility that the FT protein may represent a long-distance signal generated in photoperiodically-induced leaves (mainly in vascular tissues) and act at the shoot apex to initiate floral development.

T01-034

Isolation and characterization of secreted proteins from the inflorescence of A.thaliana.

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Plant hormones like auxin, cytokinin, GA, ABA and ethylene etc. play a very important role in long distance signal transduction in plants. Recently it became clear that plants also use proteins or peptides for short-distance signal transduction. Work from several labs including ours showed that some small proteins have important functions in intra- or inter-cellular signaling. These proteins include CLV3 (controlling meristem size, Fletcher et al, 1999), ENOD40 (Rhizobium-plant nodulation signalling), ESR (endosperm development, Opsahl-Ferstad et al, 1997), SCA (stigma-style Cysteine Rich Adhesin, Park et al, 2000, Plant Cell 12:151), SYSTEMIN (18 AA peptide in wounding response, Pearce et al, 1996 Science 253:895 and CLE19 (functions in meristem development, Fiers et al, Gene 327 (2004) 37-49).

Most of these peptides are derived from a larger pre-protein with a secretion signal like CLV3, CLE19 and ESR. In order to find other secreted small signaling molecules a protocol was developed to isolate secreted proteins from the inflorescence of A.thaliana. These proteins were purified and separated in a 2-dimensional method based on their charge, hydrophobicity and size. Protein bands were cut from a SDS-PAGE gel and digested with trypsine before analysis on a Q-TOF. After sequencing, peptides were compared with the translated genome of Arabidopsis in order to identify the mature protein. Our work proposed the feasibility of using peptideomics to identify unknown secreted peptides from plants.

Fiers et al, Gene 327 (2004) 37-49

T01-035

FIDGET (FIT), an APETALA2-like protein promotes flowering by direct activation of FT

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Arabidopsis thaliana is a facultative long-day plant, flowering earlier under long than short-day conditions. CONSTANS, a central protein in the photoperiod response pathway, promotes flowering by activating transcription of FT under long days. The induction of FT leads to the transcription of the floral meristem identity genes LFY and AP1. So far, CO is the only protein described to activate FT expression and thereby induce early flowering in long days.

We have successfully used the yeast-one-hybrid technology to identify new proteins interacting with the promoter of FT. One of these proteins is FIDGET (FIT), which interacted with a 300 bp-fragment of the FT-promoter in yeast and in vitro. FIT is an AP2-like protein that belongs to the subclass of ethylene-responsive element binding proteins (EREBP). FIT mRNA shows a circadian expression pattern peaking 16 hours after dawn.
In transient promoter studies we found that FIT is able to activate the expression of FT. Overexpression of FIT from the 35S promoter produced slightly earlier flowering plants, but expression from the SUC2-promoter in the phloem companion cells, where FT is thought to act, results in a clearly early-flowering phenotype. The expression pattern of FIT is being analyzed using promoter-GUS fusions and SUC2::FIT is being crossed into various flowering-time mutants to place it within the current model of the network of genes that control flowering in Arabidopsis.

T01-036

Inhibition of the V-ATPase leads to deformation of golgi stacks during male gametophyte development

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The V-ATPase is a highly conserved eukaryotic proton pump, which uses ATP to pump H+ from the cytosol into the lumen of different intracellular compartments. Acidification of endomembrane compartments and consequently the establishment of a proton gradient is important for various cellular functions such as secondary active transport, enzyme function, protein targeting, and vesicle trafficking.
In order to analyze V-ATPase function in vivo, we identified T-DNA insertions disrupting genes encoding Arabidopsis V-ATPase subunits. A T-DNA insertion in the single copy gene encoding the catalytic subunit VHA-A showed a severely reduced transmission rate and we failed to identify plants homozygous for this insertion. Reciprocal crosses showed that inactivation of the catalytic subunit leads to complete male and partial female sterility. Complementation of the vha-A allele rescued the sterility phenotype. Tetrad analysis of pollen from vha-A/+ plants combined with electron microscopy revealed, that the first visible result of a lack of V-ATPase activity was a deformation of golgi stacks. The observed cell death of two pollen per tetrad in later stages might be a result of this golgi abnormality. The importance of the V-ATPase for the functionality of the golgi apparatus was further confirmed by pharmacological studies using Concanamycin A, a specific inhibitor of the V-ATPase. Treatment of growing pollen tubes with Concanamycin A led to a reduction of polar cell elongation and electron microscopy showed similar deformations of golgi stacks as seen in the vha-A pollen.

T01-037

Identification and characterisation of three genes determining embryogenesis by means of T-DNA mutagenesis in Arabidopsis thaliana (L.)

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One of the possible approaches to a plant gene function study in model plant Arabidopsis thaliana leads across T-DNA mutagenesis. Collection of 2500 T-DNA lines have been obtained by A. thaliana transformation with Agrobacterium tumefaciens containing plasmid pPCVRN4 with HPT selectable gene (Koncz et al. 1994). Screening of individual lines revealed three different mutations with defects in immature seed development (marked 1265, 1293, 1321) which have been characterised and analysed in detail. One T-DNA insert was determined in each line and the mutations were confirmed to be monogenic and recessive-lethal by genetic analysis. Morphological studies of embryo development were performed by clearing in chloral hydrate. Mutant embryos were blocked in certain steps in the process necessary for embryo viability and development. In 1265 mutation defect in globular stage was observed. It was associated with abnormal pattern of cell division connected with shoot apical meristem formation during embryogenesis. In 1293 mutation defect in cotyledon formation was observed and in 1321 mutation defect was connected with late embryogenesis, seed maturation and storage products formation.
Cosegregation of T-DNA with the mutation was confirmed only in the 1265 line. The inverse PCR method was applied for isolation of flanking plant DNA. The obtained fragment of DNA was sequenced and subjected to BLAST and The Arabidopsis Information Resource database search, which revealed T-DNA insertion in At1g53330 gene coding pentatricopeptide repeat (PPR) containing protein. The hypothetical function of this gene is connected with transporter activity and probably cell - cell signalling.
The other mutations, 1293 and 1321, which did not cosegregated with T-DNA, were subjected to genetic mapping for perspective isolation of the affected genes by map-based cloning strategy. DNA markers, SSR and CAPS, linked to the mutated gene were used to delimit the region containing the gene of interest. 1321 mutation was located on chromosome 2 in tight linkage with marker nga 1126 and 1293 mutation was linked with marker nga 63 on chromosome 1. The next work will be aimed at fine mapping enabling candidate gene choice and the isolation of DNA sequence that underlines the phenotype of interest.

Acknowledgements
This research work was supported by the projects no. MSM 143100008 from the Ministry of Education of the Czech Republic and no. 521/00/D036 from the Czech Science Foundation

Koncz, C. et al. (1994) Plant Molecular Biology Manual, Kluewer Acad. Publ., B2: 1-22, Dordrecht, Boston, London.

T01-038

Influence of methylation pathway genes on FLC expression in Arabidopsis thaliana.

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Activity of the FLC gene (Flowering Locus C) plays a central role in the control of flowering time in the autonomous and vernalization pathways. FLC activity is negatively regulated by other genes of both pathways. The main goal of this work was to determine the influence of the six late-flowering genes (dn, L4, L5, L6-1, L6 a Spi) of a newly discovered methylation pathway (Lízal and Relichová, 2001) on FLC expression. Also, the effect of developmental stages, vernalization and 5-azacytidine treatment on the level of FLC mRNA in rosette leaves was analysed. Moreover, the tissue distribution of the FLC transcript was determined.
For this purpose, reverse transcription and PCR amplification of FLC was used.
All six late-flowering mutants of the methylation pathway increased the level of FLC mRNA relative to control lines S96 and Di-G. For analysis of the effect of developmental stages, vernalization and 5-azacytidine treatment, the level of FLC mRNA was examined in rosette leaves, which were collected from plants (L4 and Spi) before and after flowering. FLC mRNA levels were not affected either by vernalization, 5-azacytidine treatment or by plant age during vegetative and reproductive development. The tissue distribution of the FLC transcript was analysed in the Ler standard line and the Spi late-flowering mutant. RNA was isolated from roots, stems, rosette and cauline leaves, inflorescences and siliques. FLC was expressed in all vegetative tissues but was not detectable in reproductive tissues - inflorescences and siliques.
The late flowering phenotype of all six mutation genes is caused by an elevated level of the FLC transcript. The level of FLC transcript in rosette leaves is steady throughout life cycle of the plant and is restricted only to tissues of the vegetative phase. Vernalization and 5-azacytidine treatment did not reduce the level of FLC mRNA in rosette leaves. These results indicate that FLC expression is downregulated in the apex only after the transition to flowering.

This research was supported by project No. 521/02/P127 from the Czech Science Foundation (GACR).

P. Lízal, J. Relichová, Physiol. Plant. 113 (2001): 121-127.

T01-039

Drawing a line in the Arabidopsis fruit: How the valve margin forms at the border between the valve and the replum

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During Arabidopsis fruit development a band of cells called the valve margin differentiates at the border between the valve (seedpod wall) and the replum (central ridge of tissue that remains attached to the plant). Upon maturity, the fruit dries and the valves separate from the replum along the valve margins to release the seeds. Therefore the correct differentiation of the valve margin precisely at the border between the valves and the replum is essential for seed dispersal. The SHATTERPROOF (SHP) MADS-box genes as well as two bHLH genes INDEHISCENT (IND) and ALCATRAZ (ALC) are required for the formation of the valve margin and are all expressed in stripes at the valve margin of wild type fruit. However, SHP, IND, and ALC are ectopically expressed throughout the valves of fruitfull (ful)-mutant fruit. Consequently, ful valve cells fail to differentiate correctly and instead adopt the characteristics of valve margin cells. The ectopic activity of SHP, IND, and ALC in the ful-mutant valves is largely responsible for their failure to differentiate since valve development is almost completely restored in shp ind alc ful mutants. Therefore the FUL MADS-domain protein is not directly required for the development of most valve cells and instead the role of FUL in fruit development is to limit the differentiation of the valve margin to the edge of the valve. Similarly, SHP, IND, and ALC are ectopically expressed in the cells of the replumless (rpl)-mutant replum. Consequently the cells of the replum region fail to differentiate as replum cells and instead adopt the characteristics of valve margins cells. The ectopic activity of SHP is largely responsible for the failure of replum development in rpl mutants because removal of SHP in shp rpl mutants restores replum differentiation. Therefore the RPL homeodomain protein is not directly required for replum development, but is instead required to limit the differentiation of the valve margin to the edge of the replum. In conclusion, RPL and FUL act in parallel to negatively regulate SHP, IND, and ALC limiting them to a stripe at the border between the valve and the replum so that the fruit opens to disperse the seeds precisely at this junction.

T01-040

GeBP and LEC genes, two putative pathways to regulated trichome formation.

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Arabidopsis trichomes are single cells derived from protodermal cells of leaf primordia. We have previously shown that gibberellin hormones (GAs) up-regulate GLABROUS1 (GL1), a myb gene required for trichome initiation. To unravel GA pathway leading to GL1 activation, we have first undertaken the analysis of mutants that make ectopic trichomes on cotyledons during embryogenesis, namely mutants of genes LEAFY COTYLEDON2 (LEC2) and FUSCA3 (FUS3). We show that GL1 is ectopically expressed during mutant embryogenesis. Mutation in GL1 or in GAs biosynthesis prevent trichome formation in lec2 and fus3 embryonic mutants. We hypothesized that GL1 misexpression was due to a misactivation of the GA pathway. Using real-time RT-PCR and GUS reporter gene analysis, we have shown that one specific GA-biosynthesis gene is de-repressed in lec2 and fus3 mutants. We show evidence that FUS3, a B3 domain transcription factor, interact directly, in vitro, with a regulatory element located in the promoter of this GA-biosynthesis gene. This demonstrates that ectopic trichome formation on cotyledons of fus3 and lec2 mutants, are due to abnormal GA production during embryogenesis. We have also identified a new regulatory protein that specifically interacts with the 3' cis-regulatory element of GL1, named GeBP (GL1 enhancer Binding Protein). GeBP is the first member of a new gene family composed of 4 members in Arabidopsis, all with unknown fonctions. We identified two domains in GeBP, a putative DNA-binding domain and a leucine zipper that are necessary for trans-activation in yeast. A fusion between GeBP and the Yellow fluorescence protein shows that GeBP is a nuclear protein, localised in sub-nuclear foci. GUS reporter gene analysis indicates that GeBP is expressed mainly in the shoot apical meristem (SAM) and leaf primordia consistent with a regulation of GL1. Finally we show that GeBP transcript level is regulated by KNAT1, a KNOX gene expressed in the SAM and involved in leaf specification and repression of GA-biosynthesis. These results suggest that GeBP is a transcription factor involved in the repression of leaf cell fate.

Curaba J, Herzog M, Vachon G (2003) Plant J 33: 305-317.

T01-041

Methods to identify in vivo target genes in Arabidopsis

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Several large families of transcription factors exist in plants, among them the MADS-box gene family, which comprises a little over 100 members. In plants they are involved in e.g. flowering, flower formation, fruit dehiscence, reproduction, and leaf and root development.
MADS-box proteins do not only form dimers, but are also capable to form ternary and quaternary protein complexes in yeast. They bind as complexes to motifs in promoter sequences of target genes, called CarG-boxes (CC(A/T)6GG). To date only a few target genes from MADS-box proteins have been identified. Our aim is to isolate target genes from AGAMOUS, SEPALLATA3 and FRUITFULL, which are all involved in pistil and silique development.
Methods to identify in vivo target genes are: protein fusions with the glucocorticoid receptor in combination with microarray experiments, Chromatin Immuno Precipitation (ChIP) or Chromatin Affinity Purification (ChAP). ChIP allows purification of in vivo formed complexes of a DNA-binding protein and associated DNA. ChAP also allows isolation of protein-DNA complexes, but is based on the purification of epitope-tags that are fused to the protein of interest. The advantage of ChAP is that the same antibodies can be used to identify target genes from different transcription factors. The green fluorescent protein (GFP) is one of the epitope-tags used, which gave rise to biologically functional GFP-tagged MADS-box proteins. Plants expressing these tagged proteins will be used for protein-DNA complex isolation, as well as protein-protein complex isolation. The latest results will be presented.

T01-042

Loss-of-function mutation lhy-12 is caused by mis-splicing of the LHY gene and an intragenic suppressor mutation lhy-2 may partially restore the defect in Arabidopsis

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We have recently proposed that LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) are essential components for circadian clock function in Arabidopsis (1). Gain-of-function mutation of lhy (lhy-1) causes late flowering under long days (LD) and elongated hypocotyl phenotypes.
Loss-of-function mutations of lhy (lhy-11, 12 and 13) were isolated as intragenic suppressors and causes early flowering phenotype in short days (SD). We have screened for mutations that suppressed the early flowering phenotype of the lhy-12 mutant under SD. Here we demonstrate isolation of a new allele of lhy (lhy-2) as an intragenic suppressor of the lhy-12. The lhy-2 is quite similar to the lhy-1 in terms of late flowering under LD and long hypocotyl phenotypes. We also show the lhy-12 has a point mutation in the end of the 5th intron and this causes mis-splicing of LHY gene. The mis-splicing seems to be partially suppressed by the lhy-2. We will report details on characterization of the lhy-2.

(1) Mizoguchi et al., Developmental Cell 2002

T01-043

Identifying downstream targets of INDEHISCENT (IND), a bHLH transcription factor important for fruit dehiscence

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Arabidopsis has dry fruits that open to release their seeds through a process known as dehiscence. When the fruits are mature, the seed pod walls, or valves, separate from the central replum at the valve margins. The valve margins are composed of specialized cells that undergo cell-cell separation during dehiscence. Several genes that are important for valve margin differentiation have been characterized. One of these genes is INDEHISCENT (IND), which encodes a basic helix-loop-helix transcription factor. We are interested in identifying the downstream targets of IND and eventually unraveling the entire cascade of gene activity that leads to valve margin differentiation and fruit dehiscence. To this end we have created an inducible IND overexpression line with IND fused to the glucocorticoid receptor (GR). We are planning to use the Affymetrix microarrays to identify genes that are differentially expressed due to IND induction and due to loss of IND activity. Preliminary results from microarrays comparing the ind mutant to wild type will be presented.

Liljegren et al. (2004) Cell 116(6):843-53

T01-044

Moleculer Basis of Late-Flowering Phenotype in Dominant fwa Mutants

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Dominant late-flowering mutant fwa is an epigenetic mutant that ectopically expresses a GL2-class HD-ZIP gene due to promoter hypomethylation (Soppe et al. 2000). In wild type, however, FWA is not expressed during vegetative phase, and loss-of-function mutants of FWA are indistinguishable from wild type in flowering time. These facts suggest that FWA per se is not a component of the regulatory mechanisms of flowering. Genetic analysis suggests that FWA blocks the pathway at FT and/or downstream of FT. We envisage that FWA may provide a unique tool to dissect pathway from FT to flowering. We examined interaction of FWA protein with known flowering regulators such as FT, TFL1 and FD. FWA protein strongly interacted with FT protein through its C-terminal region and ZIP domain in yeast cells. No interaction was observed between FWA and TFL1 or FWA and FD. Interaction between FWA and FT was confirmed by the in vitro pull down assay. C-terminal truncation of FWA abolished interaction with FT. Overexpression of C-terminal truncated FWA did not cause late-flowering phenotype. These suggest that ectopically-expressed FWA inhibits floral transition by interfering with the FT function through protein-protein interaction.We hypothesize that ectopic FWA inhibits floral transition by interfering with the interaction between FT and a meristem-specific bZIP transcription factor FD through binding to FT. To test this, FWA is being expressed in various parts of plant to determine the tissue where FWA can exert its negative effect on flowering.

T01-045

Characterization of TSF, a homolog of floral pathway integrator FT

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TSF (TWIN SISTER OF FT) is a member of a small gene family in Arabidopsis, which includes FT. FT plays an important role in determination of flowering time. Since TSF is the closest homolog of FT in the family, it is likely that TSF plays a similar role as a floral promoter.

Both in long-day (LD) or short-day (SD) conditions, TSF and FT showed similar patterns of diurnal oscillation. TSF and FT mRNA levels gradually increased with time under long day conditions. When SD entrained seedling were shifted to LD, TSF and FT mRNA were immediately up-regulated. TSF and FT mRNA levels were decreased in co-2 and other late flowering mutants, such as fca-1. Upon induction of CO activity, TSF and FT expression was increased in the presence of cycloheximide. TSF and FT are regulated in a similar manner, and both are regulatory targets of the photoperiod pathway via CO. Constitutive overexpression of TSF caused early-flowering. On the other hand, a T-DNA insertion line with decreased TSF mRNA levels did not change flowering time under LD. However, a decrease of TSF activity enhanced the phenotype of loss-of-function allele of FT. These results suggested that TSF has a role as a floral promoter and that it may act redundantly with FT in the determination of flowering time.

Survey of selected accessions has revealed natural variations in and near the TSF locus, which can be classified into several types. Col-0 had a 1-kb insertion of unknown origin in 3' UTR, which has no obvious effect on expression. Ws and several accessions had a ca. 5-kb retroelement in 3' UTR and had greatly reduced levels of expression. Ler and Cvi alleles had a simple structure without large insertions found in other accessions.

T01-046

Epigenetics of seed development: what is the significance of imprinting?

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Recent work from our group and several other groups indicate that epigenetic processes play an important role in seed development. These processes include the roles of polycomb genes during endosperm development and parent of origin specific DNA methylation controlling the size of mature seed. In order to define these epigenetic processes further and also to find other genes that control the epigenetic process as well as the genetic processes that accompany it, we have undertaken in-planta gene expression studies in endosperm using reporter gene GFP.

A number of gene promoters, from different genomic regions have been used in these studies. The results suggest that a large part of the maternal genome might be repressed following pollination with hypomethylated pollen explaining the rescue of the fis mutation as well as the reduced size of seed caused by such pollination. We have mapped genomic regions undergoing hypomethylation-induced changes responsible for the rescue of fis seeds.

A novel model involving imprinting, hypomethylation induced gene repression and epigenetic rescue of fis class seeds will be described.

T01-047

Initiation of Seed Coat Development in Mutants Affecting Embryo and Endosperm Development

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The Arabidopsis seed coat is derived from maternal integument tissue and contains no tissue derived from the fertilization event. Nevertheless, following fertilization, the seed coat develops in unison with the embryo and endosperm to produce the mature seed. We have investigated the initiation of seed coat development in mutants that are either embryo lethal or gametophytic, of the Fertilization Independent Seed or FIS class. In the absence of pollination, the endosperm develops autonomously in FIS mutants but there is no embryo development. Our results indicate that seed coat development is initiated in both the embryo lethal and the FIS class of gametophytic mutations. One interpretation of this result is that the signal for seed coat initiation is triggered by endosperm development.

To test this hypothesis we have asked whether seed coat development is initiated in a novel mutant called All Dressed Up with Nowhere to Go (ADU). When adu flowers are emasculated the integuments of the ovule grow but neither the embryo nor endosperm develops. The seed coat phenotype of the adu mutant will be presented along with a model showing the roles of the embryo and endosperm in seed development.

T01-048

CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis

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Flower development at the shoot apex is initiated in response to environmental cues. Among these, daylength is one of the most important and is perceived by the mature leaves. When exposed to inductive photoperiodic conditions, these leaves produce endogenous signals that are transported to the shoot apical meristem where they cause the transition from leaf to flower morphogenesis.

Mutants impaired in the flowering response to daylength may provide a route to identifying the transmissible substances, explaining how their synthesis and transport are regulated, and defining the mechanism by which they induce flower development. In Arabidopsis, genetic analysis identified a pathway of genes required for the initiation of flowering in response to daylength. The nuclear zinc-finger protein CONSTANS (CO) plays a central role in this pathway, and in response to long days activates the transcription of FLOWERING LOCUS T (FT), which encodes a RAF-kinase inhibitor- like protein. The use of CO::GUS fusion revealed that CO is expressed widely in both the vascular tissue and the shoot apical meristem. However, the identity of the cells in which CO acts to promote flowering remains unknown.

In an attempt to identify these cells, we misexpressed CO under the control of various tissue-specific promoters (shoot, root, vasculature) in the late-flowering constans mutant and identified transgenic plants in which the late-flowering phenotype was complemented. CO misexpression from phloem-specific promoters, but not from meristem-specific promoters, was sufficient to induce early flowering and complement the co mutation.

The mechanism by which CO triggers flowering from the phloem involves partly the cell autonomous activation of FT expression since FT is also able to trigger flowering from the phloem. Genetic approaches finally indicate that CO activates flowering through both FT-dependent and FT-independent processes. However, unlike CO, FT also activates flowering when expressed in the meristem.

In conclusion, we propose that CO regulates the synthesis or transport of a floral signal in the phloem, thereby positioning this signal within the established hierarchy of regulatory proteins that control flowering. It thus remains to be determined which floral signals are under the control of CO.

T01-049

ENHANCERS OF LUMINIDEPENDENS DEFINE A ROLE FOR BRASSINOSTEROIDS IN FLORAL PROMOTION

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A critical phase change in the development of a flowering plant is the transition from vegetative to reproductive growth. In Arabidopsis thaliana, the timing of this transition is regulated by the photoperiodic, the autonomous, the vernalization, and the gibberellin (GA) pathways. In the attempt to identify additional signaling components modulating floral timing, we mutagenized the autonomous-pathway mutant luminidependens (ld) and performed a genetic screen to isolate genes that act independently of the autonomous pathway. We obtained two allelic modifiers that strongly enhanced the late-flowering phenotype of ld, as these double mutants flowered significantly later under long day-conditions. When a functional LD was introduced, the resulting single mutants under long-day-growth are only slightly late-flowering compared to wild-type plants, while under short-day-growth, these single mutants exhibit a strong late-flowering phenotype. These flowering phenotypes are similar to those seen in GA-pathway mutants. Further genetic and molecular analyses revealed that both enhancers-of-ld are mutants in BRI1, which encodes a likely brassinosteroid (BR) receptor. When various BR-biosynthetic mutants were introduced into ld, a dose-dependent defect in flowering time was observed. We also found that application of exogenous GA can rescue the late-flowering phenotype of the bri1 ld double mutant. To define further BR signaling within the flowering-promoting pathways, we are analyzing the effect of various autonomous-, photoperiod- and gibberellin-pathway mutants on the flowering time of bri1. Detailed physiological and gene-expression studies on these double mutants are being conducted. Results from these studies will be presented. We propose a model in which BRs and GAs act in concert as part of a "hormone pathway" that interacts with the other genetic pathways to promote flowering

T01-050

ECL1, which acts in parallel with CO, accelerates flowering by upregulating FT

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ecl1-1D (Early flowering and Curly Leaf1-1D) isolated from activation tagging screen (Weigel et al., Plant Physiology 122: 1003 [2000]) flowered early and showed altered leaf morphology. Various approaches showed that activation of ECL1 caused early flowering by upregulating FT (Flowering locus T). Furthermore, both ft and fd loss-of-functions partially suppressed early flowering phenotype of ecl1-1D. Interestingly, ECL1 downregulated SOC1 (Suppressor of CO overexpression 1) and a T-DNA insertional allele of soc1 is completely epistatic to ecl1-1D. ecl1-1D co-2 plants flowered earlier than co-2 plants and double overexpressor of ECL1 and CO flowered earlier than parental lines, suggesting that ECL1 acts in parallel with CO. RT-PCR analysis showed that the curly leaf phenotype was resulted from overexpression of AG, as shown in clf mutant (Uchimiya et al., Planta 206:2 [1998]). ecl1-1D was not responsive to the vernalization and GA treatment, suggesting that ECL1 acts in photoperiod-dependent pathway. A DNA chip experiment using the Affymetrix GeneChips showed that transcription of fifty-four genes including MADS box genes was significantly enhanced in ecl1-1D. In addition, thirty genes including floral repressors and SOC1 were downregulated in the chip experiment. Taken together, our data suggest ECL1 is a floral promoter that exerts its effect on FT and SOC1 oppositely in determining flowering time.

Weigel et al., Plant Physiology 122: 1003 [2000]
Uchimiya et al., Planta 206:2 [1998]

T01-051

Partial complementation of the pollen defective apyrase mutation.

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Apyrases are present in all pro- and eukaryotic organisms. These highly active enzymes hydrolyze nucleoside tri- and diphosphates. In Arabidopsis, earlier studies identified two apyrase genes (Atapy1 and Atapy2) and demonstrated a pollen-specific function of the encoded enzymes (Steinebrunner et al., 2002). T-DNA mutated apyrase genes prevented pollen germination and consequently, no double knockout (dko) plants were obtained. However, in order to further study the physiological role of apyrases dko mutants are crucial. Our approach involves complementation of double heterozygous apyrase mutants plants with a wt copy of Atapy1 and Atapy2, respectively, fused to the SPIK (Shaker Pollen Inward K+ channel) promoter to obtain fertile dko plants. It has been previously shown that the SPIK gene is specifically expressed in pollen before and during germination (Mouline et al., 2002). Progress from our partial complementation strategy will be presented.

Steinebrunner et al., 2002
Mouline et al., 2002

T01-052

Characterisation of AtNIC4, a member of the MATE family from Arabidopsis thaliana

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The multidrug and toxic extrusion family (MATE) has members in archeae, bacteria, yeast, animals and plants. The genome of A. thaliana codes for at least 58 MATE proteins. Most protein members of the ubiquitous family possess 12 transmembrane helical segments (TMS). The MATE proteins from A. thaliana are sequence homolog to the functionally characterised bacterial efflux transporters NORM, VMRA, VCMA and seem to function as secondary carriers catalyzing substrate/cation antiport.

MATE proteins mediate resistance to structurally diverse substrates like aminoglycosides, cationic dyes, fluoroquinolones and different antibiotics and drugs.
Phylogenetic analysis revealed that the MATE proteins from A. thaliana can be divided into five subgroups. Only five MATE proteins were functionally characterised so far (ALF5, AtDTX1 subgroup 1, TT12 subgroup 2, EDS5, FRD3 subgroup 3). AtNIC4 (A. thaliana novel ion carrier 4) is one of eight proteins, that constitute subgroup 4.

Our project aims to analyse the function of AtNIC4 using molecular, biochemical and physiological methods in transgenic plants including the modulation of the gene activity by antisense-, RNAi- and gene overexpression strategies, subcellular localisation by the use of AtNIC4-GFP fusion proteins and promoter-activated GUS-expression.

Heterologous expression in yeast indicated that AtNIC4 is able to transport the cations lithium and sodium. RNA blot experiments and transcriptional fusion of the AtNIC4-promoter to the ß-glucuronidase reporter gene revealed that AtNIC4 is expressed in the vascular tissue of leaves, flowers, stems and roots. Overexpression of AtNIC4 in transgenic plants causes extreme phenotypic changes, including large numbers of flowers and stalks, indicating that AtNIC4 is involved in the distribution of substances, which regulate growth and development of plants.

T01-053

Intercellular protein trafficking of TFL1 and FT is essential for inflorescence meristem identity and floral transition in Arabidopsis.

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Arabidopsis TERMINAL FLOWER 1 (TFL1) gene is required for establishment and maintenance of the inflorescence meristem (IM), since tfl1 mutants show early flowering and terminal flower phenotype. Using GFP-TFL1 fusion protein, we have shown that TFL1 protein moves from the inner region of L3 layer, where the TFL1 transcript is accumulated, to the outer layers of shoot apical meristem (SAM) in accordance with the vegetative to reproductive phase transition. That is TFL1 protein does not move in the vegetative meristem (VM), but starts to move when the SAM is switched to IM by floral induction, and TFL1 protein becomes to localize in the whole layers of the SAM in the mature IM. This protein movement also occurs from L1 to L3 direction in the IM but which does not happen in the flower stalk, mature floral organs, and leaves. Thus, TFL1 protein moves in the spatiotemporal manner.

We made immobilized TFL1 proteins by fusing multiple GFPs to increase molecular mass. This immobilized TFL1 does not complement tfl1 mutant either it is expressed in the TFL1 genomic context or by L1 specific promoter, but rescues mutant phenotype when expressed by CaMV 35S promoter. These results suggest that TFL1 protein is required localize in the whole region of IM to function properly and intercellular movement itself is not essential to gain TFL1 function. Molecular dissection of TFL1 protein revealed that 21 amino acids region is sufficient for protein trafficking. We are now making a series of amino acid substitutions in this region to obtain functional but non-trafficking TFL1 protein. This kind of protein is useful to genetic screening of cellular mechanism of protein trafficking.

One of the floral pathway integrators, FT is a homolog of TFL1 and we have found that FT protein also moves cell to cell in the SAM. The FT function is opposite to TFL1 since ft mutant shows a late-flowering phenotype. Recently, we found that FT is expressed in the vascular tissue of leaves in response to the long-day (LD) signals, but not in the SAM at the vegetative stage. ft mutant is rescued when FT is expressed in the VM, suggesting that FT function is required in the VM. We are now investigating whether the intercellular protein trafficking of FT contributes to the long distance signaling from leaves to the SAM to promote flowering.

T01-054

Characterization of Naturally Occuring Proteins Modified by the Phytohormone IAA from Bean and Arabidopsis seeds

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In bean, proteins exist that have a prosthetic group consisting of the phytohormone indole-3-acetic acid (IAA). The gene for IAP1, a protein covalently modified by IAA was isolated and cloned from bush bean (Phaseolus vulgaris) seeds. Antibodies (Ab3.6K) raised against a bean 3.6 kDa IAA peptide detected several polypeptides in bean seeds. Using immunoblotting and GC-MS analysis a major protein of 42 kDa with IAA covalently attached was found. Based on the microsequencing results of this protein the iap1 gene was found and cloned. The expression of IAP1 is correlated to a developmental period of rapid growth during seed development. Using Ab3.6K cross reacting proteins were detected in Arabidopsis seeds. GC-MS analysis confirmed the presence of IAA covalently bound to the proteins indicating the presence of IAA proteins in dicots other than bean. To investigate the physiological role of the protein modification by IAA we are currently generating transgenic Arabidopsis thaliana and Medicago truncatula plants that express the iap1 gene. GFP fusion proteins with IAP1 will help to localize it within the cell. To isolate IAA proteins from Arabidopsis, peptide antibodies against conserved amino acid regions were generated and used for immunoprecipitation of total protein from Arabidopsis thaliana.

T01-055

Ectopic expression of the proline biosynthesis genes rolD and AtP5CS affects axillary bud formation and flowering in Arabidopsis

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Flower transition is a fundamental developmental change in plant life that is controlled by a complex network of flowering genes, responding to and modulated by a number of internal and environmental stimuli. On the basis of previous work from our group, we hypothesized that one such stimulus may be proline, or a related metabolite, that may behave, directly or indirectly, as a signal molecule capable to affect flowering. The rationale of this hypothesis lies on the demonstration that the plant oncogene rolD encodes a functional ornithine cyclodeaminase - an enzyme that catalyzes the conversion of ornithine into proline - and stimulates reproductive phase transition in some plant species. To support this hypothesis we generated and analyzed, at phenotypic and histological level, Arabidopsis plants transgenic for the proline biosynthesis genes rolD and AtP5CS, the latter being the rate-limiting enzyme of proline biosynthesis in higher plants - under the expression of either the rolD or CaMV35S promoter. The phenotypic alterations revealed by this analysis are similar for both genes and affect axillary bud development, inflorescence formation, time of flowering, plant height, and senescence. The phenotypes exhibited by the P5CS plants, however, are more severe compared to those showed by rolD plants, consistent with the major role of AtP5CS in proline biosynthesis. We are currently studying the effects of these transgenic plants on the expression of the main flowering time and flower meristem identity genes. Further work is in progress to assess the relationships between proline content and phenotypic alterations, by means of northern blot and aminoacid analysis.

Trovato, M. et al. (2001). The plant oncogene rolD encodes a functional ornithine cyclodeaminase. PNAS, 98, 13449-13453.

T01-056

bHLHs transcription factors interacting with CO are potential regulators of flowering time

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CONSTANS is a nuclear protein that acts by rapidly inducing the expression of downstream flowering time genes such as SOC1 and FT in response to long-day conditions (Samach et al, 2000). The CO and CO-Like proteins contain two highly conserved segments: a zinc finger containing region near their N-terminus and a CCT domain near the C-terminus.
Further understanding of the function of CO is likely to come from identifying interacting proteins. Since CO is probably not able to bind DNA by itself, it is likely to be recruited by a DNA-binding protein and act in a protein complex. In order to gain insight into CO function, we have performed yeast two hybrid screening with both conserved domains of CO. We have identified a set of bHLH proteins as potential interactors with the CCT domain of CO. In vitro- and FRET experiments confirmed interaction between some of these proteins and CO

Samach et al, 2000 Science 288,1613-1616

T01-057

Exploiting the non-flowering fca-1 co-2 ga1-3 triple mutant and gene expression profiling to characterise the role of individual genes in the transition to flowering of Arabidopsis

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The switch from vegetative to reproductive growth is one of the major transitions in the life cycle of a plant. In Arabidopsis four main signalling pathways promote this transition: the environmental photoperiod and vernalisation pathways, and the endogenous autonomous and gibberellin pathways. The triple mutant fca-1 co-2 ga1-3 impairs three major pathways and does not flower under long or short photoperiod conditions. We have used the non-flowering triple mutant to investigate the capacity of single flowering genes to initiate flowering when expressed from the 35S promoter. The genes investigated, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), FLOWERING LOCUS T (FT) and LEAFY (LFY), are regulated at the transcriptional level by each of the pathways impaired in the triple mutant. Over expression of FT strongly promotes flowering in the triple mutant background, doing so after approximately 30 days growth when the plants have produced a total of 12 leaves; the ability of both SOC1 and LFY to initiate flowering in this background is much weaker, typically occurring after three to four months growth with around 55 leaves, additionally not all plants carrying the SOC1 or LFY overexpressors were able to flower in the triple mutant background. Northern analysis established that FT is able to act partially through the upregulation of SOC1 mRNA in this background, furthermore the use of a 35S::CO ft-7 line demonstrated that CO is able to upregulate SOC1 independently of FT.
To identify genes regulated by each of the flowering-time genes microarray experiments were carried out using RNA extracted from different genotypes and the 8K AtGenome1 or 24K ATH1 Affymetrix Arabidopsis GeneChips. Clustering analysis of a subset of these experiments, and the follow up of interesting candidate genes will be presented.

T01-058

Characterization of a late-flowering T-DNA tagged mutant of Arabidopsis thaliana

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In order to identify and isolate specific genes involved in regulating plants growth and development we have employed a gene knockout approach using T-DNA tagging and in vivo gene fusion in Arabidopsis thaliana. Screening of the T-DNA tagged lines resulted in identification of a mutant (line no. 197/4) exhibiting a significant delay in flowering time. This line also exhibits a tissue-specific expression of the promoterless gus reporter gene. GUS activity was detected predominantly in the shoot apex. Inverse PCR was used to clone the T-DNA flanking plant DNA sequence. This sequence was used as a query in a BLAST search and a candidate gene was identified. Gibberellin is a growth regulator and it is involved in many developmental processes in plants such as stem elongation and flowering time. When measuring the gibberellin content in the plants of line 197/4 we found that these plants had reduced levels of some GAs like GA4 and elevated levels of other GAs like GA9, in comparison to wild-type plants. These results suggest that the identified gene might be involved in regulating gibberellin biosynthesis in A. thaliana. To investigate this hypothesis we exposed the plants of line no. 197/4 to gibberellic acid (GA3). This treatment enhanced flowering time in the mutant plants; they flowered almost as early as the wild type plants. For further verification of these results we are now analyzing several lines of SIGnAL (Salk Institute Genomic Analysis Laboratory) mutants of A. thaliana.

T01-059

Functional characterization of MAF2: an FLC Paralogue

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The switch from vegetative to reproductive phase, culminating in flowering has been defined as a critical event in the life cycle of Arabidopsis, given the fact that it is an out-come of closely interacting multiple pathways responding to both internal and external cues. The current model on flowering, framed in four major pathways, identifies the floral repressor FLC, which encodes a MADS domain protein, as a key determinant of the vernalization pathway. Natural accessions of Arabidopsis vary greatly in their levels of FLC expression, which is reflected in widely varying flowering times.

The Arabidopsis Genome Initiative has identified at least five MADS box paralogues of FLC, amongst which MAF2 (AT5G65050) has been characterized as a moderate repressor of flowering, with a specific role of preventing premature vernalization response (Ratcliffe et al. 2002). Coupled to this is the observation that each of these paralogues expresses several splice variants, the roles of which remain undefined. Since several alleles with major indels in FLC have been described, we surveyed a set of 45 natural accessions for major indels in FLC paralogues. We found that such instances are rare and restricted to non-coding sequences. In addition, RT-PCR revealed little variation with respect to expression of transcripts corresponding to these paralogues in about 100 accessions. Currently, we are further dissecting MAF2 function by comparing the phenotypes derived from over-expressing three reported and a novel iso-form from Columbia vis-à-vis full-length genomic region in different genetic backgrounds. In a related effort, we shall be carrying out a global-expression profiling of MAF2 T-DNA insertion mutant in response to inductive day length.
This study is supported by the Max-Planck-Society

Ratcliffe et al. 2003; Plant Cell 15: 1159-1169

T01-060

VRN5, a PHD/FNIII protein, is involved in vernalization by repressing FLC

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The requirement of a long period of cold temperatures for the induction of flowering is one important factor that ensures the onset of the reproductive phase in favourable growth conditions in many plant species. The induction of flowering by long periods of cold is called vernalization and is thought to have an epigenetic basis. One major factor that is negatively regulated by vernalization in Arabidopsis thaliana is the floral repressor gene FLC. During cold treatment the chromatin structure of the FLC locus is transferred into a repressed state that is maintained during succeeding phases of growth. vrn5 mutants are not able to maintain the repression of FLC and this leads to late flowering even after vernalization. The isolation of VRN5 by map-based cloning revealed that VRN5 encodes a PHD/FNIII protein. Together with VIN3 (Sung et al., 2004), a gene previously described to be involved in the cold induced repression of FLC, it belongs to the VEL gene family in Arabidopsis. Genetic analyses and protein interaction studies between VRN5 and genes known to be involved in the maintenance of FLC repression will clarify the relationship between VRN5 and other pathway components and reveal its function in the vernalization response.

Sung S, Amasino RM. (2004) Nature 427(6970):159-64.
Bastow R, et al. (2004) Nature 427(6970):164-7.

T01-061

TRANSPARENT TESTA1 controls early and late steps of flavonoid biosynthesis in the endothelium of Arabidopsis thaliana seeds

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Wild-type seed coats of Arabidopsis thaliana are brown because they contain condensed tannin pigments. Accumulation of these pigments requires the activation of genes involved in phenylpropanoid biosynthesis. Mutations in these genes cause the transparent testa (tt) phenotype, which is characterised by yellow appearance of the seeds. While mutations in single copy genes encoding phenylpropanoid biosynthetic enzymes affect the whole plant body, some regulatory tt loci, including tt1, affect seed pigmentation only. The TT1 gene has recently been shown to encode a WIP-type zinc finger protein. In this study, we show that nuclear localisation of TT1 is a prerequisite for function. We further demonstrate that TT1 controls the activity of several genes encoding early as well as late enzymes of flavonoid biosynthesis. It exerts its activity only in the endothelium of the seed coat where most of the condensed tannins accumulate in the wild-type, whereas pigment synthesis in specialised cell types at the seed base is unaffected. Our results demonstrate that TT1 is involved in the regulatory network controlling flavonoid accumulation in endothelium cells during A. thaliana seed development.

Sagasser M, Lu GH, Hahlbrock K, Weisshaar B.
Genes Dev. 2002 Jan 1;16(1):138-49.

T01-062

Comparative Protein Profiling and Expression Analyses of the Arabidopsis Subtilisin-like Serine Protease Family

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Plant subtilisin-like serine proteases are proposed to be involved in several processes, such as general protein turnover or pathogenic defence. Forward genetics has identified two subtilases as highly-specific regulators of plant development. In the Arabidopsis sdd1 mutant (stomatal density and distribution 1) the pattern of stomata formation is disrupted, resulting in clustering of stomata as well as in a dramatic increase in stomatal density. Likewise, the gene disrupted in the ale1 mutant (abnormal leaf shape 1) was cloned and found to encode a subtilase. ALE1 is required for cuticle formation and epidermal differentiation during embryo development in Arabidopsis.
SDD1 as well as ALE1 belong to a large gene family in Arabidopsis thaliana (subtilases, AtSBTs) that comprises 56 members, identified based on homology and motif searches. Sequence analysis typed the AtSBT proteins into six distinct subgroups. 31 (53%) of the AtSBT genes are organized in tandem clusters, 18 (32%) are located in segmental duplicated genomic regions. In a mutant screen we collected and confirmed 100 T-DNA insertion mutants comprising knockouts of 54 out of the 56 AtSBTs. Except for SDD1, none of these confirmed homozygous mutants revealed any obvious visible phenotypic alteration. This suggests either highly specific functions for subtilases in respect to developmental processes and/or environmental conditions, or a high degree of functional redundancy within this gene family.
To gain insight into the roles of the Arabidopsis SBTs we started a collaborative functional genomics analysis program carried out by The Arabidopsis Subtilase Consortium (http://csbdb.mpimp-golm.mpg.de/association/PSDB/PSDB_Home.html). It covers expression analyses by sqRT-PCR, promoter-GUS and in situ hybridisation, the collection and evaluation of k.o. mutants, overexpression of selected subtilase genes and comparative protein and metabolite profiling. Furthermore, we apply computational analyses of gene expression profiles to infer hypotheses about the respective functional involvements of AtSBTs.
Here we present results from our comparative protein profiling approach applying 2-DE as well as from the expression and computational analyses of the AtSBT gene family.

Berger, D., and Altmann, T. (2000)
von Groll, U., Berger, D., Altmann T. (2002)
Tanaka et al. (2001)

T01-063

Control of Arabidopsis petal size by a novel RING finger protein

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The species-specific size of plant organs is under tight genetic control as evidenced by the very low variability in size when genetically identical individuals are grown under the same environmental conditions. However, the genetic and molecular basis of organ size control in plants is largely unknown.
To begin to dissect the underlying regulatory mechanisms, we are characterizing a novel Arabidopsis mutant, big brother2 (bb2), that shows a dramatic overgrowth of petals and to a lesser extent of sepals and the stem. This appears to be due to an extended period of growth, rather than to faster growth. The enlargement of petals is independent of the known growth promoter AINTEGUMENTA (1), suggesting that bb2 defines a novel regulatory pathway in growth control.
The phenotype is due to the deletion of an uncharacterized gene encoding a protein with a RING-finger domain. Intriguingly, heterozygous bb2 mutants also show an intermediate enlargement of organs, suggesting that BB2 regulates organ growth in a dosage-dependent manner.

(1) Mizukami and Fisher (2000), PNAS 97, 942-947

T01-064

Functional analysis of armadillo repeat-only (ARO) proteins in Arabidopsis thaliana

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The armadillo domain (Arm) was first identified in the Drosophila segment polaritiy gene armadillo. It codes for beta-catenin, wich functions in cell to cell adhesion but also as a component of the wnt-signalling pathway, regulating cell fate and polarity. Proteins containing Arm repeats possess tandem copies of a degenerated protein sequence motif of about 42 amino acids that form a conserved three-dimensional structure mediating protein-protein interactions [1]. Most of these proteins are involved in intracellular signalling or regulation of gene expression during developmental processes. In contrast to animals, only two Arm repeat containing proteins have been functionally characterized in plants [2,3]. Nevertheless, 108 predicted Arm repeat proteins have been identified in the Arabidopsis genome [4], which can be subdivided on the base of their homology and the occurrence of additional motifs (e.g. U-box, F-box, S/T kinase, etc). Besides those subfamilies containing defined motifs of known function adjacent to the Arm domain, there is a subgroup of at least 26 Arm genes without any further known protein motif and with yet unknown function (ARO). Three of these genes encode proteins with significant homology to a wheat arm repeat protein from unfertilized egg cells. The wheat homolog (TaAro1) shows female and male gametophyte-specific expression. Likewise, one of the corresponding A. thaliana genes, AtAro1, can be detected specifically in female and male gametophytic tissues of Arabidopsis by RT-PCR studies. The other two genes (AtAro2 and AtAro3) are also expressed in vegetative tissues. Promoter activity of AtAro1 was analyzed using GUS as a reporter gene. Since database searches revealed the existance of a putative transmembrane domain for AtAro1, subcellular localization of the proteins was investigated using GFP fusion proteins in transient expression studies. T-DNA insertion lines of all three genes are currently examined for phenotypes, especially in respect to gametophyte formation and function. Future studies include the ectopic overexpression af AtAro1 and a screening for interacting proteins.

[1] Coates, J.C. (2003); [2] Stone, S.L. et al. (2003); [3] Amador V. et al. (2001) [4] Mudgil, Y. et al. (2004)

T01-065

STY genes and Auxin in Arabidopsis development

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Gynoecia of the Arabidopsis loss-of-function mutant sty1-1 display an abnormal style morphology and altered vascular patterning. These phenotypes, which are enhanced in the sty1-1 sty2-1 double mutant, suggest that polar auxin transport (PAT) or auxin signalling might be affected by mutations in the related genes STY1 and STY2. Transient chemical inhibition of PAT severely affects the apical-basal patterning of the gynoecium as do mutations in the auxin transport/signalling genes PID and PIN1. By transient treatments with the PAT inhibitor 1-N-naphtylphtalamic acid (NPA), we show that the apical-basal patterning of sty1-1 and sty1-1 sty2-1 gynoecia is hypersensitive to reductions in PAT and that sty1-1 enhances the PAT inhibition-like phenotypes of pid-8 and pin1-5 gynoecia. STY1 and STY2 are active not only in gynoecia but also in root primordia and root tips. The lateral root production in 35S::STY1 plants are dramatically reduced and becomes restored to that of wild-type by exogenous auxin application. This could be due to an suboptimal level or distribution of auxin in these plants. Two auxin induced GH3-like Arabidopsis genes are dramatically upregulated by transient induction of STY1 in sty1-1 sty2-1 double mutants. Overexpression of one of the GH3 genes results in similar phenotypes as overexpression of STY1; reduced number of lateral roots, dwarfism, reduced apical dominance and epinastic cotyledons. We therefore suggest that this GH3 gene mediates most of the phenotypic changes identified in 35S::STY1 lines.

T01-066

Floral induction by ambient growth temperature in Arabidopsis thaliana

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Photoperiod, light quality, vernalization and ambient growth temperature are the four major environmental factors that modulate floral transition in Arabidopsis. Compared to our understanding of the molecular basis of photoperiodic and vernalization response, the regulation of floral transition by ambient growth temperature is still largely at a fledgling stage (1, 2). In an effort to understand the growth temperature mediated effects on floral transition, we analyzed the known flowering time mutants and a few wild strains of Arabidopsis for their flowering responses at varying temperature conditions under both long and short photoperiods.

Under long day conditions, the effects of temperature were modest and high genetic correlations were observed between varying temperature conditions suggesting that the same genetic pathway (possibly the photoperiodic pathway) may play a predominant role even at different temperatures in long days. However, under short day conditions, the effects of temperature were more pronounced. Higher temperatures in short days induced floral transition (Thermo-floral induction) to a similar extent to that of the photoperiodic floral induction under lower temperatures. This effect was not mediated through the photoperiodic pathway since the thermo-floral induction was unaffected in the constans (co) and gigantea (gi) mutants. Mutants of the autonomous pathway failed to induce floral transition in response to higher temperature. However, the floral induction by ambient growth temperature can still be seen in flc-3 mutants, suggesting that the floral repression under lower temperatures is not exclusively dependent on FLC.

By analyzing wild strains of Arabidopsis we have identified accessions that are impaired in thermo-floral induction under short day conditions. We have also identified accessions that display a temperature specific flowering response under long day conditions (3). Using both Mendelian and quantitative genetic approaches we are mapping the genetic loci underlying thermo floral responses in Arabidopsis thaliana.

This work is supported by Max-Planck Society and EMBO.

1. Blazquez etal (2003), Nature Genetics.
2. Halliday etal (2003), Plant J
3. Poster by Lempe etal in natural variation

T01-067

Using microarrays to identify genes implicated in pollen and anther development

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The application of microarray technology along with the availability of the full genome sequence of Arabidopsis has been demonstrated to be a powerful tool for analysing gene expression profiling. The greatest advantage of this approach is that around 24,000 genes can be monitored on a global scale, compared with the traditional methods of expression analysis (e.g. northern hybridisation) where only a few genes can be examine at a time. We have been using the Arabidopsis Affymetrix arrays (NASC) to analyse the process of pollen and anther development in the male sterile1 (ms1) mutant. In the ms1 mutant the process of pollen development begins normally, with pollen mother cells meiosis and tetrad formation progressing as in the wild type. However, just after the microspores are released from the tetrads, the immature pollen begins to breakdown and the anther tapetal tissue becomes abnormally vacuolated. Degradation of the locule contents continues, resulting in empty anthers with unviable pollen (Wilson et al., 2001). The MS1 gene is therefore critical for the production of viable pollen.

Our analysis has focused mainly on the genes that MS1 may regulate at early stages of pollen development. RNA extracted from floral tissue at developmental stages during and post-MS1 expression has been used to screen the Arabidopsis Affymetrix gene array. Evaluation of the data generated by these experiments will be presented.

Wilson ZA, Morroll SM, Dawson J, Swarup R and Tighe P (2001). Plant Journal 28: 27-39.

T01-068

Developmental regulation of transcription factor genes in Arabidopsis seeds

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Seed storage compounds, such as proteins and lipids, are crucial for human nutrition. Synthesis of storage compounds is developmentally regulated in seeds, and controlled, at least in part, at the level of gene transcription (Ruuska et al., 2002). Transcription factors (TFs) that orchestrate these changes are likely to be developmentally regulated also. However, few TF genes involved in seed metabolism have been discovered. Genetic identification of important seed TFs may be hampered by functional redundancy and/or their absolute requirement for seed viability. Therefore, we have embarked on a reverse-genetics approach that utilizes a genome-scale real-time RT-PCR platform (Czechowski et al., 2004) to identify developmentally regulated TF genes in Arabidopsis, which may regulate storage compound synthesis.

Using gene-specific primer pairs for over 1400 TFs and SybrGreenTM to measure cDNA amplification kinetics in real-time, we identified 56 TF genes that are over fifty-fold more highly expressed in developing siliques than in shoots. To facilitate identification of TFs that control seed storage compound synthesis, we determined the timing of key metabolic transitions. Fatty acid (20:1) was measured by gas chromatography, glycerol-3-P by an enzyme cycling assay, and storage protein by SDS-PAGE. Measurements were made on seed extracted from siliques numbered from the top of the plant (i.e. from developing to mature siliques/seeds). Seed glycerol-3-P levels exhibited a local minimum in silique number 6, which increased to a maximum at silique 10. This increase preceded, and presumably fueled 20:1 fatty acid biosynthesis, which began in silique 10 and continued until silique 22. Synthesis of the legumin-type and napin-type proteins began in siliques 14 and 17, respectively. We now plan to measure transcript levels for all Arabidopsis TF genes in developing seeds both prior to and after the onset of storage compound synthesis. This will help us to reduce the list of TF suspects that may control synthesis of storage lipid, protein, and other seed compounds of nutritional interest.

Czechowski et al. (2004). Plant J. 38: 366-79.
Ruuska et al. (2002). Plant Cell 14: 1191-206.

T01-069

DAG1 and DAG2: two Arabidopsis transcription factors that play a maternal role in controlling seed germination

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The Dof proteins DAG1 and DAG2 are plant transcription factors, characterised by a strikingly conserved (Dof) domain containing a single zinc finger (C2-C2) and a downstream basic region. The DAG1 and DAG2 proteins share an identical Dof domain and a high degree of aminoacid identity outside the domain, conversely to other proteins of this family that show extensive homologies only in the Dof domain. All Dof proteins bind similar DNA target sequences with a CTTT core. DAG1 and DAG2 are both involved, with opposite roles, in the control of Arabidopsis seed dormancy and germination. In fact, inactivation of DAG1 considerably increases the germination capability of the seeds, while mutation of DAG2 results in seeds with a substantially lower germination potential than the wt. In particular, dag1 seeds show an increased sensitivity to both GA and light, whereas dag2 seeds show a reduced sensitivity to the same stimuli.

DAG1 and DAG2 show an identical RNA profiling, limited to the vascular system of the mother plant, but absent in the developing embryo as well as in the mature seed. This is in good agreement with the maternal effect of both mutations. Thus, DAG1 and DAG2 are supposed to act, with opposite roles, on the same target genes and to have an effect on seed germination through transport of some molecules that regulate germination in the seed. Analysis of the cellular and subcellular localization of these two Dof proteins is being performed by confocal microscopy studies of DAG1-GFP and DAG2-GFP transgenic plants. Moreover, in order to understand the relationship between the DAG proteins and the signalling for germination activated by PhyB, we produced dag1 and dag2 transgenic plants overexpressing PhyB. We are also performing an analysis of PhyB-GFP subcellular localization in seeds respectively in a dag1 and dag2 background compared to a wt control.

T01-070

Antagonistic role of the bZIP transcription factors FD and FDP in controlling flowering and plant architecture

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FT and TFL1 are two closely related molecules that have opposite effects on flowering time. FT strongly promotes the floral transition, while TFL1 is a floral repressor. Despite extensive knowledge of the genetic interactions of FT and TFL1, it has been unclear until now how FT and TFL1 signal in the plant, since they have no known signaling domains and are not transcription factors. We have identified a pair of closely related bZIP transcription factors, FD and FDP, which interact with FT and TFL1, and are necessary for their activity.

Loss of function alleles of FD are late flowering, and can partially suppress the phenotype of 35S:FT. Furthermore, in 35S:FD plants, there is ectopic induction of AP1, a major FT target. Until now it has not been clear how FT induces AP1 transcription, since ectopic FT expression is insufficient to activate AP1. This induction is photoperiod sensitive, occurring when plants are shifted from short days to long days, in a way that closely mirrors the induction of FT. FD is expressed in young flowers., supporting a role for FD as an important factor upstream of AP1. Since FT is expressed more widely, but temporally regulated, we propose that FD provides the positional information for executing FT function.

In contrast to FD, loss of FDP activity causes early flowering and formation of terminal flowers, similar to that of tfl1. fdp tfl1 double mutants show a strongly enhanced terminal flower phenotype, terminating with only a single flower at the apex and even earlier flowering than the single mutants. Conversely, an fdp mutation partially suppresses 35S:TFL1. FDP is expressed in a domain similar to that of TFL1. We propose therefore that the interaction of FDP and TFL1 is required to prevent inappropriate activation of the floral program triggered by FD and FT in the shoot apical meristem.

T01-071

Regulation of the circadian expression of GI

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TOC1, LHY and CCA1 are involved in a negative feedback-loop that has been proposed to form the central oscillator of the Arabidopsis circadian clock. The transcription of GIGANTEA is under the control of this circadian clock, but GI itself is controlling the expression of the central oscillator components. GI is an evening gene, peaking about 10h after dawn in a 16h long day. In the double mutant lhy cca1, GI peaks about 6h earlier than in the WT. A motif found in the promoter of many evening-genes, the evening element (EE), is required for conferring circadian rhythmicity on a reporter gene (Harmer et al, 2000). This EE has also been shown to be a binding site in the TOC1 promoter for the LHY and CCA1 transcription factors, suggesting that the EE is involved in the repression of TOC1 expression by these two factors.
Since the promoter of GI contains three EE and since the expression of GI in long day peaks 6h earlier in the lhy cca1 double mutant, we have started a GI promoter analysis to determine in which proportion each EE affects the timing of GI expression. Promoter fragments obtained by successive deletion of the EEs were fused to a luciferase reporter gene and transformed in the WT and the lhy cca1 double mutant. The promoter fragment with all EE deleted still resulted in a strong circadian expression and an unmodified timing of the peak in long day.

Harmer et al. (2000) Science, 290, 2110-2113

T01-072

Identification and analysis of genes mediating the vernalization response

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Vernalization, the promotion of flowering by a prolonged period of cold, is an important factor in the control of flowering time of plants from temperate regions. While seeds or young seedling are exposed to low temperature the effect of the cold on flowering time is seen much later in the adult plant. The isolation of FLOWERING LOCUS C (FLC) has provided insight into the molecular mechanisms involved in vernalization. FLC encodes a MADS-box protein which acts as a repressor of flowering, with the level of expression of FLC correlating with the time taken to flower. Vernalization promotes flowering by causing a decrease in FLC expression. Using a genetic approach, four genes have been identified that mediate the vernalization process; VRN2 encodes a nuclear localized zinc-finger protein with similarity to polycomb group proteins, while VRN1 encodes a DNA-binding protein that binds DNA non-sequence-specifically in vitro. VIN3 and VRN5 encode proteins with PhD (plant homeodomain) and fibronectin type III domains. To identify other components required for the repression of FLC, we generated a FLC:luciferase fusion which behaves like native FLC. A population of plants was mutagenized and the M2 seedlings were screened at immediately after vernalization (to identify plants that have not repressed FLC during vernalization) and 2 weeks later (to identify plants that initially repress FLC but are unable to maintain the repression, i.e. vrn mutants). Here we report some new mutants that have an altered vernalization response.

T01-073

Characterization of The vanguard1 Mutant That Is Involved in Stabilization and Growth of Pollen Tube in Arabidopsis

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The Arabidopsis vanguard 1 (vgd1) mutant was identified by its reduced fertility. The homozygous vgd1 plant produced much less seeds than wild-type plant. Outcross of homozygous vgd1 plant with wild-type plant indicated that vgd1 was a male gametophytic mutation. The vgd1 pollen tube was much less stable in vitro and grew much more slowly in style and transmitting tract. However, vgd1 mutation did not affect the morphology and in vitro germination rate of pollen grain. Molecular cloning of the VGD1 gene showed that it encoded a protein that shared high homologies with a group of pectin methylesterases (PMEs). The total PME enzyme activity in the vgd1 pollen grain was reduced about 20% compared to that in wild-type pollen grain. Several studies have suggested that PMEs act on the modification of cell wall (Futamura, et al., 2000, Plant Cell Physiol. 41: 16-26; Micheli, 2001, Trend Plant Sci. 6: 414-419). The VGD1 protein may take an important role in the stabilization and growth of pollen tube, possibly by modification of pollen tube cell-wall.

T01-074

Analysis of flowering time in Arabidopsis and Lolium by micro-array analysis and heterologous overexpression

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Vernalization is the exposure of the imbibed seeds or vegetative plants to a period of cold temperature in order to flower. Our aim is to investigate if the vernalization- dependent flowering process in Arabidopsis is comparable with some of the mechanism of the vernalization-pathway in monocots (Lolium perenne). A cDNA-microarray with about 1500 unique PCR-amplified clones of Lolium perenne was prepared and gene expression changes were detected during the period of vernalization.
Among the genes responding to vernalization some transcription factors were identified including the APETALA1(AP1)-like MADS-box genes that seem to play a role in determining the floral transition in Lolium at an early time point, which is in contrast to the function of AP1 in Arabidopsis. The expression during flower induction of LpMADS1, LpMADS2 and LpMADS3 appeared to be gradually and strongly increased, which is distinct from Arabidopsis AP1-subfamily members. To assess the function and the response to vernalization of the clones, differentially expressed during vernalization, overexpression lines are generated in wildtype Arabidopsis (Col0 and H51) and in fca-1 and flc3 flowering mutants. Currently, these lines are analyzed under short-day conditions with and without a vernalization period. The latest results of these experiments will be presented.

T01-075

Cloning of ISE1 gene, which regulates plasmodesmatal function during embryogenesis in Arabidopsis

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Plasmodesmata (PD) are plasma membrane-lined dynamic channels that provide a pivotal role in cell-to-cell communication in plant cells. Recent studies demonstrate the functional significance of intercellular RNA and protein trafficking through PD during plant development. Numerous studies are underway to characterize the function of PD using a variety of different methods. Our current approach utilizes genetics to uncover mutant genes that affect PD function during embryogenesis. Here we report the molecular cloning of the ISE1 (INCREASED SIZE EXCLUSION LIMIT of PIASMODESMATA 1) gene and the localization of its gene product to the cell periphery. The results support that ISE1 is an important regulator of PD critical to embryonic development in Arabidopsis.
We also studied the spatial and temporal limits of macromolecular transport during embryo development using various sizes of GFP expressed by endogenous promoters. These results will be presented as well.

T01-076

Regulation of egg cell identity in the female gametophyte

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Contrasting the complex sporophyte, the gametophytes of flowering plants are highly reduced and have a relatively simple structure that is well suited for studies of cell fate determination and differentiation. The female gametophyte of Arabidopsis consists of only seven cells. These cells develop from one haploid mother cell and differentiate into four different cell identities, one of which is the egg cell. We are studying mechanisms that underlie the specification of egg cell identity. To monitor egg cell identity, we made use of a marker line that confers GUS expression specifically to the egg cell. Following EMS mutagenesis M1 plants were screened for deviations in the GUS expression pattern. We have isolated three mutants that show ectopic expression of the egg cell marker in both the synergids and the central cell, indicating that the affected genes play a role in the restriction of egg cell identity. We discuss our morphological, functional and molecular data in relation to mechanisms of cell specification in the female gametophyte.

T01-077

Functional analysis of SBP box gene SPL8

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SPL8 is one of the SBP-box genes, which encode plant specific transcription factors. Although SBP-box genes are known to be present in many higher plants, little is known about their roles in plant development. SPL8 was identified for its role in anther development, such that SPL8 knockout mutant have reduced male fertility. This work is aimed to get a deep insight into the functional mechanism of SPL8. Dominant-negative mutants were created to identify important residues within DNA binding domain. Yeast two hybrid was used to find out interactors of SPL8. What's more, SPL8 is significantly upregulated by gibberellic acid according to microarry data, the involvement of GA in the proper function of SPL8 is to be delved.

T01-078

Investigating the role of GABA in pollen tube growth and guidance in Arabidopsis

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The POP2 gene in Arabidopsis encodes a transaminase that modulates ?-amino butyric acid (GABA) levels. GABA is elevated up to 100 fold in pop2 flowers, resulting in pollen tube growth defects in the septum and misguided pollen tubes during the final stages of growth. In wild type pistils, a concentration gradient of GABA exists from the stigma to the micropyle—the final target of the pollen tube; pop2 pistils maintain a gradient only from the septum to the micropyle, however at much higher overall levels. in vitro, high GABA impairs pollen tube growth, while lower levels are stimulatory. Consequently, elevated GABA levels in pop2 pistils reduce growth of pop2 pollen. Based on these results, we hypothesize that because pop2 pollen tubes are unable to turn GABA over, they cannot sense a GABA gradient and hence cannot guide to their targets. In order to identify additional components of a GABA growth and guidance pathway, we performed a screen for suppressors of the fertility defect. Suppressors have been identified that have restored pollen tube growth, but a seed set less than wild type, indicating that the guidance defect still exists. Suppressors have GABA levels between that of wild type and pop2. We are currently mapping several suppressor lines. Additionally, we are generating plants deficient in GABA by 1) overexpression of POP2 and 2) knockouts of the glutamate decarboxylase genes that synthesize GABA. Since single mutants do not show fertility defects, combinations of multiple glutamate decarboxylase mutants are being generated.

T01-079

Regulation of flowering time by gibberellins

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In Arabidopsis, reduction in the level of active gibberellin (GA) delays flowering in long days and prevents flowering in short days. The failure of gibberellin-deficient ga1-3 mutants to flower under short-day conditions is caused by a failure to upregulate the LEAFY promoter activity. We have shown that GA4 is the active gibberellin responsible for LEAFY regulation and that floral initiation under non-inductive short day conditions is preceded by a dramatic increase in the shoot apical content of GA4 and sucrose. We have also in detail analyzed the regulation of LEAFY by GA4. RT-PCR analysis reveals that GA4 addition up-regulates LEAFY transcription very rapidly and that the activation occurs in the presence of the translational inhibitor cycloheximide, suggesting that the GA regulation of LEAFY transcription does not require translation. Surprisingly, the translational inhibitors Cycloheximide, Anisomycin, Emetine and Verrucarin A alone were able to superinduce LEAFY expression. This effect is, at least partly, dependent on the activity of the proteasome, indicating that LEAFY is under control of a labile repressor. Furthermore, among many tested genes involved in flowering time regulation, this effect appears to be specific to LEAFY. Finally, we have also characterized the genetic interactions among various late flowering mutants and plants with a constitutive GA response. This analysis has revealed new interactions between the GA pathway and the long-day and autonomous pathways controlling flowering time.

T01-080

Identification of enhancers of elf3-7 through activation tagging

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The control of floral initiation in Arabidopsis thaliana is regulated in part by photoperiod, and a number of components in this pathway have been identified through mutagenesis screens, including EARLY FLOWERING 3. ELF3 is necessary for proper photoperiodic control of flowering and photomorphogenesis, and may gate light input to the circadian oscillator. In order to identify new components of the photoperiodic floral induction pathway, we have performed an activation tagging screen in the elf3-7 mutant background. While elf3-1 null alleles exhibit photoperiod-insensitive early flowering and increased hypocotyl elongation, elf3-7 alleles exhibit early flowering and increased hypocotyl elongation, but maintain photoperiod sensitivity. We have created a large screening population by Agrobacterium-mediated transformation of elf3-7 mutant plants, and have screened for T-DNA transformants that show earlier flowering in non-inductive short day conditions, or enhancement of the elf3-7 phenotype. Thus far, we have identified 18 transgenic lines with alterations in flowering time, and our preliminary results suggest that these phenotypes are heritable. In the future, we will further characterize these mutant lines and identify genes whose increased expression results in the phenotypes we observe.

T01-081

Characterization and mapping of photoperiod-sensitive suppressors of elf3-1

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Flowering time in Arabidopsis is coordinated with daylength in order to optimize growth and reproduction. Although many genes involved in the photoperiodic control of flowering have been identified and characterized, much remains unknown about this pathway, and it is unlikely that all components have been identified. In order to identify additional genes that regulate flowering, we have performed a screen for suppressors of the elf3-1 mutation, which causes photoperiod insensitive early flowering and elongated hypocotyls. These suppressors delay flowering of elf3-1 and restore photoperiod sensitivity; thus, they were designated photoperiod-sensitive suppressors of elf3-1. We have determined the phenotypes caused by several pse mutations in an otherwise wild-type background, and have identified two pse mutants with weak single mutant phenotypes, pse7 and pse21, which we are continuing to characterize. Using mapping, sequence analysis, and a complementation test, we have found pse13 to be allelic to LUMINIDEPENDENS, supporting the integration of signals from the photoperiodic and autonomous pathways. Current models can explain how the pse13 mutation could correct the early-flowering phenotype of elf3-1, but not how pse13 could correct the lack of photoperiod sensitivity in elf3-1 plants.

T01-082

EMB Genes of Arabidopsis with Unknown Cellular Functions

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Many genes of Arabidopsis thaliana are annotated to encode proteins with unknown functions. Determining what functions these proteins perform is a long-term objective of genomics efforts worldwide. We describe here a collection of Arabidopsis genes with unknown functions required for normal embryo development. These genes represent a valuable subset of the Arabidopsis unknowns because they are known to be essential. Included in this collection are proteins with defined motifs but uncertain cellular functions and proteins with uncertain functions based on marginal BLASTP matches. From an initial collection of 56 candidate unknowns with a knockout seed phenotype (www.seedgenes.org), we have confirmed 30 gene identities through the recovery of duplicate alleles derived from a combination of forward and reverse genetics. Another four genes have been confirmed through molecular complementation. Approximately half of the confirmed genes have no paralogs in Arabidopsis and most do not appear to have counterparts outside of plants. RT-PCR analysis confirmed that gene expression is for the most part not embryo-specific, consistent with general functions throughout the life cycle. We conclude that EMB genes represent a valuable resource for identifying novel proteins associated with important plant processes.

Funded by the NSF 2010 Program and the S.R. Noble Foundation.

T01-083

Identification of Genes Required for Embryo Development in Arabidopsis

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A long-term goal of Arabidopsis research is to define the minimal gene set needed to produce a viable plant with a normal phenotype under diverse conditions. This will require both forward and reverse genetics along with novel strategies to characterize multigene families and redundant biochemical pathways. Here we describe an initial dataset of 250 EMB genes required for normal embryo development in Arabidopsis. This represents the first large-scale dataset of essential genes in a flowering plant. Analysis of these genes has been the primary objective of our NSF 2010 Project (www.seedgenes.org). When compared with 550 genes with other knockout phenotypes, EMB genes are enriched for basal cellular functions, deficient in transcription factors and signaling components, have fewer paralogs, and are more likely to have counterparts among essential genes of yeast and worm. EMB genes also represent a valuable source of plant-specific proteins with unknown functions required for growth and development. Many of the estimated 500-1000 EMB genes in Arabidopsis have nevertheless escaped detection to date. Based on sequence comparison with essential genes in other model eukaryotes, we have identified 244 candidate EMB genes without paralogs that represent promising targets for reverse genetics. Salk lines containing insertions within these genes are currently being screened for seed defects. These efforts should facilitate the recovery of additional genes required for embryo development in Arabidopsis.

Funded by the NSF 2010 Program and the S.R. Noble Foundation.

T01-084

Disruption of abh1, the Arabidopsis mRNA cap binding protein, causes early flower development by affecting the transcript abundance of photoperiod and vernalization pathway regulators.

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ABH1 encodes the large subunit of a dimeric Arabidopsis mRNA cap binding complex (CBP80) and its mutation in the Col ecotype causes ABA-hypersensitive regulation of seed germination, stomatal closing and cytosolic calcium increases in guard cells (Hugouvieux et al., 2001, Cell 106, 477-487). Abh1 disruption in the C24 ecotype also results in ABA hypersensitive seed germination and stomatal closure. Moreover, abh1 plants in the C24 ecotype exhibit an early flowering phenotype under long day and short day growth conditions. Both mutant and wildtype plants respond to stepwise prolonged cold treatment by gradually reducing rosette leaf numbers to an equal quantity at the time of flowering. A semi quantitative RT-PCR approach on RNA isolated from untreated and cold treated plants identified changes in the transcript abundance of key regulators of flowering time in the photoperiod and vernalization pathways. Intron specific RT-PCR analyses revealed no significant influence of abh1 on pre mRNA maturation processes of flowering-associated MADS box transcription factors. A model for abh1 effect on flowering time will be presented, in which modulation of transcript levels of positive and negative regulators affect the flowering promotion network.

T01-085

Patterns of Gene Expression during Arabidopsis Flower Development

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We are using DNA microarrays to identify genes that are expressed only at certain stages during flower development or specifically in certain parts of the flower. These spatially and/or temporally regulated genes may play important roles in the regulatory processes that pattern the flower or in the differentiation of the various floral cell types. We have initiated our study by comparing the gene expression profiles of wild-type flowers with those of mutants that show homeotic transformations. In these homeotic mutants, certain types of floral organs are absent or are replaced by other types of organs. By combining the data sets obtained from these experiments, we were able to identify a large number of genes that are specifically expressed or are strongly enriched in one of the four different types of floral organs.
We are also trying to identify the target genes of several of the many transcription factors that have been implicated in flower development. To this end, we have generated inducible systems that allow us to do time-course experiments and to observe changes in gene expression that occur shortly after the activation of the factors as well as later changes that are presumably downstream of the primary events.

T01-086

Novel developmental mutants of Arabidopsis thaliana

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A number of interesting developmental mutants of Arabidopsis thaliana (Ler) were isolated following mutagenization with ethylmethane sulphonate. These mutants were initially screened on the basis of resistance to spermine, NAA or BA, but many of these exhibited no resistance in next generations. The phenotypes of these mutants included a number of developmental abnormalities affecting all growth stages from seed germination to seed formation, such as aberrant seed development, altered seed shape, transparent testa, vivipary, affected root and/or hypocotyls gravitropism, absence or abundance of root hair, short root hair, long hypocotyls, 1-3 cotyledons, dwarf or semi-dwarf stature, spirally-twisted growth of whole shoot, variation in leaf shape/size, twisting of rosette and cauline leaves, absence of trichomes, increased number of leaves, inflorescences and lateral branches, reduced apical dominance, malformed flowers, variation in the number and size of floral organs, homeotic conversions of floral organs, male or female sterility, reduced number of stigmatic hair, bifid or sunken stigma, crinkled or club-shaped to globose siliques, and pendulant or horizontal siliques. The phenotypes of these mutants are controlled by single recessive nuclear mutations. Some of the mutants are allelic to existing ones; others appear to be unique. Consequently, 12 mutant phenotype symbols have been registered with TAIR.

T01-087

Cell separation in Arabidopsis flowers and fruit

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Specialized cell types allow plants to shed entire organs—such as leaves, flowers and fruit—through a carefully orchestrated process of cell separation (abscission). We are investigating the molecular mechanisms that control cell separation in the Arabidopsis flower. As in many other higher plants, Arabidopsis flowers have pattern elements which allow distinct separation events like floral organ shedding and fruit opening to take place. Through forward and reverse genetic approaches, we have uncovered a nonlinear transcriptional network including the redundant SHATTERPROOF MADS-box genes and an atypical bHLH gene, INDEHISCENT, that controls differentiation of three fruit-specific cell types essential for Arabidopsis fruit opening and seed dispersal. Currently, we are characterizing a predicted G-protein regulator, NEVERSHED, that is required for floral organ shedding and may regulate vesicle trafficking during the cell separation process. Our studies suggest that fruit dehiscence and floral abscission are independently regulated during flower development and provide the basis for future studies exploring the pathways that control cell separation in plants.

T01-088

A mutation in the TILTED1 locus uncovers the interplay of cell division and patterning during embryogenesis in Arabidopsis

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Patterning and morphogenesis require the coordination of cell division rates and orientations with the developmental signals that specify cell fate. Both processes are intertwined and there has been a long debate about how they interact, particularly about how the length of the cell cycle affects patterning and morphogenesis. A number of experiments have addressed this issue in plants, but only during post-embryonic development, and with conflicting results (Beemster et al., Trends in Plant Sci. 8: 154-158, 2003).
In Arabidopsis, embryonic patterning, including the placement of the future shoot- and root-poles, takes place during the pre-globular and globular stages. We have analyzed a mutant in the catalytic subunit of the replicative DNA polymerase epsilon, tilted1 (til1), that affects both cell cycle length and the proper positioning of the root pole. The cells in embryos homozygous for a partial loss of function allele divide slower than those of their wild type siblings, yet the embryos are larger than wild type embryos. The embryos are particularly delayed in their passage through the globular stages. In spite of this, morphogenesis and patterning are normal, except at the basal end. At the root pole, the hypophyseal cell divides inappropriately, resulting in an asymmetrically positioned lens-shaped cell. The expression patterns of marker genes reflect this asymmetry. The lens cell then organizes the hypophyseal derivatives into a relatively normal-looking root pole, which is displaced from its wild type position on top of the suspensor. Mature mutant embryos are undistinguishable from wild type ones, although the number of cells that will give rise to the hypocotyl and the root meristem are reduced. Putative null alleles of til1 are lethal, arresting at the mid-globular stage.
Our observations on til1 lead to several interesting ideas: 1) Except for the root pole, patterning follows cell division. 2) At the root pole, slowing down cell division affects the reception of a developmental signal (maybe from the maternal tissue) and results in inappropriate divisions. 3) The globular stages seem to be a “checkpoint” for embryo development, and until patterning is not properly set up, the embryo does not proceed to the following stages. 4) The lens-shaped cell (future quiescent center, QC) functions as an organizing center of the embryonic root pole, the same role the QC plays post-embryonically.

T01-089

LOV1 is a floral repressor that negatively regulates CO in Arabidopsis

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We isolated a lov1-1D (LOng Vegetative phase 1-1D) mutant that showed late flowering phenotype in Arabidopsis from activation tagging screening (Weigel et al., Plant Physiology 122:1003 [2000]). Late flowering of lov1-1D is mainly contributed by prolonged all growth phases. In lov1-1D, a T-DNA was inserted adjacent to a gene that encodes a NAC domain protein that is homologous to petunia NAM (No Apical Meristem) (Souer et al., Cell 85:159 [1996]). RNA blot analysis showed that 35S enhancers in SKI015 increased transcription level of the NAC domain gene. Furthermore, overexpression of its cDNA recapitulated the original late flowering phenotype, confirming that the gene is responsible for the late flowering phenotype. LOV1 was expressed in early embryogenesis and in the vegetative tissues including shoot apex later on. Because lov1-1D showed delayed flowering and LOV1 expression was controlled in a circadian rhythmic manner, we examined expression levels of flowering time genes within photoperiod pathway. Semiquantative RT-PCR showed that LHY (Late Elongated Hypocotyl) and CCA1 (Circadian Clock-Associated 1) were not affected, but expression of CO (CONSTANS) was downregulated in lov1-1D. Furthermore FT (Flowering locus T) and SOC1 (Suppressor of CO overexpression 1), the two floral integrators downstream of CO, were also downregulated. Constitutive expression of CO, FT, and SOC1 completely suppressed the late flowering of lov1-1D, suggesting that LOV1 is a floral repressor that negatively regulates CO in photoperiod pathway. The role of LOV1 in determining flowering time will be further discussed.

Weigel et al., Plant Physiology 122:1003 [2000]
Souer et al., Cell 85:159 [1996]

T01-090

Annual plant for a perennial problem

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The life cycle of annual plants is completed in one growing season and perenniation is achieved through the seed. Perennial plants, not only set seed, but produce structures (buds) that lie dormant during adverse conditions and resume growth the following season. For both seeds and buds the timing of growth is critical; new shoots must appear during conditions that are environmentally acceptable. There are striking similarities between germination in seeds and bud break in perennial plants including for example chilling requirements and the effects of plant growth regulators, including ABA and gibberellins. Our hypothesis is that the genes that regulate germination in seeds are the same as those that regulate bud break.
We have shown the amino acid proline accumulates in breaking kiwifruit buds prior to leaf emergence (1). Proline has been shown to accumulate in germinating Arabidopsis seeds and that added proline reduces or slows germination. Preliminary results indicate that the expression patterns of most of the genes in the proline biosynthetic pathway are similar in breaking kiwifruit buds and germinating Arabidopsis seeds. There is a shift from the pentose-phosphate pathway (PPP) to glycolysis during seed germination and bud break concurrent with the transition from heterotrophic to autotropic growth. We are investigating the potential for Arabidopsis seed germination to be used as a model for bud break in perennial plants.

(1) Walton et al (1998). Physiologia Plantarum 102: 171-178.

T01-091

Functional analysis of a phosphatidic acid in ABA signaling during germination

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The hormone abscisic acid (ABA) regulates developmental processes and stress responses in plants. In this study we analyzed a role of a phosphatidic acid (PA) in ABA signal transduction during seed germination. A physiological analysis showed PA triggers early signal transduction events that lead to the ABA responses during seed germination.
To examine the possible function of PA during germination, we measured PA production, and found that PA increased. Phosphatidic acid phosphatase (PAP) is an enzyme that catalyzes PA to diacylglycerol. We analyzed a role of PAP in PA signaling during germination. There are four genes for Arabidopsis genome. To identify functional PAP genes during germination process, we analyzed expression of the four PAP genes and phenotypes of their knockout mutants. The PAP-knockout plant revealed a hypersensitive phenotype to ABA and accumulated PA during germination. These results suggest that PAP is involved in ABA signaling during seed germination.

T01-092

Analysis of sepal and petal development using fl51 mutant of Arabidopsis

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Arabidopsis flowers are composed of four types of floral organs, four sepals, four petals, six stamens, and two fused carpels. Each type of organ forms in a concentric whorl. It is well known that the organ identity is established in concentric pattern by floral homeotic genes. On the other hand, floral organ position is defined in each whorl in relation to a putative axis in the floral meristem, indicating regulatory mechanisms controlling the position of floral organ within each whorl. For proper development of floral organs, primordia formation and growth such as differentiation and proliferation of cells are to be strictly controlled. However, these developmental processes are not well understood. To identify the genes and mechanisms controlling primordia formation and growth, we are analyzing fl51 mutant showing defects in sepal and petal development.

In fl51, four sepals and petals are narrower and longer than those of wild type, though their identities are normal. Sepals are sometimes fused along their edges towards the base. Lateral sepal primordia are smaller than those of wild type, and their position shifted toward either the abaxial or adaxial sepal primordium. By positional cloning, we found that FL51 gene encoded a protein that was a component of the spliceosome. This suggests that FL51 protein is required for mRNA splicing of genes involved in the formation and growth of primordia of sepals and petals. An RT-PCR assay revealed that FL51 gene was expressed in almost all tissue, though the abnormalities in fl51 are confined to sepals and petals. In fl51, a nucleotide change occurred at the splice donor site, resulting in miss splicing. From database search, in addition to FL51 gene, one FL51-related gene was found in Arabidopsis.

We are examining the spatial and temporal expression patterns of FL51 gene in inflorescences by mRNA in situ hybridization. We are also analyzing the phenotype of T-DNA insertion lines of FL51 to investigate differences in the effect to floral organ development between severe alleles with weak ones of fl51 mutants. In addition, we investigate the relationship between FL51 gene and other floral genes by using double mutants. The phenotype of fl51, expression and function of FL51 will be presented.

T01-093

SHI family genes redundantly regulate gynoecium and leaf development in Arabidopsis

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The SHI gene family comprises nine expressed members in Arabidopsis, STY1, STY2, SHI, LRP1 and SRS3 to SRS7, and one putative pseudogene, SRS8 (Fridborg et al. 2001; Kuusk et al 2002). These genes are highly divergent in sequence, except for in two conserved regions; one encoding a RING finger-like zinc finger domain and the other encoding a domain of unknown function. At least six of the SHI-related genes redundantly regulate the development of gynoecia, stamens and leaves. In sty1-1 mutants, the gynoecia form aberrant apical tissues and exhibit distorted vascular patterning (Kuusk et al 2002) whereas mutations in STY2, SHI, SRS3, SRS4, SRS5 and LRP1 have no apparent effect on gynoecium development. The sty1-1 gynoecia phenotype is, however, enhanced in the sty2-1, shi-3, srs4-2, srs5-1 and lrp1 mutant backgrounds, and triple, quadruple and pentuple mutants show that the consecutive knockout of SHI-related genes correlates with increases in gynoecium abnormalities. In sty2-1 mutants, the leaves are more serrated compared to the leaves of wild type and other SHI family mutants studied. Quadruple and pentuple mutants reveal that several of the SHI-related genes redundantly affect leaf morphogenesis. In accordance with the gynoecium and leaf phenotypes, these genes are active in developing gynoecia and young leaves, but exhibit distinct temporal, and/or spatial, expression patterns. The genes are also expressed in other organs such as lateral root primordia and root tips. Lateral root formation in 35S::STY1 plants is dramatically reduced and becomes restored to that of wild type by exogenous auxin application. One interpretation could be that the level or distribution of auxin, and not the auxin sensitivity or perception, in 35S::STY1 roots are suboptimal. Moreover, sty1-1 sty2-1 mutants are hypersensitive to reductions in polar auxin transport (PAT) in the gynoecia and STY1 activates at least two auxin inducible genes. These data suggest that SHI family genes affect auxin regulated processes.

Fridborg et al. (2001) Plant Physiol. 127, 937-948
Kuusk et al. (2002) Development 129, 4707-4717

T01-094

Mutations in the Arabidopsis FLAKY POLLEN gene cause both sporophytic and gametophytic male sterility

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A recessive male-sterile mutant of Arabidopsis was isolated from a T-DNA-mutagenized population and is designated as flaky pollen (flk), since the pollen grains lack the pollen coat, resulting in a defect of the pollen recognition by the stigma. Under a high humidity condition, however, the pollen grains can germinate and elongate pollen tubes into the stigmatic papillae, suggesting the pollen grains are viable. The FLK gene encodes the HMG-CoA synthase that is a single-copy gene in Arabidopsis. From Northern and RT-PCR analyses, the gene is expressed at high levels in flower buds and roots, and weakly expressed throughout the body. In anthers in the flower buds, strong expression in the tapetum and relatively weak expression in the microspores are observed by a promoter-GUS experiment and an in situ hybridization. From a biochemical analysis, the flk pollen grains lack the sterols, the major components of pollen coats. It is consistent with that the HMG-CoA synthase is an essential enzyme in the mevalonate pathway required for the sterol biosynthesis. In the flk tapetal cells, development of elaioplasts are not observed. The elaioplasts present in the wild-type tapetum accumulate granules designated plastoglobuli, that are mainly made from sterols. The remnants of plastoglobuli are thought to be deposited on the surface of maturing pollen grains after the tapetal cells are broken down. These results indicate that the FLK gene is essential in tapetal cells for the biosynthesis of sterols which then change into pollen coats.

In contrast with the above-described sporophytic defects of primarily isolated flk alleles (flk-1 and flk-3) which have T-DNA insertions in the FLK promoter region, recently identified null alleles of flk mutants (flk-4 and flk-5) show a gametophytic male sterility, whereas no defects in the function of female gametophyte. These observations suggest that the requirement of FLK gene (i.e. the requirement of mevalonate pathway) is variable depending on the cell types.

T01-095

Identification and Characterisation of Genes that Control Petal

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The role of homeotic genes in determining floral organ identity is relatively well understood. Conversely; little is understood concerning the downstream events that lead to floral organ development and senescence. A small number of genes involved in petal development or senescence have been cloned, however, there is little information concerning their function. We are interested in improving the understanding of the molecular basis of petal senescence. We are using the combination of two model species: the rose, as an applied ornamental model species and Arabidopsis thaliana. We are currently using three approaches to search for senescence associated genes (SAG's). (1) A small scale targeted transcriptional survey of rose petals at different developmental stages. (2) A target search for genes that are differentially expressed in senescing and non-senescing rose petals. A number of genes have been identified that specifically accumulate at the onset of petal senescence. Arabidopsis thaliana is currently being employed to determine functionality of some of these selected genes. (3) Forward genetic approach to isolate novel petal senescent mutants. Mutagenised senescent marker line, SAG-GFP, are being screened for mutants that display altered fluorescence pattern. Several mutants have been isolated that show attenuated, early or late fluorescence pattern when compared to the parental marker line.

T01-096

The transcript profile of cytoplasmic male sterile Brassica napus

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Floral organ development is influenced by nuclear-mitochondrial interactions. This is demonstrated by cytoplasmic male sterility (CMS), a maternally inherited trait manifested as inhibited pollen production. In addition, homeotic-like conversions of the anthers into carpel-like structures with ovules and stigmas are often observed in CMS-plants.

CMS-lines derived from B. napus (+) A. thaliana somatic hybrids, which have been produced in our laboratory, display the aberrations described above, a phenotype very similar to ap3/pi mutants in A. thaliana. RFLP analysis has shown that the nuclear and plastid genome consist of B. napus DNA, while mitochondria contained rearranged DNA from both A. thaliana and B. napus with frequent rearrangements.

The transcriptome of the CMS-line was compared to fertile B. napus on cDNA microarrays. The results from these investigation showed that 90 genes displayed a different expression in the CMS-line in comparison to B. napus. Several of these genes are involved in stamen and pollen formation. They displayed a lower expression in the CMS-line compared to fertile B. napus. The opposite is true for genes involved in gynoecia formation. In accordance with the phenotype the AP3 and PI expression is reduced in the CMS-line.

Two mitochondrial genes (orf139 and atp9) have a much higher expression in the CMS-line compared to B. napus. Furthermore, two mitochondrial processing-peptidases displayed a lower expression. These enzymes are nuclearly encoded and they also function as the Core 1 and 2 proteins of the cytochrome bc1 complex.

Several pectinesterase and polygalacturonase genes displayed a lower expression in the CMS-line in comparison to B. napus. Some of these genes are pollen specific according to previous studies.

This result indicates a link between the mitochondria and the nuclear encoded genes, e.g. the two B-genes, since the CMS-phenotype is due to aberrations in nuclear-mitochondrial interactions. In the poster we will discuss this further.

T01-097

The Arabidopsis formin AtFH5 is a potential effector of Polycomb group activity in endosperm polarity

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The Polycomb group (Pc-G) proteins are widely conserved transcriptional repressors. They act as a modular complex that maintains expression patterns epigenetically through chromatin remodeling. In Arabidopsis, mutations in any of the three fertilization independent seed (FIS) Pc-G members result in an aberrant endosperm development: over-proliferation of the endosperm nuclei, enlargement of cysts in the posterior pole and an absence of the developmental transition from syncytial to cellularized endosperm.

We have previously reported the characterization of an enhancer trap line, KS117, whose GFP expression in the posterior pole is disrupted in a fis background. The T-DNA responsible for KS117 expression was localized to the formin coding gene AtFH5. Formins are actin nucleating agents whose conserved function in cytokinesis and cell polarity makes them likely candidates as targets of FIS pathways. in situ hybridization in the wild-type seed revealed that AtFH5 expression is limited to the cyst and nodules of the posterior endosperm. To test the biochemical function of the AtFH5 gene product, actin assembly was characterized using a combination of fluorescence spectroscopy and light microscopy. Purified recombinant AtFH5 was able to nucleate and cap actin filaments in vitro. Finally, an AtFH5 insertion line was identified that truncates the AtFH5 transcript within the conserved Formin Homology 2 domain. These mutant atfh5 plants delay endosperm cellularization. In addition, over 20% of atfh5 seeds lack posterior cyst structures, suggesting a role for AtFH5 in nuclei migration to the posterior pole. Thus, localization, activity and mutant phenotype are all consistent with a model in which FIS activity promotes endosperm polarity by targeting the Arabidopsis formin AtFH5 to the posterior pole.

T01-098

Establishment of fruit patterning in Arabidopsis

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Determining the mechanisms that establish shape and identity in organs has long been a goal for developmental biology. In plants, while many gains have been made uncovering the genetic pathways that specify organ identity, little is known about the downstream processes that actually regulate morphology and cell type. Work focusing on the development of the Arabidopsis fruit, however, has begun to elucidate some of these processes. The fruit is composed of three domains, the valves, or seed pod walls, the replum which develops in between the two valves, and the valve margin which develops at the valve/replum border. Seed dispersal is promoted by the valve margin, which undergoes a process of cell-cell separation that facilitates the detachment of the valves from the replum. Valve margin formation is dependent on the activation of the valve margin identity genes, SHATTERPROOF1,2, ALCATRAZ and INDEHISCENT. In addition, the restricted activation of these identity genes to the valve margin is controlled by the repressive activities of FRUITFULL in the valves and REPLUMLESS in the replum. (See poster by Roeder et al.) While much work has been done defining the regulatory network that controls the definition of the valve margin, very little is known about the mechanisms which establish this network. We will present work that uncovers new layers of regulation controlling the development of the fruit which unites genetic pathways that control lateral organ shape and polarity with those that control valve margin identity.

T01-099

Graft transmission of floral signalling in Arabidopsis is dependent on long-distance action of genes in the photoperiod pathway

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Photoperiodic regulation of flowering requires light perception in leaves, followed by transmission of mobile 'florigen' signals from leaf to shoot apex. However, no universal florigens have been discovered, and the genetics of florigen signalling is largely unknown except in pea where several mutations are associated with graft-transmissible positive or negative effects on flowering. Using Arabidopsis micrografting (1), we demonstrate that certain flowering time mutants can be rescued by long-distance signalling. Experiments with [14C]sucrose showed that two-shoot 'Y-grafted' wild-type plants had effective phloem continuity. If one of the grafted shoots was held in long days (LD), it accelerated flowering of the second shoot which received only short days, indicating that the LD signal is probably a positive regulator. We then grafted wild-type plants to late-flowering, photoperiod-insensitive gi-2, co-2 or ft-7 mutants held under LD. Flowering times of gi-2 and co-2 were dramatically accelerated relative to ungrafted controls, but a much smaller effect was seen with ft-7. We conclude that native GI, CO and probably FT genes can act in the leaf upstream of florigen signal generation. This is consistent with recently published work showing that phloem-specific over-expression of CO or FT was sufficient to rescue flowering time in corresponding mutant backgrounds (2). The partial rescue of grafted ft-7 shoots may be explained by the possible requirement of FT to be expressed in apex and leaf. Finally we discuss analytical approaches to discovering the nature of the signal(s) regulated by CO and/or its downstream target genes.

(1) Turnbull et al. (2002) Plant Journal 32, 255-262. (2) An et al. (2004) Development in press.

T01-100

The Genetic and Molecular Network of SOC1 for Flowering in Arabidopsis

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Flowering is regulated by integrated network of several genetic pathways in Arabidopsis. The key genes integrating multiple flowering pathways are FT, SOC1 and LFY. To elucidate the interactions among them, genetic analyses were performed using both loss-of-function mutants and gain-of-function transgenics of the three integrators. Double mutant analysis showed that SOC1 acts partially independently of FT for determination of flowering time and acts in parallel with LFY for floral initiation, suggesting the three integrators have both overlapping and independent functions. Furthermore, the expression analysis showed that FT regulates the SOC1 expression, and SOC1 regulates the LFY expression but not vice versa, which is consistent with the fact that FT and LFY have the least overlapping functions among the three integrators. The two integrators FT and SOC1 share a common upstream negative regulator FLC, a flowering repressor integrating vernalization and autonomous pathways. The flowering of ft soc1 is further delayed by an increase of FLC expression, showing additional targets are regulated by FLC. In addition, vernalization caused acceleration of flowering in the flc ft soc1 triple mutant, suggesting that the vernalization pathway also has targets other than FLC, FT, and SOC1. Finally, the triple mutant of ft soc1 lfy failed to produce flowers and the triple overexpression of FT, SOC1, LFY caused flowering right after germination with only two cauline leaves, which is very similar to the phenotype of the embryonic flower mutant. This result suggests that the integrative function of FT, SOC1, LFY are necessary and sufficient for flowering.

T01-101

Interaction of Polycomb-group proteins controlling flowering in Arabidopsis

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The Drosophila Polycomb-group genes Suppressor of zeste12 (Su[z]12) and Enhancer of zeste (E[z]) genes encode two components of a protein complex involved in histone methylation. In Arabidopsis, each is represented by a small gene family. The Su(z)12 family has three members, FERTILISATION INDEPENDENT SEED2 (FIS2), EMBRYONIC FLOWER2 (EMF2), and VERNALISATION2 (VRN2). The three genes have distinct developmental roles in seed development, flowering time control and vernalization response, respectively. The E(z) family also has three members: MEDEA (MEA) which has a similar function to FIS2; CURLY LEAF (CLF) which, like EMF2, represses flowering; and a poorly characterised third member, SWINGER (SWN) (also known as EZA1). We show that these similarities reflect interactions between the plant E(z) and Su(z)12 class proteins. The interactions are mediated by two novel domains that are conserved between the plant and animal proteins. Yeast two-hybrid studies also show that the CLF and SWN proteins can interact with VRN2, suggesting that they may also mediate the vernalization response. Characterisation of SWN reveals that it acts redundantly with CLF, and therefore that CLF has a broader developmental function than was evident from analysis of single mutant phenotypes. Mis-expression studies indicate that MEA has diverged from CLF and SWN both in expression pattern and protein function. We suggest that the plant Pc-G proteins form at least three complexes with discrete developmental roles. These complexes are likely similar to the animal PRC2 with respect to composition and biochemical function, but have diverged with respect to target gene specificity.

T01-102

Isolation of novel mutants defective in pollen tube growth

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We have generated more than 2000 T-DNA transformed Arabidopsis lines which carry a Basta resistance cassette and a GUS-GFP marker driven by the pollen specific LAT52 promoter. In a first round we have screened the primary transformants for abnormal segregation to identify mutations in genes that lead to gametophytic deficiencies. In a second round we have used the expression of the GUS-GFP protein in mutant pollen to select tagged lines that show specific defects in pollen growth.

Using this screen we have so far isolated two lines that show interesting defects in pollen growth. Line A segregates at a ratio of approximately 1:1.5 and pollen can still grow, albeit to a decreased length, and they can lead to fertilisation in relatively few cases. We have identified a T-DNA insert in a gene encoding a subunit of the 26S proteasome.

A second line (Line B) segregates at a ratio of 1:1 indicating that no fertilisation occurs by mutant pollen. The pollen tubes stained for GUS do not penetrate the style in vivo and show very poor growth in vitro. A T-DNA insertion was identified in the GAD2 gene which encodes a protein involved in the g-amino butyric acid (GABA) biosynthesis pathway. In vitro experiments show that tube growth can not be rescued by the exogenous addition of GABA.

T01-103

Components of the Arabidopsis autonomous floral promotion pathway, FCA and FY, are conserved in grasses

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Flowering time is an important trait agriculturally. To investigate if the same genetic pathways that control the flowering time of the model dicot Arabidopsis are also present in monocots, two components of the Arabidopsis autonomous floral promotion pathway, FCA and FY, were isolated from rice (Oryza sativa) and ryegrass (Lolium perenne). The predicted FCA proteins are highly conserved over the RNA-binding and WW protein interaction domains. In Arabidopsis, FCA limits its own production by promoting the polyadenylation of FCA pre-mRNA within intron 3 to form a truncated transcript called FCA-b. FCA-b transcripts were found in rice and ryegrass. A comparison of Arabidopsis, rice and ryegrass intron 3 sequences, as well as ESTs representing FCA-b transcripts from a range of plants, revealed the presence of conserved sequence that may be required for FCA autoregulation. FCA's autoregulation and flowering time functions require FCA to interacts with the 3' end-processing factor, FY. FY was identified from rice and ryegrass and encodes proteins with highly conserved WD repeats and a less well-conserved C-terminal region containing Pro-Pro-Leu-Pro (PPLP) motifs. The FCA WW domain, which is thought to recognise PPLP motifs, interacted with ryegrass FY protein in GST-pulldown assays. These experiments suggest that the FCA and FY genes from monocots may have similar functions to the dicot flowering-time genes.

T02 Development 2 (Shoot, Root)

T02-001

Root Hair Tip Growth Requires the Arabidopsis COW1 Gene which Encodes a Phosphatidyl Inositol Transfer Protein

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Root hairs present an important model system for development studies in higher plants, since root hairs are a major site for the uptake of water and nutrients into plants, and their tip growth is a major requirement for growing. The cow1 mutant in Arabidopsis thaliana is impaired in root hair tip growth. The N-terminus of the COW1 protein is 32% identical to an essential phosphatidylinositol transfer protein (PITP), the yeast Sec14 protein (sec14p), while the C-terminus is 34.5% identical to a late nodulin of Lotus japonicus, Nlj16. In good agreement with the role of Nlj16 in Lotus japonicus we show that GFP fusion with the COW1 protein is targeted to the plasma membrane of root hairs.
Furthermore, the growth defect associated with Sec14p dysfunction in yeast is complemented by expression of the COW1 lipid-binding domain in our studies. PITPs play important roles in promoting the activities of various inositol lipid-signaling pathways by regulating the production of certain phosphoinositides.
We conclude that the COW1 protein is essential for proper root hair growth, that it has a PITP function, and that it is targeted to the plasma membrane. The potential role of COW1 in PLC signaling required for the tip Ca2+ gradient will be discussed.

T02-002

Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root

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In an attempt to isolate novel factors that modulate quantitative aspects of root development and are responsible for intra-specific morphological variation, we exploited natural genetic variation in the model plant Arabidopsis thaliana. Quantitative trait locus analysis of a cross between isogenized accessions revealed that a single locus is responsible for approximately 80% of the variance of the observed difference in root length. We succeeded in isolating the corresponding gene, which we named BREVIS RADIX (BRX), by map-based cloning. BRX controls the extent of cell proliferation and elongation in the growth zone of the root tip and is a member of a small group of highly conserved genes. This family of BRX-like genes is only found in multicellular plants. Analyses of Arabidopsis single and double mutants suggest that BRX is the only gene of this family with a role in root development. The BRX protein is nuclear localized and activates transcription in a heterologous yeast system. BRX family proteins contain three distinct highly conserved domains that are predicted to form alpha-helical structures. Two of the domains are highly similar to each other and appear to mediate the transcriptional activation in the yeast system. The combined data indicate that BRX family proteins might represent a novel class of transcription factors. Further details on the genetics and biochemistry of this gene family will be reported.

T02-003

Arabidopsis auxin influx proteins AUX1 and LAX3: a tale of two carriers

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Auxin represents a key regulator of plant cellular and developmental processes. The coordinated movement of IAA within or between plant cells is essential to execute many developmental programmes. Plants employ specialised influx and efflux carriers to mobilise the major form of auxin, indole-3-acetic acid (IAA) from cell to cell. Molecular genetic studies in Arabidopsis thaliana have identified putative auxin influx carrier component AUX1. AUX1 is most closely related to three Arabidopsis sequences termed LAX1, LAX2 and LAX3 (Like AUX1), which share between 73% and 82% identity at the amino acid level. Phenotypic characterisation of lines carrying dSpm insertions within each LAX gene has identified auxin-related developmental defects, consistent with LAX proteins performing an auxin transport function. Combinations of aux1 and lax mutations exhibit additive auxin-related phenotypes. For example, the aux1 lax3 double mutant exhibits an additive lateral root phenotype, reducing the number of primordia by over 90%, but which can be reversed by the addition of the membrane permeable auxin, 1-NAA. AUX1 and LAX3 expression studies have provided a spatial explanation for the additive lateral root phenotype of the double mutant. AUX1 and LAX3 promoter GUS and functional protein-YFP fusions have revealed that AUX1 is expressed in developing lateral root primordia, whereas LAX3 is expressed throughout the stele with the exception of dividing pericycle cells. In summary, AUX1 and LAX3 perform distinct auxin transport related functions; LAX3 appears to facilitate the polar transport of IAA in root stele tissues, whereas AUX1 facilitates IAA uptake into newly initiated lateral root primordia.

T02-004

Length and width: Both cell proliferation and cell elongation are controlled in a polar-dependent manner in a leaf, two-dimensional and determinate organ.

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The leaf is a fundamental subunit of the shoot system; thus, the leaf is the key organ for a full understanding of shoot morphogenesis. In a leaf, number of leaf cells is not necessarily reflected on leaf shape (Tsukaya, 2003: Curr. Opin. Plant Biol. 6: 57). Genetic analyses of leaf development in arabidopsis shows that a compensatory mechanism(s) act in leaf morphogenesis and an increase of cell volume might be triggered by a decrease in cell number. Thus, leaf size is, at least to some extent, uncoupled from the size and number of cells by the compensatory mechanism(s) (Tsukaya, 2003). However, shape of plant organs has been thought to be fundamentally regulated by polar cell elongation. In fact, focusing on mechanisms that govern polarized growth of leaves in arabidopsis, we have showed that two genes act on the processes of polar cell elongation in leaves: the AN gene, a member of CtBP gene family (Kim et al., 2002: EMBO J. 21: 1267), regulates width of leaf cells and the ROT3 gene, a member of cytochrome P450 family (Kim et al., 1998: Genes & Dev. 12: 2381), regulates length of leaf cells.
On the other hand, in seed plants, natural diversity of leaf shape is mainly attributable for diversity of cell number along a particular axis of leaf lamina, not for diversity of cell shape. Thus, polarity-dependent control of cell proliferation must be involved in the processes of leaf-shape control. Recently, we identified novel genes for polarity-dependent regulation of cell proliferation in leaves. ROT4 gene is involved in control of cell proliferation in the leaf lamina only along the longitudinal axis (Narita et al., 2004: Plant J., in press). ROT4 encodes a peptide which localizes to the plasma membrane. On the other hand, AN3 gene, a gene encoding a co-activator, regulates leaf width via regulation of leaf cell proliferation (Horiguchi et al., this meeting) Interestingly, although the an3 mutant has defect in cell number both in the leaf-length and leaf-width direction, leaf lamina of the an3 mutant shows a specific defect in the length. This is because above-mentioned compensatory system works in the an3 leaves to increase cell volume. Taken together, both cell proliferation and cell elongation in the leaf lamina are controlled in the two-dimensional, polar-dependent manner. Based upon our above results, genetic mechanisms for two-dimensional growth of leaves will be discussed.

T02-005

Leaf Vascular Patterning Mutants

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Organized vascular patterning in the leaf is integral to leaf development and function. In the model organism Arabidopsis thaliana the leaf venation pattern is a closed, continuous reticulate system of veins and is heritable. The pattern arises as cells within the leaf sense their position relative to existing veins cells and differentiate accordingly, eventually creating the regularly spaced succession of vein size orders characteristic of the pattern. The genetic nature of this pattern makes it ideal to study how positional information is perceived and translated into a stably maintained vascular network.

My work aims to identify, clone, and characterize genes unique to vascular patterning in Arabidopsis thaliana. I have performed brute-force forward genetic screens searching for vascular pattern defects in juvenile leaves of transposon and activation mutagenized Arabidopsis thaliana lines. I have identified ~25 vascular pattern mutants from my screens and plan to characterize and positionally clone at least five mutants. Interestingly, all of the mutants I recovered have an associated change in leaf shape. The one exception is a mutant I found to contain a mutation in CVP2, a gene previously cloned and characterized in our lab. I have created mapping populations for a number of these mutants and I am map based cloning them to determine their molecular identity.

I have also positionally cloned three parallel venation mutants recovered from a chemical mutagenesis screen. I have initially characterized the defects in these three mutants and positionally cloned each of them to a region spanned by less than six BACs. These mutants have veins aligned more in a proximal/distal direction with more veins exiting the petiole than wild-type, which is reminiscent of monocot venation patterns. Two mutants, #70 and #111, have defects in root growth in addition to vascular patterning defects in the first pair of true leaves. Although the plant hormone auxin has been strongly implicated in vascular patterning, surprisingly none of the parallel venation mutants have auxin-response defects as determined by 2,4-D root elongation, polar auxin transport, or PIN1 immunolocalization studies.

T02-006

ANGUSTIFOLIA3 encodes a homolog of synovial sarcoma translocation protein and mediates local cell proliferation for lateral expansion of leaf blade in Arabidopsis thaliana

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Polar leaf expansion is dependent on not only cell expansion but also cell proliferation and plays a central role in the determination of leaf shape. Here we identified a cell-proliferation-dependent pathway that controls lateral expansion of leaf blade by using a narrow leaf mutant of arabidopsis, angusitfolia3 (an3). We compared leaf development of an3 with that of wild type in relation to cell numbers along leaf-length and -width directions, angle of cell division plane and frequency of cell division per leaf primordium. Based on these data, we divided leaf development into two phases. The narrow leaf phenotype of an3 originates from the reduced activity of cell proliferation during Phase II where lateral expansion of leaf blade and longitudinal growth of the leaf primordium concomitantly take place. The earlier phase (Phase I) is not significantly affected in an3. Noticeably, the frequency distribution of the angle of cell division plane is similar in both an3 and wild type in Phase II. Rather, the frequency of cell division per leaf primordium is smaller in an3 than in wild type. These observations suggest that AN3 would be required to maintain or promote cell proliferation rather than to control the polarity of cell proliferation. Interestingly, the reduced cell number is associated with an increased expansion of each leaf cell in an3, resulting in the partial compensation of the final leaf area.

We also cloned AN3 and found that it encodes is a homolog of a transcription coactivator, synovial sarcoma translocation protein (SYT), in human. Transgenic plants harboring an AN3 promtoer::?-glucronidase construct shows strong AN3 promoter activity in a basal portion of Phase II leaf primordia. Overexpression of AN3 stimulates cell proliferation and creates larger leaves with normal shape rather than wide leaves, supporting our proposed role of AN3. We also isolated genes for putative transcription factors, GROWTH-REGULATING FACTOR (AtGRFs), that bind to AN3 in yeast two-hybrid system. These results suggest that coordination of the dynamic changes in the direction of cell proliferation with local maintenance and/or promotion of cell proliferation by AN3 is crucial for the lateral expansion of leaf blade. Now we are investigating expression patterns and overexpression phenotypes of AtGRFs.

T02-007

A novel class of Arabidopsis response regulator genes, the ectopic expression of which results in phenocopy of the wol cytokinin-receptor deficient mutant

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Arabidopsis thaliana has a number of response regulators (ARRs) implicated in the histidine (His) to aspartate (Asp) phosphorelay signal transduction. According to the current consistent model, both the type-A and type-B ARR family members play crucial roles in the cytokinin signaling circuitry. However, this higher plant has a few extra ARRs, on which no attention has been paid so far. Characterization of these extra ARRs might provide us with new insight into the His-Asp phosphorelay signal transduction in plants. For this reason, in this study we extensively examined the natures of such a representative (named ARR22). Transcripts of ARR22 were expressed predominantly in reproductive organs, and a GFP::ARR22 fusion protein was localized in the cytoplasmic space in onion epidermal cells. The purified ARR22 protein had the ability to undergo phoshorylation in vitro, when incubated with phospho-AHP5, indicating that ARR22 has the fundamental ability to participate into a His-Asp phosphorelay pathway in its own right. In plants, transgenic lines overexpressing ARR22 were characterized (referred to as ARR22-ox), which showed the characteristic dwarf phenotypes with poorly developed root systems. The results of Northern blot hybridization with selected sets of hormone-responsive genes suggested that cytkinin responses are selectively attenuated in ARR22-ox, while other hormone responses (auxin, ABA and ethylene) occur normally. The results of microarray analyses with cytokinin-treated wild-type and ARR22-ox plants further supported the view that cytokinin responses are globally attenuated in ARR22-ox, at least, at the level of gene regulation. Finally, we demonstrated that the dwarf phenotypes of ARR22-ox appear to be phenocopies of the wol mutant, which has a sever lesion in the AHK4/CRE1 cytokinin-receptor of histidine protein kinase. These results suggested that ARR22 might also be implicated, directly or indirectly, in the cytokinin-responsive His-Asp phophorelay signal transdution.

T02-008

Localization and activity of the embryo pattern regulator BODENLOS

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Embryogenesis in plants transforms the fertilized egg cell into a multicellular organism with many distinct cell types, organized in a defined pattern. During this pattern formation, establishment of the embryonic root meristem requires regulated activity of the transcriptional activator MONOPTEROS (MP/ARF5), and its repressor BODENLOS (BDL/IAA12), both involved in auxin-dependent gene activation. Loss of MP function, or stabilization of BDL leads to the same phenotype early in embryo development. The root meristem precursor (hypophysis) does not divide properly, and no root is formed. In situ hybridization revealed that MP and BDL mRNAs are not expressed in the hypophysis, but in the adjacent proembryo cells, suggesting non-autonomous action of MP and BDL, that could involve movement of the proteins themselves, or cell-to-cell communication through a mobile downstream signal. We performed a series of experiments to probe the accumulation pattern and the spatio-temporal activity of the BDL protein. As expected, translational fusions of BDL to GUS showed the protein to be nuclear and unstable, targeted for proteasome-dependent degradation by auxin. We will present a detailed analysis of accumulation patterns of BDL:GUS in the early embryo. To determine spatial requirements for BDL activity, a dominant stabilized mutant bdl protein was expressed in restricted embryo domains using GAL4/UAS expression technology. Expression of the stabilized bdl protein within the endogenous BDL expression domain reconstitutes the bdl mutant phenotype. We will present the results of controlled expression of bdl in a variety of cell types within the early embryo.

T02-009

Shoot stem cells: not naive at all

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Stem cells renew themselves while at the same time contributing daughter cells to form differentiated tissues. A general question in stem cell biology is the extent to which the progeny of the stem cells is directed to alternative fates by signals from surrounding tissues, or whether they have an intrinsically limited range of fates.

In plants, most of the shoot originates from a small group of stem cells, which in Arabidopsis are specified by WUSCHEL (WUS). To test whether these cells have an intrinsic potential to generate shoot tissues, or whether shoot identity is promoted by signals from more mature tissues, we studied the effects of expressing WUS outside shoots. We saw that in the absence of additional cues, WUS expression in the root acted non-cell-autonomously to activate CLAVATA3 (a marker for shoot stem cell identity), followed by AINTEGUMENTA expression (a marker for early shoot organogenesis) and leaf development. This suggested that WUS-specified cells have intrinsic shoot identity. The ability to change from root to shoot identity in response to WUS, however, was limited to a subset of the cells in the primary and secondary root meristems.

In response to WUS expression, root cells could also be diverted to floral fate (in response to LEAFY) or embryo fate (in response to increased auxin), We are now interested in how WUS promotes this developmental flexibility. Ectopic floral and embryo identity in roots have been described in mutants affecting genes that encode chromatin regulators (PICKLE and in FERTILIZATION INDEPENDENT ENDOSPERM), so we are testing the interaction between WUS and these genes. We also investigating why the competence to respond to ectopic WUS is restricted to only a subset of root meristem cells.

Gross-Hardt, R. & Laux, T. (2003). J. Cell Sci. 116, 1659-1666.
Gallois, J.-L. et al. (2004). Genes Dev. 18, 375-380.

T02-010

Rice Lectin-Receptor Kinase (OsLRK) senses galactose and plays a role in root development.

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An Oslrk1 mutant was obtained in a phenotype screen of Ds insertion lines. The mutants showed increased number of adventitious roots and longer lateral roots as well as bigger leaves, increased number of panicle branches and as a result higher seed yield. Segregation analysis of Basta resistance and phenotype confirmed that mutant phenotype was caused by Ds insertion. Southern hybridization on the genomic DNA isolated from the mutant plant showed that it had a single copy of Ds. Database searches using the nucleotide sequence of Oslrk1 revealed that it is a member of a multi-gene family comprising of at least 15 homologues in rice genome. By crossing homozygous mutant lines with plants harboring transposase, Ds was mobilized and revertant with the footprint was obtained. The expression of Oslrk1 was analyzed by RT-PCR, Northern-blot hybridization and by using T-DNA lines carrying promoter of Oslrk1 fused with either beta-glucoronidase (GUS) or green fluorescent protein (GFP). The Oslrk1 is expressed intensively in roots of seedlings, less in panicles, callus, leaves and stem. A gradient of GUS staining was observed in roots with distal elongation zone stained intensively and less expression in mature zone. Cross-section of GUS-stained root revealed expression of Oslrk1 in vasculature and pericycle cells. Prediction analysis of OsLRK1 protein sequence revealed the presence of trans-membrane spanning domain and confocal microscopy on Oslrk1 Promoter-GFP transgenic plants confirmed the expression of Oslrk1 in cell and nuclear membranes. Monocot mannose/galactose specific motif (Q89-D91-N93-Y97) was found in beta-chain of lectin domain. Two cleavage sites (NDT and NGT) were located between two chains of lectin domain which might determine specificity to galactose. When mutant and wt seeds were germinated in MS containing either galactose or mannose, both showed similar response to mannose, while mutant was more sensitive to galactose. Transcript of Oslrk1 was also induced by galactose. Potential galactose-sensing role of OsLRK1 in root development is proposed.

Nichiguchi M., et. al., Mol. Genet. Genomics, 2002, 267, 506-514;

Herve C. et. al., J. Mol. Biol., 1996, 258, 778-788

T02-011

A model of Arabidopsis leaf development

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There are internal genetic differences in leaf development between species, superimposed upon this, regulation of leaf growth responds to environmental signals. The aim of this work was to investigate the regulation of leaf growth in Arabidopsis thaliana by genetic (mutation) and environmental (light) factors. Two leaf development mutants were selected from EMS-induced mutants (Berná et al., 1999). One with increased (ron2-1) and one with reduced (ang4) leaf area. Wild type (Landsberg erecta, Ler) and mutant plants were grown in rigorously controlled environmental conditions and subjected to various light treatments.
In all light conditions, ron2-1 produced leaves with significantly higher areas than the wild type while ang4 produced leaves with significantly lower areas. In all genotypes, final leaf area was reduced by reduced incident light. Finally, the internal and external regulatory factors exploited in this study produced a 16-fold difference between the largest and smallest mean final leaf 6 area. They also caused differences in all leaf growth variables such as duration of leaf expansion, relative and absolute leaf expansion rates, leaf initiation rate, leaf emergence rate and, at the cellular scale, epidermal cell size and epidermal cell number. The presence of any relationships between the leaf growth variables was investigated in this large range of growth curves and a model of leaf development was created from these relationships.
Our results indicate that conditions affecting early events of leaf development (initial relative expansion rate, cell division) have an impact on late processes such as the duration of expansion or final cell size. Such a model could help to resolve the existing controversy about the role of cell division in organ formation (Fleming, 2002) and also to identify a role for endoreduplication in leaf development.

Berná et al., 1999, Genetics, 152:729-742
Fleming, 2002, Planta, 216 :17-22

T02-012

Synergistic interaction of ERECTA-family receptor-like kinases regulate cell proliferation, patterning, and organ growth

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Growth of plant organs relies on coordinated cell proliferation followed by cell growth, but nature of cell-cell signal that specifies organ size remains elusive. The Arabidopsis receptor-like kinase (RLK) ERECTA regulates inflorescence architecture. Our previous study using a dominant-negative fragment of ERECTA revealed the presence of redundancy in the ERECTA-mediated signal transduction pathway. We found that Arabidopsis ERL1 and ERL2, two functional paralogs of ERECTA, play redundant but unique roles in a subset of the ERECTA signaling pathway and that synergistic interaction of three ERECTA-family RLKs define aerial organ size. While erl1 and erl2 mutations conferred no detectable phenotype, they enhanced erecta defects in a unique manner. Overlapping but distinct roles of ERL1 and ERL2 can be largely ascribed to their intricate expression patterns rather than their functions as receptor kinases. Loss of the entire ERECTA family genes led to striking dwarfism, reduced organ size, and abnormal flower development, including defects in petal polar expansion, carpel elongation, and anther and ovule differentiation. These defects are due to severely reduced cell proliferation. We propose that ERECTA-family RLKs act as redundant receptors that link cell proliferation to organ growth and patterning. The specific roles of ERECTA-family RLKs during epidermal cell-type specification and patterning will be discussed.

Shpak et al. (2003) Plant Cell 15: 1095-
Shpak et al. (2004) Development 131: 1491-

T02-013

The NAC gene family in Zea mays: evidence for the conservation of NAM- and CUC-like functions during SAM development in monocots

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All aerial parts of a higher plant originate from the shoot apical meristem (SAM) which is established during embryogenesis as part of the basic body plan. Genetic and molecular studies have revealed that members of the NAC gene family of plant-specific transcription factors have crucial functions in the initiation of the SAM in dicots:
both mutations in the NO APICAL MERISTEM gene (NAM) from Petunia or mutant combinations of the CUC1 and CUC2 and/or CUC3 genes (CUP-SHAPED-COTYLEDON) in Arabidopsis result in seedlings which lack a SAM and show fusion of cotyledons to a cup-shaped structure. In Antirrhinum, mutations in the homologous CUP (CUPULIFORMIS) gene have recently been shown to cause similar effects. Expression of NAM and the CUC genes is initiated early during embryogenesis and marks the region where the SAM will form between the two cotyledons. In later stages, expression becomes confined to the boundary region between the cotyledon margins and the SAM. It has been suggested that these genes may function in inhibiting cell proliferation to confer establishment of organ boundaries. Early gene activity may lead to the formation of a niche of slower dividing cells which could be essential for the formation of the SAM.
In contrast, pattern formation processes underlying meristem development in monocots are largely unknown. Unlike in dicot species, the SAM in Zea mays is established in a lateral position of the root-shoot axis at the adaxial side of the embryo at a distance from the emerging single cotyledon (scutellum). As an approach to address how meristem development is initiated in an evolutionary distant monocot species, we screened for potential NAM- and CUC-homologues in maize. Several highly related genes of the maize NAC gene family were isolated and characterized. Based on phylogenetic analysis and the study of expression patterns we present evidence for the conservation of NAM- and CUC3-like functions during SAM establishment in early maize embryogenesis. Based on our results SAM development in Zea mays will be compared to Arabidopsis.

Randolph, 1936; Souer et al., 1996; Aida et al., 1997; Takada et al.,2001; Vroemen et al., 2003; Weir et al., 2004

T02-014

The DP-E2F-like DEL1 gene is a suppressor of the endocycle in Arabidopsis thaliana

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Although endoreduplication is widespread among eukaryotes, the molecular mechanisms that regulate the endocycle remain unclear. Recently, a novel class of E2F-like proteins have been described, nominated E2F7 in mammals and DELs in Arabidopsis thaliana. While DEL proteins were demonstrated to act as negative regulators of the E2F-DP pathway, their physiological role remains to be established. Here we demonstrate that plants that overexpress the DEL1 gene are slightly smaller than wild type plants. Transgenic plants display a strong inhibition of their endocycle, whereas the mitotic cell cycle is unaffected. Similarly, DEL1 was proven to specifically inhibit the endoreduplication phenotype but not eh ectopic cell divisions triggered by the co-expression of E2Fa and DPa. The specific expression of DEL1 in mitotic dividing cells suggests that DEL1 acts as a negative regulator of the endocycle. Interestingly, although ploidy levels were severely lower in the transgenic plants, the ploidy-cell size relationship was maintained whereas simultaneously the size distribution of cells with an 2C and 4C DNA content was increased, genetically proving the existence of both ploidy-dependent and ¯independent cell growth.

T02-015

Micro-RNA targeted TCP genes are regulated at several levels

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TCPs are a plant specific transcription factor family with 24 members in Arabidopsis thaliana, sharing a TCP domain involved in DNA binding. We have previously reported that five TCP genes are regulated by miRNAs. The jaw-D mutant overexpresses miR-JAW (miR-319a), which causes degradation of this group of TCPs, leading to crinkly leaves .
We show here that KOs for TCP2, 4 and 10 have larger leaves than wild type. The TCP2/4 double KO has an even more pronounced leaf phenotype, resembling that of weak miR-JAW overexpressing lines. In addition, TCP4 mutants flower late, as does the jaw-D mutant.
We have analysed in more detail the regulation of TCP2 and TCP4. The TCP2 promoter is active in leaves and floral organs, while the TCP4 promoter is active in the vasculature of cotyledons and young leaves. Expression of the reporters is increased in jaw-D, suggesting that promoter regulation involves a miRNA-dependent feed back loop. The miRNA-regulated TCPs also contain a long 5' UTR, with several AUGs. Deletion of the 5' UTR causes both an enhancement of reporter expression and ectopic activation. Taken together these results suggest that this group of TCPs is regulated at multiple levels, including transcriptional and post-transcriptional steps.
In a second approach to understand the biological role of the TCPs, miRNA-resistant TCPs (rTCPs) were generated. Expression of rTCP4 under the control of TCP4 regulatory sequences is mostly lethal. rTCP2 plants survive, with smaller and rounder leaves, which is roughly the opposite of TCP2 KO plants. rTCP2 plants also have longer hypocotyls and reduced fertility.
The gain- and loss-of-function phenotypes of TCP2 and TCP4 highlight both common and differential activities of the miRNA-regulated TCPs.

T02-016

The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis

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Hormones are important regulators of plant growth and development. In Arabidopsis perception of the phytohormones ethylene and cytokinin is accomplished by a family of sensor histidine kinases including ethylene-resistant (ETR) 1 and cytokinin-response (CRE) 1. We identified the Arabidopsis response regulator 2 (ARR2) as a signalling component functioning downstream of ETR1 in ethylene signal transduction. Analyses of loss-of-function and ARR2-overexpressing lines as well as functional assays in protoplasts indicate an important role of ARR2 in mediating ethylene responses. Additional investigations indicate that an ETR1-initiated phosphorelay regulates the transcription factor activity of ARR2. This mechanism may create a novel signal transfer from endoplasmatic reticulum (ER)-associated ETR1 to the nucleus for the regulation of ethylene-response genes. Furthermore, global expression profiling revealed a complex ARR2-involving two-component network that interferes with a multitude of different signalling pathways and thereby contributes to the highly integrated signal processing machinery in higher plants.

T02-017

Does the universally conserved eukaryotic release factor 1 have an additional function in Arabidopsis?

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AteRF1-1 encodes an Arabidopsis eukaryotic translation release factor 1 homolog, involved in the termination of translation. It has 60-70% nucleotide sequence identity to a diverse range of eukaryote release factors including human, frog and yeast. Complementation studies of rabbit, syrian hamster and human eRF1 in yeast have shown that this sequence conservation also translates into functional conservation.
However, co-suppression of eRF1-1 in Arabidopsis yields an unexpected phenotype. Co-suppressed plants exhibit a significant reduction in height and produce a ‘broomhead’-like cluster of malformed siliques at the tip of the inflorescence. Light microscopy on these ‘bunched-up’ regions indicates that elongation of the inflorescence has been suppressed. Interestingly, AteRF1-1 promoter-GUS studies show that the cells that normally express AteRF1 are also the cells that appear most altered in the co-suppressed plants. Furthermore microarray data on ‘broomhead’ inflorescences hint at another role for this universally conserved translation release factor.

T02-018

Identification and Characterisation of an AHP1-interacting protein from Arabidopsis thaliana

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The multi-step phosphorelay, a sophisticated version of the two-component signalling systems, is used for perception, integration and termination of environmental stimuli in plants (reviewed in Grefen and Harter, 2004). Amongst the involved protein groups the histidine-containing phosphotransfer proteins (HPt) play an important role in handing down signals from upstream histidine kinases (HKs) to downstream response regulators (RRs). In Arabidopsis five HPt proteins (AHPs) have been identified and for AHP1 and AHP2 a putative function in cytokinin and ethylene signalling is probable. However due to their ubiquitous expression any AHPs are more or less able to interact with HKs or RRs. We identified an AHP1-interacting protein (AIP7) via a yeast two-hybrid screen that shows no sequence similarity with known histidine kinases or response regulators. Furthermore there was no positive interaction detectable with some HKs and RRs in yeast. RT-PCR experiments revealed that AIP7 is expressed in all tissues but in leaves of 30 days old Arabidopsis the level of AIP7 transcript is significantly decreased whereas a slightly higher expression could be observed in flowers. Physiological characterisation of corresponding loss-of-function lines will provide further insights into the role of AIP7 in two-component signalling in Arabidopsis.

Grefen C, Harter K (2004) Plant two-component systems:principles, function, complexity and cross talk; PLANTA in press

T02-019

Modulation of light (phytochrome B) signal transduction by the response regulator ARR4

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Within the last years it became evident that the canonical elements of His-to-Asp two-component signaling pathways are conserved in higher plants and play a crucial role in hormone and light signalling as well as in developmental processes (Grefen and Harter, 2004).
During our recent studies we characterized in detail the molecular function of the Arabidopsis A-type response regulator ARR4. ARR4 interacts with the extreme NH2-terminus of the red light photoreceptor phytochrome B (phyB), thereby stabilizes the active Pfr form of phyB under inductive and continuous light conditions and elevates the level of phyB-Pfr in planta. In agreement with these observations overexpression of wildtype ARR4 results in plants hypersensitive to red light. Mutation of the conserved Asp95 to Asn creates an ARR4 version (ARR4D95N) which could not longer be phosphorylated in vitro, but still interacts with phyB in vivo, and confers red light hyposensitivity and a phyB mutant-like phenotype in transgenic Arabidopsis plants. These results indicate that the function of ARR4 on phyB depends on its phosphorylation state. Further data will be presented suggesting that ARR4 phosphorylation and activity is regulated by a hormone-driven two-component signalling cascade. In summary, we propose a working model, in which ARR4 acts as an output element of a two-component system that modulates red light signalling and the light responsiveness of Arabidopsis directly on the level of phyB dynamics.

Grefen C, Harter K (2004) Plant two-component systems:principles, function, complexity and cross talk PLANTA in revision

T02-020

Growth control of the Arabidopsis root meristem by Cytokinin.

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Two plant hormones, auxins and cytokinins, have long been recognized as essential signaling molecules in diverse processes of plant growth and development. They are believed to act synergistically or antagonistically to control fundamental developmental process such as cell division and cell differentiation which ultimately lead to shoot and root organogenesis. Although, molecular, genetic and biochemical studies have already provided evidence for a role of auxin in controlling cell specification, cell division and cell polarity, the role of cytokinin at the cellular and tissue level during plant development has remained elusive. Recently a number of molecular and genetic tools have been developed to study cytokinin signal transduction pathway in Arabidopsis. We will take advantage of these tools to shed light on the role of cytokinin in planta by focusing on the well-characterized Arabidopsis root meristem.

T02-021

Plant organ growth involves the chromatin modifying complex Elongator

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The leaf is used as a model to study the genetic control of organ formation and growth. Mutational analysis and reverse genetics have been used in Arabidopsis to identify genes important for growth in lamina width and a number of interesting genes have been cloned with an impact on either cell expansion or more interestingly on cell division in the lateral direction. EMS mutations were fine-mapped using AFLP, Indel and SNP marker technology and cloned by a candidate gene approach (Cnops et al., in press). Tagged alleles were cloned through PCR-based methods. To date we cloned eleven genes of which 7 encode yeast homologues of the chromatin modifying complex Elongator with histone acetyl transferase activity and with a function in RNAPII-mediated transcriptional control (Nelissen et al., 2003 and unpublished data; Otero et al., 1999). Knock-out mutants in components of this complex resulted in plants with narrow leaves due to reduced cell number and with reduced primary root growth. We determined the genome-wide transcription profiles of the knock-out mutants using cDNA-AFLP and microarray analyses in order to obtain their detailed molecular phenotypes. The data indicated specific processes that were disturbed in the mutants and currently we validate the expression data with biological assays. These results indicate that the activation status of chromatin is important in the regulation of organ growth and development in plants.

This work was supported by the European Community's Human Potential Programme under contract HPRN-CT-2002-00267 (DAGOLIGN) and the Marie Curie Training site HPMT-CT-2000-00088 (PREDEC).

Cnops et al. J. Exp. Bot. in press
Nelissen et al. (2003) Plant Cell 15, 639-654
Otero et al. (1999) Mol. Cell 3, 109-118

T02-022

Comparitive protein profiling: Effects of ethylene and cytokinin on the proteome of Arabidopsis

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The plant hormones ethylene and cytokinin regulate many aspects of plant growth and development. Ethylene is not only involved in fruit ripening but also regulates seed germination, seedling growth and stress responses. Cytokinins regulate cell division and are involved in shoot initiation and growth. The effects of these hormones upon transcriptional regulation have been elucidated through various means, including microarrays, however proteomic changes in response to these hormones have only begun to be examined. While the proteomic changes will reflect transcriptional regulation, it will also reflect post-transcriptional levels of regulation. Recently the proteomics technique of two-dimensional electrophoresis (2DE) has been enhanced through the use of powerful software packages that facilitate automated comparison of multiple 2DE gels and that are able to reliably detect subtle changes in protein levels. We have used 2DE to map the changes in the Arabidopsis (var. columbia) proteome in response to ethylene and cytokinin. Candidate hormone-regulated proteins were subjected to mass-spectrometry for identification. Among the ethylene-regulated proteins, several enzymes were identified that are involved in the pathway for ethylene biosynthesis (e.g. ACC-oxidase and SAM synthetase 2). These are proteins known to be regulated by ethylene at the transcriptional level, and thus confirm the ability of this 2DE-based experimental approach to accurately identify proteins involved in the ethylene response.

T02-023

FIC, a Factor Interacting with CPC, as a Putative Partner for Cell-to-Cell Movement

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Intercellular communication is a crucial process for building a multicellular organism such as a higher plant. Cell-to-cell movement of macromolecules has been thought to play an important role in this communication. Recently SHORT-ROOT (SHR), a putative transcriptional factor, was shown to move from stele cells to the endodermis and function as an activator of endodermal cell fate and cell division in Arabidopsis. A maize homeobox protein, KNOTTED1, that controls leaf formation was also shown to move from inner cells to epidermal cells possibly through plasmodesmata (Zambryski et al., 2004). But their molecular mechanisms of cell-to-cell movement are not known yet.

To understand the mechanism of cell-to-cell movement of regulatory protein, we are studying Myb protein, CAPRICE (CPC), which positively regulates root hair formation in Arabidopsis.

Through previous study we found that CPC mRNA is expressed exclusively in hairless cells, and that CPC:GFP fusion protein can move from cell-to-cell from hairless cells to hair cells in Arabidopsis root epidermis (Wada et al., 2002). Truncated CPC proteins fused to GFP demonstrated that two motifs are responsible for cell-to-cell movement of CPC protein. Two motifs in CPC, one in the N-terminal region and the other in the Myb domain, are required for cell-to-cell movement. Amino acid substitution experiments on CPC:GFP indicated that both W76 and M78 in the Myb domain are critical for this cell-to-cell movement. The W76A mutation also reduced the nuclear localization of CPC:GFP

To elucidate the molecular machinery for cell-to-cell movement of CPC, we used yeast two-hybrid screening to isolate the Factor Interacting with CPC (FIC). FIC gene encodes the novel protein. A data base search revealed that Arabidopsis has one homologue, and one homologue is found in Oryza sativa and in Hevea brasiliensis, respectively. We observed reduced interaction between FIC and CPC with either mutation W76A or M78A, suggesting that this protein was involved in cell-to-cell movement of CPC. The FIC gene had two transcripts, named a and b. Only FICa interacted with CPC in a yeast two-hybrid assay. In vitro interaction between CPC and FICa was observed by using a pull down assay. RT-PCR analysis revealed that both FICa and b mRNA were expressed in various tissues, including the root, leaf, stem, flower bud, and silique. Now we are analyzing the function of FIC in the plant.

Zambryski, J.Cell.Biol. 162, 165-168 (2004). Wada et al., Development 129, 5409-5419 (2002)

T02-024

Role of sterols in the integration of shoot and root meristem function

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The shoot apical meristem (SAM) is a site of cell division activity that leads to the formation of lateral organs, which in the case of the vegetative SAM are leaves. This is in contrast to the root apical meristem (RAM), which does not generate lateral organs directly. Activity and cellular patterning of the RAM is dependent on complex hormonal interactions which in turn require correct sterol biosynthesis, possibly for the correct trafficking, recycling or localization of membrane-bound proteins required for controlled signalling. To investigate the roles of sterols in activity of the SAM, we characterized leaf development in the sterol mutants hydra1 (hyd1) and fackelhyd2. These mutants have dramatic cellular patterning defects in the shoot, with abnormal leaf phyllotaxy, stomatal patterning and leaf dorsoventrality as marked by abnormal expression patterns of YABBY genes. In the hypocotyl, cortical microtubule organization is irregular, consistent with a degeneration of anisotropic cell growth and shape. To distinguish ethylene effects from other effects on development, we constructed double mutants between hydra1 (hyd1) and fackelhyd2 respectively and ein2-1, resistant to ethylene. Many of the developmental defects seen in the sterol mutants are rescued in each of the double mutants, notably stomatal patterning, leaf cell expansion, and vascular patterning. However, defective YABBY gene expression was not rescued. We propose that sterols are required for two major and independently regulated developmental pathways in Arabidopsis, one mediated by defective auxin and ethylene signalling and a second involving sterol ligand-dependent transcription factor activity.

Souter et al. (2002) Plant Cell 14, 1017-1031.

Souter et al. (2004) Planta (in press).

T02-025

ROOT HAIR PATTERNING AND THE REGULATION OF ROOT HAIR-SPECIFIC GENES

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Arabidopsis expansin (the cell wall-loosening factor) 7 gene (AtEXP7) expresses specifically in the root epidermal cells from which root hairs differentiate, resulting in a striped expression pattern along the root hair-forming cell files. The gene expression is closely correlated with root hair initiation, suggesting that expansin-mediated wall loosening is required for hair emergence from the epidermal cell. Promoter analyses showed the essential cis-element for this root hair-specificity and also for the responsiveness to hormones and environmental factors of the gene expression. A putative transcription factor capable of binding this element was identified by yeast one-hybrid screening. Three different types of root hair cell arrangement are recognized in vascular plants, where different cell fate machineries should direct the distinct patterns. Intriguing questions on this matter are '(1) Do the different hair-patterning species have orthologs of AtEXP7? (2) Are the AtEXP7 orthologous genes expressed in the same manner as in Arabidopsis? This casts a fundamental question on the evolution of cell differentiation: 'Does patterning unilaterally dictate cell differentiation?' or 'Does cell differentiation hire patterning as to serve in a special multicellularity situation?' We present some preliminary results to answer these questions. (This research was supported by the grants from Plant Diversity Research Center, 21C Frontier R&D Programs and from Environmental Biotechnology Research Center.)

T02-026

The AtMYB11 gene is a possible regulator of development in Arabidopsis thaliana

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MYB proteins are transcription factors sharing a characteristic DNA-binding domain shown to bind DNA in a sequence specific manner. MYB proteins in animals contain three repeats (R1, R2, R3), while in plants this domain generally consists of two imperfect repeats of about 50 residues (R2, R3). In Arabidopsis, more than 125 R2R3-MYB genes have been identified, representing one of the widest family of plant transcription factors described. The information available on the function of a few plant MYB proteins suggest an important role of this family in various processes like regulation of metabolic pathways, control of cell division and plant morphogenesis, response to different stresses and involvement in hormone signal transduction.
RT-PCR analyses showed that one of the genes under study in our laboratory, AtMYB11, was expressed throughout flower development and in 4 days-old seedlings, where the activation is mediated by light. Subsequent analyses by in situ hybridization and promoter-GUS fusions revealed that AtMYB11 is specifically expressed in meristems and primordia of Arabidopsis. In fact, the AtMYB11 transcript is found mainly in the shoot and root apical meristems of seedlings, but also in young cotyledons and secondary root primordia. In addition, AtMYB11 is expressed in the inflorescence meristems, in axillary meristems of stem and in flower primordia. During flower development the transcript is present in ovule primordia, in the ovary wall of mature ovules and in the embryo epidermis of developing seeds.
Two dSpm insertion mutants has been isolated from the Wageningen collection and for one of them homozygous knock-out plants has been analysed in all growth phases. These mutants are morphologically similar to wild-type plants, however they show an accelerated germination and morphogenesis. In particular, they show an earlier development of seedling organs and an accelerated emergence and differentiation of leaves, inflorescences and roots. This acceleration leads to early flowering plants with more lateral inflorescences, flowers and more adventitious and lateral roots. Furthermore, flowers produce shorter siliques and less seeds than wild-type.
Since three additional dSpm insertions are present in the background of the mutant plants analysed, a second independent insertion mutant, but also RNAi and overexpression plants are currently under analysis, in order to confirm that the accelerated growth depends on the mutation in the AtMYB11 gene.

T02-027

HORMONAL MEDIATION OF KNOX ACTIVITY IN THE DISSECTED LEAF OF TOMATO

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Leaves arise from the activity of the shoot apical meristem. The regulation of KNOTTED1-like homeobox (KNOX) transcription factors is central to the transition from meristem to leaf identity. In simple leaf species, expression of KNOX genes is excluded from leaf primordia and confined to meristematic areas. In contrast, the compound leaves of tomato retain KNOX gene expression, indicating that meristematic activity persists in such leaves. This expression pattern, with the increased leaf dissection obtained by overexpressing KNOX genes in tomato, suggested that differential regulation of KNOX expression may be responsible for the dissected leaf form.
Studies done in simple leaf species suggest that KNOX function may be mediated by the plant growth regulators (PGRs) cytokinin and gibberellin. However little is known on the relationship between KNOX activity and PGRs in compound leaves. To investigate whether and to what extent KNOX dependent leaf dissection in tomato is mediated by PGRs we are studying genetic interactions between tomato GA mutants, and lines with altered levels of KNOX expression and altered leaf dissection. We are also studying expression of GA biosynthetic and catabolic genes in such backgrounds. Transcript levels of the LeGA20ox1 gene are reduced in KNOX overexpressing tomato mutants suggesting that similar links as those observed in Arabidopsis may exist in tomato. We are currently analysing the expression of other GA biosynthetic and catabolic genes in response to induced KNOX gene expression. We are also trying to determine the relative contribution of cytokinin and GA to KNOX function.

T02-028

THE RKS FAMILY OF TRANSMEMBRANE RECEPTOR KINASES

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The Receptor Kinases like SERK (RKS) family of transmembrane receptor kinases is represented by 14 different members in Arabidopsis thaliana. Together with other extracellular and/or transmembrane receptors these RKS receptors are involved in transmitting extracellular signals towards intracellular compartments. Recently, one member of this receptor complex, BAK1/RKS10, proved to be involved in brassinosteroid perception. Our results indicate that other RKS gene products are also involved in brassinosteroid perception and subsequent modulation of plant development. The extracellular domain of the 14 different RKS gene products consists of 4-5 leucine rich repeats. A family of 14 different extracellular peptides that represent the presumed candidate ligands for RKS interactions will be presented together with the functional analysis of the RKS receptor complexes in Arabidopsis thaliana.

T02-029

Role of the UGF protein family during Arabidopsis thaliana development

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During postembryonic development plants undergo several dramatic phase changes. A good example is the switch from vegetative growth to flowering. During this event, the shoot apical meristem switches fate and starts producing flowers instead of leaves. We have studied this process on a global scale by expression profiling and found several genes that are highly responsive to changes in photoperiod (Schmid et al., 2003).
Among these genes we have identified a small, plant specific family, now called UGF, which comprises four members in Arabidopsis. Two of the UGF genes (UGF1 and UGF2) are induced by long-day conditions while the other two genes (UGF3 and UGF4) are repressed. We are studying in detail how the UGF genes contribute to meristem identity and floral induction. To that end we have been analysing the expression of the UGF genes by in situ hybridization. The subcellular localization of the gene products is being studied by UGF:GFP fusion proteins. We are further creating and analysing 'loss-of-function' and 'gain-of-function' alleles for all four UGF genes. In order to understand UGF protein function, we have identified interacting proteins in a yeast-two-hybrid screen. Preliminary data suggest that UGF genes participate in several developmental processes, including meristem maintenance and shoot branching. In the end we hope to integrate the UGF genes and interacting proteins in the network of meristem maintenance and floral transition.

Ref: Schmid et al. Development (2003), 130, 6001-6012.

T02-030

CYTOKININ INDEPENDENT 2 (CKI2), a putative receptor histidine kinase of Arabidopsis

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CYTOKININ INDEPENDENT 2 (CKI2) is a member of the functionally diverse family of plant receptor histidine kinases, that includes both hormone (CRE1 and ETR1) and light (PHY) receptor kinases. CKI2 was first identified in an activation tag screen in Arabidopsis; although poorly penetrant, cki2-1 mutant callus could initiate shoot growth in the absence of exogenously applied cytokinin. The endogenously expressed CKI2 coding sequence was isolated and the predicted amino acid sequence contains highly conserved residues indicative of both histidine kinase and response regulator regions. In addition, an amino terminal region, with sequence homology to a cyanobacterial PAS domain, was identified and is present in a putative CKI2 ortholog from rice. Based on RT-PCR and confirmed by a P-CKI2::GUS transcriptional fusion, CKI2 is expressed in several tissues. Unlike other hormone receptors, CKI2 lacks detectable transmembrane regions based on computational algorithms and fluorescence from a CKI2:GFP fusion protein is localized to the cytosol. A putative functionally null mutant allele, cki2-2, was identified. Homozygous cki2-2 plants have an overall delay in plant growth, reduced root growth rate, and chlorotic appearance. In the presence of exogenously applied cytokinin, root growth and reporter gene expression of cki2-2 plants behave as in wild type. The putative role of CKI2, in relation to the canonical cytokinin-dependent signal transduction pathway, will be discussed.

T02-031

A suppressor screening of jaw-D, a microRNA overexpressing mutant

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MicroRNAs (miRNAs) are small RNAs with regulatory function in both plants and animals. Their mode of action requires interaction with target mRNAs through complementary basepairing. In plants, most miRNAs seem to guide their mRNA targets to cleavage, causing downregulation of target transcripts.

miR-JAW (miR-319a), like several other miRNAs, plays important roles in plant development. The jaw-D mutant, which constitutively overexpresses miR-JAW, shows pleiotropic phenotypes, including epinastic cotyledons, crinkly leaves and a delay in flowering time. This is due to the simultaneous downregulation of five genes belonging to the TCP class of plant-specific transcription factors, which are thought to be involved in the regulation of cell proliferation.

To gain further insight into the molecular mechanisms responsible for miR-JAW activity and function, we carried out a suppressor screen in jaw-D background. We mutagenized 20.000 jaw-D seeds with EMS and screened the M2-population for plants in which the jaw-D associated phenotypes are suppressed. 50 putative suppressors were isolated. In some, all phenotypes of jaw-D mutants are affected, while in others only a subset is affected, like leaf shape or flowering time. RT-qPCR of the TCPs allows a further classification of the suppressors, e.g. a low TCP level would imply that the suppressor acts downstream of the transcription factors. We will describe the phenotypic and molecular characterization of several suppressor mutants.

T02-032

CUC2 and CUC3 are involved in axillary meristem formation and post-embryonic organ separation.

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A shoot apical meristem (SAM) formed in embryo constructs a main shoot after germination and axillary shoot meristems formed in each leaf axils develop lateral shoots. These meristems are essential for the development of shoot architecture.
CUC1 and CUC2 were identified as factors regulating SAM formation and cotyledon separation during embryogenesis.
We have isolated CUC3 (CUP-SHAPED COTYLEDON3) from screening of cuc2 enhancer mutants. CUC3 have a highly conserved NAC domain comparable with CUC1, 2 but have no conserved C terminal element and micro RNA recognizing site found in CUC1, 2.
cuc3 single mutant showed an abnormal shoot, lateral shoots occasionally uncoupled from cauline leaf and there is no axillary shoot meristem at a low frequency. Additionally, in cuc2 cuc3 double mutant, lateral organs such as leaves, stems and floral organs were fused each other, indicating that mutation of CUC2 enhanced cuc3. However, mutation of CUC1 didn't enhance cuc3 in aerial part. These indicated that CUC2 and CUC3 act redundantly to regulate axillary meristem formation and organ separation post-embryonically.
CUC2 and CUC3 expressed between SAM and lateral organ after germination. This expression profile and post-embryonic phenotype are similar to that of LAS (LATERAL SUPPRESSOR). To understand the genetic interaction among CUC2, 3 and LAS, we analyzed the double and triple mutants. As a result, mutation of LAS enhanced both cuc2 and cuc3 single mutant, especially cuc3 las double mutant showed strong abnormality such as axillary shoot defect and lateral organ fusion.

T02-033

A new GAL4-based activation tagging system for Arabidopsis root developmental study

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Efficient induction of gain-of-function mutations is essential for functional assignment of genes for which loss-of-function mutations do not cause obvious phenotypic defects. In order to identify genes responsible for root morphogenesis, we are establishing an activation tagging system that utilizes a yeast transcriptional activator GAL4 and its recognition sequence (UAS). In this system, a T-DNA containing five copies of 17-mer UAS at its left-border end was randomly inserted into the genome of GAL4:VP16-expressing Arabidopsis plants that had been established by Jim Haseloff and coworkers. Simple interaction of well-characterized GAL4:VP16 transcription activator and UAS is expected to efficiently induce ectopic expression of tagged genes in a pattern defined by the GAL4:VP16 expression. Since the host lines also harbor a GFP reporter gene placed under the UAS, plants defective in root patterning can be screened in the primary transformants based on altered GFP expression. We have so far generated 4,200 lines in the J0571 background that expresses GAL4:VP16 in the root endodermis and cortex. T1 plants of these lines were screened for abnormal root morphology as well as altered GFP expression pattern by confocal observation. We have also generated 3,800 transformants in the Q2610 background that expresses GAL4:VP16 in all root cells. These lines were allowed to set seeds to establish an activation-tagging seed pool that can be used in future screening. We are in the process of identifying tagged genes for some mutant candidates by TAIL-PCR.

T02-034

Analysis of pale-green mutant apg6 using Ac/Ds transposon system in Arabidopsis.

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To study Arabidopsis nuclear genes responsible for chloroplast development and pigment synthesis, we systematically analyzed chloroplast albino and pale-green (apg) mutants isolated from Ac/Ds transposon tagged mutant lines. A large number of apg mutants that affect early plastid growth and thylakoid membrane development and result in a low levels of chlorophyll were identified. We examined one of the apg mutants, designated apg6-1. Molecular characterization of the apg6-1 established that the APG6 gene encodes for a Hsp101 homolog (ClpB1) of heat shock protein, a member of the diverse group of Clp polypeptides that function as molecular chaperons and/or regulators of energy-dependent proteolysis. We have isolated its somatic revertants, and also identified two Ds tagged and one T-DNA tagged mutant alleles of apg6 including apg6-1. All these three alleles showed same phenotype, pale-green. The APG6 protein contains a transit peptide that functions in chloroplast localization, but no transmembrane domain. Since chloroplasts of apg6-1 plants are smaller than those of wild type, and contain undeveloped internal thylakoid membranes, APG6 is important for chloroplast development. The expression of APG6 gene was strongly increased by heat stress but is less abundant in the other environmental stress. The apg6-1 mutants contained few chloroplast proteins related to photosynthesis by immunoblot analysis. These results suggest that the APG6 protein may function as a chaperon involved in internal stroma formation.

T02-035

ead1, an orthologue of a human oncogene, is required for ethylene and auxin responses in Arabidopsis.

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In the past decade, a number of interesting studies have presented experimental evidences for the existence of a complex network of interactions between ethylene and auxin response pathways. Nevertheless, our current understanding of the molecular components involved in this cross-talk is very limited. Towards the identification of the molecular elements involved in ethylene-auxin interactions, we have performed a two-step genetic screen obtaining several mutants with general defects in both ethylene and auxin responses. Molecular and physiological studies of ead1 (ethylene and auxin defects1) indicate that mutations in the EAD1 locus result in a reduced response to ethylene as well as in a number of auxin phenotypes, including increased number of lateral roots, decreased apical dominance, altered venation, pin-like inflorescences, and ettin-like flowers. The analysis of the phenotypic data suggests that the EAD1 function is required for both normal ethylene response and auxin levels/distribution. This conclusion is further supported by the finding that ead1 mutants show abnormal expression of the synthetic auxin reporter DR5-GUS.

A combination of T-DNA tagging and map-based approaches was used to clone EAD1. Sequence analysis of three independent alleles indicates that EAD1 encodes an Arabidopsis orthologue of a novel human oncogene. Although it is well established that the miss-regulation of this human gene is associated with certain types of cancer, the molecular function of the corresponding protein remains unknown.

To address the role of EAD1 in plants, a translational fusion with GFP has been made. Analysis of the pEAD1-EAD1::GFP reporter in wild-type seedlings revealed that the EAD1 protein is expressed in tissues undergoing rapid cell division, i.e. root tips and developing leaves, but not in mature organs. Our progress in the functional characterization of EAD1 in Arabidopsis will be presented.

T02-036

Understanding the Molecular Mechanism of TFL1

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During Arabidopsis development the shoot apical meristem (SAM) generates lateral primordia which display stage-specific traits.
In the initial vegetative phase (V), leaves are produced. The V phase is followed by the I1 phase, which consists of 2-3 leaves (cauline) subtending secondary shoots (coflorescences). Upon integration of environmental and endogenous signals, the SAM enters the reproductive phase (I2) and produces flowers on its flanks.
The TFL1 gene is a key component of the phase change machinery as mutations in TFL1 affect the timing of phase switching. Also tfl1 mutants enter a novel phase (terminal flower) which is normally absent in wild type.
In order to understand the mechanism underlying TFL1 function we attempted to identify protein interacting with TFL1. A functional TAP tag version of TFL1 was expressed in plants under the 35S promoter to allow affinity purification of TFL1 protein complex. So far no obvious protein appears in conjunction with TAP tag TFL1.
To reveal essential downstream functions required for TFL1 signaling, suppressor mutants of plants ectopically expressing TFL1 were isolated. One of them has been characterized and mapped within a confined region on the bottom of chromosome 3.
In order to follow TFL1 protein expression throughout development we raised antibodies to TFL1. These antibodies recognize TFL1 in vivo and can be used to detect TFL1 protein distribution in the SAM at various stages of growth in wild type and mutant backgrounds. Detailed subcellular localization of TFL1 should give us some clues to its function.

T02-037

Transcriptome analysis reveals an alternative mechanism for habituation

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For nearly 50 years, scientists have recognized that varying ratios of the plant hormones cytokinin and auxin induce plant cells to form particular tissues: undifferentiated callus, shoot structures, root structures, or a whole plant. Proliferation of undifferentiated callus tissue, greening, and the formation of shoot structures are all cytokinin-dependent processes. Habituation refers to a naturally occurring phenomenon whereby callus cultures, upon continued passage, lose their requirement for cytokinin. Studies of calli derived from plants with a higher-than-normal cytokinin content, indicate that overproduction of cytokinin by the culture tissues is a possible explanation for acquired cytokinin-independence. In the course of a transcriptome analysis of the well-established T87 Arabidopsis cell culture line, we have discovered an alternative explanation for the phenomenon of habituation: aberrant expression of the cytokinin receptor protein CRE1. Results of the full-genome transcript analysis of habituated and non-habituated callus cultures, using the Arabidopsis thaliana 60mer microarray (NimbleGenTM Systems, Inc., Madison, WI), will be presented. Progress toward quantification of the cytokinin content and absolute quantification of the CRE1 protein, in the habituated cell line, will be discussed.

T02-038

Molecular genetic analysis of three bHLH genes involved in root hair and trichome differentiation

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Genetic analysis demonstrated that Arabidopsis GLABRA3 (GL3), mutations which result in smaller trichomes with fewer branches, encodes a bHLH type transcription factor (Payne et al., 2000). It was also reported that plants with mutations both in GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3), another bHLH gene closely related to GL3, have more root hairs than normal (Bernhardt et al., 2003). We have investigated GL3 and three homologues, EGL3, AtMYC1, and TT8, and found that AtMYC1 was also involved in root hair and trichome differentiation.
In situ RNA hybridization showed that the GL3, EGL3, and AtMYC1 genes were preferentially expressed in root-hair cell files, which is consistent with the result shown in promoter::GUS plants. Detailed observations established that there were some differences among the 3 genes in their strength and pattern of expression around the root apical meristem. These mRNA expression patterns, however, could not simply account for their function as negative regulators of root hair initiation, so we examined the localization of the 3 bHLH proteins through the use of GFP fusion proteins. Under control of the promoter of each gene, AtMYC1:GFP fluorescence was observed mainly in the cytoplasm of root-hair cells, similar to the results with the promoter::GUS; whereas GL3:GFP (and also EGL3:GFP) fluorescence was observed clearly in the nuclei of both root-hair and hairless cells. When the GL3:GFP protein was expressed under control of the AtMYC1 promoter, GL3:GFP fluorescence was also found in the nuclei of all root epidermal cells, in contrast to AtMYC1:GFP. This result confirmed that GL3 is capable of the cell-to-cell movement. We also examined the characteristics of the 3 bHLH genes with regard to TTG1. Yeast interaction assays revealed that GL3 and EGL3 showed strong interactions with TTG1, but AtMYC1 did not. Promoter::GUS staining of the 3 bHLH genes in ttg1 showed that TTG1 regulated GL3 expression positively and EGL3 expression negatively, but had no influence on AtMYC1 expression. These results indicate that AtMYC1, GL3, and EGL3, which have similar mRNA expression patterns and cooperatively regulate epidermal cell differentiation, have rather different properties from each other. We are now analyzing the expression profiles of each single mutant of the 3 bHLH genes using the Affymetrix GeneChip. We will discuss the differences in regulation between GL3, EGL3, and AtMYC1.

Payne, C. T., et al. (2000), Genetics 156(3): 1349.
Bernhardt, C., et al. (2003), Development 130(26): 6431.

T02-039

"Overexpression of CDK inhibitors at the SHOOTMERISTEMLESS domain causes precocious exit of cell cycle and affects morphogenesis in Arabidopsis"

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The shoot apical meristem is the source of cells that will compose the aerial parts of the plant. A perfect orchestration of cell division rates and cell fate spatially controlled within the SAM is required for normal organ formation and plant development. This coordination is achieved by the activity of genes that maintain SAM function such as STM, WUS and the CLV loci. On the other hand, fundamental control of cell division is performed by the activity of the CDK/Cyc complexes. The integration of cell cycle control and development is still poorly understood. Plants overexpressing core cell cycle genes had their morphology altered as in the case of CycD3;1 and KRP2 overexpressing lines (Dewitte et al; 2003 and De Veylder et al; 2001). These and other works suggest that plant development is also controlled by the correct balance of cell cycle regulators and their activity. To further investigate this matter we opted for a tissue specific overexpression approach. Known cell division inhibitors KRP2 and CDKA;1.N146 were overexpressed at STM domain of the SAM. Molecular and phenotypic analysis of trangenics will be shown as well as a discussion based on the control of CDK activity as a determinant of cell differentiation switch.

Dewitte W, Riou-Khamlichi C, et al.(2003) Plant Cell 15:79-
De Veylder L, Beeckman T, et al.(2001) Plant Cell 13:1653-

T02-040

Identification and functional characterization of brassinosteroid-responsive genes

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Detailed analysis of brassinosteroid (BR)-regulated genes can provide evidence of the molecular basis of BR effects. Classical techniques (such as subtractive cDNA cloning) as well as cDNA and oligonucleotide microarrays have been applied to identify genes, which are upregulated or downregulated after BR treatment or are differently expressed in BR-deficient mutants. Current work focuses on genomic BR effects in different organs of Arabidopsis (in particular roots) and tomato plants (sink leaves, source leaves, and roots). Expression profiling experiments suggest large differences in genomic BR effects in shoots as compared to roots. Several BR-responsive genes encoding transcription factors (e.g. TF55, see poster by Lisso et al.) or potential mediators of BR responses (e.g. EXO) are subject to detailed functional characterization. A macroarray (termed ‘development macroarray’, currently comprising 190 genes, inclusive more than 50 BR-responsive genes) was established for the characterization of these candidate genes. Furthermore, the macroarray is used to analyse phytohormone interactions on the gene expression level.

Coll-Garcia et al. (2004) FEBS Let. 563: 82-86
Müssig et al. (2003) Plant Physiol. 133: 1261-1271

T02-041

Functional analysis of the CLE40 signal in Arabidopsis root meristem development

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Stem cell activity of the initial cells of the root meristem is controlled directly by the adjacent cells of the quiescent centre. They maintain cells in their neighbourhood, comparable with WUSCHEL-expressing cells in shoot- and floral meristems. CLE40, a member of the CLE protein family, encodes a potentially secreted protein that is distantly related to CLV3. While CLV3 transcripts are confined to stem cells of the shoot meristem, CLE40 is expressed at low levels in all tissues, including roots. Although different in their expression patterns, CLV3 and CLE40 are functionally equivalent proteins, as already shown by promoter swap and misexpression experiments. High level expression of CLV3 or CLE40 results in the premature loss of root meristem activity and differentiation of meristem cells, indicating that activation of a CLV-like signaling pathway may restrict cell fate also in roots. Cle40 insertion mutants show developmental defects that are probably due to the premature loss of stem cell activity in the root. Our aim is to study how the CLE40 signal is transmitted and which genes and functions are regulated by CLE40.

T02-042

Genetic analysis of the SCABRA and RUGOSA genes

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We have performed a large-scale screening for EMS-induced mutants displaying aberrantly shaped leaves in the model system Arabidopsis thaliana. Some of the recessive mutations found were named scabra (sca) and rugosa (rug), which cause the vegetative leaves to be rounded, with protruded laminae and irregularities on the epidermis that may be due to the perturbation of cell division or cell expansion. In addition, necrotic sectors are visible on the surface of rug1 leaves, probably as a result of local processes of cell death. Transverse sections and confocal microscopy studies revealed that internal leaf architecture is dramatically perturbed in the sca1, sca4 and rug1 mutants, whose palisade mesophyll cells are extremely reduced in number. Scanning electron microscopy analyses indicated that rug1 epidermal cells are larger than the wild type ones, although they are similar in shape to those of the wild type. We are positionally cloning the SCA and RUG genes, which map at chromosome 2 (SCA1 and SCA3), 3 (SCA4) and 5 (SCA5 and RUG1), respectively. We have delimited candidate regions of 1 Mb, 100 kb and 0.9 Mb, respectively, for the SCA3, SCA4 and RUG1 genes. To study genetic interactions, the sca and rug mutants have been intercrossed and crossed to other leaf mutants previously isolated in our laboratory. The identification and characterization of the corresponding double mutants is in progress. Further details of the study of sca and rug mutants will be presented at the meeting.

T02-043

Analysis of the distribution of Arabidopsis thaliana amidase1, an enzyme capable of forming indole-3-acetic acid from indole-3-acetamide.

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The biosynthesis of the major plant growth hormone indole-3-acetic acid (IAA) has not yet been fully uncovered. The only clarified pathway of IAA synthesis which has formerly been known to exclusively exist in phytopathological bacteria, e.g. in the genera Agrobacterium, leads from tryptophan to IAA via indole-3-acetamide (IAM). This reaction sequence is catalyzed by a tryptophan-2-monooxygenase (TMO) and an indole-3-acetamide hydrolase (IAH). As IAM could be elucidated as an endogenous compound of Arabidopsis thaliana seedlings (Pollmann et al., 2002), this pathway might also be valid in this model plant. With IAM as a precursor of IAA and the finding of a specific IAM hydrolase (AMI1), catalyzing the conversion of IAM to IAA (Pollmann et al., 2003), the function, distribution and histological localization of this enzyme has become matter of particular interest.
For this reason the A. thaliana amidase1 was fused to the green fluorescent protein (GFP) of the jellyfish Aequorea victoria and its subcellular localization was monitored utilizing transiently transformed plant cells and confocal laser scanning microscopy.
To further investigate the expression pattern of AMI1 in whole plants we performed semiquantitative RT-PCR using different plant tissues. In addition, transgenic Arabidopsis lines carrying the ami1-promoter sequence fused to the b-glucuronidase (GUS) reporter gene were created. Here we present the first experiments, pointing out the expression of AMI1 in meristematic tissue in planta, thus emphasizing the putative AMI1 function in auxin biosynthesis.

Pollmann et al. (2003) Phytochemistry 62, 293-300
Pollmann et al. (2002) Planta 216, 155-161

T02-044

Altered Cytokinin Sensitivity 1 (AtACS1) encodes a cytokinin-binding protein involved in cytokinin perception

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In the 50 years since cytokinins were first characterised as promoters of cell division they have been shown to influence almost every plant developmental process. The use of biochemical techniques to identify cytokinin receptors is an important development in our understanding of this essential group of plant growth regulators, given the deficiency of mutants with lesions in cytokinin perception machinery. AtACS1 has been identified as a thaumatin-like cytokinin-binding protein in Arabidopsis and disruption of AtACS1 expression alters plant perception of cytokinin.

Analysis of mutant and misexpressing lines has been used to show that AtACS1 expression is important for normal responses to applied cytokinin. A T-DNA insertion in the 3'UTR of AtACS1 (Atacs1-1) is sufficient to perturb root and leaf callus responses, root elongation on exogenous cytokinin and nutrient stress responses. The location of AtACS1 has been demonstrated to be extracellular and this cumulative evidence indicates that AtACS1 is a novel cytokinin receptor.

T02-045

Interactions between Lateral Organ Boundary gene family members (LBDs) and KNOX genes : new clues from the analysis of the lollo mutant

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Here we describe a new serrated leaves mutant, called lollo. This mutant was found after a transposon activation tagging screen, and the gene responsible for it belongs to the Lateral Organ Boundary (LOB) gene family. In situ analysis results underline the involvement of the LOLLO gene in the establishment of organ boundaries: the LOLLO expression is detectable between meristems and organ primordia. This expression pattern is similar to the one of the LOB gene. Transgenic plants that ectopically express the LOLLO show serrated leaves and short petioles, flowers lacking or with undeveloped organs, carpels that are bending outside of the corolla. The overall appearance of lollo mutants is packed and bushy. One previously characterized member of the LBD family, ASYMMETRIC LEAVES2, is preventing KNOX gene expression outside of meristematic regions. The serrated and lobed leaves in lollo mutants, and in plants where the LOLLO gene is ectopically expressed, suggest that LOLLO plays a role opposite to AS2 in controlling KNOX genes expression.

T02-046

Characterization of A and B-type cyclins in Arabidopsis

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Cyclins are activators of the cyclin dependent kinase (CDK) complex, which regulates cell division onset and progression. The fluctuation and change in sub-cellular localization of cyclin proteins during the cell cycle are the key factors that control CDK activity, making them important regulatory proteins. Sequencing and subsequent annotation of the Arabidopsis genome has identified thirty cyclins. This is an unexpectedly high number of cyclins, and raises the possibility that different cyclins carry out specialized functions during Arabidopsis development. In mammals the G2 to M phase transition is controlled by CDKs that interact with A and B-type cyclins. Nineteen A and B-type cyclins have been identified in Arabidopsis, however, their functions are poorly understood. Single mutants representing knock-outs of fifteen of these mitotic cyclins (A1, A2, A3, B1 and B3 classes) have been isolated from T-DNA collections (SIGnAL, SAIL and Wisconsin knock-out facility) or generated through RNAi constructs. These mutants have no obvious phenotypes, suggesting redundancy within this class of genes. To help with the identification of subtle phenotypes and to help us understand how these genes are regulated throughout development, we have generated GFP and GUS fusions. These fusion constructs include the destruction box, which is required for the degradation of mitotic cyclins during the cell cycle. Therefore, accurately reflect the expression and turn over of the mitotic cyclins during development. Preliminary results indicate that A2-type and B1-type cyclins have overlapping expression patterns, helping to clarify the high level of redundancy within this group of genes.

T02-047

At1g36390 is highly conserved, and may play a role in shoot development

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At1g63690 was identified as a gene expressed preferentially in the shoots of leafy spurge (Euphorbia esula) as shown by hybridizing labeled shoot vs leaf cDNA populations from leafy spurge to Arabidopsis cDNA arrays. Subsequent expression analysis of this gene demonstrated that it was preferentially expressed preferentially in actively growing shoot apices of leafy spurge. A search of the various DNA sequence databases identified putative orthologues of this gene in all plant species examined, and similar genes of unknown function in mammals, yeast, insects, and roundworms. Sequence analysis of the gene indicated that it encodes a protein with a growth factor receptor signature. The encoded protein contains a putative leader sequence, PA domain (Protease Associated), and a series of trans-membrane domains through the carboxy terminal half of the protein, suggesting it may play a role in signal transduction processes. Arabidopsis plants putatively containing insertion mutations in this gene display the formation of aerial rosettes and/or a very late flowering phenotype. Tobacco plants transformed with RNAi constructs designed to knock out expression of the orthologous gene display a rolled and wavy leaf phenotype. Combined, these results suggest that At1g63690 encodes a previously uncharacterized protein involved in meristem development.

T02-048

Regulation of Lateral Root Formation by SLR/IAA14, ARFs, and Chromatin Remodeling Factor, SSL2/CHR6

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Auxin promotes lateral root (LR) formation in higher plants. To elucidate the molecular mechanisms of auxin-regulated LR formation, we are studying the solitary-root (slr) mutant, which has a gain-of-function mutation in IAA14, a member of Aux/IAA family in Arabidopsis. The slr mutation blocks the early cell divisions in the root pericycle for LR initiation, suggesting that the mutant IAA14 protein repress the activity of the Auxin Response Factors (ARFs), which are required for LR initiation. To understand the roles of SLR/IAA14 and ARFs for LR formation, we are characterizing; 1) the roles of several ARFs in LR formation, 2) the transgenic plants expressing the mutant IAA14 protein or mutant IAA14-GR (glucocorticoid receptor) under the tissue specific promoters in roots, and 3) the target genes regulated by SLR/IAA14. The data from these analyses will be presented.

In addition, to identify the new factors involved in auxin-regulated LR formation, we isolated the extragenic suppressor mutants of slr from EMS-mutagenized slr seedlings. The ssl2 (suppressor of slr 2) is a single recessive mutation, and we have four ssl2 alleles (ssl2-1 ~ ssl2-4). The ssl2 slr double mutants produce LRs but still have the other slr defects, indicating that the slr phenotype in LR formation is partially dependent on SSL2. We observed that the ssl2 mutation enhances cell divisions in the slr pericycle in both the presence and the absence of exogenous auxin, suggesting that SSL2 acts the repression of pericycle cell divisions in the slr roots. SSL2 encodes CHR6/PICKLE/GYMNOS, one of the chromatin remodeling factors. SSL2/CHR6 promoter::GUS reporter gene is expressed in the root stele tissues in the slr as well as in the wild-type. These results suggest that transcriptional regulation through SSL2/CHR6-mediated chromatin remodeling might be important for auxin-regulated LR formation.

T02-049

Effectors of SHOOTMERISTEMLESS function.

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The SHOOTMERISTEMLESS gene in Arabidopsis is the orthologue of KNOTTED1 in maize and is one of the major genes responsible for the formation and maintenance of the shoot apical meristem (SAM). Although much is known about the function of STM, little is known about its regulation. Strong mutants at the STM locus completely fail to initiate a functional SAM. We have performed an EMS mutagenesis screen using a line expressing a pSTM-GUS construct, to identify second-site effectors of SHOOTMERISTEMLESS (STM) function. Two putative mutants were isolated showing an altered STM expression domain. The phenotype of one of the mutants, 3010 shows an enlarged STM expression domain in vegetative apical meristems, extending into leaf primordia. However, STM expression is reduced in inflorescences. The mutant also shows disrupted organ growth, with almost all organs such as roots, leaves, stems and siliques being twisted and dwarf. This mutation was mapped to the bottom arm of chromosome 5 and found to cosegregate with markers also cosegregating for the TORNADO1 locus. Allelism tests are currently being performed to show whether our mutation is a trn1 allele. The genomic region spanning the trn1 locus has been published to be contained by a single YAC clone. We are currently analysing the sequence in this region further and attempting to identify candidate transcripts which may be mutated in our mutant. A further descriptive characterisation of the 3010 mutant in terms of its altered meristem organisation at the molecular and morphological levels will be presented.

T02-050

KNAT3 and KNAT4: two KNOX genes control aspects of plant development and are active in the shoot apical meristem.

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The KNAT3 and KNAT4 genes belong to the Class II family of KNOX (knotted-like homeobox) transcription factors. A role for Class I genes such as SHOOTMERISTEMLESS, KNAT1/BREVIPEDICELLUS and KNAT2 in meristem development and plant architecture has been established. However, no function has been assigned to date to Class II KNAT genes. In order to understand further the function of KNAT3 and KNAT4, we have taken a dominant-negative approach and a knock-out mutant approach. We have used CHRIS (Chimeric Repressor Interference System; Chandler & Werr, TIPS 8:279-285, 2003) to generate mutant phenocopies for both genes. Plants expressing transdominant negative engrailed repressor domains for both KNAT3 and KNAT4 have long hypocotyls and petioles, due to more elongated cells. We have also isolated knockout mutants containing a T-DNA insertion for both genes. knat3 mutants have an identical phenotype to that of CHRIS-KNAT3 plants. However, knat4 mutants appear wild type except for a reduction in the plastochron. The difference between the knockout mutant knat4 phenotype and CHRIS-KNAT4 phenotype suggests that since dominant-negative effects probably operate at the protein level, KNAT3 and KNAT4 share a subset of interacting proteins whose function can be repressed by CHRIS. Both genes have a function in the shoot apical meristem in controlling primordium initiation as evidenced by a plastochron phenotype in both mutants and we have shown via in situ hybridisation that KNAT3 is expressed at least in the inflorescence meristem. Consistent with a known function of KNOX genes in regulating gibberellin concentrations in the meristem, the knat3 mutant has decreased transcript levels of GA3ox1 which we infer to represent increased gibberellin levels and cause the cell elongation phenotype. We have used a pKNAT4-GUS construct to analyse the expression pattern of KNAT4 which will be presented. Expression of KNAT4 is down-regulated in knat3 mutants, suggesting that it acts downstream of KNAT3. We are currently constructing a knat3 knat4 double mutant and we will present our current model for understanding the function of these two genes in Arabidopsis development.

T02-051

CYTOKININ RESPONSE GENES OF ARABIDOPSIS IDENTIFIED BY GENOME-WIDE TRANSCRIPTOME ANALYSIS REVEAL NOVEL CYTOKININ-SENSITIVE PROCESSES

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Cytokinin is a key regulatory molecule for shoot and root development, but to date only a limited number of cytokinin response genes is known. A better knowledge about its target genes could contribute to a better insight into the molecular mechanisms of its regulatory functions. Therefore, we have performed a genome-wide trancriptome analysis with the Affymetrix 22k GeneChip microarray to discover cytokinin-induced changes in steady-state transcript levels. More than 80 immediate early response genes showed significant changes 15 min after cytokinin treatment. Immediate early cytokinin response genes include a high proportion of transcriptional regulators, at later time points genes coding for signaling proteins, protein degradation, light reactions, primary metabolism and redox regulation were more prevalent. Parts of the regulated genes are known response genes and regulators of other hormones, indicating partial overlap of signalling pathways. Analysis of cytokinin-deficient 35S::AtCKX seedlings has revealed long-lasting cytokinin-sensitive transcriptional changes and indicates processes for which cytokinin is a limiting and possibly a regulatory factor. Comparative overlay-analysis with the software tool MapMan was used to identify metabolic reactions that were differently regulated after cytokinin addition and in cytokinin-deficient plants. Several previously unknown cytokinin-sensitive metabolic steps were identified.

T02-052

DORNRÖSCHEN/ESR1 is putatively involved auxin-regulated embryonic development and interacts with PHAVOLUTA.

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The DORNRÖSCHEN gene is a member of the ERF-type (Ethylene Response Factor), AP2-domain family of transcription factors and its overexpression affects many aspects of meristem development and influences lateral organ development in a pathway which is independent of STM, CLV and WUS (Kirch et al. The Plant Cell 15:694-705, 2003). In addition to the activation of these genes to activate and maintain meristem activity, organogenesis at the SAM is dependent on a prepatterning in auxin concentration which is established in the early embryo and modified by various auxin influx and efflux carriers throughout further development. Evidence is accumulating that DRN may be involved in auxin-regulated embryo development. Firstly, DRN is reported to be positively regulated by auxin. In addition, there are several putative auxin response elements in the promoter sequence and also, the phenotype of a drn knock-out mutant phenocopies that of several auxin transport mutants, with an alteration in cotyledon number and fusion of cotyledons at a low penetrance.

A Yeast Two Hybrid screen using the DRN-AP2 domain as a bait identified three potential protein interaction partners, one of which is PHAVOLUTA (PHV), which is involved in the establishment of leaf polarity (McConnell et al. (2001) Nature 411:709-713). Another partner is a bHLH protein and a third partner putatively involved in auxin signaling. In situ hybridisation for DRN and PHV reveals they have overlapping expression domains in the globular and early heart stage embryo. We have also performed split YFP expression studies which confirm the interaction in planta between DRN and PHV. Results from interaction studies for the other putative interaction partners in planta will be presented.

A paralogue of DRN exists in the Arabidopsis genome, DRN-like, with a similar knockout phenotype to that of drn, although it a distinct and non-overlapping expression pattern to that of drn in the embryo, and also contains putative auxin responsive elements in its promoter. We will elaborate the approaches we are taking to understand further the function of DRN and DRN-LIKE and to position them within the current model of auxin-regulated organogenesis.

T02-053

Homeodomain Interactions at the Shoot Apex

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The BELL class of homeodomain protein physically interact with KNOX1 proteins and this interaction allows transport of the BELL class to the nucleus in a mechanism similar to that uncovered in Drosophila between Homothorax and Extradenticle. This interaction is likely to have important role in meristem maintenance and this is described for the BLR/RPL/PNY BELL protein. A role in meristem partitioning is also discussed.

Bhatt, A.M., Etchells, J.P., Canales, C., Lagodienko, A. and Dickinson, H.G. (2004). Gene Vol. 328, pp 103-111.

T02-054

A family of single MYB domain proteins redundantly inhibits trichome initiation on the epidermis of shoot organs

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Trichome patterning on the Arabidopsis leaf epidermis is a unique model system to study de novo pattern formation. Though many components of the trichome selection mechanism were previously identified, we are still far from a comprehensive understanding of this process, which requires isolation and characterization of each component of the patterning machinery. The molecular characterization of the patterning genes revealed a suit of conserved genes that promote or inhibit trichome selection. Both trichome inhibitors, TRIPTYCHON and CAPRICE, encode single-MYB repeat proteins and they share redundant functions during trichome patterning. Here we show that two additional single-MYB domain genes, ETC1 and ETC2, also act as inhibitors of trichome initiation revealing a high degree of redundancy in the inhibition of the trichome cell fate.

T02-055

Vascular bundle differentiation in stems of the auxin mutants pin1, pinoid and monopteros

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Vascular bundle formation is a highly regulated developmental process, which requires that cells obtain positional information. Auxin has been proposed to direct vascular patterning by an increased flux of auxin in procambium cells (Sachs, 1981). In addition, it was proposed that auxin is involved in xylem formation, as well as in the regulation of cambium activity (Uggla et al., 1998). The multiple tasks for auxin require a strict control of its transport, as well as a highly regulated responsiveness of single cells to auxin. Several HD-ZIP class III transcription factors are expressed early in xylem differentiation and their expression may partially be regulated by auxin (Ohashi-Ito and Fukuda, 2003). Overexpression of the Arabidopsis HD-ZIPIII gene AtHB8 promoted xylem and interfascicular fiber differentiation (Baima et al., 2001). In order to investigate on the function of auxin and AtHB8 in vascular bundle formation, I have chosen the three auxin mutants pin1, monopteros (mp) and pinoid (pid). Although these three mutants have similar defects in organ formation at the inflorescence meristem, the formation of the vasculature varies considerably. Here I present a detailed analysis of vascular bundle anatomy in the stem of WT and the mutants at different developmental stages. In addition, gene expression of HD-ZIPIII transcription factors has been analysed. pin1, mp and pid were crossed with plants expressing AtHB8::GUS, and the expression pattern of AtHB8 was visualized in the mutants.

Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G (2001) The Arabidopsis ATHB-8 HD-Zip protein acts as a differentiation-promoting transcription factor of the vascular meristem. Plant Physiol. 126: 643-655
Ohashi-Ito K, Fukuda H (2003) HD-Zip III homeobox genes that include a novel member, ZeHB-13 (Zinnia)/ATHB-15 (Arabidopsis), are involved in procambium and xylem cell differentiation. Plant Cell Physiol 44: 1350-1358
Sachs T (1981) The control of patterned differentiation of vascular tissues. Adv. Bot. Res. 9: 151-262
Uggla C, Mellerowicz EJ, Sundberg B (1998) Indole-3-acetic acid controls cambial growth in scots pine by positional signalling. Plant Physiol. 117: 113-112

T02-056

Phenotypical analysis of the cytokinin receptor mutants ahk2, ahk3 and ahk4 unveils partially redundant functions in shoot and root development

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Cytokinins are regulating several aspects of plant development and physiology, e.g. cell division, shoot and root growth, chloroplast development, senescence and stress response. In Arabidopsis the cytokinin signal is percepted by the three cytokinin receptors AHK2, AHK3 and AHK4/CRE1/WOL. These receptors are histidine kinases, which show high similarity to histidine kinases of the bacterial two component system. So far the mutants wooden leg (wol) and cytokinin response1 (cre1) have been isolated, which are mutant alleles of AHK4. To elucidate the function of the other two histidine kinases in cytokinin signalling we have isolated ahk2 and ahk3 mutant lines. Phenotypical analyses of single and double mutants indicate partly redundant receptor functions in shoot and root development. The phenotype of the triple ahk2, ahk3, cre1/ahk4 mutant corroborates the crucial role of cytokinins in regulating organ growth.

T02-057

Genetic and biochemical evidence for the function of phospholipase A in auxin signal transduction

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The plant cytosolic form of phospholipase A (PLA) is the patatin-related PLA or iPLA2 (HOLK A, ET AL. (2002) PLANT PHYSIOL. 130, 90-101). Knockout lines for the PLA genes AtPLA I, AtPLA IVA, and AtPLA IVC were isolated and found to be damaged in typical auxin-related functions. The knockout for AtPLA I is defect in nutation, gravitropism and phototropism. Expression is found around the bundles in shoots, in the stele in roots, pollen and trichomes. A defect in (lateral) auxin transport is suggested by a slower bending reponse due to lateral auxin application in the knockout. The knockout for AtPLA IVA is defect in root growth under iron deficiency and low nitrate. Expression of the gene is found exclusively in roots. Iron deficiency and nitrate deficiency pathways use proably auxin-related functions/components. The third knockout line for gene AtPLA IVC shows a defect root growth under water stress or ABA treatment. The gene is up-regulated by ABA in the root and seems necessary for auxin-dependent root development in drought conditions. Constitutive expression is also found in the young gynaecium and flowers are female-defect in fertility. When plants, containing the auxin-activated DR5 promoter-GUS construct, were treated with 10 µM 2,4-D plus increasing concentrations of PLA inhibitors HELSS and ETYA, they blocked auxin-induced promoter activation, similarly as they inhibited auxin-induced elongation. This provides evidence that activation of PLA precedes auxin-induced gene activation and growth. Inhibitor studies with isolated enzyme indicate a post-translational activation mechanism. The iPLA-specific inhibitor HELSS binds covalently to the active center. Our studies with prrified enzymes point out that in the plant iPLA isoform AtPLA IVB the C-terminus covers the active center from binding HELSS (insensitive enzyme) whereas the enzyme is HELSS-sensitive when the C-terminus is deleted. The C-terminus may be mobile influencing thus the active center. In conclusion, AtPLA genes are involved in typical auxin-related functions as evidenced by insertinal mutants. Enzymatical studies indicate a position upstream of early auxin-induced genes in signal transduction.

T02-058

Mutations in the RETICULATA gene strongly modify internal architecture but not organ shape in vegetative leaves

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A number of mutants have been described in Arabidopsis thaliana whose leaf vascular network can be clearly distinguished as a green reticulation on a paler lamina. One of these reticulate mutants was named reticulata (re) by Redei in 1964 and has been used for years as a classical genetic marker for linkage analysis. We identified five novel recessive alleles of the RE gene, which we have characterised together with the original re-1 recessive mutation. The re alleles studied here are null or hypomorphic mutations causing a marked reduction in the density of mesophyll cells in interveinal leaf regions, which does not result from perturbed plastid development in specific cells but rather from a dramatic change in internal leaf architecture. Our results suggest that loss-of-function mutations in the RE gene specifically perturb mesophyll cell division in the early stages of leaf organogenesis. The morphology of vascular and mesophyll cells is apparently normal in re plants, but the density of the vascular network of their leaves is reduced, suggesting that a reduction in the proliferation of mesophyll cells during leaf development affects recruitment to vascular cell fate. It is interesting that the leaves of re mutants are of almost normal shape in spite of their extremely reduced mesophyll cell density, which suggests that the epidermis plays a major role in regulating leaf shape in Arabidopsis thaliana, whereas the correct development of the mesophyll tissue is more important in the control of leaf thickness. The RE gene was positionally cloned and found to be identical to the recently cloned LCD1 gene, which was identified based on the increased sensitivity to ozone and virulent Pseudomonas syringae caused by its mutant allele lcd1-1. The RE (LCD1) gene is ubiquitously expressed and encodes a protein of unknown function.

T02-059

ROT3 and ROT3 homolog, which fine-tune the biosynthesis of brassinosteroids in Arabidopsis, play critical roles in plant morphogenesis

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Brassinosteroids (BRs) are plant hormones that are essential for a wide range of developmental processes in plants. Many genes responsible for the early and later reactions in the biosynthesis of BRs have recently been identified. However, the genes for enzymes of several steps in the biosynthesis of BRs remain to be characterized, and none of the genes responsible for the reaction that produces bioactive BR have been identified. Here we found that the ROTUNDIFOLIA3 (ROT3) gene, which is involved in the specific regulation of leaf length in Arabidopsis, encodes the enzyme CYP90C1, which is required for the conversion of typhasterol to bioactive castasterone in BR biosynthesis. We also analyzed the gene most closely related to ROT3, ROT3 homolog/ CYP90D1, and found that double mutants for ROT3 and for ROT3 homolog have a synthetic dwarf phenotype, whereas cyp90d1 single knockout mutants do not, suggesting that these two cytochrome P450s act independently at different steps in BR biosynthesis. BR profiling in these mutants revealed that ROT3 homolog is also involved in the early steps of BR biosynthesis. ROT3 and ROT3 homolog were expressed differentially in leaves of Arabidopsis, and the mutants for these two genes differed in defects in elongation of hypocotyls under various light conditions. Dark induced the expression of ROT3 homolog, especially in leaf petiole. These results provide evidence that these two cytochrome P450s, ROT3 and ROT3 homolog, not only play critical roles in BR biosynthesis, but also connect BR biosynthesis to the responses of plants to light.

This research was supported by a grant (PF0330503-00) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government and by a grant from the KOSEF to the Environmental Biotechnology Research Center (R15-2003-002-01002-0).

T02-060

Identification of trichome specific promoter regions of GLABRA1 and TRIPTYCHON

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The spacing of trichomes in the leaf epidermis of Arabidopsis thaliana is a well studied epidermal patterning system, in which nearly all involved genes are identified.
The current model to explain de novo pattern formation is based on interdependent positive and negative regulators that can enhance small fluctuations to form a stable pattern. It is assumed that the relative concentration of the different factors determines the cell fate. As almost all patterning genes encode putative transcription factors it is conceivable that the regulation takes place on the transcriptional level.

To specify the transcriptional regulation we want to determine trichome specific regulatory elements which affect the localisation, the concentration and the timing.
We made promoter deletion constructs of the activator GLABRA1, a myb R2R3 transcription factor, and the inhibitor TRIPTYCHON, a myb R3 transcription factor. Both genes show the same expression pattern with strong expression in trichomes.
We identified for both regulatory regions, which are necessary for trichome specific transcription. In the case of GL1 it could be shown that one region is sufficient to rescue the gl1 mutant background, whereas the other is not. For the TRY fragment the improvement of the sufficiency is still in work. A fine mapping and functional analysis of the relevant fragments will be presented.

T02-061

Non-cell autonomous action of TTG1 during trichome pattern formation

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The generation of the trichome spacing pattern on the leaf surface of Arabidopsis thaliana is based on a gene cassette of the positive factors GLABRA1, TRANSPARENT TESTA GLABRA1 (TTG1), GLABRA3, ENHANCER OF GLABRA3 and the negative factors TRIPTYCHON and CAPRICE. TTG1 appears to be involved in lateral inhibition as weak mutants exhibit clusters of trichomes. As lateral inhibition is likely to involve non-cell autonomous action, we studied whether TTG1 functions in a non-autonomous manner and whether this is relevant for trichome patterning.
Cre-Lox experiments show that TTG1 acts non-cell autonomously and in addition we can show that a TTG1-YFP fusion is able to move between cells. Further experiments suggest that this non-cell autonomous action is relevant for the trichome patterning process.

T02-062

Cytokinin Regulated Transcription Factors

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Recent work in understanding the role of the plant hormone cytokinin in growth in development has focused on the aspects of the cytokinin signaling pathway, including the receptors (AHKs) and type-A and type-B cytokinin response regulators. In order to identify novel cytokinin responsive genes, we conducted global expression analyses on seedlings after application of exogenous cytokinin for various times. From this study we identified two highly related AP2-like transcription factor genes that were induced by exogenous cytokinin application at several time points over 24 hours. Both of these genes are members of the ethylene response factor (ERF) subgroup of the AP2 family of genes and reside in the same clade of eight genes within the ERF subgroup. No function has yet been ascribed to any of the genes in this clade. We have begun characterization of the members of this clade, including the kinetics of cytokinin induction of all 8 members. We have characterized leaf and root phenotypes of single and multiple knockout mutants for several members of this clade under standard conditions and in response to cytokinin. Additionally, over-expression of one of these cytokinin responsive transcription factors suggests a link to members of the cytokinin signaling pathway. This poster will discuss the work to date on these cytokinin responsive transcription factors.

T02-063

The cell-autonomous ANGUSTIFOLIA-gene regulates organ size and form in a non-cell-autonomous way

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The ANGUSTIFOLIA-mutant plants have two striking phenotypes. First, an-mutant plants have underbranched trichomes and less-lobed pavement cells. Second, the leaf width is reduced due to a reduced cell number.
The analysis of periclinal chimera has shown previously that in particular the L2 layer determines the leaf shape. In this study we used ANGUSTIFOLIA as a tool to assess how cell shape and number in different tissues contributes to organ shape. We expressed AN under a L1- (AtML1) and a L2- (pPCAL) specific promoter in an-mutant plants. Expression of AN in the epidermis rescued the epidermal phenotypes. Leaf shape was mildly rescued. Sub-epidermal expression of AN resulted in a recue of leaf shape but not the epidermal morphogenesis phenotype.
Our results suggest that sub-epidermal driven leaf expansion is compensated by the leaf epidermis through increased cell division.

T02-064

Totipotency of pericycle cells in Arabidopsis thaliana root and hypocotyl explants for both root and shoot regeneration

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The capacity of Arabidopsis root and hypocotyls explants to regenerate new shoot apical meristems (SAMs) in tissue culture has been analysed using the two-step method(1) which gives rise to indirect shoot regeneration. Looking precisely at the successive events of the dedifferentiation that occurs during the first 5 days on the callus-inducing medium (CIM) then following the subculture of the shoot-inducing medium (SIM), it was shown that the structures previously named "calluses" or CIM(2) are in fact root-like structures arising from the pericycle. All pericycle cells enter dedifferentiation on CIM, giving rise either to root-like meristems behind the xylem poles or to rings of pericycle-derived cells dividing periclinally. Such active divisions of internal cells lead to exfoliation of external cells. SAM regeneration that occurs 7 to 9 days after transfer on SIM was detected at various locations, either from the superficial pericycle derivatives or from superficial cells located at the base of root-like structures. More surprisingly, some regenerated SAMs originate at the tip of the root-like structures. This last origin suggested a redetermination of root-apical meristem cells into SAM cells. All the SAM origins resulting more or less directly from pericycle dedifferentiation, it could be suggested that pericycle in Arabidopsis is a potential source of both root and shoot stem cells as previously found in Rorippa sylvestris, another member of the Crucifereae family(3). The kinetics of expression of a range of marker genes involved in SAM identity (WUS, CLV1, CLV3, CUC1, STM, KNAT2) as well as in mitotic activity (CYC B) or endogenous auxin (DR5::GUS) or cytokinins (ARR5::GUS) was established. The timing of expression of these genes was not similar to what was reported during zygotic embryogenesis when embryonic SAM cells take place. In addition, SAM specific genes were not found to be solely expressed in the morphogenetic field involved in shoot regeneration suggesting that their expression could be controlled by local changes in endogenous hormones and cell cycling activity.

(1) Valvekens D et al., 1988 ¯ P.N.A.S.
(2) Cary AJ et al., 2002 - Plant J.
(3) Projetti ML et al., 1986 ¯ Can. J. Bot

T02-065

Role of CHAYOTE in root hair development and epidermal cell patterning

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The Brassicaceae root epidermis has been used as a simple model to understand how cells become specified in multicellular organisms. The Arabidopsis root epidermis has two kinds of cells: trichoblasts, located over the intercellular space between two underlying cortical cells and atrichoblasts located over a single cortical cell (Dolan et al., 1993). Root hairs are cylindrical outgrowths from trichoblasts and exhibit a form of polarised growth called tip growth.

We isolated chayote (cht) from a EMS mutagenised population in a screen for root hair defective plants. cht root-hairs are longer than wild-type and have a wavy appearance, implicating CHT in root hair elongation and morphogenesis. In addition, hair density is higher in cht roots and suggests that CHT plays a role in epidermal cell patterning. More detailed phenotypic analysis identified an alteration in the quiescent centre (QC; slowly-dividing cells controlling root meristem organisation). CHT mutants display a higher frequency of division in these cells leading to disorganisation of cells in the meristem after several days.

Dolan, L., K. Janmaat, et al. (1993). "Cellular organisation of the Arabidopsis thaliana root."Development 119(1): 71-84

T02-066

GONZO1 regulates leaf polarity in Arabidopsis

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Lateral organs arise from the shoot and floral apical meristem and exhibit an adaxial/abaxial polarity. E2023 is an enhancer trap line that expresses GFP preferentially in the abaxial tissue of the hypocotyl and stem. The enhancer trap is inserted in the promoter of a transferase (GONZO1) and results in a reduction in the expression of this gene. Plants homozygous for the E2023 insertion and kan1 resemble kan1kan2 mutants. Our results indicate that GONZO1 promotes abaxial identity, perhaps by directly or indirectly regulating members of the KANADI gene family.

T02-067

TYPE-B RESPONSE REGULATORS FUNCTIONALLY OVERLAP IN THE REGULATION OF CYTOKININ RESPONSES

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Two-component signaling systems involve histidine kinases, histidine-containing phosphotransfer proteins, and response regulators, and have been implicated in plant responses to hormones and environmental factors. Genomic analysis of Arabidopsis thaliana supports the existence of 22 response regulators (ARRs) that can be divided into at least two distinct groups designated type-A and type-B. Phylogenetic analysis indicates that the type-B family is composed of one major and two minor subfamilies. The expression of the type-B ARRs was examined by using both RT-PCR and GUS fusion constructs. The major subfamily of type-B ARRs showed particularly high expression in regions where cytokinins play a major role, including cells near the apical meristem and in young leaves that would be undergoing cell division. Type-B ARRs were also found near the root tip with highest expression in the root elongation zone. Based upon the analysis of T-DNA insertion mutant lines, we found that the members of the major subfamily of type-B ARRs not only show similarities in expression but also appear to functionally overlap. Of the single mutants, only arr1 plants displayed clearly reduced cytokinin sensitivity. Progressively greater insensitivity to cytokinin was observed in higher order mutants. Our data support a role for multiple type-B ARRs in modulating cytokinin responses with ARR1 playing the most significant role.

T02-068

Analysis of TRANSPARENT TESTA GLABRA2 involved in trichome differentiation

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Trichomes and root hairs are specialized cells differentiated from the epidermis. Several genes have been identified as regulators of epidermal cell differentiation, playing a role in the development of these special cells. The Arabidopsis TRANSPARENT TESTA GLABRA2 (TTG2) gene encodes a WRKY transcription factor. The ttg2 mutant has fewer trichomes than the wild type, its trichomes are less branched, and it has defects in tannin and mucilage production in its seed coat. TTG2 is expressed in leaf primordia, trichomes, seed coats, and hairless cells of developing roots. It is proposed that TTG2 functions downstream of TRANSPARENT TESTA GLABRA1 (TTG1) and GLABRA1 (GL1), and shares functions with GLABRA2 (GL2) in trichome development (Johnson et al., 2002).

To further elucidate the relationship between TTG2 and other genes that regulate epidermis differentiation, we analyzed TTG2 expression in various trichome and root hair mutants. We found that TTG2 expression in roots was suppressed in the werewolf-1 (wer-1) mutant, and that TTG2 is ectopically expressed in root-hair cells in the caprice-1 (cpc-1) mutant. These results suggest that TTG2 is positively regulated by WER and negatively regulated by CPC. WER encodes a MYB protein homologous to GL1, and negatively regulates root-hair cell differentiation. CPC encodes a small MYB protein and promotes root-hair cell differentiation. There are several putative Myb binding sites in the TTG2 promoter. We are analyzing transgenic plants carrying a deletion series of TTG2 promoter::GUS to determine whether or not Myb proteins directly regulate TTG2.

We also analyzed phenotypes of double mutants of ttg2-1 which had some trichome mutations. In the glabra3-2 (gl3-2) mutant, the number of trichomes is reduced, and its trichomes are less branched than those of the wild type. In the ttg2-1 gl3-2 double mutant, the number of trichomes was much reduced, and trichomes were detected as small pointed outgrowths. In the triptychon-82 (try-82) mutant, trichomes are often clustered and more branched than those of the wild type. In the ttg2-1 try-82 double mutant, the number of trichomes was intermediate between those of its parental mutants, and its trichomes were similar to those of the ttg2-1 mutant. We will discuss the genetic interaction of TTG2 with other regulators of epidermal cell differentiation.

Johnson, C. S. et al. (2002) Plant Cell 14: 1359-1375

T02-069

Arabidopsis BROS is involved in cell expansion-related organ development

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The final size of later organs in plant is determined by coordinate cell division and expansion during organogenesis. We have previously reported that the Arabidopsis ARGOS gene transduces auxin signal to regulate the duration of cell proliferation, thereby affecting organ size. Here, we show that another Arabidopsis putative gene, BROS, is involved in organ size control. Reduced- or over-expression of BROS in Arabidopsis results in enlarged or smaller cotyledons and leaves, as well as other lateral organs, respectively. Histological analysis indicates that this alteration is not caused by changes in organ cell number, but by cell size. BROS is expressed highly in cotyledon but at a moderate level in roots, expanding leaves and flowers, and is induced by brassinosteroid, suggesting that it may mediate brassinosteroid-related cell expansion during organ development.

T02-070

Quantitative Trait Loci for Root Architecture in Arabidopsis

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The architecture of a plant root system is influenced by a multitude of factors including light, gravity, nutrient gradients and proximity to symbiotic or pathogenic organisms. The primary root is partly formed in the embryo. After germination, the primary root grows and post-embryonic lateral roots initiate and elongate. The remarkable plasticity of root architecture points to a unique biological system underscored by complex genetics.

Isolates of different lines of Arabidopsis thaliana show different root system architecture. Segregation of the alleles has been analysed in recombinant inbred progeny lines. This data has given information on the degree of genetic complexity of root architecture.

T02-071

Diverse activities of Mei2-like RNA binding protein genes

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We describe an unusual class of RNA binding genes, termed mei2-like, that may function to regulate cell fate in plants. Named for their similarity to the Mei2 gene of Schizosaccharomyces pombe (1), these genes share 3 RNA recognition motifs (RRMs), with the third RRM providing the hallmark of the family (2). Analyses of transcript accumulation for plant mei2-like genes show diverse tissue specific patterns with one subfamily, termed TEL for terminal ear-like, showing expression in both the shoot and root apical meristems from early embryogenesis onward. During vegetative development, TEL2 transcripts accumulate over the central zone of the SAM while TEL1 transcripts show a broader, more patchy distribution over the SAM. In the RAM, TEL1 transcripts are concentrated over the QC and stele initials, but not root cap, epidermis, or cortex/endodermis initials. In developing inflorescences, TEL2 expression shifts from a central position to patches that correspond to anlagen of axillary branches. We have begun an analysis of UTR sequences to gain insight into the basis for these intriguing expression patterns.

While the expression of TEL genes in both the root and shoot apical meristems might suggest an activity related to the function or maintenance of stem cells, our analysis of loss of function phenotypes for these genes suggest only subtle phenotypes. This contrasts with the phenotypes observed for loss of function mutations to maize orthologue, terminal ear 1, (3), which involve more frequent and abnormally positioned leaf initiation events. To address the possibility of genetic redundancy, Arabidopsis plants carrying multiple knockouts to mei2-like genes are being analysed. A second strategy utilises the LhG4 system to ectopically express TEL genes in a variety of different patterns. Results of these experiments are discussed in relation to models in which TEL genes act to inhibit cells from entering terminal differentiation pathways.

1)Watanabe and Yamamoto(1994).Cell 78:487-98
2)Alvarez et al PMB (in press).
3)Veit, B. et al(1998). Nature 393:166-8

T02-072

An Arabidopsis dwf8 mutant displays pleiotropic phenotypes that may or may not be associated with typical brassinosteroid dwarf mutants

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Brassinosteroids (BRs) collectively refer to the growth-promoting plant steroids. Isolation and characterization of Arabidopsis dwarf mutants have been instrumental in characterization of BR biosynthetic enzymes and further validation of the biosynthetic pathways. However, currently all the genuine BR dwarfs from Arabidopsis seem to be comprehensively isolated and characterized . Interestingly, a dwf8 mutant that was identified by the Arabidopsis Biological Resource Center shows pleiotropic phenotypes that may or may not be associated with a typical BR dwarf mutant. Similar to BR mutants, dwf8 shows round and curled leaves, reduced fertility, and slightly altered endogenous BR levels. However, differently from BR dwarfs, roots are much shorter and thicker, and have many more root hairs than those of a wild type and typical BR dwarf mutants. In addition, the vascular pattern in an inflorescence is in relatively good order, whereas BR mutants display irregular positioning and underdeveloped phloem tissues. We are performing a map-based cloning experiment to clone the DWF8 gene. We have tested 389 mapping lines, and found that dwf8 is located on the bottom of Chromosome 1, close to dwf5. Results from the cloned gene and various physiological, biochemical, and anatomical analyses would make it clear how the DWF8 gene plays a role in BR pathways.

T02-073

RHD6-like transcription factors involved in root hair development

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The Arabidopsis root epidermis is a well-characterised model system for analysing cellular differentiation using genetic approaches. It is composed of two cell types: trichoblasts that produce root hairs by polarised outgrowth, and atrichoblasts that do not produce hairs. Once trichoblast/atrichoblast cell fate has been determined under the control of transcriptions factors like WER, GL2 and CPC, another point of regulation occurs at the initiation of root hair growth controlled by RHD6. The rhd6-1 mutant shows signs of trichoblast differentiation but produces almost no root hairs.
A screen of enhancer trap lines for root hair phenotypes resulted in the identification of a new rhd6 allele. RHD6 was cloned and encodes a member of the basic-Helix-loop-Helix (bHLH) transcription factor family. The gene is expressed specifically in trichoblast cells. Five other rhd6 alleles with similar phenotypes have been isolated.
A phenotypic analysis of available mutants for the five closest homologues of RHD6 has been undertaken. This revealed that at least one homologue is also involved in root hair development because mutants have shorter hairs than wild type. Expression analysis shows that 4 of the homologues are specifically expressed in roots and that 3 of them are strongly downregulated in the rhd6-1 mutant. This indicates that they are positively controlled by RHD6.
The results suggest that the RHD6 subfamily of transcription factors form a network to regulate the initiation of root hair growth.

T02-074

Isolation and characterization of gulliver mutants that are defective in the light and brassinosteroid signaling pathways

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Brassinosteroids are plant steroidal hormones that play essential roles during plant growth and development. Although understanding of brassinosteroid signaling pathway has been greatly improved via isolation and characterization of the signaling mutants such as bri1, bzr1, bes1, dwf12/bin2, and bsu1, signaling cascade still harbors the questions needed to be answered. In order to uncover new components in brassinosteroid signaling pathways, we used brassinazole (Brz) as a screening reagent: Brz is a brassinosteroid biosynthetic inhibitor that had been successively employed to isolate bzr1 and bes1 mutants. We have identified three loci from either EMS mutagenized or T-DNA activation population, and named gulliver mutants since they all display the characteristic phenotypes of elongated hypocotyls and petioles. Since gulliver mutations suppress the bri1-5 mutant phenotype as revealed by double mutant analysis, these mutants are believed to be associated with brassinosteroid signaling pathway. Interestingly, gul2 respond abnormally to red light but not to the far-red and blue light, however, gul3 and gul4 respond normally to the all light regimes tested. In addition, gul3 seems to be involved in feedback regulation of the brassinosteroid biosynthesis at the transcriptional level by altering the expression level of the brassinosteroid biosynthetic enzyme such as DWARF4. Here we report the progress of the map-based cloning of the gulliver mutants and discuss their possible roles in plant growth and development.

T02-075

Large-scale analysis of nuclear-encoded chloroplast proteins using Ac/Ds transposon system in Arabidopsis.

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Only 100 plastid proteins are encoded on the plastid genome. Most of plastid proteins are encoded by the nuclear genome, synthesized as precursors in the cytosol, and then transported to the proper regions for their functions within chloroplasts. Plastid proteins can be identified by computational prediction of the N-terminal presequences (chloroplast transit peptides, cTPs) of their cytoplasmic precursor proteins (Richly and Leister 2004). About 2,100 plastid proteins with a cTP are predicted to be encoded by nuclear genomes in Arabidopsis thaliana.
To study function of nuclear genes involved in chloroplast development and photosynthesis, we have started to analyze their functions using Ds-tagged lines. We took two approaches as follows.
(1) To determine essential nuclear-encoded genes for chloroplast development, we have screened 9425 Ds-tagged lines to isolate 38 mutants with albino or pale green (apg) phenotypes. Identified APG genes have sequence homology with housekeeping proteins involved in photosynthesis, translation, transcription, translocation and so on.
(2) To screen mutants with wild-type phenotype but defective in photosystem, we isolated a Ds-tagged mutant showing different fluorescence kinetics from that of wild type using chlorophyll fluorescence monitoring system.

T02-076

STRUBBELIG defines a novel receptor-mediated signaling pathway regulating meristem development in Arabidopsis

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Above-ground plant organs originate postembryonically from the shoot apical meristem. It remains an open question how meristem size is regulated. Maintenance of the apical meristem depends on the balanced development of centrally-located, self-renewing stem cells, and the peripheral zone, from where lateral organs initiate. In Arabidopsis, control of the stem cell population includes a feedback loop involving the negative regulation of the stem cell identity gene WUSCHEL (WUS) by the CLAVATA (CLV) signaling pathway. We provide evidence that STRUBBELIG (SUB) defines a novel receptr-mediated signaling pathway directly regulating meristem size. The sub mutant phenotype suggests that SUB plays a positive and negative role in this process. SUB seems to affect particularly the peripheral zone. SUB encodes a putative receptor kinase with a possibly inactive kinase domain. Genetic analysis suggests that SUB and CLV belong to different pathways. Our data indicate that fine-tuning meristem size involves a transmembrane-receptor-mediated signaling mechanism, which acts in a bimodal fashion.

T02-077

Axillary bud growth: one pathway or many?

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The maintenance of meristems that are able to control the production of new organs throughout the plant's life cycle, enables the prevailing environment and physiological status of the plant to be integrated into the developmental program. This is an important adaptation to the sessile habit.

Axillary meristems are formed within the leaf axils of plants. Tissue may eiter immediately forms a axillary shoot or form a few leaves before arresting, as a bud, until conditions are optimal for outgrowth. The control of axillary bud growth is a useful model for investigation of how plants integrate signals because various classes of inputs have been implicated in its regulation. The branching process is environmentally responsive, nutrient deprivation and crowding both causing a reduction in bud out growth. A large body of physiological studies implicate plant hormones in regulating axillary bud growth. An apical auxin signal acting remotely inhibits bud outgrowth, which is moderated by basally applied cytokinin and abscisic acid1,2. Abscisic acid inhibits bud growth and cytokinin promotes it. Novel mutants showing aberrant branching patterns have been identified. In Arabidopsis the More AXillary branching mutants(max1 ¯ max4) show increased lateral branching relative to wildtype3,4. Grafting experiments suggest that MAX1, MAX3 and MAX4 work in a pathway that produces of a graft transmissible substance that is synthesised throughout the plant axis.

While all these stimuli have been implicated in moderating axillary bud outgrowth the level of integration between these pathways is unknown. Do all these stimuli act on a common pathway to moderate bud out growth or do they act independently? In Arabidopsis clear morphological and molecular defininitions of bud out growth exist. This makes it is possible to link what is happening locally within the bud to the physiological environmental and genetic influences within the plant. We are using these definitions to investigate signal intergration in the control of bud outgrowth; specifically how the max mutants genetically interact, how the MAX genes relate to the auxin signalling pathway and how the mutants respond when environmentally stressed.

1 The Plant Journal 24:159-169
2 Plant Cell 15:495-507
3 Development 129:1131-1141
4 Genes and Development 17:1469-1474

T02-078

Discrete heterodimers direct nuclear import of the transcription factor SHOOT MERISTEMLESS in the shoot apical meristem of Arabidopsis

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The shoot apical meristem (SAM) of angiosperm plants gives rise to new leaves and stem in a predictable and regular pattern. The SAM arises during embryogenesis and is a highly organized group of cells divided into morphologically distinct domains with different functions. The central zone at the tip of the SAM provides a permanent source of stem cells. This group of cells is surrounded by the so-called peripheral zone, where new primordia continuously are generated. Although the meristem itself is extremely stable, its component cells are dividing, expanding, and differentiating. The Arabidopsis SHOOT MERISTEMLESS (STM) gene is required for the initiation and maintenance of the SAM. The STM gene encodes a Knotted-like homeodomain containing protein (Long et al., 1996). Transcriptional activity is confined to meristematic cells and STM is downregulated in founder cells (P0) of lateral organ primordia.

To elucidate the biological function of STM we isolated interacting protein partners in a yeast-two-hybrid screen. Among several transcription factors and transcriptional coregulators, 3 BLH (BelI-Like-Homeodomain) proteins and one ARF (Auxin-Response-Factor) were identified. Whereas the BLH proteins interact with both STM and the closely related KNAT1 protein, the ARF interaction discriminates between STM and KNAT1. All four putative interaction partner genes are transcribed in the SAM and the BLH expression domains mark different zones of the SAM. By use of the split-YFP system (collaboration with Klaus Harter) we have shown that the BLH or ARF proteins interact with the STM gene product in plant cells. Only interaction with BLH proteins targets the STM gene product into the plant cell nucleus, whereas STM/ARF heterodimers remain cytoplasmatic. Our results suggest that the formation of different heterodimers in distinct zones of the SAM targets the STM protein into the plant cell nucleus, which may be relevant to understand STM functions.

Long et al.(1996) Nature 379:66-9.

T02-079

Biological function studies of RHD6-like transcription factors involved in root hair development

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The specification of root hairs in Arabidopsis provides a useful model for the study of pattern determination in plants. Root-hair cell distribution arises in a position-dependant pattern over the inter-cellular space between underlying cortical cells. We are investigating the molecular basis of root-hair cell differentiation.
We have undertaken a genetic approach to investigate the molecular mechanisms of epidermal specification, patterning and cell morphogenesis. Previous studies suggest that a complex of three transcription factors, WEREWOLF (WER), GLABRA3 (GL3) and TRANSPARENT TESTA GLABRA (TTG), is required for non-hair fate whereas hair fate is induced by the action of CAPRICE (CPC) that inhibits the WER/GL3/TTG complex.
We characterized ROOT HAIR DEFECTIVE 6 (RHD6), a new basic helix-loop-helix (bHLH) transcription factor. RHD6 is expressed in the cells undergoing hair growth initiation and the rhd6 mutants are root hair less or produce only few normal hair-cells. This suggests that RHD6 is necessary for root hair growth initiation.
The first aim of my research is to perform expression analysis, two hybrids studies and subcellular localization experiments to understand the biological functions of RHD6 in root hair development and particularly to determine how RHD6 could interact with the WER/GL3/TTG complex. In addition, I am also investigating the implication of five other RHD6-like transcription factors in Arabidopsis root development.

T02-080

Genetic analysis of regulators of axillary meristem initiation

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The primary axis of growth in plants arises from the primary shoot apical meristem (SAM) which is formed during embryonic development. However, the overall aerial architecture of a plant is established by formation of secondary axes of growth that are initiated post-embryonically by new meristems formed in the axils of all leaves. These are called axillary meristems. Several mutants showing defective axillary meristem formation have been identified in different plant species. The tomato lateral suppressor (ls) mutant and its Arabidopsis ortholog (las) are characterized by non-initiation of axillary meristems during the vegetative phase of development. The pattern of LAS transcript accumulation in the meristems corresponds to its mutant phenotype. We have analysed the role of Arabidopsis LAS in axillary meristem initiation and searched for new genes involved in this process. EMS mutagenesis has been carried out on las-4 to look for modifiers, and suppressors and enhancers of the mutant phenotype have been identified. The number of side shoots formed in the suppressors of las-4 (sol) show a partial restoration of the wild type phenotype. The enhancers of las-4 (eol) display a reduction in the number of side shoots arising from the axils of cauline leaves. sol- and eol mutants are currently being characterized.

1. Greb,T et al, Genes Dev. 2003
2. Schumacher,K et al, Proc Natl Acad Sci USA. 1999

T02-081

FEZ and SMB encode two-plant specific transcription factors required for the orientation of cell division and cell specification in the Arabidopsis root cap.

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The root cap at the distal end of the Arabidopsis root is composed of continuous tissue layers of two cell populations with distinct clonal origin, the columella root cap and the lateral root cap (LRC). Post-embryonically, anticlinal divisions of the columella stem cells (initials) maintain the columella. The LRC is derived from periclinal divisions of a collar of cells located in a ring around the columella stem cells, called epidermal initials (Scheres B. et al, 1994, Development 120: 2475).
To identify genes involved in patterning the root cap, a large-scale genetic screen was performed for changes in the expression of two different root cap specific enhancer traps, J1092 (GFP, www.plantsci.cam.ac.uk/Haseloff/gene_expression/geneExpFrameset.html) and LRC244 (GUS, J.E. Malamy and P.N. Benfey, Development 124, 33-44(1997)).
Mutations in two genes, FEZ and SOMBRERO (SMB), were isolated. The fez and smb mutants have specific defects in lateral root cap specification but influence stem-cell specific divisions in opposite ways. Both genes were cloned using a map-based approach and encode two related members from the same family of putative transcription factors containing a highly conserved domain in their N-terminal region. Consistent with their mutant phenotype, both genes are specifically expressed in the root cap stem cell domain.

T02-082

VND7, a NAC-domain protein regulates xylem vessel formation in Arabidopsis

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Xylem vessels comprised of tracheary elements (TEs) transfer water, nutrients, and also signal molecules through body of plants. To investigate the genes required for vascular formation, we have identified genes expressed in association with in vitro TE transdifferentiation of Zinnia using cDNA microarray. The expression of Z567 encoding a NAC-domain protein was up-regulated prior to TE differentiation. We searched Arabidopsis genome for genes with sequence similarity to Z567 and identified 7 genes that were designated as VASCULAR RELATED NAC-DOMAIN PROTEIN 1 to 7 (VND1 to VND7). By promoter analysis, the VND genes were shown to be expressed in vascular cells. The VND1 to VND4 were expressed mainly in procambial cells and VND5 and VND7 were specifically expressed in developing immature vessels. To elucidate the VND function, we observed the phenotypes of VND overexpression (ox) lines. Although VND1ox to VND5ox lines did not show any significant change compared to wild-type, VND7ox line showed ectopic xylem vessel formation in non-vascular cells, such as epidermis of hypocotyls and cortex of roots. On the other hand, dominant repression of VND7 function caused inhibition or delay of protoxylem vessel formation in roots. These results suggest that VND7 acts as a positive regulator and is needed for xylem vessel formation.

T02-083

Isolation and characterization of the genes interacting with VND7 (Vascular-related NAC Domain Protein 7)

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We have isolated a number of cDNAs of Zinnia elegans that are expressed in association with in vitro transdifferentiation from photosynthetic mesophyll cells to xylem cells. One of these clones, termed Z567, which encodes an ORF containing NAC domain, transiently expresses prior to the formation of visible characteristic secondary cell wall structure. NAC gene family encodes plant specific transcription factors and contains 90 genes in Arabidopsis genome. We designated 7 genes closely related to Z567 as VND (Vascular-related NAC Domain Protein) 1 to 7. Surprisingly, overexpression of VND7 ectopically induces xylem cells on the various tissues, clearly indicating that VND7 plays a pivotal role in promoting xylem differentiation. To understand how VND7 regulates xylem differentiation, we tried to isolate VND7-interacting factors, and characterize their functions. There has been reported that several NAC proteins can make a homo-dimer or hetero-dimer with other NAC via each NAC domain. So, we analyzed whether VND7 can interact with VND7 itself and/or the other VND proteins by using yeast two-hybrid system. The yeast cells in which the GAL4-BD-fused VND7 and the GAL4-AD-fused VND1, 2, 3 or 7 were introduced, respectively, did express the reporter genes. By contrast, the yeast cells in which VND7 and NAC1 are co-transformed, could not show the transcriptional activity. These data suggest that VND7 can make dimers with VND family but not with all NAC family. We also isolated a few cDNA clones interacting with VND7 by yeast two-hybrid screening. Now, efforts are underway to elucidate their expression pattern in the various organs and their functions.

T02-084

Identifying Targets of Inducible PLETHORA Root Identity Genes

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In plants, patterning and differentiation initiate in the embryo and continue in the meristems throughout lifetimes that can span hundreds of years. The main-tenance of these functional meristems requires delicate coordination between the loss of stem cells through differentiation and the replacement of these cells through division. In the Arabidopsis root, stem cells are maintained in a niche by a mitotically inactive organizing center. This so-called quiescent center (QC) con-tacts the stem cells of all root tissues patterned in the radial and apical/basal di-mension, and controls their differentiation status. Preservation of the QC requires input from several independently acting transcription factors, among which the PLETHORA (PLT) genes.
The redundantly acting PLT1 and PLT2 genes belong to a class of AP2-type putative transcription factors. Both plt1 and plt2 single mutants display a slight but significant reduction in root growth as well as in the number of meris-tematic cells. The plt1 plt2 double mutant phenotype consists of a rapidly differen-tiating root meristem. In addition, marker analysis in plt1 plt2 double mutants and PLT overexpressors show that the PLT genes are essential and sufficient for root development, QC specification and stem cell maintenance. To identify down-stream PLT targets we generated transgenic plt1 plt2 double mutant lines harbor-ing a steroid-inducible form of PLT2 under the control of its own promoter. Induc-tion of PLT2 activity rescues the double mutant phenotype resulting in a mutant phenotype. The immediate transcription response monitored on a microarray will be presented.

T02-085

The HEMIVENATA gene encodes a TIP120 (CAND1) protein and is required for venation pattern formation

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The vascular systems of multicellular organisms define a family of three-dimensional, hierarchized tree-like branched biological structures, which also includes the nervous and respiratory systems of many animal species. The generative basis of such characteristic topologies remains to be explained in spite of efforts made to propose theoretical models and to identify the genes governing the patterning mechanisms underlying their morphogenesis. The venation pattern of insect wings and plant leaves provide two-dimensional, simple models for dissecting such cellular and molecular processes. In a search for natural variations in Arabidopsis thaliana leaf vein pattern, we have already identified the spontaneous hemivenata-1 (hve-1) recessive allele, which causes an extremely simple venation in leaves and cotyledons. Here, we report the positional cloning of the HEMIVENATA (HVE) gene, which was found to encode a CAND1 (cullin-associated and neddylation-dissociated, also named TIP120, for TATA-binding protein-interacting protein 120) protein. The hve-1 recessive mutation causes missplicing of the HVE transcripts, and its associated phenotype is indistinguishable from that of other two putatively null alleles, hve-2 and hve-3, which have been found in a publicly available library of T-DNA insertions. Our results indicate that HVE is involved in a developmental pathway dependent upon auxin signalling, and suggest that an early defect in the venation patterning mechanism in hve/hve leaves leads to a decrease in the number of cells recruited for a vascular fate. In addition, our results strongly suggest that in Arabidopsis thaliana, as previously shown in human cells, the CAND1 (HVE) protein regulates the assembly of SCF complexes by sequestering the CUL1 protein.

T02-086

Mutual antagonism between SHOOT MERISTEMLESS (STM) and (YABBY3)

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The shoot apical meristem provides continuously new cells to be recruited for organ formation. In the meristem, SHOOT MERISTEMLESS (STM) plays an important role in maintaining these cells undifferentiated. We have previously reported that a steroid-inducible fusion between STM and the rat glucocorticoid receptor (STM-GR) caused ectopic activation of meristem marker genes (Gallois, Woodward et al. 2002). To identify additional genes controlled by STM, we screened for changes in gene expression after activation of STM-GR, using a cDNA array with probes for approximately 1200 Arabidopsis transcription factors. Gene expression was monitored in the aerial part of seedlings, four or eight hours after treatment with dexamethasone (compared with mock-treated controls). From these experiments, YABBY3 (YAB3) appeared to be repressed by STMGR. This was consistent with the expression pattern of YAB3, which is activated in leaf primordia, coincident with down-regulation of STM (Siegfried, Eshed et al. 1999). In addition, YAB3 is known to act directly or indirectly down regulate meristematic genes during lateral organ development (Kumaran, Bowman et al. 2002), suggesting that YAB3 and STM could be mutually antagonistic. Northern analysis showed that rather than simply reducing mRNA levels, STM-GR induced cleavage of YAB3 mRNA. Site-specific cleavage was confirmed by 5' RACE. We are currently investigating whether STM-GR-induced cleavage of YAB3 is mediated by microRNAs.

Gallois, J. L., C. Woodward, et al. (2002). "Combined SHOOT MERISTEMLESS and WUSCHEL trigger ectopic organogenesis in Arabidopsis." Development 129(13): 3207-17.
Kumaran, M. K., J. L. Bowman, et al. (2002). "YABBY Polarity Genes Mediate the Repression of KNOX Homeobox Genes in Arabidopsis." Plant Cell 14(11): 2761-2770.
Siegfried, K. R., Y. Eshed, et al. (1999). "Members of the YABBY gene family specify abaxial cell fate in Arabidopsis." Development 126(18): 4117-4128.

T02-087

Regulation of LATERAL SUPPRESSOR ¯ a gene involved in the formation of axillary meristems

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The architecture of plants is determined by the number, arrangement and growth intensity of their sideshoots. Sideshoots are initiated by the formation of axillary meristems in the axils of leaves. We are interested to understand at the molecular level the mechanisms which control the formation of axillary meristems.

The las-4 mutant of Arabidopsis fails to initiate axillary meristems during vegetative development, whereas in the reproductive phase sideshoots are formed in the axils of cauline leaves. The LAS gene belongs to the GRAS gene family and encodes a putative transcription factor. RNA in situ hybridization experiments have demonstrated that LAS is expressed in the axils of all primordia originating from the SAM. The aim of our work is to identify and characterize upstream regulators that delimit the LAS expression domain.

To understand the control of LAS expression we are analyzing the LAS promoter. Complementation experiments with deletion constructs indicated that at least 820 bp upstream of the ATG are necessary for a restoration of the wildtype phenotype. In addition, T-DNA insertion lines were characterized to identify important regulatory elements in the promoter region. Currently these cis regulatory elements are being used to screen for trans acting factors .

Greb T. et al., (2003) Genes Dev.;
Schumacher K. et al., (1999) Proc Natl Acad Sci USA

T02-088

Genetic analysis of procambial development in the Arabidopsis root

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We have determined the cell lineage relationships for phloem and xylem during procambial development in Arabidopsis root. Subsequently, we have been searching for and characterizing mutations that are informative for formative and/or proliferative functions related to procambial development. A recessive mutation, wooden leg (wol), in the CRE1/WOL/AHK4 gene coding for a cytokinin receptor, results in reduced cell proliferation and exclusive xylem differentiation (1). This indicates the involvement of a specific cytokinin mediated morphogenetic pathway during early stages of vascular development. Another recessive mutation, altered phloem development (apl) results in defective phloem related asymmetric cell divisions as well as differentiation (2). APL encodes a MYB-like transcription factor active specifically in developing phloem cells, consistent with a key role as a regulator of phloem development. Furthermore, ectopic APL expression in the stele results in inhibition of xylem development. We present a model of procambial development involving cytokinin signaling and the phloem promotive and xylem repressive functions of APL. We also discuss current forward and reverse genetics approaches to identify new key pathways in phloem and xylem cell differentiation.

1 Mähönen et al. 2000, Genes Dev 14:2938; 2 Bonke et al. 2003, Nature 426:181.

T02-089

Altered meristem patterning and hormone signaling in the cellulose deficient tsd1 (tumorous shoot development1) mutant, an allele of the KORRIGAN1 endo-1,4-β-glucanase

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Cellulose presents the major polymer of the higher plant cells. The orientation of the cellulose microfibrils within the plant cell wall determines the direction of cell expansion and is, therefore, a major determinant of cell shape and plant morphology. In the screen for mutants showing hormone autonomous callus-like growth in vitro, tsd (tumorous shoot development) mutants were identified. The tsd1 mutant shows severe changes of shoot and root development. The shoot of tsd1 converts into callus-like tissue, which is able to grow on medium without hormone supply, and the root ceases division shortly after germination. We have identified the TSD1 gene by a map-based approach and found it to be allelic to KOR1, which encodes a membrane-bound endo-1,4-β-glucanase necessary for cellulose synthesis. Employing marker gene expression of key regulators of the shoot apical meristem (SAM) (e.g. STM::GUS, CLV1::GUS, KNAT1::GUS), markers for organ formation (LFY::GUS) and L1 layer identity (ML1::GUS), developmental changes in the SAM accompanying the formation of apical callus were monitored in the tsd1 mutant. Similarly, markers of the QC (quiescent centre, e.g. QC185), the root cap and the auxin status (DR5::GUS) were utilized, in order to follow changes leading to the cessation of root growth. Finally, reporter gene analysis of auxin and cytokinin early response genes and double mutant analysis with hormone mutants were examined in the tsd1 mutant.
Our results indicate that the defect in cellulose synthesis caused by the tsd1 mutation leads to formation of multiple SAMs, accounting likely for the hormone autonomous growth of the tsd1callus-like tissue. In the root proper cellulose synthesis is necessary for the maintenance of QC activity and root meristem function. Changed patterning of the early auxin and cytokinin response genes suggests an altered hormone sensitivity and/or transport, which may partially account for the developmental defects of the tsd1 mutant.

Frank et al. 2002 Plant J, Lane et al. 2001 Plant Physiol, Nicol et al. 1998 EMBO J, Sato et al. 2001 Plant Cell Physiol

T02-090

Identification of permeable leaves mutants that exhibit surface defects in leaves using a new method

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The epidermis of higher plants generates the cuticle layer that covers the outer surface of each plant. The cuticle plays important roles in retention of water and prevention of organ fusion. We have shown that genes encoding a subtilisin-like protein (ALE1) and receptor-like protein kinase (ACR4) are required for formation of the cuticle [1,2]. These results suggest that a signaling pathway(s) is involved in differentiation of epidermis, which is prerequisit for cuticle formation. Toward further understanding of the molecular mechanism that governs the differentiation of epidermis, we have developed a new method for visualization of cuticular defect, designated toluidine-blue (TB) test [3]. We demonstrated the validity of the TB test using mutants of Arabidopsis thaliana, including abnormal leaf shape1 (ale1), fiddlehead (fdh) and five eceriferum (cer) mutants, in which the structure and/or function of the cuticle are abnormal. We performed a genetic screening for mutants using the TB test and identified six loci named permeable leaves (pel1 through 6) as well as fdh. The cuticle-defective regions of leaves of the mutants revealed five intrinsic patterns of surface defects, suggesting that formation of functional cuticle on leaves involves various spatially regulated factors.

[1] Tanaka et al. (2001) Development 128, 4681
[2] Watanabe et al. (2004) Plant J. (in press)
[3] Tanaka et al. (2004) Plant J. 37, 139

T02-091

Isolation of two novel putative effectors of polar auxin transport in Arabidopsis thaliana

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During the last years evidence accumulated that PIN genes from Arabidopsis thaliana are crucial for establishment and maintenance of auxin gradients in the plant. The predominantly polar localization of PIN proteins in cell membranes and the phenotypes of corresponding mutants makes them likely candidates for being polar transporters of auxin. Nevertheless, regulatory mechanisms that affect expression and/or activity of the putative auxin carriers are essentially unknown. A genetic approach has been initiated in our laboratory in order to characterize effectors of PIN genes in A. thaliana. Recessive mutations, representing two complementation groups (soe2-1 and soe3-1, for SUPPRESSOR OF EIR1), could be identified in the screen. Both soe mutants appear to be defective in the control of auxin transport, as both mutants exhibit altered responses to inhibitors of polar auxin transport such as TIBA and NPA, while responsiveness to auxin and other growth regulators, is not altered. Soe mutants are also agravitropic like eir1-1 but - unlike eir1-1 - show a pronounced inhibition of root elongation. In addition, defects in shoot phototrophism could be demonstrated for both mutants. Moreover, both mutants have a pronounced tendency to form pin-like inflorescences, resembling those of pin1 and pid mutants. Finally, analysis of auxin inducible marker lines and cell identity markers support a scenario, in which the SOE genes are required for the ordered distribution of auxin in Arabidopsis. Progress in the genetic and functional analysis of the SOE loci will be presented.

This work is supported by grants P-13948 and P-16311 from the Austrian Science Fund

T02-092

Modulation of GA biosynthesis by other plant hormones in Arabidopsis thaliana

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Gibberellins (GAs) are plant growth regulators involved in a wide variety of developmental processes, including seed germination, hypocotyl growth, leaf expansion, stem growth, flowering and fruit and seed development (1). The importance of GAs in these processes is evident from the phenotypic abnormalities caused by GA-deficiency and over-accumulation in plants. The enzymes involved in the final stages of GA metabolism, including the biosynthetic enzymes, GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox) and the inactivating enzyme GA 2-oxidase (GA2ox), have been shown to be important in the regulation of GA content (2,3). These enzymes are dioxygenases, which are encoded by small multi-gene families in Arabidopsis thaliana, the members of which show distinct tissue-specific patterns of expression as well as being differentially regulated by environmental signals, such as light and temperature. Their expression is also regulated by the action of GAs themselves, as a mechanism to achieve GA homeostasis. In addition, it has been demonstrated that GA biosynthesis is modulated by the plants hormones auxin and brassinosteroids, and it is likely that other plant hormones may also affect this process. As part of our studies on the regulation of GA biosynthesis in Arabidopsis we have produced reporter lines for many of the GA, dioxygenase genes. We are using these reporter lines to study the effects of other plant hormones on GA biosynthesis, both by treating plants with hormones or inhibitors of hormone biosynthesis/transport/action, and by crossing the reporter lines into known hormone mutant backgrounds. Our results suggest that expression of some gene family members is indeed modulated by several hormones, including ethylene, cytokinin, auxin and jasmonate. Their effects on gene expression appear to be dependant on the tissue and developmental state of the plant, indicating processes where the interaction between the hormone signalling pathways may be important in development control.

1-Hooley PMB 26:1529-1555
2-Hedden, Phillips Trends Plant Sci 5:523-530
3-Olszewski et al Plant Cell supp S61-S80

T02-093

rol mutations suppress the root hair cell wall formation mutant lrx1

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The development of the plant extracellular matrix is still largely unknown both in respect to the structure and the biosynthesis of the different polysaccharidic components of the cell wall. Also, it is not well understood how the process of cell wall development is regulated. We have identified LRX proteins in Arabidopsis, which are involved in the formation of the cell wall in root hairs. These proteins consist of an N-terminal LRR domain and a structural extensin moiety at the C-terminus. While the extensin moiety is presumably important for anchoring of the LRX protein, the LRR domain might serve a regulatory or signaling function. LRX1 and its paralog LRX2 are both specifically expressed in root hairs and both lrx1 mutants and the lrx1/lrx2 double mutants are strongly affected in the structure of the cell wall. As a consequence, these plants develop a strong mutant root hair phenotype.
To identify components that are involved in the LRX1-dependent process, we have isolated suppressors of the lrx1 mutant. These rol (repressor of lrx1) mutants develop wild type-like root hairs in an lrx1 mutant background. Except one, all rol mutants are silent suppressors, i.e. do not develop an aberrant root hair phenotype in the LRX1 wild-type background. Genetic analysis has shown that several rol mutants depend in their suppressive activity on LRX2, indicating that LRX2 is involved in a similar process as LRX1 and that the rol mutants might function in this process. We have identified a point mutation in the recessive rol1 mutant, which interrupts a gene that presumably is involved in the biosynthesis of a cell wall component. This result suggests that LRX1 is indeed involved in the regulation of the cell wall formation process and that changes in the composition of the cell wall can compensate for the lack of LRX1.

Baumberger et al., Genes & Development 15: 1128-1139

Baumberger et al., Plant J 35: 71-81

T02-094

Investigating the roles of the Arabidopsis MAX genes in shoot branching control

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The MAX genes of Arabidopsis define an important pathway involved in the auxin mediated inhibition of bud outgrowth. Although the MAX genes have been cloned, much remains to be learnt about their detailed function. Grafting of wild-type and max4 roots and shoots was used to examine the nature of the MAX signal. Data suggest MAX4 is involved in production of a mobile signal which inhibits bud outgrowth. Metabolite profiling of the max mutants has identified candidate signalling molecules, which are being tested for their ability to restore a wild-type phenotype to mutant plants. A promoter:: GUS construct was used to investigate whether shoot branching is regulated by changes in MAX gene expression. The reporter gene is expressed in root tips and in nodal tissue surrounding buds, but not in the buds themselves. Evidence of auxin regulation of MAX4 was seen following 8 hours auxin application, when staining distal to the root tip and in the hypocotyl becomes apparent. This upregulation is reduced in an axr1-12 mutant background.

T02-095

PLETHORA1 and PLETHORA2 are involved in the formation and maintenance of Arabidopsis root stem cells

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In the Arabidopsis root, stem cell activity is dependent on a short-range signal from the quiescent center (QC), a group of mitotically less active cells surrounded by the stem cells. Putative transcription factors of SHORT-ROOT (SHR) and SCARECROW (SCR) as well as the phytohormone auxin are involved in QC and stem cell patterning. We identified the PLETHORA1 (PLT1) and PLT2 genes encoding AP2-class putative transcription factors, which are redundantly required for QC specification and stem cell activity. Accordingly, expression of the PLT genes is detected in the distal region of the root meristem including the QC and the surrounding stem cells. The PLT genes act independently of the radial pathway represented by SHR and SCR and their expression pattern is correlated with auxin accumulation. Distal PLT transcript accumulation creates an overlap with the radial expression domains of SHR and SCR, providing positional information for the QC. Furthermore, the PLT genes are activated in the basal embryo region that gives rise to hypocotyl, root and root stem cells, and when ectopically expressed, transform apical regions to these identities, indicating a key role for these genes in establishment of basal organ identities during embryonic pattern formation.

T02-096

Dynamic growth maps modelling for Arabidopsis leaves

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In the past few years, there have been many molecular biology studies that have unravelled genes involved in cell fate determination or regional patterning in the establishment of organ identity and polarity. However, there has been no integrative approach considering the dynamics of organ shape establishment. We want to bridge that gap by using a dynamic and integrative approach to model organ growth. This approach has already been successfully settled for Antirrhinum petals in the lab (Rolland-Lagan et al., 2003) and we want to extend it to Arabidopsis to take advantage of its genetics tools, including transformation facilities and mutant availability. We chose the leaf as a model as it can be considered as a nearly flat organ and is accessible all throughout the plant life cycle.
Leaf shape establishment can be modelled by using four regional growth parameters that are growth rate, direction, anisotropy and rotation (Coen et al., 2004). Growth rate describes how the considered area is increasing in size ; direction the angle at which the preferred growth orientation occurs ; anisotropy the extend to which growth arises in any preferred direction and rotation defines how each region is 'moving' relatively to its neighbours. To measure those growth parameters, we can use either landmark tracking or clonal analysis. Here we describe how clonal analysis will be used to study leaf growth in Arabidopsis. This approach relies on following sectors expressing cell autonomous marker in different areas of the leaf. To do so, we are taking advantage of an inducible CRE-LOX system to trigger GFP expression in cells at different time points during leaf development (Gallois et al., 2002). For the moment, the work is held in a wild-type background, in the future this approach will be extended to the analysis of different mutant leaf sectors.

Coen et al., PNAS, 101, 4728-4735.
Gallois et al., Dev. 129, 3207-3217.
Rolland-Lagan et al., Nature, 422, 161-163.

T02-097

MGOUN3, AN ARABIDOPSIS GENE WITH PROTEIN INTERACTION MOTIFS IS ASSOCIATED WITH MERISTEM ORGANIZATION AND REGULATION OF FLOWERING TIME

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Plant growth and development depend on the activity of continuously replenished pools of undifferentiated cells so-called meristems are complex whose functionning is tightly regulated.
We have isolated a new Arabidopsis gene, MGOUN3 (MGO3), whose mutation affects the structural organization and the functional regulation of both shoot and root meristems. Four mutant alleles for this gene display severe alterations in the regulation of the apical meristem dynamics. Indeed, during post-embryonic development, the cell identity patterning are impaired. The expression pattern of basic regulatory genes of the shoot apical meristem functioning is also misshaped, consistently with the phenotypic alterations (1). Another key feature of mgo3 mutants is their early flowering phenotype in short days, associated with a misexpression of flowering time regulators.
MGO3 gene is unic in the Arabidopsis genome. The protein deduced from the cDNA sequence contains TetratricoPeptide Repeats (TPR) and Leucine-Rich Repeats (LRR), two motifs that are thought to act in protein-protein interactions. Although MGO3 protein presents TPR as in others Arabidopsis proteins, the MGO3 motifs are more similar to those present in LGN-related proteins, which are regulators for some of the asymmetric cell divisions in animal development.
Give that that mgo3 mutants are allelic to bru1 mutants which are affected in the stability of heterochromatin organization and epigenetic gene silencing (2), MGO3 appears as a new type of key regulators for epigenetic control of Arabidopsis development and especially meristematic functioning and flowering transition.

(1) Guyomarc'h S. et al. (2004) J. Exp. Bot. 55, 673-684.
(2) Takeda S. et al. (2004) Genes Dev 18, 782-93.

T02-098

Analysis of the transcriptional regulation of cell specialisation during leaf development in Arabidopsis thaliana

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The objective of our project is to study the transcriptional regulation during leaf development of Arabidopsis thaliana. The SERRATE gene which appears to be involved in this process will be given particular emphasis within the project. The SERRATE gene for which a mutant is already available encodes a protein with a single C2H2 zinc finger motif. Characterisation of the serrate mutant revealed defects in both vegetative and inflorescence phase lengths, the timing of phase transitions, leaf shape, leaf number and phyllotaxy. The SERRATE gene is also required for normal embryo development. The effects of reducing or eliminating SERRATE expression on genes involved in leaf development will be established by comparing transcript profiles of the serrate mutant with profiles of wild type plants. Inducible expression of SERRATE in a serrate mutant background followed by microarray-based expression profiling will be used to identify direct targets of SERRATE. Cell-specific expression patterns of SERRATE will be determined, firstly in wild type and then in various mutants affecting genes which show changed expression in the serrate mutant. The aim of these experiments will be to determine the regulatory hierarchies controlling leaf development in Arabidopsis. A two component expression system is being used to assess the effects of altering SERRATE protein levels in different tissues and at different stages of plant development. The analysis of protein-protein interactions between SERRATE and a number of transcriptional regulators will provide us further indications of the function of SERRATE.

Clarke J.H. et al. (1999) Plant Journal, 20: 493-501
Prigge M.J. & Wagner D.R. (2001) The Plant Cell, 13: 1263-1279

T02-099

Regulatory Mechanisms in Shoot and Root Development

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Plant architecture is the product of shoot and root apical meristems, which form from opposite poles during embryogenesis and remain active throughout the life of the plant. Shoots and roots grow in very different environments, and thus respond to different exogenous and endogenous cues. They also have different ways of making lateral organs, with shoots producing leaves and flowers in stereotypical arrangements at the very tip, and primary roots forming lateral roots in a stochastic fashion at some distance from the apex. However, recent experiments have revealed that shoots and roots share some common molecular mechanisms for regulating their growth and development, and their responses to their environments. As part of our ongoing effort to understand the molecular processes that regulate Arabidopsis shoot apical meristem function, we are characterizing a dominant, activation-tagged mutant called jabba-1D (jba-1D) that has defective shoot apical meristem (SAM) activity and lateral organ polarity. The SAMs of jba-1D plants enlarge progressively beginning during embryogenesis, leading to the splitting of the shoot apex during the vegetative phase and eventually to inflorescence stem fasciation. This phenotype is associated with broadening of the WUS and CLV3 expression domains, and with abnormally high levels of WUS transcription in the SAM. jba-1D plants also develop extra vascular bundles in their stems, and form radialized leaves and gynoecia. We show that the jba-1D phenotypes are caused by over-expression of miR166g, one of nine microRNAs encoded in the Arabidopsis genome that are complementary to the class III homeodomain-leucine zipper (HD-Zip) developmental regulatory transcription factor family. miR166g over-expression in jba-1D plants requires DICER-LIKE1 (DCL1) activity, and alters the expression of the HD-Zip genes REVOLUTA, PHABULOSA, and PHAVOLUTA.

T02-100

Comparative chloroplast proteomics of a cpSRP54 deletion mutant in Arabidopsis thaliana

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Chloroplasts are essential organelles containing some 3000 nuclear-encoded proteins, ~120 chloroplast-encoded proteins and an internal thylakoid membrane system. Protein sorting, assembly and proteolytic disposal within this plastid are critical for plant development and function. Specifically, the targeting machinery must ensure proper coordination and insertion of hundreds of known and unknown nuclear and chloroplast-encoded thylakoid proteins, while the proteolytic machinery must maintain cellular housekeeping and remove aggregated and damaged proteins. Different protein sorting routes and their protein components have been discovered within the chloroplast, with just a handful of identified substrates for each of these routes.

This poster will describe our preliminary data on the effect of deletion of the cpSRP54 subunit on the chloroplast proteome in Arabidopsis thaliana. cpSRP54 has been demonstrated to be involved in membrane targeting of selected nuclear-encoded hydrophobic chlorophyll a/b binding thylakoid membrane proteins. cpSRP54 is also implied in insertion of several hydrophobic chloroplast-encoded membrane proteins. However, the deletion mutant is viable, showing slightly pale leaves, reduced biomass and a delay in flowering time. Given that the thylakoid membrane proteome contains hundreds of membrane proteins, it is expected that other proteins also depend on cpSRP54. However, as the deletion mutant is viable, with a relative mild chloroplast phenotype, it is logic to postulate that other sorting pathways or mechanisms can compensate for the loss of cpSRP54. Also, protein aggregates resulting from miss-targeting are possibly removed by up-regulation of proteolytic activity.

I will present data concerning the thylakoid proteome, as well as the stromal proteome, using different fractionation techniques, mass spectrometry and Western blotting. In addition, I will discuss the relation between expression of protein targeting components and the developmental stage of the leaf.

Amin, et al. (1999). Plant Physiol 121, 61-70.

T02-101

Identification and characterization of mutations suppressing the wol mutation in the CRE1/WOL cytokinin receptor gene

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The developmental ontogeny of the vascular system (consisting of xylem, phloem and [pro]cambium) is poorly understood despite its central role in plant physiology. We are studying the genetic control of vascular patterning during root development in Arabidopsis. The primary effect of the wooden leg (wol) mutation is the lack of the formative cell divisions required for the organization of the vascular tissue. We have determined that the WOL gene codes for a putative signal transducer with a histidine kinase activity (Mähonen et al. 2000: Genes&Dev. 14, 2938-2943). Moreover, Inoue et al. (2001: Nature 409, 1060-1063) showed that CRE1/WOL is a true cytokinin receptor. In order to more systematically approach the genetic control of cell proliferation during vascular development, we have carried out an EMS based screen for suppressors of wol based on root growth pattern. 13 strong and 7 weak suppressor lines were identified representing both extra- and intragenic suppressors, which seemed to restore vascular development. This indicates a negative activity for mWOL receptor. Moreover, overexpression of 35S:: mWOL results in inhibition of vascular cell proliferation also in the aerial part. Additionally, the strongest extragenic suppressors phenocopy the root growth pattern of the weakest intragenic suppressors, indicating that these mutations may identify novel genes related to the control of cell proliferation during procambial development. Further analyses focus on the mapping of the extragenic suppressors and characterization of their physiological responses.

T02-102

A genetic interaction analysis of incurvata mutants identifies microRNA targets and microRNA machinery elements

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Recent studies have expanded the spectrum of important developmental processes that are controlled in metazoans and plants by microRNA-mediated repression of gene function. This regulatory mechanism involves three classes of genes, which encode microRNAs, elements of the microRNA machinery, or microRNA targets. The observation of phenotypic synergy, as opposed to additivity, has been used as a criterion for identifying functional interactions in developmental processes. Here, we present a genetic interaction analysis involving 9 Arabidopsis thaliana mutants, which we isolated and initially named incurvata (icu) and considered potentially useful for the identification of genes required for the making of a leaf, since they present incurved, rather than flattened, leaves. Based on the phenotypes observed in the 35 double mutants obtained, two groups of synergy were defined, composed of 5 and 2 mutants that yield pairwise synergistic interactions. A total of 24 double mutants involved mutations that gave rise to merely additive phenotypes, as would be expected if their gene products act in an independent manner. Further genetic and molecular analyses confirmed that the functions of the genes damaged in synergistic double mutants are related, and that some of the mutations under study represent novel alleles of genes studied by others. A number of the synergistic phenotypes found were caused by interactions between genes encoding elements already known or suspected to belong to the microRNA machinery, and between these genes and genes encoding microRNA targets. The ICU4 gene was found to encode the ATHB-15 transcription factor, and to be probably regulated by a microRNA (see the communication presented by Ochando et al. in this congress) and to synergistically interact with HASTY (HST), ARGONAUTE1 (AGO1), HUA ENHANCER1 (HEN1) and HYPONASTIC HYPOCOTYL1 (HYL1), as seen in double mutants involving novel hst, ago1, hen1 and hyl1 hypomorphic alleles. On the other hand, we found that the ICU2 gene encodes a DNA polymerase subunit putatively required for chromatin-mediated cellular memory (see the communication presented by Barrero et al. in this congress), and that it synergistically interacts with CURLY LEAF.

T02-103

A mutational analysis of the ABA1 gene of Arabidopsis thaliana highlights the involvement of ABA in vegetative development

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Much of the literature on the phytohormone abscisic acid (ABA) describes it as a mediator in triggering plant responses to environmental stimuli, as well as a growth inhibitor. ABA-deficient mutants, however, display a stunted phenotype even under well-watered conditions and high relative humidity, which suggests that growth promotion may also be one of the roles of endogenous ABA. Zeaxanthin epoxidase, the product of the ABA1 gene of Arabidopsis thaliana, catalyzes the epoxidation of zeaxanthin to antheraxanthin and violaxanthin, generating the epoxycarotenoid precursor of the ABA biosynthetic pathway. We describe here the characterization of a series of nine mutant alleles of the ABA1 gene, which cause different degrees of ABA deficiency, four of them previously isolated (aba1-1, aba1-3, aba1-4 and aba1-6) and the remaining five novel (sañ1-1, sañ1-2, sañ1-3, sañ1-4 and sre3). The size of the leaves, inflorescences and flowers of these mutants is reduced, and their rosettes have lower fresh and dry weights than their wild types, as a result of a diminished cell size. Low concentrations of exogenous ABA increase the fresh weight of mutant and wild-type plants, as well as the dry weight of the mutants. The leaves of aba1 mutants are abnormally shaped and fail to develop clearly distinct spongy and palisade mesophyll layers. The aba1 mutants are partially de-etiolated when grown in the dark, and display reduced hypocotyl elongation, which is promoted by exogenous ABA, as it is in their wild types. Taken together, these phenotypic traits indicate that ABA acts as a growth promoter during vegetative development and that it is required for skotomorphogenesis. The morphogenetic effects of ABA cannot merely be explained by the modulation of water loss, given that exogenous ABA increases the dry weight of aba1 mutants, suggesting a growth-promoting role which is not dependent on its effect on the hydric balance.

T02-104

Regeneration of shoots through the action of ESR genes

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Plant cells from almost any organs are able to de-differentiate, divide, and re-differentiate to generate new shoot or root organs. However, the molecular mechanisms controlling plant totipotency remain yet to be elucidated. This process depends mainly on the concentration of the hormones cytokinin and auxin.

Through functional screening, we have previously identified a gene, designated ENHANCER OF SHOOT REGENERATION 1 (ESR1) of Arabidopsis, which encodes an AP2/ERF (ethylene response factor) transcriptional factor (Banno et al). Screening of the Arabidopsis genome uncovered a related gene, ESR2, with the same function as that of ESR1when overexpressed in vitro. It is also suggested that transcriptional activity of ESR2 is necessary for increased shoot regeneration efficiency.

Transgenic seedling overexpressing ESR2 by estradiol inducible system (XVE) strongly inhibited cell differentiation and developed green calli from hypocotyls and occasionally from roots, when grown in the light. In the dark XVE-ESR2 seedlings exhibited de-etiolated phenotypes. Estradiol-induced expression of either ESR1 or ESR2 in cre1/ahk4 mutant hypocotyl and root explants conferred cytokinin-independent shoot regeneration, suggesting that the two ESR genes are downstream component of cytokinin signaling pathway. Combination with microarray technique and estradiol receptor mediated translocation system enabled to identify direct target genes of ESR2. Direct targets of ESR2 were found to be CYCLIN D1;1, NPK1 (nucleus- and phragmoplast- localized protein kinase 1)-related MAPKKKs, AP2 domain transcriptional factors, and nonfunctional phosphotransmitter AHP6. Expression of CUC1 (CUP SHAPED COTYLEDON 1) is found to be mainly upregulated by indirect effect of ESR2.These results demonstrate that upregulation of cell cycle related genes is associated with disorganized cell proliferation in ESR2 overexpression plants. Determination of meristem identity is, in part, due to the induction of CUC1. Expression of nonfunctional AHP6 could be responsible for the reduced expression of A-type response regulators in 35S::ESR2 transgenic plants. Our results suggest that downstream cytokinin events leading to shoot regeneration through the action of ESR genes.

Banno et al (2001) Plant Cell 13, 2609

T02-105

The INCURVATA4 gene encodes the ATHB-15 transcription factor and is probably regulated by a microRNA

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Recent genetic studies in Arabidopsis thaliana have identified a number of genes expressed in lateral organs, such as PHABULOSA (PHB) and PHAVOLUTA (PHV), which are probably involved in the specification of lateral organ dorsoventrality, and encode transcription factors of the class III homeodomain/leucine zipper (HD-Zip III) family. We have previously identified two mutants carrying semidominant alleles of the INCURVATA4 (ICU4) gene, which display incurved vegetative leaves, abaxial trichomes in juvenile leaves, and abnormal fusion at the apex of the gynoecium. These weak adaxializing traits are largely enhanced in the icu4-1 hst-1 double mutant, whose phenotype is synergistic and includes a partial adaxialization of rosette leaves and the transformation of the abaxial replum into an adaxial placenta with associate ovules. The HASTY gene is known to encode a nucleocytoplasmic transporter. We positionally cloned the ICU4 gene, which was found to encode the ATHB-15 transcription factor, another member of the HD-Zip III family. The icu4-1 and icu4-2 semidominant, gain-of-function alleles carry the same point mutation, which is identical to those of dominant alleles of PHB and PHV, and affects the binding site of two microRNAs that differ in only one nucleotide, miR165 and miR166. Our results suggest that the ICU4 gene is negatively regulated by a microRNA and lend support to the hypothesis that HASTY carries microRNA precursors from the nucleus to the cytoplasm.

T02-106

SCHIZORIZA is required for root patterning in Arabidopsis

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Cell layers of the arabidopsis primary roots are organised in a simple radial pattern. The root comprises concentric rings of tissue with lateral root cap outside the epidermis, which surrounds the ground tissue (cortex and endodermis).This cell pattern is set up during embryogenesis and is maintained by regular divisions of stem cells in the meristem of the developing root. schizoriza (scz) was identified in a screen for genes involved in the development of the root epidermis. scz develops hair cells in the subepidermal cell layer (ground tissue) while in wild type roots they are only formed in epidermal cells. Additionally, scz roots show supernumerary ground tissue layers due to extra periclinal divisions in the root meristem. These mutant phenotypes indicate that SCZ repress epidermis identity and periclinal divisions in the ground tissue of wild type roots and that SCZ is required for the establishment of radial organisation of tissues in the root

T02-107

Characterization of cell type specific transcription factors and their regulatory network in Arabidopsis roots

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Unraveling gene expression patterns at the cellular level can facilitate linking the role of a gene to the differentiation of the certain cell or tissue types. We are interested in understanding how transcription factors regulate the developmental processes in Arabidopsis roots using genomics approach. About 22,000 genes were analyzed from sorted GFP expressing protoplasts of Arabidopsis roots using Affymetrix Arabidopsis genome array and their expression patterns in six cell or tissue types have been compiled. From expression data of stele, endodermis, endodermis and cortex, atrichoblast, lateral root cap, and quiescent center, putative cell-type specific transcription factors were identified. To compile their regulatory regions and cross-compare to high-throughput microarray data, their gene expression patterns have been examined with transgenic Arabidopsis carrying GFP reporter gene fused either transcriptionally or translationally to the genes of interest. Most of expression patterns of transcriptionally fused GFP to 5' UTR fit well to the data from microarray experiments though we already found few genes putatively regulated by 3' UTRs or introns. Using GFP and microarray data, endodermis specific transcription factors were ectopically expressed in other cell types, and gene expression profiles altered by ectopic expression are being built to find the gene regulatory network.

T02-108

Radial Patterning in Arabidopsis: Networks and movement

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Plant embryos consist primarily of two stem cell populations known as meristems, one that will make the root and the other that makes the shoot. Determining how the cells in these meristems are able to control their own division and the differentiation program of their progeny to form organs is one of the major questions of plant development. We have uncovered evidence for a signaling center located in the internal tissues of the Arabidopsis root that provides pattern information through cell-cell movement of a transcription factor to the surrounding cell layer.

In the root of Arabidopsis, we have characterized mutations in which specific meristem cells fail to divide, or their progeny acquire the wrong identity. Analysis of mutations in the SCARECROW (SCR) and SHORT-ROOT (SHR) genes indicates that they are key regulators of radial patterning in the root. Analysis of tissue-specific markers provides evidence that SCR is primarily required for the asymmetric division that gives cortex and endodermis. The SHR gene is required for the asymmetric cell division responsible for formation of ground tissue as well as specification of endodermis. Both SHR and SCR are members of the GRAS family of putative transcription factors. SHR acts in a non-cell autonomous fashion to regulate the amount of RNA that is made by the SCR gene. Analysis of SHR localization revealed protein both in the stele and in the cells immediately adjacent to it indicating that SHR is able to move from the stele to the adjacent layer. Ectopic expression of SHR revealed a broad competence of cells to respond to SHR indicating that tight regulation of movement was essential for proper organogenesis. Efforts to identify the mechanism of this highly regulated protein movement will be discussed.

In a complementary effort, we are using sorted cell populations to analyze global gene expression patterns at cell-type specific resolution. The long-term goal of this project is to identify transcriptional networks that control root development.

T02-109

Root hair development in adaptation to Fe and P deficiency

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During post-embryonic development of Arabidopsis, all root epidermis cells lying over the clefs of underlying cortical cells (H position) develop into root hairs. In the mature primary root and in laterals this number is significantly reduced and the epidermal cell patterning becomes sensitive to environmental cues. Phosphate deficiency evokes an increase in the number and length of root hairs. In iron-deficient plants a high percentage of root hairs are branched. Mutant analysis showed that predominantly genes that are involved in root hair specification are differentially affected by the abundance of Fe and P. The gl2-1 and cpc mutants had significantly different phenotypes from the wild-type in hair (H) and non-hair (N) position. We investigated the expression patterns of these genes in H and N position in response to Fe and P nutrition. The homoeostasis of Fe and P has been shown to be regulated by systemic and local signalling pathways, which are yet unknown. We present a detailed analysis into the regulation of root hair branching under Fe deficiency and the increased number of root hairs under P deficiency. Furthermore, we report the characterisation of mutants that are not able to produce root hairs in the absence of P and display the wild-type phenotype in the presence of P.

T02-110

Expression of the bHLH genes GL3 and EGL3 during cell fate specification in the Arabidopsis root epidermis

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A central question in developmental biology is how cells become determined to adopt a specific cell fate. In the Arabidopsis root epidermis only two cell types, root hair cells and non-hair cells, arise in a distinct position-dependent manner and therefore provide an excellent system to study cell fate specification.
Several genes encoding putative transcriptional regulators that influence this cell fate decision have been identified and the final epidermal cell pattern appears to be determined by positive and negative regulatory interactions between these components . We have previously shown that two bHLH genes, GL3 (GLABRA3) and EGL3 (ENHANCER OF GLABRA3), are also involved in this process and act in a redundant manner to specify root epidermal cell fates. Mutations in both genes cause the formation of ectopic root hair cells, while overexpression of GL3 and EGL3 promotes the non-hair cell fate. Like WER and TTG, both bHLH genes are active at an early stage during root epidermal development before the first visible differences in root hair and non-root hair cell characteristics occur. GL3 and EGL3 are shown to be required for the position-dependent expression of both, the non-hair cell specification gene GL2 (GLABRA2), and the hair cell specification gene CPC (CAPRICE). Thus, GL3 and EGL3 seem to be involved in the specification of both epidermal cell types. The function of GL3 and EGL3 is dependent on WER activity and yeast two-hybrid data indicate that GL3 and EGL3 are able to interact with both the WER and CPC proteins. Therefore, the GL3 and EGL3 bHLH proteins might act as binding partners for the MYB protein WER in the N-position and the truncated MYB protein CPC in the H-position to activate or inhibit GL2 expression, respectively, thereby mediating the cell patterning process.
We are currently further investigating the role of GL3 and EGL3 during root epidermal cell specification by studying the expression of GL3 and EGL3 and their regulation by other components of the patterning pathway. We are also analyzing whether these genes are active in the embryo and whether GL3 and EGL3 play a role during the epidermal cell specification process in the hypocotyl.

T02-111

The cyclophilin AtCyp95 regulates root system development

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Little is known about how plants regulate their morphology at the organismal level. For instance, proper development of a plant's root system architecture (RSA) is critical to its ability to obtain water and nutrients, yet its regulation is poorly understood. We have found that a mutation in an Arabidopsis cyclophilin, AtCyp95, confers altered RSA. Mutant plants have increased rates of lateral root initiation, development and growth under all conditions we have examined. AtCyp95-1 mutant plants also show a slight increase in auxin sensitivity in the roots, but no other auxin-related phenotypes. AtCyp95 is the largest of the 29 Arabidopsis cyclophilins (Cyps) (1,2). Cyps are a class of peptidyl-prolyl isomerases found in all organisms, and are proposed to function in protein folding and stability, and in protien complex formation. Despite the large number of Arabidopsis Cyps, our results show that AtCyp95 is essential for correct regulation of RSA development.
The AtCyp95 protein contains an isomerase domain characteristic of all Cyps, and an extended SR-rich C-terminus of unknown function. A single basepair change in AtCyp95-1 is predicted to create an early stop codon, leaving the isomerase domain intact, but deleting most of the C-terminus of AtCyp95. SR-rich domains have been proposed as sites of protein-protein interaction, and our work demonstrates the importance of the SR-domain for correct AtCyp95 function. This is consistent with the proposed role of Cyps in forming and stabilizing protein complexes. Progress in purification of AtCyp95-containing complexes, and identification of complex components, will be reported.

1. He et al, Plant Physiology 2004 134:1248-1267
2. Romano et al, Plant Physiology 2004 134:1268-1282

T02-112

TCP transcription factors control cell division and differentiation in patterning of organ development.

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We recently developed an effective system for gene silencing using a chimeric repressor (the CRES-T system), in which a transcription factor is converted into a dominant repressor by fusion with the EAR-motif repression domain (ref.1). This system allows us to identify the functions of redundant plant transcription factors by suppressing the expression of the target genes resulted in a induction of phenotype similar to that induced by the corresponding loss-of-function allele in transgenic plants.
TCP is a transcription factor specific for plants. 24 genes that code TCPs have been identified in Arabidopsis genome. Although some of TCPs were shown to regulate development of organ asymmetry, growth of axillary bud and leaf morphogenesis, their biological functions remain to be identified.
To clarify function of the TCP transcription by using the CRES-T system, we converted 10 different Arabidopsis TCP transcription factors into dominant repressors and expressed them respectively in transgenic Arabidopsis plants. The transgenic plants that express the chimeric TCP repressor showed various abnormal phenotypes: asymmetrical cotyledon positioning, ectopic formation of shoots and trichomes, leaves with lobes and sinuses, excess growth of axillary shoots and crinkled siliques. The SEM analysis of leaves showed that excess cell divisions were observed in the restricted region of the lobes. Wild type plants form files of long straight cells found in the leaf margin, while such differentiated cell files were not observed in the transgenic plants but replaced by undifferentiated cells in the sinuses. These results suggest that TCP family control cell division and differentiation in patterning of organ development.

(1) Hiratsu K, Matsui K, Koyama T, Ohme-Takagi M (2003) Plant J. 34 733-739

T02-113

In planta functions of the Arabidopsis cytokinin receptor family

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Arabidopsis has three related histidine kinases, CRE1/WOL/AHK4, AHK2 and AHK3, which function as cytokinin receptors. We have previously reported that cre1 mutants are less responsive to cytokinins than the wild-type. To understand redundant and specific functions of cytokinin receptors, we have examined cytokinin responsiveness of mutants that lack one, two, or three of the receptor-genes. Double mutants are less responsive to cytokinins than corresponding single mutants in most assay systems. These results indicate that three cytokinin-receptors have partially overlapping functions. Surprisingly, triple mutants were recovered, which were very small and infertile yet carrying basic organs-roots, leaves and inflorescences. The triple mutant did not show cytokinin responses, including inhibition of root elongation, inhibition of root formation, cell proliferation in and greening of calli, and induction of cytokinin primary-response genes. These results confirm that cytokinins are a pivotal class of plant growth regulators, but raise the question of whether cytokinins are required for the formation of a minimal vegetative body plan.

Higuchi et al., (2004) Proc Natl Acad Sci USA in press.
*These authors contributed equally to this work.

T02-114

Isolation and analyses of a thick-leaved mutant N692

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Leaves have polarities of a thickness besides a length and a width. Diversity in leaf thickness reflects morphological adaptation of leaves to various environments. In addition to it, it is known that the thickness of leaves varies even in a particular species in response to environmental factors, but the mechanisms for the leaf-thickness control is still unknown. As the first step of the study of thickness control in leaves, we developed an instrument that can measure the thickness of an Arabidopsis living leaf reproductively by a laser displacement sensor. The previous measurement method requires several complicated steps, such as a fixation of leaves, an embedding of the samples, a slicing by cutters and an observation by microscopes. These works can be omitted by using our instrument that measures about 1 leaf thickness per minute. To evaluate the performance of the instrument, we measured 10 of glabra1 leaves by instrument. As a result, it gave 103.5 ± 10.8 μm as mean ± SD.

Using the instrument, thick-leaved mutants were screened from a T-DNA activation-tagged library of C24. From screening of more than 1500 lines, we found one mutant N692. The thickness of N692 leaves was 158.0 ± 10.4 μm, while that of wild-type C24 leaves was 126.4 ± 5.68 μm. Leaves of N692 are tight, while those of wild type are waved and curly. The stem of N692 is thicker than that of wild type. Since the segregation ratio of thick-leaved phenotype is 1:1, it is possible that N692 has a sterility of gametes. Anatomy of the mutant showed that mesophyll cells of N692 were larger than those of wild type. We are in progress of cloning of the N692 gene. Investigations of N692 will bring new knowledge for controls of the leaf thickness and the cell expansion.

T02-115

Genetic Analysis of Vascular Development in Arabidopsis

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Leaves of terrestrial plants have diverse and complex patterns of leaf venation. Although many descriptive studies of venation exist, little is known about the molecular mechanisms underlying the formation of venation pattern. We are taking genetic approach to understand the mechanisms of vein patterning in Arabidopsis. The vascular-enhancer-trap line, in which GFP is expressed specifically in the procambium and young vasculature, was mutagenized with EMS. By observing GFP-lit-up veins in leaves, we are screening mutants defective in vascular development. We obtained mutants and classified them into at least three groups according to their phenotypes. In group 1 mutants, there are fewer veins in leaves. These include 4-47 mutant that develops narrow leaves with no vein; 1B-22 and 3B-55 mutants with fewer veins in leaves, which tend to be asymmetric; 2B-17 mutant whose secondary veins in leaves are not connected in the margin; 1B-11 mutant which has narrow leaves with reduced numbers of veins. In group 2 mutants, veins in leaves are fragmented. 621C-6 mutant, which looks normal in appearance, has fragmented tertiary veins. As a group 3 mutant, 1D-1 mutant has extra xylems in veins, which have irregular shape. Characterization of phenotypes of these mutants and mapping the mutant loci are in progress. We will discuss possible functions of the affected genes in vascular development.

T02-116

Transcription profiling with the Complete Arabidopsis Trancriptome Microarray (CATMA): analysis of cell elongation in the hypocotyl.

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The "Arabidopsis Genome Initiative" has predicted more than 27000 genes in the Arabidopsis genome sequence, the physiological function of the majority of which remain largely unknown. Transcription profiling with microarrays can provide first insights in these functions. The Complete Arabidopsis Transcriptome MicroArray program has the objective to create a versatile Arabidopsis microarray covering almost all the annotated genes containing specific probes, thus avoiding cross-hybridization within the gene families. The current version of CATMA contains 24500 « Gene Sequence Tags » (Crowe et al., 2003) designed with a specifically designed software: SPADS, Specific Primers & Amplicons Design Software, (Thareau et al., 2003). Our laboratory has developed a transcript profiling platform based on CATMA microarrays. Normalization and statistical methods have been specifically developed for this type of microarrays. We report here on the study of changes in the transcription profiles associated with cell wall deposition and cell elongation in dark-grown Arabidopsis hypocotyls. Hypocotyls at different growth stages treated or not with isoxaben, an inhibitor of cellulose biosynthesis, were isolated and transcript profiles were compared In this way specific transcripts respectively involved in the cell wall synthesis and cell wall loosening and which specifically responded to isoxaben treatment could be identified.

Crowe et al., Nucleic Acids Res, 2003
Thareau et al., Bioinformatics , 2003

T02-117

Study of miRNA targeting

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microRNAs (miRNAs) are endogenous small RNAs of about 22 nucleotides in length that have been found in different organisms including plants. They have been implicated to negatively regulate genes required mainly for developmental processes.
To study the biological role that miRNAs play when they interact with their targets genes and miRNA localisation, chimeric transgenes carrying predicted miRNA targets have been generated and the phabulosa target gene has been used as a starting model system. These constructs express a transcript in which either a native or a mutant miR165 target sequence is present in a GFP mRNA at the 3' end (GFP:PHB and GFP:PHBm, respectively).
Transient assay experiments in Nicotiana benthamiana have demonstrated that the chimeric GFP:PHB transcript can be down regulated by the corresponding miRNA in the same way as the native mRNA target, whereas the mutant target version is not subject to this regulation. Arabidopsis thaliana transformants with these constructs confirm the GFP expression pattern and give the possibility to use this system to study miRNA targeting. Based in these results we have generated constructs for 20 other miRNA targets and introduced them into Arabidopsis. The phenotypes of these transgenes are being investigated in both Columbia and sde1 mutant Arabidopsis. A difference in these genotypes indicates that SDE1, a putative RNA-dependant RNA Polymerase, mediates transitivity and amplification of the silencing mechanism that is activated by the miRNA.

T02-118

Redundant PIN gene activity as a major control mechanism in patterning and cell division in Arabidopsis root development.

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Polar auxin transport inhibitors have been in use for decades in plant research and their effects suggested that auxin distribution is a major developmental control mechanism. More recently, genetic analyses have revealed molecular components of polar auxin transport process: PINs, AUXs, MDRs In contrast to drug studies, many single pin mutants display mostly subtle phenotypes. Recent analysis of redundant PIN gene function reveals a collective requirement for auxin accumulation in the apical cell of the 2-cell embryo, and in the tips of embryonic and post-embryonic organ primordia; these auxin maxima are required for proper outgrowth and development of the cells within which they reside. An important question that emerges is how regulation of auxin distribution can regulate organ patterning and growth. Here, we analyse combinations of multiple pin mutants with a focus on root development, in which pattern formation was previously shown to strongly dependent on polar auxin transport. Dramatic phenotypes demonstrate that PIN genes, through their fine-tuned effects on auxin distribution, are major players in size control of the mitotic cell pool and in embryonic pattern formation. PIN2 emerges as a major player in cell division control. In addition, PIN4 and PIN7 have a major role in a patterning process to focus the expression domain of the PLETHORA1 gene, required for root and root stem cell specification, which can be partially compensated by PIN1and PIN3. This major role in patterning the basal pole of the embryo and post-embryonic maintenance of this pattern contrasts with the transient PIN dependence on establishment of expression domains of the apical embryonic patterning gene WUS. Our analysis dissects the roles of a regulatory network that mediate auxin transport and regional fate determinants to orchestrate patterning and cell division.

T02-119

AtRaptor and meristem activity

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Plant development depends on the regulated growth and division of root and shoot apical meristem cells. We describe the disruption and characterization of the Arabidopsis Raptor homologues, two genes whose protein products play a critical role in regulating meristem activity. Raptor is a ~1,300 residue protein comprised of multiple HEAT and WD-40 protein interaction domains. In yeast and mammalian cells, Raptor forms a nutrient-sensitive complex with the protein kinase TOR, a major regulator of protein synthesis and cell growth (1,2). Disruption of the Arabidopsis homologue AtTOR is recessive lethal; embryos undergo unstructured cell division but do not gain volume (3).
We have disrupted AtRaptor1A and AtRaptor1B, the two Arabidopsis Raptor homologues. AtRaptor1B knockout plants show a broad range of subtle shoot and root developmental phenotypes. Root growth is slower than wild-type. Roots are often branched or curled, and show a defect in the ability to penetrate agar. Leaf initiation is slower than wild type, bolting and senescence are delayed, and there is a reduction in primary shoot apical dominance, resulting in a highly branched shoot architecture. AtRaptor1A insertion plants show no conspicuous phenotype. AtRaptor1A1B double mutants arrest development as seedlings after minimal shoot apical meristem activity.
Raptor is thought to act by recruiting substrates to TOR for phosphorylation; known substrates include S6K, 4EBP, and E2F. We show that AtRaptor1B interacts with AML1, a homologue of the fission yeast meiotic differentiation regulator mei2 (4), suggesting that Arabidopsis mei2-like proteins act downstream of an AtTOR signaling cascade (see T02-072).
Collectively, these results point to a role for AtRaptor / AtTOR signaling in the regulation of cellular differentiation in the meristem.

1 Kim (2002) Cell110:163
2 Hara (2002) Cell110:177
3 Menand (2002) PNAS99:6422
4 Shinozaki-Yabana (2000) MCB20:1234

T02-120

Profiling Primary Auxin Responses and Transcriptional Regulation Mediated by AXR1 and SCFTIR1 Functions

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Rapid changes in gene expression occur in response to auxin when members of the Aux/IAA family of transcriptional repressors are modified by ubiquitin attachment and subsequently degraded. Root development and other auxin-mediated processes are influenced critically by AXR1 modulation of activities that initiate this selective elimination of repressors. AXR1-dependent modification of ubiquitin-protein ligase SCF complexes influences the stability of a variety of substrates, and it is the variable F-box protein component of the SCF that determines recognition specificity.
As a starting point for examining a broader set of early auxin responses than characterized previously, we profiled the root transcriptome after 30 minute treatments with IAA. Microarray analyses using Affymetrix oligonucleotide arrays representing >22,000 genes allowed the identification of numerous early response genes beyond those previously known to be derepressed by auxin-induced Aux/IAA protein degradation. We expanded this study to examine influences of AXR1 function on the auxin-affected expression profiles. axr1 plants exhibit defects in all known auxin-mediated processes, suggesting that comparative transcriptional profiles would reveal genes involved in each aspect of root growth and development, including cell elongation, gravitropism, lateral root and root hair formation, and auxin-regulated metabolic processes that influence total plant development. Transcriptional profiles of axr1 roots allowed us furthermore to identify sets of genes regulated by AXR1 function in the absence of auxin.
To deliniate specificities of function among SCF complexes downstream of AXR1, we continued similar microarray studies with mutants in F-box proteins involved in auxin response. SCFTIR1 acts downstream of AXR1 to promote the degradation of Aux/IAA proteins. Mutations in TIR1 confer auxin resistance that is less severe than observed in axr1, consistent with a smaller set of effectors downstream of TIR1. Within the TIR1 subclade of the F-box protein family are two proteins that share 84% identity and are 59% identical to TIR1. These appear to act additively in auxin-sensitive processes in the root (Dharmasiri et al., in preparation). Transcriptional profiles of tir1 and of the triple mutant were analyzed and integrated into the above datasets. Datasets presented here are of high statistical significance and detail the extensive influence auxin exerts on the root transcriptome.

del Pozo JC, et al., 2002. Plant Cell, 14: 421-433.
Gray WM, et al., 2001. Nature, 414: 271-276.

T02-121

GRAS Proteins involved in a variety of developmental Processes

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GRAS proteins are a recently discovered family of plant-specific proteins named after the first three of its members isolated from Arabidopsis (GAI, RGA and SCR). Although the Arabidopsis genome encodes at least 33 GRAS protein family members only a few GRAS proteins have been characterized regarding their biological function. However, it becomes clear that the functional role of GRAS proteins range from meristem maintenance to regulation of hormone- and light signal transduction. GAI and other “DELLA” proteins are involved in gibberellic acid signal transduction, PAT1 in phytochrome signal transduction, and SCR, SHR, HAM and Ls are involved in morphological development. With the help of reverse genetics we are elucidating the biological pathways GRAS proteins are involved in. Knock-out mutants lead to the identification of novel developmental processes GRAS proteins are involved in, such as lateral root formation and hormone signal transduction. GRAS proteins have highly variable N-termini that differ in length and sequence, but share significant homologies throughout their C-termini. The different domains of GRAS proteins do not allow to attribute a biochemical function to the protein family. Therefore we are characterizing these proteins according to their structure, their cellbiological function and biochemical properties. Recent results of the functional analysis will be presented.

T03 Cell Biology

T03-001

Mitochondrial Biogenesis in Arabidopsis

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We have used a sequence similarity based approach to identify genes involved in mitochondrial protein import in Arabidopsis. Many components are encoded in small multi-gene families and the predicted proteins from these families display significant structural differences. Expression profiling in various tissues, during development and under various stress treatments indicated that there was one gene most prominently expressed from each family, and that the other member(s) were stress inducible. Proteomic characterisation of mitochondria indicated that the proteins detected correlated with transcript abundance. Surprisingly we found that many genes encoding import components were more highly expressed in senescing tissue, in contrast to nuclear encoded plastid gene expression, which had significantly decreased. We are characterising novel components of the plant mitochondrial protein import apparatus by matching the presence of proteins and expression profiles of unknown proteins with that of known components in plants. To elucidate the function of the various protein isoforms we are using knock-out mutants, over-expressing selected isoforms and monitoring gene expression of mitochondrial import components and some other mitochondrial and plastid components. Reconstitution import assays will be used to assign functions and determine mechanisms. We are analysing the promoters of key import components to elucidate the factors and signals that activate gene expression responsible for mitochondrial biogenesis and retrograde regulation.

Murcha et al (2003) Plant Physiol 131: 1737-1747
Lister et al (2004) Plant Physiol 134 (in press)

T03-002

An Arabidopsis Mitochondrial Proteome

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Mitochondria are the principle sites for energy production within the cell but also undertake a range of other essential biochemical processes. Plant mitochondria code for approximately 100 distinct open reading frames, although a fully functioning organelle is presumed to contain between 1000 and 2000 distinct proteins. We have been utilising 2D-PAGE and non-gel techniques in an attempt to define the Arabidopsis mitochondrial proteome through mass spectrometry. Thus far we have identified approximately 400 proteins using this approach (1). These data have been used to populate an Arabidopsis Mitochondrial Proteomic Database (AMPDB) (http://www.mitoz.bcs.uwa.edu.au) for further analysis. Homology based comparisons with recently available mitochondrial proteomes from human, yeast and the closest living mitochondrial progenitor Rickettsia prowazekii, indicate that the majority of homologous mitochondrial proteins from these diverse species belong to the broad functional groups of energy and metabolism. In contrast, many of the less represented functional categories shared little cross-species homology with the other species which included a large set of approximately 70 unknown proteins. This analysis indicated that plant mitochondria (and likely human mitochondria, yeast mitochondria and Rickettsia prowazekii) contain many unique processes that are not evolutionarily shared with these other diverse species. The identification of such a large protein organelle set has also provided us with the capability to assess sub-cellular prediction programmes. Using the relational querying capabilities of the AMPDB we evaluated the performance of five prediction programmes. Generally these programmes were capable of predicting between 40 and 50% of the experimentally determined mitochondrial proteins. However these findings must be considered in the context that over 10,000 proteins (~35% of the Arabidopsis proteome) are predicted to be localised to the mitochondria by any one of these prediction programmes. More recently in an attempt to obtain a greater proteomic depth we have been sub-fractionating the mitochondria prior to mass spectrometric analysis.

(1) Heazlewood, JL et al., (2004). Plant Cell 16, 241-256.

T03-003

Regulation of signaling and membrane dynamics by RAC GTPase in Arabidopsis

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Plants have a family of RAC GTPases implicated in regulation of numerous signaling processes involved in growth, development and defense. Yet, very little is known at the molecular level how RACs function. Results from transgenic and mutant plants, yeast and mammalian cell lines will be presented. Co-localization immuno assays in mammalian cells show that similar to the mammalian RACs, activated Arabidopsis RACs co-localize with actin at the plasma membrane, localize at cell-cell junctions and induce formation of membrane ruffles. These results suggest that plant RACs compose a subfamily of RAC proteins and not a divergent group in the Rho superfamily of small GTPases. In transgenic Arabidopsis, activated RACs induce vesicle fusion at the plasma membrane regulating cell shape and growth. This activity of the plant RACs is actin-independent and presents a new dimension of Rac function. To elucidate RAC signaling networks we searched for proteins that interact with GTP or GDP-bound RACs. Several novel interacting proteins were identified in yeast two-hybrid assays. Because type-II RACs are posttranslationally palmitoylated in plants but not in yeast, we sought to determine RACs protein-protein interactions in plants. To this end, we developed an assay based on reconstitution of fluorescent YFP chromophore by interaction of fused proteins. Using this assay, we have observed differences in RAC protein-protein interaction between yeast and plants. These results raise interesting possibilities on regulation of RAC activity, and signaling downstream of RACs.

T03-004

A role for ubiquitin in plant cell death

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A role for ubiquitylation in apoptosis is well established in animal cells. In plants, however, many components of the mammalian apoptotic machinery have no recognizable homologs, so that mechanistic similarity can not be taken for granted. We therefore initiated a genetic approach to identify and study the role of ubiquitin in plant cell death processes. An earlier result of our lab indicated that expression of a ubiquitin variant with Lys 48 replaced by Arg (ubR48) can induce cell death in plants (Bachmair et al. 1990, EMBO J. 9, 4543; Schlögelhofer et al., submitted). We constructed an inducible poly-ubR48 gene for expression in Arabidopsis thaliana. Mutants were selected that can withstand inducing conditions that lead to death of wild type plants. The mutants are currently being analyzed and shall be used for positional cloning of the affected genes. In parallel, a chemical genetics approach was initiated to find substances that prevent death of plants under selection conditions similar to those used for the genetic screen. Substances that prevent cell death in our assay shall be used for further studies.

T03-005

The N-end rule pathway for protein degradation

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The N-end rule pathway is a ubiquitin-dependent proteolysis pathway that exists in all eukaryotes. The degradation signal contained in substrates of this pathway is a bulky first amino acid residue. Recently, a role for this pathway in senescence of plants was established (Yoshida et al. 2002, Plant J. 32, 129). In Drosophila, the pathway is involved in the fast cell death process of apoptosis (Ditzel et al. 2003, Nat. Cell Biol. 5, 467). We have been characterizing ubiquitin ligases of the N-end rule pathway. PRT1 (At3g24800), an apparently plant-specific component, exclusively ubiquitylates proteins with aromatic amino-terminal residues (Stary et al. 2003, Plant Physiol. 133, 1360). Another gene of Arabidopsis, At5g02310, is homologous to ScUBR1, the ubiquitin ligase of the S. cerevisiae N-end rule pathway. At5g02310 is annotated as the ECERIFERUM3 gene. However, we find that T-DNA insertion mutants in this gene do not have the typical cer3 waxless phenotype. We are currently analyzing the involvement of At5g02310 in the plant N-end rule pathway, its possible contribution to the senescence process, and potential reasons for the lack of the cer3 phenotype.

T03-006

Comparative and functional genome analysis corroborate the existence of ESCRT (endosomal protein sorting complexes required for transport) in Arabidopsis thaliana

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In contrast to the increasing attention on protein sorting via multi-vesicular bodies (MVBs) in yeast and mammalian cells, knowledge in plants remains limited and nothing is known about the ESCRT complexes -I, -II and -III which are involved in cargo recognition, complex assembly, sorting, vesicle formation and recycling of membrane proteins.
MVBs are recognized by their intralumenal vesicles/membranes and belong to a subset of late prevacuolar endosomes. MVBs are crucial for the partitioning of proteins either for degradation or recycling and are at the crossroad of the biosynthetic and endocytic protein transport routes between the trans-Golgi network (TGN), the vacuole and the plasmamembrane.
For instance in yeast and mammals some monoubiquitinated membrane-bound proteins as cell surface receptors or the precursor of carboxypeptidase S are targeted to the vacuole via the MVB pathway for degradation or activation, respectively. Their sorting is executed by class E Vps proteins which assemble into ESCRT complexes -I, -II, and -III, and the AAA-type ATPase Vps4. Here we show that all class E VPS genes involved in MVB sorting are existing in the Arabidopsis genome and present initial data about their function.

This project is supported by the Austrian Science Fund (FWF) project nos. P16420-B12 to MTH

T03-007

Molecular characteristics of REP (Rab Escort Protein) subunit of Rab prenyltransferase from Arabidopsis thaliana

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Intracellular vesicular transport in eukaryotic cells involves the activity of more than 60 GTPases of the Rab family. Targeting of Rab proteins to the membrane requires prenylation by Rab geranylgeranyltransferase (RabGGTase), the enzyme consisting of catalytic α/β heterodimer and an accessory Rab Escort Protein (REP). Prenylation by RabGGTase results in addition of two geranylgeranyl groups bound via thioether linkage to the carboxylterminal cysteine containing CC or CXC motifs (where X is any amino acids) of Rab proteins. On the other hand REP is the product of the choroideremia gene (CHM) that when deleted in hetereditary disease leads to loss of vision. The cDNA encoding REP obtained in the RT-PCR reaction was cloned in pQE vector. The 6〈His-tagged REP was expressed and purified from E.coli extracts on Ni-agarose. Identification of thus obtained protein was performed using anti-human REP1 antibody. Biochemical tests are in progress. The protein will be also used for preparation of antibodies. A. thaliana insertion mutants in the REP gene (seeds obtained from Nottingham Arabidopsis Stock Centre, UK) are analyzed in order to characterize the plant fenotype, changes in Rab GGTase activity and the profile of prenylated proteins.

T03-008

From genomics to cellular dynamics: Dissection of guard cell ABA signal transduction mechanisms

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Guard cells are a well-suited model for dissecting early signal transduction mechanisms. Relatively few signal transduction components have been identified from recessive ABA insensitive disruption mutants known to function during early ABA signal transduction upstream of transcription. The limited number of genetically identified positive ABA transducers is most likely due to redundancy in genes encoding ABA signaling components. To overcome this limitation and to dissect redundant signal transduction proteins, we have developed an alternative “single cell-type genomics” approach. This approach includes gene chip experiments performed with Arabidopsis guard cell RNA and degenerate oligo-based PCR screening of Arabidopsis guard cell cDNA libraries. Data obtained from detailed molecular genetic and cell biological analyses demonstrate that two guard cell-expressed NADPH oxidase catalytic subunit genes play central roles as positive signal transducers in guard cell ABA signal transduction. In addition, comprehensive analyses of microarray experiments with Arabidopsis guard cell and mesophyll cell RNA will be presented. From the microarray results, we identify a strongly ABA-induced protein phosphatase 2C gene in guard cells. A T-DNA disruption mutation in this gene confers ABA-hypersensitive regulation of stomatal closing and seed germination. The presented data provide a basis for cell-type specific genomic scale analyses of gene function.

T03-009

Cellulose biosynthesis and cell elongation

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Cellulose plays a central role in plant development. The orientation of microfibrils is regulated and controls growth anisotropy and cell shape. The acquisition of the ability to control the orientation of microfibrils appears to have been a crucial event in the colonisation of terrestrial ecosystems. Understanding cellulose synthesis and deposition is therefore essential for understanding plant growth, development and evolution. Cellulose-deficient mutants have been isolated (Mouille et al., 2003), including mutants in cellulose synthase isoforms (Fagard et al., 2000), a membrane-bound endo-1,4-ß-glucanase (EGase, Nicol et al., 1998) and a novel predicted integral membrane protein (Pagant et al., 2002). Besides regulation at the transcript level, cellulose synthesis is potentially regulated at several other levels such as enzyme activity, protein turnover, dimerization via disulfide bridge formation under redox control, assembly and disassembly of the cellulose synthase complex and trafficking of the complex between intracellular compartments (golgi, endosome) and the plasma membrane. Mutations in KOR, encoding a membrane-bound EGase cause a deficiency in cellulose and do not affect xyloglucans, indicating that the enzyme is directly involved in the synthesis of microfibrils. We show that the enzyme is a part of high molecular weight complex that can be observed in Arabidopsis seedlings, but also in cotton fibres. Interestingly, the molecular weight of the complex changes during cotton fibre development and herbicide treatments, suggesting that KOR interacts with different partners at different growth stages. According to the sequence, KOR is predicted to be an integral membrane protein. Cellulose is produced at the plasma membrane and KOR is expected to act at the plasma membrane-cell wall interface. Using immunofluorescence on Arabidopsis root cells, AtKOR was detected in intracellular patches that are different from the Golgi apparatus. We further examined the intracellular trafficking of AtKOR using different GFP-KOR proteins. Potential roles for the enzyme in the synthesis of cellulose will be discussed.

Nicol et al. 1998 EMBO J., 17, 5563-5576. Fagard et al. 2000 The Plant Cell, 12, 2409-2423. Pagant et al. 2002, The Pla

T03-010

Uncovering COP9 signalosome-dependent processes in plants through the isolation of new CSN interacting factors

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In the last few years the multi-subunit COP9 signalosome complex (CSN) has been shown to be able to regulate a number of distinct developmental pathways in virtually all eukaryotes. This is achieved mainly by the ability of CSN to control the activity of ubiquitin ligases both in animals and plants. The ubiquitin/proteasome pathway is one of the most important ways to control plant responses to external and internal stimuli. In fact, in Arabidopsis, CSN has been already implicated in the control of a broad array of cellular and physiological responses, such as light response, hormone signalling, flowering and pathogens response. In an attempt to identify novel CSN-dependent processes we have isolated several plant specific factors, which co-purify with CSN. Their molecular and functional characterization is underway and will provide new insights into the complex CSN function.

Serino G and Deng, XW (2003), Ann. Rev. Plant Biol., 54: 165-182.

T03-011

The family of conserved glycoproteases from higher plants and bacteria

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Glycoproteases (Gcp), called also O-sialoglycoprotein endopeptidases, are putative Zn-metalloproteases belonging to the M22 peptidase family (http://merops.sanger.ac.uk) whose members might contain an additional chaperone activity. Interestingly, Gcps show no sequence similarity to any known class of proteolytic enzymes, both of the prokaryotic or eukaryotic origin, suggesting their unique physiological role. Progress in genome sequencing revealed that Gcps are highly conserved in taxonomically diverse species from bacteria to man. While all eukaryotic organisms contain two gcp genes (called here gcp1 and gcp2), the prokaryota have only one, either of the gcp1- (Bacteria) or gcp2-type (Archaea). We cloned gcp1 genes from bacteria and higher plants and demonstrated that encoded products are integral membrane proteins with two predicted transmembrane helices located in the inner mitochondrial membrane of Arabidopsis thaliana or plasma membrane of Escherichia coli. Topology studies revealed that the catalytic center of Gcp1 in A. thaliana is directed toward the mitochondrial intramembrane space and the preliminary data obtained for E. coli homologue suggest a periplasmic location of this domain. We demonstrated that A. thaliana Gcp1 is expressed only transiently at the early stages of the seedling development with the maximal expression level reached between 1-3 weeks from the seed germination. Using immunocytochemistry we showed that Gcp1 is strongly expressed in axially meristems. Also young developing organs, such as roots, leaves, flowers and seed pods expressed significant amounts of Gcp1. In fully differentiated tissues or fully developed organs Gcp1 was not detected under normal growth conditions. Although the physiological role of Gcp1 is not yet known, this protein is essential for cellular life, as proven by the lethality of E. coli deletion mutants. Therefore, we constructed various conditional Gcp1 mutants in E. coli, where the gcp1 gene was inserted into a plasmid encoding an inducible arabinose promoter and where the chromosomal gcp1 gene was deleted. While depletion of a plasmid-encoded gcp1 expression lead to a cessation of E. coli cell division its induction by arabinose rescued the wild type phenotype. These data suggest that the role of Gcp1 might be connected to cell division and/or differentiation.

T03-012

Analysis of RNase Z proteins from Arabidopsis thaliana

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In all organisms tRNA genes are transcribed as 5' and 3' extended precursor molecules that have to be processed to become functional tRNAs. The maturation of these precursors is accomplished by several processing reactions that include removal of 5'-leader and 3'-trailer sequences. In all organisms analysed, the 5' end is generated by an endonucleolytic cleavage catalysed by RNase P, whereas 3' end maturation of tRNA seems to be more variable. In bacteria, generation of mature 3' end is initiated by an endonucleolytic cut downstream of the discriminator nucleotide before exonucleases remove the residual 3' trailer nucleotides. In eukaryotes, 3' termini of tRNAs are generated through an endonucleolytic pathway mediated by RNase Z. Despite of the prokaryotic origin of organelles, several studies on 3' end maturation of tRNA showed that they also follow the endonucleolytic, eukaryotic mechanism. RNase Z proteins belong to the Elac1/2 protein family. The C-terminal sequences of Elac1 and Elac2 are conserved. The Elac1 proteins are about 250- 380 amino acids long, whereas eukaryote- specific Elac2 proteins contain 800- 900 amino acids.
In Arabidopsis thaliana four RNase Z genes were identified: AthZ1 (nuz) and AthZ2 (cpz) are short forms homologous to the Elac1 protein. AthZ3 (mtz) and AthZ4 (cp2z) are long forms homologous to the Elac2 protein. AtZh1 was the first identified RNase Z.
To characterise the individual functions of RNase Z homologs in Arabidopsis thaliana we first analysed the subcellular localisation of all four proteins. The N-terminal parts of the RNAse Z proteins were fused to the GFP protein in psmGFP vector and these constructs were transformed into protoplasts of Nicotiana tabacum. As an alternative approach we transformed Nicotiana bentamiana by injection of Agrobacterium tumefaciens harbouring respective constructs. We analysed tissue- and development specific transcription activity by GUS-promoter fusions. T-DNA insertion mutants and RNAi knock down mutants were used for further functional analysis of RNase Z.

T03-013

Has the Arabidopsis NAC domain protein ATAF1 a regulatory function within stress and glucose signaling?

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Members of the plant-specific NAC (NAM (no apical meristem) ATAF1/2, CUC2 (cup-shaped cotyledons)) transcription factor family have been implicated in the regulation of development and differentiation. They share a conserved N-terminal region ("NAC domain") and a highly diverged C-term, which is specific for each member. The Arabidopsis NAC-domain protein ATAF1 was identified in the two-hybrid system and in vitro as a binding partner of SNF1-related kinases (SnRK´s) AKIN10 and AKIN11. Out of the NAC proteins only the ATAF1 was identified as interacting partner and the binding domain was mapped to the C-terminal end indicating a specific interaction of these proteins.
SnRK´s are essential in stress and glucose signaling, which is involved in pleitropic regulation of metabolic, hormone and morphological stress responses. SnRK activity is inhibited by the WD protein PRL1. Arabidopsis plants over expressing an epitope-tagged ATAF1 protein show dwarfism, changed leaf morphology and growth arrest. Expression in actively dividing cell cultures succeeded only in case of the prl1 null mutant background. Thus the possible regulatory function of ATAF1 within stress and glucose signaling will be discussed.

T03-014

Changes in local auxin concentrations control valve margin formation in the Arabidopsis fruit

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Seed dispersal is an essential process for most plants to ensure successful reproduction. Formation of specialized valve margin tissue at the valve/replum borders of Arabidopsis fruits allows for the valves to detach from the replum (a process called fruit dehiscence) and for dispersal of the seeds when fruits are fully matured. Valve margin cell specification is dependent on the actions of the SHATTERPROOF1 and 2 (SHP1/2) genes and of the INDEHISCENT (IND) gene such that shp1/2 double mutant fruits and ind single mutant fruits are indehiscent. Here we show that local changes in auxin concentrations control valve margin cell differentiation. In fact, we demonstrate that ectopic production of auxin in valve margin cells of Arabidopsis fruits results in a complete loss of valve margin specification and dehiscence. Conversely, ectopic inactivation of auxin at the valve margin is sufficient to rescue the indehiscent phenotypes of the shp1/2 and ind mutations. Our data pinpoint the importance of auxin removal for valve margin formation, and furthermore show that the gene products of SHP1/2 and IND function upstream of the auxin removal process.
To gain additional knowledge about events downstream of IND, we performed a suppressor screen of the ind phenotype. Interestingly, we isolated a line with a nonsense mutation in the gene encoding UNUSUAL FLORAL ORGANS (UFO). UFO is a member of the F-box family involved in ubiquitin-mediated protein degradation, and ufo mutants have defects in floral organ identity. Here, we show that besides suppressing the ind phenotype, ufo mutant fruits dehisce prematurely even before the seeds are fully matured. Preliminary data on the role of UFO in valve margin formation will be presented.

T03-015

Knock-out of the Mg-protoporphyrin IX methyltransferase in Arabidopsis : effects on chloroplast development and chloroplast-to-nucleus signalling.

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Chloroplast development is dependent upon the coordinated synthesis of chlorophylls and cognate proteins and upon their specific integration into photosynthetic complexes. Beside their direct role in photosynthetic complex formation, chlorophyll intermediates have also been proposed to play a role in intracellular signalling. Several biochemical and genetic studies have implicated both Mg-protoporphyrin IX (the product of the Mg-chelatase enzyme) and Mg-proto IX monomethyl ester (the product of the Mg-protoporphyrin IX methyltransferase enzyme) as inhibitors of the transcription of nuclear genes encoding photosynthesis-related proteins. However, due to the possibility of substrate channelling occurring between these two enzymes, it was not possible to fully discriminate the specific contribution of either chlorophyll intermediate in the repression of the nuclear genes encoding photosynthesis-related proteins. As an initial approach to address this point, we have previously characterized the Arabidopsis gene At4g25080 encoding the Mg-protoporphyrin IX methyltransferase. In the present work, we took advantage of an Arabidopsis thaliana Mg-protoporphyrin IX methlytransferase knock-out mutant and analyzed the role of this enzyme and its product in chlorophyll synthesis and chloroplast-to-nucleus signalling.

T03-016

Identification and characterization of proteins that interact with an Arabidopsis kinesin

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Kinesin-like calmodulin-binding protein (KCBP), a microtubule (MT) motor protein involved in regulating cell division and trichome morphogenesis, was first isolated in Arabidopsis. KCBP is unique among all known kinesins in having a myosin tail homology-4 and talin region in the N-terminal tail and a calmodulin-binding region following the motor domain. Calcium, through calmodulin (CaM), has been shown to negatively regulate the interaction of KCBP with MTs. Genetic studies have shown that KCBP interacts with several other proteins. Using the yeast two-hybrid system, we have identified three interacting proteins, two that interact with the N-terminal region of KCBP and one that interacts with the C-terminal region. A novel calcium-binding protein (KIC, KCBP interacting CCD-like protein) with a single EF-hand motif interacts with the CaM-binding domain of KCBP. Although both Ca2+-KIC and Ca2+-CaM are able to interact with KCBP and inhibit its MT-binding activity, the concentration of Ca2+ required to inhibit MT-stimulated ATPase activity of KCBP by KIC is three-fold less than that required for CaM. Over expression of KIC in Arabidopsis resulted in trichomes with reduced branch number resembling kcbp/zwi phenotype. These results suggest that KIC modulates the activity of KCBP in response to changes in cytosolic Ca2+ and regulates trichome morphogenesis. A protein kinase (KIPK, KCBP interacting protein kinase) related to a group of kinases specific to plants interacts with the tail region of KCBP. The interaction of KCBP with KIPK was confirmed using coprecipitation assays. We have shown that the catalytic domain is capable of auto-phosphorylation. The third interacting protein identified through the yeast two-hybrid system is a zinc-finger protein. Characterization of this interaction is ongoing. These studies together with the results from genetic studies suggest that KCBP acts as a protein complex.

T03-017

PAS1 immunophilin targets a NAC-like transcription factor to the nucleus during the cell cycle

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Immunophillins represent a large family of proteins characterized by their ability to bind immunosuppressive drugs and by their peptidylprolyl cis-trans isomerases (PPiases) activity (Harrar et al. 2001). Immunophilins have been involved in the regulationof the activity or the targetting of several signal transduction protein complexes in mamals. PASTICCINO1 gene (PAS1) is required for proper cell division and cell differentiation since mutation in this FKBP (AtFKBP70) is associated with ectopic cell division leading to tumorous growth in presence of the plant hormone cytokinins (Vittorioso et al. 1998). Loss of PAS1 function is associated with cell dedifferentiation, ectopic cell proliferation and profound developmental abnormalities (Faure et al. 1998). Here, we show that PAS1 associates with PAN (PAS1 Associated NAC) a member of a large plant specific family of transcription factors called NAC. In vitro assays and FRET experiments in live cells demonstrate a direct interaction between PAN and the C-terminal calmodulin binding domain (CaMBD) of PAS1. This domain is required for the nuclear targeting of PAS1 at the G2/M transition of the cell cycle. Through their association, PAS1 targets also PAN to the nucleus during mitosis. In absence of the CaMBD, PAS1 presents both a nuclear and cytosolic localisation and PAN does not colocalize with PAS1 in the mitotic nucleus. Together our results suggest that the immunophilin PAS1 plays a role in cell proliferation through the targeting of transcription factor to the nucleus during the cell cycle.

Faure (1998) Dev, 125, 909-918
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T03-018

Molecular and Functional Characterization of Metacaspases in Arabidopsis

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Increasing evidence indicates that many cases of plant programmed cell death (PCD) proceed through a mechanism that is functionally conserved between animals and plants. Recent studies have provided evidence for the participation of caspase-like proteases (CLPs) during the activation of PCD including HR cell death. However, to date no functional homologues of animal caspases, which are known to play a crucial role in signaling and executing of animal apoptosis, have been identified in plants. Metacaspases, a family of CLPs found in plants, fungi and protozoa, have recently been shown to have significant tertiary structure homology to animal caspases. However, enzymatic properties and physiological functions of plant metacaspases, especially whether they are involved in the regulation of plant PCD, remain unknown. To determine whether the two predicted family members of Arabidopsis metacaspase genes (AtMCP1a-1c and AtMCP2a-2f) are expressed during development, we performed standard RT-PCR using gene specific primers. Our RT-PCR analysis indicated that all members of AtMCP transcripts, with the exception of AtMCP2e, are differentially expressed and accumulate in all tissues tested. Furthermore, northern blot analysis revealed that transcripts of all type-I metacaspases (AtMCP1a-1c) and two type-II metacaspases (AtMCP2b and AtMCP2d) are rapidly up-regulated at a similar fashion upon infection of leaves with bacterial pathogens, suggesting their possible involvement in the activation of cell death. We have also carried out functional characterization of two AtMCP genes, representing the two subtypes of Arabidopsis metacaspase families, using the well-characterized yeast metacaspase-1 (YCA1) null mutant. Our results indicate that both of these two classes of plant metacaspases could partially replace YCA1 in mediating PCD activation by oxidative stress as well as aging in yeast.
Acknowledgement: our research is supported by a grant from the USDA.

T03-019

Characterization of CULLIN3 in Arabidopsis thaliana

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Cullin proteins belong to a multigene family that include at least three members in budding yeast, six in human, five in C. elegans and five in Arabidopsis thaliana (Shen et al., 2002). These proteins all share the C-terminally located conserved so-called “cullin domain”. All cullins analyzed so far directly interact with RBX1/HRT1/ROC1, a RING finger protein, thereby forming the core module of different ubiquitin ligase complexes. The ubiquitin ligases specifically recruit substrate proteins to ubiquitylation and thereby often target the substrates to subsequent degradation by the proteasome.
CULLIN1, the only cullin protein studied so far in plants, is the scaffold protein of the SCF (SKP1-Cullin-F-box protein) ubiquitin ligase complex. In recent years, the SCF has been shown to be essential for plant development and to play a role in multiple signaling cascades.
Recently, it was reported that CULLIN3 proteins of C. elegans and fission yeast are able to bind proteins containing a BTB domain, and thus assemble in SCF like ubiquitin ligase protein complexes (Krek, 2003). In silico analysis of the Arabidopsis proteins revealed that the genome encodes for more than 50 BTB domain proteins.
In Arabidopsis thaliana two closely related CULLIN3 proteins exist, CULLIN3A and CULLIN3B. Northern analysis and promoter-GUS studies revealed a ubiquitous expression of CULLIN3A/B in Arabidopsis plants.
We aim to investigate if Arabidopsis CUL3A and CUL3B form also ubiquitin ligases containing BTB domain proteins. In order to determine the role of CUL3A/B in plant development, we used a reverse genetic approach and identified T-DNA insertion mutants in each gene andd will analyse the phenotypes of the mutants.

Shen et al (2002) Mol. Biol. Cell 13: 1916 ¯ 1928.
Krek (2003) Nat Cell Biol. 2003 11: 950-951.

T03-020

Mechanisms generating specificity within the Arabidopsis CBL-type calcium sensor protein / CBL interacting protein kinases signaling network

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Calcium ions have been firmly established as ubiquitous second messengers functioning in diverse signaling and adaptation processes in plants. Calcium signal deciphering and signal-response-coupling often involve calcium-binding proteins as responders or relays in this information flow. We have recently described a new family of calcineurin B-like (CBL) calcium sensor proteins from Arabidopsis and identified a specific group of serine-threonine protein kinases (CIPKs, CBL-interacting protein kinases) as targets of these sensor proteins. Comparative CBL-CIPK interaction studies suggested preferential complex formation as one of the mechanisms generating the temporal and spatial specificity of calcium signals within plant cells. Thus, various combinations of different CBL/CIPK proteins can form a complex network that connects extracellular signals to defined cellular responses. Here we present our analysis of additional factors contributing to the required specificity within this signaling network. Comparative expression analyses of different CBL genes revealed a rather specific expression pattern of each calcium sensor. Lipid modification of certain CBL proteins appears to provide an additional signaling switch, since four out of the ten Arabidopsis CBL proteins harbor a potential N-terminal myristoylation motif. In vitro myristoylation assays confirmed the functionality of these motifs. Accordingly, myristoylated CBL1 is attached to the plasma membrane, while non-myristoylated CBL1 is not targeted to the membrane. Moreover, the observed differential subcellular localization of other CBL proteins provides an additional mechanism to spatially separate different calcium signaling processes in the cell.

Kolukisaoglu U., Weinl S., Blazevic D., Batistic O., Kudla J. (2004) Plant Physiol, 134, 43-58.

T03-021

Localization of an ascorbate-reducible cytochrome in the plant tonoplast. Possible involvement in iron metabolism

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Ascorbate (ASC) is a key player in the regulation of cellular redox processes. It is involved in responses to biotic and abiotic stresses and in the control of enzyme activities and metabolic reactions. Despite its importance, key players in ASC metabolism remain to be determined.
Cytochromes b561 (Cyts b561) are a class of newly identified membrane proteins, that catalyze ASC-driven trans-membrane electron transport, and contribute to ASC-mediated redox reactions in subcellular compartments. For example, the chromaffin granule Cyt b561 in the mammalian adrenal gland mediates intravesicular ASC regeneration, supporting the biosynthesis of noradrenaline (Menniti et al. 1986). Biochemical evidence has demonstrated the presence of at least one ASC-reducible Cyt b561 in PM-enriched fractions from plants, including Arabidopsis thaliana (Asard et al. 1989, Bérczi et al. 2001). Putative genes encoding Cyts b561 have been identified in A. thaliana (L.) Heynh. (ecotype Columbia) on the basis of sequence similarity to their mammalian counterparts. However, little is known about the function or subcellular localization of this unique class of membrane proteins.
We have expressed one of the putative A. thaliana Cyt b561 genes (AtCB1) in yeast and demonstrate that this protein encodes an ASC-reducible b-type Cyt with absorbance characteristics similar to that of other members of this family. Several lines of evidence demonstrate that AtCB1 is localized at the plant tonoplast (TO). Isoform-specific antibodies against AtCB1 indicate that this protein co-sediments with a TO marker on sucrose gradients. Moreover, AtCB1 is strongly enriched in TO-enriched membrane fractions, and TO fractions contain an ASC-reducible b-type Cyt with α-band absorbance maximum near 561. The TO ASC-reducible Cyt has a high specific activity, suggesting that it is a major constituent of this membrane.
We have recently isolated a homozygous T-DNA insertion AtCB1 mutant (Atcb1-1), lacking detectable levels of the protein on Western blots. Preliminary evidence suggests that these mutants have an impaired iron metabolism. In addition, transcript levels for AtCB1 were found to be strongly upregulated under specific iron-deficiency conditions in wild type A. thaliana. Our results provide evidence for the presence of a trans-membrane redox components in the plant TO this membrane. Moreover ASC-reducible Cyts b561 may be involved in vacuolar iron metabolism.

Menniti FS, et al. (1986) J Biol Chem: 261; 16901
Bérczi A, et al. (2001) Protoplasma 217: 50

T03-022

"Light regulation of cell cycle progression in living plants"

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The circadian clock regulates rhythmically cyclic physiological phenomena such as photosynthesis, nitrogen and CO2 fixation, nutrient uptake and/or flowering time. Lately an effort has being made in order to understand at molecular level the circadian clock and its interaction with different biological processes. In both prokaryotes and eukaryotes, the circadian clock machinery relies mainly on a negative feedback loop of certain classes of transcription factors which are kingdom-specific (Yanovsky and Kay, 2003). Similarly the cell division cycle (CDC) core machinery is conserved among eukaryotes. Some studies have shown that a control of the CDC by the circadian clock exists in unicellular algae (Goto and Johnson, 1995) and recently it was demonstrated for the first time, that the circadian clock directly controls the expression of cell cycle-related genes regulating the entry into mitosis of regenerating mice liver cells (Matsuo et al., 2003). Few evidences indicate that such a control may also exist in plants, as in the case of root meristem cells of Luffa cylindrica where an increase of mitotic activity has been observed during the night (Castilhos and Diehl-Fleig, 1992). However such interactions between the circadian clock and the CDC remain to be shown in plants, especially at the molecular level. We chose a transcriptional regulation approach in which regulatory region of CycB1;1 (M phase marker) and histone4 (S phase marker) were fused to the luciferase reporter gene. A. thaliana transgenics entrained by different light/dark cycles were assayed in vivo for reporter gene activation. Reporter gene expression profile obtained suggests a control of CDC by the circadian clock.

CastilhosR,Diehl-FleigE 1992BJGen
GotoK,JohnsonCH 1995JCBiol
MatsuoT,YamaguchiS..2003Sci
YanovskyM,KayS 2003NatMolCBi

T03-023

Systematic determination of protein localisation in Arabidopsis cells

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We have developed and applied a novel streamlined approach for studying intracellular localisation of GFP-fusion proteins in Arabidopsis cell suspensions. High throughput transient transformation of cell suspensions by constructs containing constitutive promoters and a N-terminal GFP fusion, delivered by a hypervirulent strain of Agrobacterium, allowed processing of large batches of constructs. A set of Arabidopsis full-length trimmed ORF clones (obtained from the SSP consortium, http://signal.salk.edu/SSP/index.html) have been used for semi automated cloning to convert to GATEWAY expression vectors in a 96 well format. The selection of ORFs has been biased towards genes involved in regulation of growth and development. Localisation patterns of 155 expressed fusion proteins have been studied and the patterns classified into five main categories: nucleus, nucleolus, cytoplasm, organelles and endomembrane compartments, and cell wall. Representative members of several functional groups of proteins included protein kinases (CDK and cyclins, MAPK), protein phosphatases, transcription factors and DNA-associated proteins (AP2, GTFII, HAP, WRKY, MYB, bHLH, DRT families), RNA processing proteins, translation factors, metabolic enzymes and signal transduction factors. Several genes annotated in GenBank as unknown have been ascribed a protein localisation pattern. The localisation patterns for a number of functional groups of proteins were compared with the bioinformatic predictions of subcellular target domains based on the amino acid sequence motifs (using PSORT software). The set of proteins analysed so far have provided some interesting insights into possible biological functions, and in some cases have indicated regulation by control of localisation. This approach can be extended for functional studies including precise cellular localisation and prediction of function of unknown proteins, confirmation of bioinformatic predictions, co-localisation and FRET analysis of interactions, and proteomic experiments.

T03-024

Chloroplast division site placement requires dimerisation of the ARC11/AtMinD1 protein in Arabidopsis

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Chloroplast division is mediated by the coordinated action of a prokaryote-derived division system(s) and a host eukaryote-derived membrane fission system(s). The evolutionary conserved prokaryote-derived system comprises several nucleus-encoded proteins two of which are thought to control division site placement at midpoint of the organelle: a stromal ATPase MinD and a topological specificity factor MinE. Here, we show that arc11, one of 12 recessive accumulation and replication of chloroplasts (arc) mutants in Arabidopsis, contains highly elongated and multiple-arrayed chloroplasts in developing green tissues. Genomic sequence analysis revealed that arc11 contains a missense mutation in alpha-helix 11 of the chloroplast-targeted AtMinD1 changing an Ala at position 296 to Gly (A296G). Introduction of wild-type AtMinD1 restores the chloroplast division defects of arc11 and quantitative RT-PCR analysis demonstrated that the degree of complementation was highly dependent on transgene expression levels. Overexpression of the mutant ARC11/AtMinD1 in transgenic plants results in inhibition of chloroplast division demonstrating that the mutant protein has retained its division inhibition activity. However, in contrast to the defined and punctate intraplastidic localization patterns of an AtMinD1-YFP fusion protein, the single A296G point mutation in ARC11/AtMinD1 results in aberrant localization patterns inside chloroplasts. We further show that AtMinD1 is capable of forming homodimers and that this dimerisation capacity is abolished by the A296G mutation in ARC11/AtMinD1. Our data demonstrate that arc11 is a loss-of-function mutant of AtMinD1 and suggest that the formation of functional AtMinD1 homodimers is paramount for appropriate AtMinD1 localization ultimately ensuring correct division machinery placement and chloroplast division in plants.

Fujiwara, M.T., Nakamura, A., Itoh, R., Shimada, Y., Yoshida, S. and Møller, S.G. (2004). J Cell Sci. 117. 2399-2410.

T03-025

Genetic dissection of mucilage secretory cell differentiation in Arabidopsis

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The mucilage secretory cells of the Arabidopsis seed coat provide an excellent model system in which to study not only complex polysaccharide biosynthesis and secretion, but also the regulation of these processes. Mutations in MUM4 lead to a reduction in the amount of pectin produced by these cells. Cloning of MUM4 has shown that it encodes a key enzyme in pectin biosynthesis (putative UDP-L-rhamnose synthase). Expression studies have shown that MUM4 transcription is upregulated in developing seeds through the action of several epidermal cell transcription factors (TTG1, GL2, AP2). While these studies have allowed the construction of a preliminary framework for the regulation of mucilage secretory cell differentiation, there are many questions still to be answered. To begin to address these questions, we are cloning and characterizing two novel genes: PRAIRIE and PATCHY. While PRAIRIE may be involved in the regulation of mucilage production, PATCHY appears to be acting in cell wall modification. Furthermore, we are performing an enhancer/suppressor screen using mutagenized mum4 lines that should reveal genes acting in parallel with MUM4 and genes whose redundant function would be masked in a wild-type background.

T03-026

GIANT CHLOROPLAST 1 is essential for correct plastid division in Arabidopsis

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Plastids are vital plant organelles involved in many essential biological processes. Plastids are not created de novo but divide by binary fission mediated by nuclear-encoded proteins of both prokaryotic and eukaryotic origin. Although several plastid division proteins have been identified in plants limited information exists regarding possible plastid division control mechanisms. Here we report the identification of GIANT CHLOROPLAST 1 (GC1), a new nuclear-encoded protein essential for correct plastid division in Arabidopsis. GC1 is plastid-localised and is anchored to the stromal surface of the chloroplast inner envelope by a C-terminal amphipathic helix. In Arabidopsis, GC1-deficiency results in mesophyll cells harbouring 1-2 giant chloroplasts whilst GC1 overexpression has no effect on division. GC1 can form homodimers but does not show any interaction with the Arabidopsis plastid division proteins AtFtsZ1-1, AtFtsZ2-1, AtMinD1 or AtMinE1. Further analysis reveals that GC1-deficient giant chloroplasts contain densely packed wild-type-like thylakoid membranes and that GC1-deficient leaves exhibit lower rates of CO2 assimilation compared to wild-type. Although GC1 shows similarity to a putative cyanobacterial SulA cell division inhibitor our findings suggest that GC1 does not act as a plastid division inhibitor but rather as a positive factor at an early stage of the division process. GC1 represents a to-date unrecognized plastid division component in Arabidopsis.

Maple, J., Fujiwara, M., Kitahata, N., Lawson, T., Baker, N., Yoshida, S. and Møller, S.G. (2004) Curr Biol. 14. 776-781

T03-027

AtNAP7 is a plastidic SufC-like ABC/ATPase essential for Arabidopsis embryogenesis

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In bacteria, yeast and mammals iron-sulfur (Fe-S) cluster-containing proteins are involved in numerous processes including electron transfer, metabolic reactions, sensing, signaling and regulation of gene expression. In humans iron-storage diseases such as X-linked sideroblastic anaemia and ataxia are caused by defects in Fe-S cluster availability. The biogenesis of Fe-S clusters involves several pathways and in bacteria the SufABCDSE operon has been shown to play a vital role in Fe-S biogenesis and repair during oxidative stress. Although Fe-S proteins play vital roles in plants, Fe-S cluster biogenesis and maintenance and physiological consequences of dysfunctional Fe-S cluster assembly remains obscure. Here we report that Arabidopsis plants deficient for the SufC homolog AtNAP7 show lethality at the globular stage of embryogenesis. AtNAP7 is expressed in developing embryos and in apical, root and floral meristems and encodes an ATP-Binding Cassette (ABC)/ATPase that can partially rescue growth defects in an E. coli SufC mutant during oxidative stress. AtNAP7 is plastid-localized and mutant embryos contain abnormal developing plastids with disorganized thylakoid structures. We found that AtNAP7 can interact with AtNAP6, a plastidic Arabidopsis SufD homolog and because Arabidopsis plastids also harbor SufA, SufB, SufS and SufE homologs, plastids probably contain a complete SUF system. Our results imply that AtNAP7 represents a conserved SufC protein involved in the biogenesis and/or repair of oxidatively damaged Fe-S clusters and suggests an important role for plastidic Fe-S cluster maintenance and repair during Arabidopsis embryogenesis.

Xu, X.M. and Møller, S.G. (2004). Proc. Natl. Acad. Sci. USA. in press

T03-028

Analysis of a putative monoubiquitination-mediated protein degradation pathway in Arabidopsis

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In recent years ubiquitin emerged as a versatile modifier for a variety of different processes. One well-studied function in yeast and mammals is the down-regulation of plasma-membrane receptors in a non-proteasomal fashion. The target protein is covalently modified by a single ubiquitin (monoubiquitination) that serves as a signal for entering the endosomal sorting pathway where it is guided by the ESCRT-complexes into the internal vesicles of multi-vesicular bodies (MVB). Upon fusion with the vacuole/lysosome the internal vesicles of the MVBs and their cargo become accessible to lytic enzymes in the lumen of the vacuole/ lysosome. In plants the existence of a similar protein degradation and sorting pathway is not known. Here we molecularly characterize the ELCH gene, which encodes the key protein of the ESCRT-I complex (Vps23/TSG101 in yeast/mammals). Our genetic and molecular work of the ELCH gene suggests that a similar mechanism exists in plants.

(Katzmann et al., 2001) (Babst et al, 2002) (Bassham, 2000) (Xie, 1998) (Bishop, 2002)

T03-029

Comprehensive oligo-microarray analysis of gene expression profiles during cell elongation

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Cell elongation is essential for plant morphogenesis and includes a lot of biological processes such as cortical microtubule organization and cell wall biosynthesis. Plenty of genes involved in these processes have been identified using molecular biology and genetics. However, the comprehensive information of gene expression during cell elongation is poorly understood. To focus on cell elongation process, we took advantage of the temperature dependence of hypocotyl cell elongation. The Arabidopsis plants were germinated and grown at 21C for 7 days and then grown at 31C for 1 day. The hypocotyl cells stopped elongation in the 7-day-old plants. The wild type cells elongated again after the temperature shift, but this effect was suppressed in the temperature-sensitive cellulose deficient acw (altered cell wall) mutants (Sato et al. 2001). With oligo-microarray analysis, we compared the gene expression profiles among Arabidopsis wild type and the acw mutants.
For microarray experiments, RNA samples were prepared from the hypocotyls. Then, the RNAs were used as templates for Cy3- or Cy5-labeled cRNA synthesis. We used the Agilent Arabidopsis 2 Oligo Microarray and analyzed the data using the computer software “Luminator” (Rosetta Biosoftware).
We have already collected several gene expression profiles of wild type and mutant hypocotyls. More than three thousand genes were up-/down-regulated during cell elongation in wild type hypocotyl. In the mutants, over six thousands of genes were up-/down-regulated, suggesting that the mutations affect the normal gene expression. We are currently analyzing the data to identify the genes involved in cell elongation.

Sato et al. 2001 Plant Cell Physiol., 42, 251-263.

T03-030

A novel CDK-phosphorylation cascade in Arabidopsis thaliana

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The full activation of cyclin-dependent kinases (CDKs) requires phosphorylation of the threonine residue within the T-loop by a CDK-activating kinase (CAK). In addition to the role in CDK phosphorylation and activation, CAK usually functions in phosphorylating the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II as a component of the basal transcription factor TFIIH. While a single CAK has been identified in vertebrates, Arabidopsis encodes four CAK-related kinases (CAK1At-CAK4At) on the genome. Recently we have revealed that CAK2At and CAK4At-immunoprecipitates of Arabidopsis crude extract exhibited both CDK- and CTD-kinase activities. Although their primary sequences are very similar to each other, they phosphorylated CDK and CTD with different preferences. We previously reported that CAK1At is a distinct type of CAK in terms of its primary structure and enzyme activity. CAK1At possessed a high CDK-activating kinase activity but no CTD-kinase activity. Here we found that CAK1At efficiently phosphorylated the potential activation sites of CAK2At and CAK4At, and that the CTD-kinase activity of CAK4At was up-regulated by its phosphorylation in vitro. Genetic complementation experiments with fission yeast mutants supported the idea that CAK1At is an upstream kinase of vertebrate-type CAKs. Moreover, when CAK1At and CAK4At were transiently expressed in Arabidopsis protoplasts, we could observe CAK4At phosphorylation by CAK1At and an enhancement of its CTD-kinase activity. Our data showed that CAK1At functioned as a CAK-activating kinase (CAKAK) in yeast and plant cells, indicating that a novel phosphorylation cascade mediated by multiple CAKs may regulate the cell cycle and transcription machineries in Arabidopsis.

T03-031

Functional analysis F-box protein family using antisense lines

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F-box proteins play roles in SCF complexes or non-SCF complexes. In Arabidopsis, F-box proteins have been reported to be involved in important biological processes such as flower formation, light signal transduction and phytohormone signal transduction by mutant analysis. By database analysis, we found 568 F-box protein genes in Arabidopsis genome, however, biological functions of most of the genes have not been cleared (Kuroda et al. PCP 2002). To understand functions of the F-box protein genes, we have produced about 800 antisense transgenic lines by bulk transformation using 82 F-box protein cDNAs. Phynotype of the antisense lines were analyzed throughout their lifecycle. We found several lines showing abnormal phenotype in leaves, flowering time and other processes of growth and development. We identified transferred cDNAs from the putative mutant lines. Retransformation of the identified cDNAs is in progress to check if the cDNAs are responsible for the mutant phenotype.

T03-032

ACTIVATION TAGGING AS A TOOL TO IDENTIFY AND DISSECT A NEW GENE FAMILY MEMBERS AND THEIR FUNCTIONS IN A.THALIANA

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Activation tagging in Arabidopsis is a powerful tool to identify dominant mutants with various phenotypes and to dissect gene functions. About 1.500 individual T-DNA activation tagged lines from Arabidopsis containing an immobilised element (4x35S Enhancer, Basta resistance, inverted repeats) have been screened for high phenylpropanoid contents by HPLC-analysis using fluorescence and UV techniques. About ten mutant lines were selected, which showed modified HPLC profiles under greenhouse conditions in the T1 generation. To identify genes associated with phenotypes, flanking regions on the T-DNA border of these lines have been amplified and sequenced. In eight out of ten mutants, genes next to the insertion were up-regulated and presumably responsible for the molecular phenotype. Interestingly, most of them turned out to be transcriptional factors and members of large gene families, namely two putative HLH transcription factors, a putative MYB factor, an UDP glycosyltransferase, a calcium dependent protein kinase, a putative RING zinc finger protein, or a bZIP factor. Molecular phenotypes of these lines appeared in a dominant or semi-dominant manner; in some lines, phenotypes observed in the T1 generation did not appear in the next generation, probably because of silencing of the target genes (Ichikawa et al., 2003). Two mutants overexpressing putative MYB and HLH factors have been studied in detail. Complementation experiments of wild type plants using sense constructs of the candidate genes driven by a constitutive 35SCaMV have been performed. Comparative analysis of HPLC profiles of overexpressing lines in comparison with the wild type showed that the observed alterations in plant secondary metabolism are caused by overexpression of the corresponding genes.

Ichikawa T., Nakazawa, M., Kawashima, M. et al. Plant J. 2003, 36, 421-429

T03-033

The Arabidopsis co-chaperone ROF2 encodes a new heat-shock induced FKBP immunophilin, which is developmentally regulated.

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One of the mechanisms for adapting to stressful conditions is the induction of heat shock proteins (HSPs) that one of their related functions is solving problems caused by protein misfolding and aggregation.
The plant co-chaperones FK506 binding proteins (FKBPs) belong to the large family of peptidyl prolyl cis-trans isomerases (PPIases) that have been shown to assist in protein folding. The Arabidopsis plant was shown to possess 22 FKBPs, which have different molecular weights and are present in all subcellular organelles. Several FKBP Arabidopsis mutants have been characterized and it was found that these genes are essential for normal plant development.
We are studying the Arabidopsis large FKBPs Rof1 and Rof2. From sequence comparison it was shown that these genes are similar in structure to the wheat large FKBPs, possessing: FKBP domain, two FKBP-like domains, TPR and calmodulin domains. The expression profiles of the FKBPs have been characterized by RNA and protein analyses as depending on age and biotic stresses. From these results we have seen that while Rof1 is constitutively expressed, Rof2 is heat stress induced. Detailed characterization of the Rof2 expression pattern in 14days old Arabidopsis seedlings revealed that it is induced only after 2h exposure at 37ºC, but in contrast to Rof1, Rof2 is not detected at 42ºC. In seedling that were pretreated at 37ºC and there after exposed to 42ºC, it was found that Rof2 is stably expressed.
In four-week-old Arabidopsis plants, tissue specificity analysis showed that Rof1 and Rof2 are highly expressed in young flowers and siliques. These results were further demonstrated by GUS assay in transgenic plants expressing the GUS reporter gene under the control of Rof2 promoter.
In order to further understand the role of FKBPs in plants, Arabidopsis FKBP knockout mutants and over expression plants are used. Arabidopsis plants over expressing the wheat FKBP73 were analyzed by microarray analysis. Classification of the up regulated genes revealed several important groups: transcription factors, Heat shock proteins such as HSP101 and transporters. Characterization of Rof1, Rof2 and the Rof1,2 double mutant, generated in our lab by the RNAi technique, will further elucidate the role of these genes in planta.

T03-034

Regulation of signal transduction by nucleo-cytoplasmic partitioning of proteins in Arabidopsis thaliana

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In eukaryotic cells the nucleus and the cytoplasm are divided by the nuclear envelope. As a result, transcription and translation are spatially separated, which creates the need for nucleo-cytoplasmic trafficking of macromolecules between these two cellular compartments. In addition, it offers a powerful tool for the regulation of signalling by nucleo-cytoplasmic partitioning of transcription factors.

We characterised the nuclear export receptor Crm1/ Xpo1 from Arabidopsis that specifically recognises proteins with a leucine-rich nuclear export signal (NES) and mediates their export to the cytoplasm. As a novel genomic approach to plant signal transduction we screened for Arabidopsis transcription factors that contain a NES. We have identified several proteins that possess both, a nuclear localisation signal (NLS) and a NES. They belong to different transcription factor families, including MYB factors, bZIP proteins, zinc finger proteins, heat shock factors and others.

We characterised the localisation signals of several of these proteins in detail and provide evidence that they show nucleo-cytoplasmic partitioning. Our results suggest that nuclear export by Xpo1 provides an important mechanism for regulated nucleo-cytoplasmic partitioning of transcription factors and hence for the control of signalling in plants.

Merkle T (2004) Curr Genet 44, 231-260

T03-035

Identification of SNARE molecules involved in the post-Golgi network pathways in Arabidopsis

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In all eucaryotic cells, specific vesicle fusion during vesicular transport is mediated by membrane-associated proteins called SNAREs (soluble N-ethyl-maleimide sensitive factor attachment protein receptors). Sequence analyses revealed 54 SNARE genes (18 Qa-SNAREs/Syntaxins, 11 Qb-SNAREs, 8 Qc-SNAREs, 14 R-SNAREs/VAMPs and 3 SNAP-25) in the Arabidopsis genome. RT-PCR analyses revealed that all SNARE genes but AtYKY62 were differentially expressed among tissues. A series of transient expression assays using GFP (green fluorescent protein) fused proteins revealed the subcellular localization of the SNARE proteins involved in various vesicular transport pathways; 6 ER-localized SNAREs, 9 Golgi apparatus-localized SNAREs, 7 TGN-localized SNAREs, 2 endosome-localized SNAREs, 18 PM-localized SNAREs and 9 vacuole-localized SNAREs. The data showed that the trans-Golgi network (TGN) was an independent organelle separated from the Golgi apparatus. The results obtained in this study suggest that combinations of SNARE molecules are involved in each transport pathway, indicating the complexity of the post-Golgi transport pathways.

T03-036

In vitro evolution of telomerase-deficient tissue cultures of Arabidopsis thaliana

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Telomerase is the reverse transcriptase responsible for the extension of telomeric repeat sequences in most species studied. Telomerase inactivation causes telomere shortening and results in the loss of the telomere's protective function, which in mammals leads to cell cycle arrest and apoptosis. Experiments performed on Arabidopsis thaliana mutants lacking telomerase activity showed their great tolerance for genome instability. Plants survived up to ten generations of selfing, but during the last five generations they suffered from developmental defects and genome rearrangements manifested by anaphase bridges, nuclear fragmentation, and aneuploidy.
In this study we present karyological analysis of two in vitro cell lines (A-G8 and B-G8) derived from seeds of the 8th generation of telomerase-deficient A. thaliana. On slides prepared by enzymatic squashing of fixed tissue cultures, nuclear cytometry and FISH with centromeric, 25S-rDNA, and telomeric probes was performed. As expected, in wild type in vitro cultures, genomes were stable. In the two G8 cell lines the absence of telomeric repeats (TTTAGGG) at the ends of chromosomes was demonstrated by Southern blot analysis and FISH with a telomeric PNA probe. The B-G8 line, which was cultured for 2 years in vitro, suffered from severe growth irregularities, including a loss of typical callus morphology (friable structure) and a high mortality in numerous callus sectors. This line also harboured frequent anaphase bridges and a high variation in chromosome number. A great proportion of nuclei were polyploid and displayed an increased number of 25S-rDNA signals. The other line A-G8 was cultured for 3 years in vitro survived a growth crisis, and is now phenotypically stable and fast-growing. Despite being cultured longer in vitro, the A-G8 line possesses the standard callus phenotype, and is much more homogenous in all parameters (ploidy level, number of FISH signals). These findings imply its genome is stabilised. However anaphases bridges were still detected in A-G8 samples. We speculate that a partial stabilization of the A-G8 genome occurred from restructuring chromosome terminal regions to provide chromosome ends with partial, but not complete protection from end-joining activities.

Acknowledgements: This research was supported by the Czech Academy of Sciences (A6004304).

Riha et al., Science 291: 1797, 2001
Siroky et al., Chromosoma 112: 116, 2003

T03-037

The chloroplast SRP-pathway: Molecular analysis of protein-protein interactions

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All known cytosolic signal recognition particles (SRPs) are involved in the cotranslational insertion of membrane proteins and their minimal functional core is formed by an RNA and a conserved ~54 kD protein (SRP54). Recently, it was demonstrated that chloroplasts contain a novel type of SRP that is involved in the posttranslational targeting of the nuclear encoded light harvesting chlorophyll proteins (LHCPs) to the thylakoid membrane. Like all known cytosolic SRPs chloroplast SRP contains a 54 kD subunit (cpSRP54). However, in contrast to cytosolic SRPs chloroplast SRP contains no RNA but a novel protein subunit of 43 kD (cpSRP43). Furthermore, it is remarkable that the 54 kDa subunit of cpSRP is also involved in the co-translational transport of chloroplast encoded D1 to the thylakoid membrane (reviewed in Schünemann, 2004). These findings raise the central question of how cpSRP54 is recruited for functioning in either the cpSRP43-dependent post-translational or the cpSRP43-independent co-translational cpSRP-pathway. In order to analyse whether cpSRP43 competes with the ribosome or the nascent protein for the same binding site on cpSRP54 and to identify specific sequence characteristica of cpSRP54, that makes cpSRP54 unique in its ability to bind cpSRP43, we characterized the binding site of cpSRP54 for cpSRP43. We identified a positively charged nonapeptide located close to the C-terminus of cpSRP54 that mediates binding to cpSRP43. Furthermore, we demonstrate that two arginine residues located within this region are essential for binding cpSRP43.

Schünemann (2004), Current Genetics, 44: 295-304
Schünemann et al. (1998) PNAS, 95:10312-10316

T03-038

DOES AtWNK8 REGULATE THE V-ATPase BY PHOSPHORYLATING VHA-C?

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WNK [With No lysine (K)] kinases constitute a novel class of Serine/Threonine protein kinases found in multicellular organisms, whose members show a highly conserved kinase domain with an unusually positioned essential lysine residue.
The subunit C of the V-ATPase (VHA-C) was shown to interact with a member of the Arabidopsis WNK subfamily - WNK8 - in a yeast two-hybrid screen. We could confirm the interaction between WNK8 and VHA-C in Farwestern analyses and preliminary data suggest that WNK8 contains two independent domains capable of interacting with VHA-C. Furthermore, WNK8 phosphorylates VHA-C in in vitro kinase assays. To investigate the relevance of this interaction in vivo, we generated a vha-C/wnk8 double mutant, AtWNK8 overexpression lines, and RNAi plants and will use them to determine if varying growth conditions affect V-ATPase activity via DET3 phosphorylation. Here we will present data characterizing the interaction in vitro and in vivo.

T03-039

The Arabidopsis KLUNKER gene encodes a putative regulator of the Arp2/3 complex

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The Arp2/3 complex is a well-described modulator of the actin cytoskeleton and it was recently shown to be an important regulator of actin-controlled cell morphogenesis in plants. The klunker mutant exhibits a very similar phenotype to the arp2/3 mutants suggesting that the KLUNKER gene is involved in the same pathway. Here we show that the KLUNKER gene encodes the PIR121 homolog which is a known upstream regulator of the Arp2/3 complex in animals and yeast.

T03-040

Exocyst complex in plants

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The exocyst (Sec6/8 complex) is a conserved protein complex comprising eight distinct subunits experimentally characterized from yeast, Drosophila and mammalian cells (reviewed e.g. in Hsu et al 2004). The exocyst localizes to specific domains of the plasma membrane corresponding to local maxima of secretion, e.g. the very tip of the emerging bud or the neck region during cytokinesis in yeast, and the region of tight junctions in mammalian epithelial cells. The exocyst, in concert with the actin cytoskeleton and RHO and RAB GTPases, is believed to function as a spatial landmark and tether secretory vesicles to the plasma membrane for fusion. The exocyst from plant cells has not yet been characterised. However, recent investigation of cell plate formation by electron tomography revealed the involvement of exocyst-like particles in tethering of vesicles (Segui-Simarro et al 2004). In our in silico analysis we have found homologues of all exocyst subunits from both Arabidopsis and rice; the peculiar aspect of plant genomes is unprecedented multiplicity of paralogous genes potentially coding for the Exo70 subunit (Elias et al 2003). We have prepared a recombinant Arabidopsis Sec6 protein and raised polyclonal antibodies that have been used to detect Sec6-related protein(s) in high molecular weight fractions of cauliflower using SEL and IEC. We were also able to localize Sec6 epitopes to the growing tip of tobacco pollen tubes. GFP-tagged versions of two exocyst subunits have been prepared and their localisation is being studied; the results indicate that the GFP tag may interfere with the incorporation of tagged subunits into the complex, as described previously in mammalian cells. We will also report on current progress of exocyst reverse genetic studies. This work is supported by the grants of MSMT CR- LN00A081"SIDROS", GAAV CR -A6038410 and EU-HPRN-CT-2002-00265 RTN project "TIPNET".

Elias et al Cell Biol Int 27:199, 2003
Hsu et al Int Rev Cytol 233:243, 2004
Segui-Simarro et al Plant Cell 16:836, 2004

T03-041

Stability of Microtubules Containing Modified Alpha-Tubulin

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In plant cells, cortical microtubule (MT) arrays generally regulate the direction of elongation. The recessive spiral1 and spiral2 mutants show right-handed helical growth in roots and etiolated hypocotyls. In contrast, the semi-dominant lefty1 and lefty2 mutants show opposite left-handed helical growth in these organs, and are caused by dominant negative mutations in α-tubulin genes. In elongating root epidermal cells of spiral and lefty mutants, cortical MTs are respectively arranged in left-handed and right-handed helical arrays. However the nature of helical MT arrays remains to be determined.

When α-tubulin6 (TUA6) was tagged with GFP, HA or myc at the N-terminus and expressed under the CaMV35S promoter in transgenic plants, the fusion protein was incorporated into MT polymers and caused right-handed helical growth in petioles and petals similar to those found spiral2. Especially HA-TUA6 transgenic lines generated strong phenotypes; radial expansion in roots and had increased trichome branching. The cortical MTs in elongating root epidermal cells of HA-TUA6 were arranged in left-handed helical arrays, and the leading plus end of HA-TUA6 MTs showed slower growth and shortening velocities, increased rescue, and an overall decrease in MT turnover. GFP-AtEB1 labeling at the plus end became larger and extended into the more central part of MTs in HA-TUA6 cells. These results suggest that expression of N-terminally tagged TUA6 results in more stable MTs which are arranged in left-handed helical arrays.

T03-042

DISTORTED2 encodes for the Arabidopsis ARPC2 subunit of the ARP2/3 complex

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The “DISTORTED” mutant class in Arabidopsis thaliana consists of eight genes, ALIEN, CROOKED, DISTORTED1, DISTORTED2, GNARLED, KLUNKER, SPIRRIG and WURM, which show altered shapes in many cell types including leaf trichomes, pavement cells, hypocotyl cells and root hairs. The common trichome phenotype was phenocopied by actin interacting drugs, suggesting that the disruption of the actin cytoskeleton is responsible for the mutant phenotype. The finding that WRM, DIS1 and CRK encode ARP2, ARP3 and ARPC5 subunits of the putative ARP2/3 complex respectively supports this view. In animals the ARP2/3 complex serves as efficient modulator of the actin cytoskeleton. Here we provide molecular and cell biological evidence to prove that DISTORTED2 encodes the Arabidopsis homolog of the ARPC2 subunit of the ARP2/3 complex.

T03-043

Cellular localization of AtFH8, an Arabidopsis Class I formin

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Formins are a family of proteins sharing a core actin-nucleating domain, FH2, conserved among eukaryotes. Bioinformatic analyses have demonstrated that formins of higher plants have multiplied and diverged into two classes; one of them (Class I) consists predominantly of proteins with putative membrane insertion signals [1,2; see also Poster T06-004]. Membrane association and role in tip growth have been so far demonstrated by others for a single plant formin, AtFH1 [3,4].
We have studied the localisation of another Arabidopsis Class I formin, AtFH8, using GFP-tagged derivatives of this protein, expressed both in a heterologous transient expression system (Nicotiana benthamiana leaves) and in stably transformed Arabidopsis, where truncated, C terminally tagged AtFH8 (AtFH8':GFP) has been introduced under the control of the alcohol-inducible promoter, pAlcA. In both cases, we observed cortical localisation, consistent with plasmalemma association, and occassionally also labelling of mobile intracellular bodies, either insoluble inclusions or derivatives of the endomembrane system. However, some nuclear signal was also seen, corresponding probably to free GFP that was detected on Western blots. In stable Arabidopsis transformants, we have found that, depending on culture and induction conditions, the AtFH8':GFP mutant protein may interfere with root hair development, supporting its role in cell morphogenesis.
This work has been supported by GACR204/02/1461 and EU-HPRN-CT-2002-00265 grants and by a NATO Advanced Fellowship to V. Zarsky. We thank M. Deeks and P. Hussey for inspiring discussion and methodological advice.

[1] Genome Biol 1:res 001, 2000 [2] TiPS 7:492, 2002 [3] Plant Cell Physiol 41:617, 2000 [4] Plant Cell 16:257, 2004

T03-044

A novel function of cyclin-dependent kinase inhibitors - KRP1/ICK1 can block mitosis and trigger endoreduplication

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Control of the cell-division cycle is crucial for organism architecture of all higher eukaryotes. Cell-cycle progression requires the coordinated activation and inactivation of a class of serine/threonine protein kinases, called cyclin-dependent kinases (CDKs). CDK activity is controlled by various mechanisms including the binding of phase-specific cyclin partners and phosphorylation/dephosphorylation at specific sites. An ubiquitously present control mechanism is the binding of CDK inhibitors (CKIs). In Arabidopsis, a family of seven low-molecular weight CKIs (KRPs/ICKs) has been identified, which show homologies to the mammalian p27kip1. Previously, we have shown that misexpression of KRP1/ICK1 in endoreduplicating trichomes can reduce the number of endocycles indicating an inhibitory function at the G1/S transition point in this cellular context.

Here, we show that misexpression of KRP1/ICK1 in dividing cells can block mitosis but still allows DNA replication leading to enlarged cells with highly endoreduplicated nuclei. Endoreduplication is often associated with terminal differentiation; we observed, however, that cell-fate specification was independent from KRP1/ICK1-induced endoreduplication. Strikingly, we found that endoreduplicated cells were able to reenter a cell-division program emphasizing the high degree of flexibility in plant development.

T03-045

Identification and Characterisation of the Arabidopsis thaliana DISTORTED Mutant GNARLED

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We use Arabidopsis leaf trichomes as a model system to study plant morphogenesis. A class of eight mutants called “DISTORTED” mutants revealed insight in the role of the actin cytoskeleton in the directionality of expansion growth. DIS1, DIS2, WRM and CRK have recently been cloned and shown to encode subunits of the ARP2/3 complex, which is essential for actin filament nucleation. Here we show that GNARLED encodes a homolog of the ARP2/3 regulating gene NAP125. A phenotypic characterisation of the mutant and the potential function of the encoded protein will be discussed.

T03-046

Screen for plasmodesmal associated proteins

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In a microscopic screen of a GFP::cDNA library expressed in Arabidopsis thaliana (Cutler et al., 2000) a protein fragment was detected which showed localization consistant with plasmodesmata. These structures are unique to plants and play a crucial role in symplastic cell-to-cell and long distance communication and in systemic movement of viruses. Despite these important functions, the molecular composition and means of biosynthesis of plasmodesmata are widely unknown. The newly identified clone D41, a truncated syntaxin, is now been used to identify interacting proteins that are responsible for its localization and may be part of plasmodesmal channels. Two constructs have been synthesized for purification of protein complexes: T7-His6-GFP-D41 and His6-FLAG-GFP-D41. Both constructs were used to transform Arabidopsis plants. The GFP allows for easy screening of positive tranformants and can, like the other two tags, be used to purify D41 from the plant extract. Probable interacting proteins that are isolated together with D41 will subsequently be identified via LC-MS/MS. Promising candidates from this screen can then be tested for their possible roles in plasmodesmal synthesis, structure, or function, and in a similar manner be used to identify further components of plasmodesmata.

Cutler, S.R.; Ehrhardt, D.W.; Griffitts, J.S.; Somerville, C.R. (2000)
Proc Natl Acad Sci USA 97: 3718-3723

T03-047

Progression through meiosis I and meiosis II in Arabidopsis anthers is regulated by an A-type cyclin predominately expressed in prophase I

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Although superficially similar to mitosis, meiosis II lacks a preceding S-phase. This feature renders meiosis II a special case for testing the roles of mitotic cyclins. There are two types of mitotic cyclins, A and B. Some aspects of B-type cyclins in meiosis II have been deciphered, but the roles of A-type cyclins in meiosis II are obscure at best. In particular, high levels of A-type cyclins have not been found during the meiosis I-meiosis II transition. How a change in the activity of an A-type cyclin might affect progression of meiosis II has not been genetically tested, because such mutations result in apoptosis in prophase I in animals. We cloned the Tardy Asynchronous Meiosis (TAM) gene of Arabidopsis thaliana and found that it encodes an A-type cyclin, CYCA1;2. The tam phenotype is temperature sensitive; tam plants exhibit slowed male meiosis and consequently form dyads and triads before forming meiotic tetrads; normal tetrads are formed at 17°C but defects are increasingly seen at 22°C and 27°C. The point mutation in tam results in a substitution of an Isoleucine for a conserved Threonine in the linker region between the predicted α4 and α5 helices of the first cyclin fold. To study CYCA1;2 dynamics during meiosis we expressed a CYC1;2 GFP fusion protein under the control of the CYCA1;2 promoter. We found that the CYCA1;2-GFP fusion protein was present at highest levels at pachytene, but was undetectable from late prophase I to the end of M-phase of meiosis II. However, assessments of durations of different stages of male meiosis indicated that in tam cell cycle progression was delayed in both pachytene and meiosis II. We also found that heterochromatin regions abnormally aggregated in pachytene and early diplotene in tam, and that chromosome condensation was delayed in prophase II. We conclude that CYCA1;2 regulates progression of both pachytene and meiosis II, even though it does not show an appreciable oscillation during the meiosis I-meiosis II transition. Therefore, at least with respect to the function of CYCA1;2, meiosis I and meiosis II seem to behave as two consecutive parts of one continuous cycle in male meiosis in Arabidopsis.

T03-048

Carbon Dioxide-Induced Modulation of Cytosolic Calcium Pattern During CO2 Signal Transduction in Guard Cells.

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Guard cells have been developed as a model system for dissecting early signal
transduction mechanisms. Carbon dioxide modulates stomatal apertures, but the signal transduction mechanisms that underlie CO2 responses are relatively poorly
understood. Repetitive increases in the cytolic calcium level ([Ca2+]cyt) have been shown
to function during guard cell abscisic acid signal transduction (Allen et al., 2001). We show that Arabidopsis guard cells exposed to high carbon dioxide, which induces stomatal closing, or low carbon dioxide, which induces stomatal opening, exhibit [Ca2+]cyt elevations at different frequencies, and that switching between CO2 concentrations causes modulation of the [Ca2+]cyt elevation pattern. Furthermore, the ABA-insensitive gca2 mutant does not alter [Ca2+]cyt oscillation frequency with changes in [CO2]. In correlation, gas exchange experiments revealed reduced stomatal conductance changes in gca2 in response to changes in [CO2]. This suggests a basis for CO2 signaling in guard cells that includes modulation in the [Ca2+]cyt elevation pattern. The question how eukaryotic cells “read” specific Ca2+ elevation patterns remains largely unresolved. Advances at understanding how cytosolic Ca2+ elevations are decoded in guard cells will also be presented.
Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffman T, Tang YY, Grill E, Schroeder JI. A defined range of guard cell calcium oscillation parameters encodes
stomatal movements. Nature 411: 1053-1057 (2001)

T03-049

Isolation and characterization of Arabidopsis thaliana spiral3 mutant

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The Arabidopsis thaliana spiral (spr) 1 and spr2 mutants exhibit right-handed helical growth in roots and etiolated hypocotyls. In addition the spr2 mutant shows right-handed helical growth in other organs such as petioles and petals. In elongating epidermal cells of spr roots, cortical microtubules are arranged in left-handed helical arrays, in contrast to wild-type transverse arrays. SPR1 and SPR2 are plant-specific novel proteins that are associated with microtubules in wild-type cells. We have report a novel mutant, spr3, which display right-handed helical growth in roots, etiolated hypocotyls, and petioles. SPR3 gene was mapped on the north region of chromosome5. We found a point mutation in a gene encoding a protein homologous to gamma tubulin ring complex protein 84 (grip84). In fungi and animal cells, grip84 is a component of the γ-tubulin small complex which show a core nucleating activity of microtubules. A defect in microtubule nucleation might affect the dynamics of cortical microtubules and lead to alteration in array organization.

T03-050

Proteomic analysis of glutathione S-transferases of Arabidopsis thaliana reveals differential salicylic acid-induced expression of the plant-specific phi and tau classes

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Plant glutathione S-transferases (GSTs) are a large group of multifunctional proteins that are induced by diverse stimuli. Using proteomic approaches we identify 20 GSTs at the protein level in Arabidopsis cell culture using a combination of GST antibody detection, LC-MS/MS analysis of 23-30 kDa proteins and glutathione-affinity chromatography. GSTs identified were from phi, tau, theta, zeta and DHAR sub-sections of the GST superfamily of 53 members. We have uncovered preliminary evidence for post-translational modifications of plant GSTs and show that phosphorylation is unlikely to be responsible. Detailed analysis of GST expression in response to treatment with 0.01 - 1 mM of the plant defence signal salicylic acid (SA) has uncovered some interesting features. Firstly, GSTs appear to display class-specific concentration-dependent SA induction profiles highlighting differences between the large, plant specific phi and tau classes. Secondly, different members of the same class, while sharing similar SA dose responses, may display differences in terms of magnitude and timing of induction, further highlighting the breadth of GST gene regulation. Thirdly, closely related members of the same class (GSTF6 and GSTF7), arising via tandem duplication, may be regulated differently in terms of basal expression levels and also magnitude of induction raising questions about the role of subfunctionalisation within this family. Our results reveal that GSTs exhibit class specific responses to SA treatment suggesting that several mechanisms are acting to induce GSTs following SA treatment and hinting at class-specific functions for this large and important, yet still relatively elusive gene family.

Sappl, P. G., Oñate-Sánchez, L., Singh, K. B., Millar, A. H. (2004) Plant Mol. Biol. (in press).

T03-051

DNA replication licensing affects cell proliferation or endoreplication in a cell type-specific manner

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Plant cell cycle regulation exhibits an enormous plasticity which is revealed, for example, by the fact that organogenesis during post-embryonic growth relies entirely on cell proliferation and differentiation. This continuous process requires a strict regulation to maintain genome stability and function. The G0/G1 and the G1/S transitions of the cell cycle, where the retinoblastoma (RBR)/E2F/DP pathway and the DNA replication licensing mechanism are two of the main chekpoints, are crucial in the control of cell viability and genome stability. DNA replication licensing control acts on the pre-replication complex (pre-RC) formed by CDC6, CDT1 and MCM, among other proteins.

Information about how pre-RC function is regulated in whole organisms and whether it impinges on differenciation and development is scarce, and in particular in Arabidopsis is unknown. We previously reported on the regulation and function of CDC6 both in proliferating and endoreplicating cells (Castellano et al., 2001). Here we have focused on the isolation of its partner, CDT1, and studied its regulation, function and the consequences of overriding licensing control. We found that CDT1 availability is strictly controlled at two levels: E2F-mediated transcriptional regulation and CDK-dependent phosphorylation, a step required for CDT1 proteasome-mediated degradation. Maintenance of adequate levels of CDC6 and CDT1 are important for a correct balance between proliferation and differentiation. Furthermore, altered levels of these pre-RC components have cell type-specific consequences in developing Arabidopsis plants since cell proliferation is stimulated in leaf cells competent to divide whereas extra endocycles are triggered in cells programmed to undergo differentiation-associated endocycles. Therefore, we propose that DNA replication licensing control, mediated at least on CDC6 and CDT1, is critical for the proper maintenance of proliferative potential, developmental programs and morphogenetic patterns.

Castellano, M.M., del Pozo, J.C., Ramirez-Parra, E., Brown, S., Gutierrez, C. (2001) Plant Cell 13, 2671-2686.

T03-052

A Transcriptomic and Proteomic Characterisation of the Arabidopsis Mitochondrial Protein Import Apparatus and its Response to Mitochondrial Dysfunction

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Mitochondria import hundreds of cytosolically synthesised proteins via the mitochondrial protein import apparatus. Expression analysis of the 36 genes encoding the 20 components of the Arabidopsis thaliana mitochondrial protein import apparatus showed that although many were present in small multigene families, often only one member was prominently expressed. This was supported by comparison of real-time RT-PCR and microarray experimental data with EST numbers and massive parallel signature sequence data. Mass spectrometry-detected isoforms correlated with the most abundant gene transcript measured by expression data. The transcript abundance of all genes encoding protein import components was measured over leaf development, revealing two general patterns. The transcript abundance of numerous other genes was measured for comparison with the import components, including those encoding proteins found in the cytosol, chloroplast, peroxisome and mitochondria. Interestingly, the transcript abundance of nearly all import components increased significantly in the later stages of leaf senescence, in contrast to chloroplast gene transcript levels, which decreased dramatically during senescence.

Arabidopsis suspension cell cultures were subjected to a wide range of abiotic stresses, mostly aimed at disrupting mitochondrial function. Significant changes in the import component transcript abundance were measured by both real-time quantitative PCR and microarrays. In general, greater changes in the message levels of the minor import component isoforms were seen in response to the stress treatment. Microarray analysis revealed changes in the transcript abundance of genes involved in mitochondrial chaperone activity, protein degradation, respiratory chain assembly and division. These findings suggest that transcription of import component genes is induced when mitochondrial function is limited, and that minor gene isoforms display a greater response than the predominant isoforms.

T03-053

Global transcription analysis of Arabidopsis core cell cycle regulators in suspension-cultured cells and plants reveals multiple and highly specific profiles of gene expression

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Dispersed plant suspension cultures allow cell proliferation and growth to be analysed in the absence of developmental processes. We have established synchronisation procedures for two Arabidopsis cell lines MM1 and MM2d and used these to analyse cell cycle regulated gene expression by GeneChip arrays, Massively Parallel Signature Sequencing (MPSS) of cDNAs on immobilised microbead arrays and real-time reverse transcriptase PCR. Gene expression was analysed in cell line MM2d during synchronous re-entry of the cell cycle, during cell cycle progression from a G1/ S phase block and during normal growth from sub-culture to stationary phase. Comparative analysis of results shows that around 1100 genes show significant cell cycle regulation, and these are involved in a wide range of cellular processes. To further extend this analysis, we used synchronised samples of the alternative light-grown cell line MM1 for transcript profiling analysis using MPSS and Affymetrix GeneChip arrays to confirm the expression patterns seen previously.

From this analysis we present the expression profiles of key regulators of the plant cell cycle. These show distinctive and specific patterns throwing new light on their likely roles in the cell cycle. Almost 90% of core cell cycle regulators are expressed at detectable levels in cell suspension. Most D-type cyclins show different timing suggesting distinct roles. In contrast, five of six groups of A and B type cyclins show highly similar expression with a peak in G2/M, including two previously unrecognised B-type cyclins. The CYCA3 group alone shows a peak in S phase. 80 genes share the G2/M regulatory pattern, about half being new candidate mitotic genes of previously unknown function. This unified and global model of transcriptional timing of all plant cell cycle regulators reveals new patterns of cell cycle regulation, identifies novel candidate mitotic genes and reveals CYCA3 as S phase cyclins. These data strongly suggest that cell cultures can be used to model many cellular processes.

In addition, available transcript profiling analysis results of core cell cycle genes using various plant tissues is currently being subjected to bioinformatic analysis to identify tissue-specific gene expression of key regulators, which will be presented.

T03-054

AraPerox: A Database of plant peroxisomal proteins

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Our knowledge on plant peroxisomal metabolism is mostly limited to the most abundant enzymes that play a role in photorespiration and fatty acid beta oxidation. Most matrix proteins from peroxisomes are targeted to the organelle by a peroxisome targeting signal type 1 or type 2 (PTS1 or PTS2): The PTS1 is a C-terminal tripeptide of the prototype SKL>, whereas the PTS2 is a nonapeptide of four conserved residues (e.g., RLx5HL) located close to the N-terminal end of the protein. We applied a bioinformatics approach to specify the PTS peptides for plants by analyzing semi-quantitatively plant ESTs that are homologous to PTS-targeted plant peroxisomal proteins for the nature of their PTS. Accordingly, nine major PTS1 and two major PTS2 peptides have been defined that are considered to indicate targeting to peroxisomes with high probability. These PTS peptides including the minor PTS peptides were applied to screen the Arabidopsis genome for unknown peroxisomal matrix proteins. About 220 and 60 proteins were identified that carry a putative PTS1 or PTS2, respectively, of which about 80% are unknown. To further support postulated targeting to peroxisomes, several prediction programs were applied and the putative targeting domains analyzed for properties conserved in peroxisomal proteins and for PTS conservation in homologous plant ESTs. Novel non-hypothetical proteins include several enzymes involved in β-oxidation of unsaturated fatty acids and branched amino acids, 2-hydroxy acid oxidases with a predicted function in fatty acid beta oxidation as well as NADP-dependent dehydrogenases and reductases. In addition, large protein families with many putative peroxisomal isoforms were recognized, including a large number of mostly unknown acyl-activating enzymes and largely unknown GDSL lipases and small thioesterases. Putative regulatory proteins of plant peroxisomes include protein kinases, small heat-shock proteins, and proteases. The information on targeting prediction, homology and in silico expression analysis for these Arabidopsis proteins has been compiled in the public database “AraPerox” to accelerate discovery and experimental investigation of novel metabolic and regulatory pathways of plant peroxisomes.

T03-055

Subcellular and functional analyses of regulatory proteins from plant peroxisomes

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Plant peroxisomes are known for their recycling function of glycolate produced as a by-product during photosynthesis and fatty acid beta oxidation during seed germination. Apart from these fundamental pathways our knowledge on post-transcriptional regulation of these pathways is rather limited. We have applied a bioinformatics approach to identify regulatory proteins, such as protein kinases and proteases, and identified novel genes in the Arabidopsis genome that encode proteins with a putative peroxisome targeting signal type 1 or type 2 (PTS1 or PTS2). Some of these genes have been cloned and their expression been analyzed by RT-PCR. To verify predicted targeting to plant peroxisomes, the genes were expressed in S. cerevisiae and in onion epidermal cell fused to spectral variants of green fluorescent protein and their targeting was studied by fluorescence microscopy. T-DNA knock-out mutants have been identified for some peroxisomal proteins and are currently being analyzed for a phenotype under specific stress conditions.

T03-056

Endocytosis of the receptor like kinases AtSERK1 and BRI1 in Arabidopsis.

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Brassinosteroid Insensitive 1 (BRI1) is the receptor essential for the perception of brassinosteroids (BR's). BRI1 interacts with BAK1 (AtSERK3), which in turn is a close homolog of Somatic Embryogenesis Receptor Kinase 1 (AtSERK1) (Li et al., 2002)(Hee Nam and Li, 2002) Fluorescent BRI1-CFP/YFP fusion proteins have been shown to interact in Cowpea protoplasts with both AtSERK1- CFP/YFP and AtSERK3 ¯ CFP/YFP proteins. Besides being present in the plasmamembrane, AtSERK3 and BRI1 are also found in endosomes. Expression of AtSERK3 and BRI1 in the same cell resulted in an increased rate of endocytosis of both receptors. Arabidopsis lines stably expressing a GFP fusion of BRI1 and an YFP fusion of AtSERK1 were analyzed. Endocytosis of BRI1 was observed in the root meristem. Both BRI1 and AtSERK1 undergo BFA-sensitive endocytosis similar to that shown for PIN1 (Geldner et al., 2001). Endocytosis of BRI1 is not dependent on exogenous application of BR. Current experiments are aimed at demonstrating the importance of AtSERK3 endocytosis in BR signaling.

Hee Nam and Li. 2002 Cell. 110 203
Li et al. 2002. Cell 110 213
Geldner et al. 2001 Nature 413 425

T03-057

A Proteomic Analysis of Leaf Peroxisomes

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The soluble matrix proteome of purified leaf peroxisomes from spinach and Arabidopsis was partially characterized by two-dimensional electrophoresis followed by mass spectrometry (MS). Apart from well-known peroxisomal proteins, such as those involved in photorespiration, fatty acid beta oxidation and metabolism of reactive oxygen species, some proteins were identified, including a short chain reductase, two enoyl-CoA hydratases/isomerases and a small heat shock protein (sHSP) as novel protein components of leaf peroxisomes. Because fractions highly enriched in leaf peroxisomes still tend to be contaminated by proplastids and mitochondria, the subcellular localization of the novel proteins was verified by differential 2D gels. Further support for peroxisomal localization is provided by cloning of the corresponding Arabidopsis genes and analyzing subcellular targeting of fusion proteins with spectral variants of green fluorescent protein in onion epidermal cells. Post-translational modifications (PTMs) including phosphorylation appeared to a salient feature of several peroxisomal proteins, even though not reported yet for any peroxisomal protein. Some novel proteins and/or isoforms appeared to be induced during conditions of stress. The MS identification of the novel proteins and the characterization of the PTMs are currently underway and are likely to contibute to a better understanding of the metabolic capacity of leaf peroxisomes.

T03-058

Genetic analysis of the AtRabGDI family

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Rab GDP dissociation inhibitors (RabGDIs) are required for proper recycling of small RabGTPases by their retrieval from target membrane compartments and for cytosolic binding of RabGTPases in the inactive form. Therefore, RabGDIs activity is essential for the control of vesicular transport. Sequencing of Arabidopsis thaliana and Oryza sativa genomes suggested that angiosperm possess three isotypes of RabGDI. We have started our analysis of the AtRabGDI family using L59 mutant containing T-DNA insertion in the first intron of AtRabGDI1 (provided by dr. Klaus Palme (Köln, Germany)). Detailed analysis of this insertion showed that homozygous plants have no observable phenotype. The same hold true about the AtRabGDI2 insertional mutant (from the Salk Institute database) with T-DNA insertion in the fifth exon. There are no convenient candidates for any insertion in AtRabGDI3 in public collections. We have obtained no double homozygous mutant from genetical analysis of reciprocal crosses between AtRabGDI1 and AtRabGDI2 mutants suggesting that disruption of both AtRabGDIs function is lethal. Female and male gamets harboring both AtRabGDI1 and AtRabGDI2 mutated alleles are functional. Specific expression of AtRabGDI3 (according to Affymetrix data) may complement this defficiency in male gamets.
This work was supported by GACR 206/99/1138 and MSMT CR-LN00A081"SIDROS" grants.

T03-059

Regulation and compartmentation of glutathione biosynthetic enzymes

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Higher plants respond to biotic and abiotic stress factors with an increase in glutathione (GSH) content. While cDNAs for the enzymes catalyzing GSH biosynthesis, GSH1 (g-glutamylcysteine synthetase) and GSH2 (glutathione synthetase), have been cloned for many plant species, the complexities of regulation and subcellular compartmentation are only partially understood. Also, as GSH biosynthesis appears to be limited to the plastids, other cellular compartments requiring GSH for maintenance of the redox status have to import GSH, emphasizing a crucial role for intracellular GSH transport. Here we summarize recent research on the compartmentation of the GSH1 enzyme in A. thaliana and B. juncea. The analysis of multiple GSH1 transcripts, in vivo targeting studies with GSH1::GFP(RFP) fusions, and the immunolocalization of GSH1 protein all confirmed an exclusive plastidic localization of the GSH1 enzyme. Conversely, a similar analysis for GSH2 indicated the presence of transcripts encoding plastidic and cytosolic proteins. As GSH is not rapidly exchanged between plastids and cytosol, the dipeptide g-glutamylcysteine is a likely candidate for plastid exit. The promoter of the AtGSH1 gene is strongly up-regulated in response to Cd and jasmonic acid exposure, confirming that the increase of GSH1 protein observed under these treatments results, at least in part, from a transcriptional up-regulation of the GSH1 gene.

T03-060

det3: Life with 50% V-ATPase activity

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The V-ATPase is a highly conserved eukaryotic proton pump present in endomembrane compartments. The V-ATPase consists of two subcomplexes, the membrane embedded V0-complex and the cytosolic V1-complex. Numerous processes such as secondary active transport, enzyme activity, protein targeting and vesicle transport are dependent on the proton gradient build up by the V-ATPase.
The det3 mutant shows a de-etiolated phenotype caused by a two-fold reduction in mRNA and protein levels of VHA-C (V-ATPase subunit C) leading to a reduced activity of the V-ATPase of approximately 50%.
However, when grown in the dark in the absence of nitrate, the mutant shows a long hypocotyl, indicating that the phenotype is inducible by nitrate or lowering the temperature and therefore conditional. We postulate that in det3 unstable V-ATPase complexes lacking the VHA-C subunit are formed under permissive conditions and that these complexes would easily disassemble under restrictive conditions leading to reduced V-ATPase activity. To test our model we are using both biochemical methods and patch-clamp analysis of det3 vacuoles. Furthermore we have investigated the transcriptome of det3 seedlings grown under permissive and restrictive conditions using microarray analysis and will present recent data.

T03-061

Isolation of mutants affecting endoreduplication by an enhancer/suppressor screen of multicellular Arabidopsis trichomes

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Endoreduplication, i.e. DNA synthesis without cell division, is a common cell cycle mode in plants often associated with cell differentiation. Arabidopsis trichomes are single-celled leaf hairs, which undergo approximately four rounds of endoreduplication. Due to their accessibility and their well-defined developmental program, we use trichomes as a model system to investigate cell-cycle control in a developmental background. In the past, we have shown that the expression of B- and D-type cyclins is sufficient to induce mitosis in trichomes. However, we found that at least a few cells of a multicellular trichome underwent endoreduplication arguing that additional factors are involved in promoting endoreduplication and/or repressing mitosis. To identify further factors involved in the control of mitosis and the transition to an endoreduplication program, we conducted an EMS second site screen of plants misexpressing B-type cyclins in trichomes. A total of 75 000 seeds were mutagenized and from the descendants of 3600 M1 plants screened 5 complementation groups with altered cell numbers and/or cell sizes of the individual cells in multicellular trichomes were isolated. Here, we present a first characterization of the recovered mutants. Mapping and a detailed morphological analysis of the mutant lines is in progress.

T03-062

An Arabidopsis mutant that has a defect in organization of endomembranes

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Plant cells highly develop complicated and sophisticated endomembrane systems including endoplasmic reticulum (ER), Golgi complex and vacuole. To understand the molecular mechanisms underlying the precise organization of the endomembrane systems, we generated a transgenic Arabidopsis plant that exhibited GFP fluorescence throughout the endomembrane systems. We mutagenized the plants to obtain mutants, which exhibited abnormal structures of endomembrane organization. We identified an Arabidopsis mutant, in which endomembranes formed an aggregate, and designated it katamari1 (kam1), which. means 'aggregate' in Japanese. The aggregates contained almost all of ER, Golgi, endosomes, peroxisomes and vacuoles in the mutant cells. We found that some of actin filaments were associated with the aggregates. The KATAMARI1 gene encoded a type II membrane protein. Our findings suggest that the KATAMARI1 protein is essential for a proper organization of the endomembrane system in plant cells.

T03-063

CDKA;1 is essential for Arabidopsis embryo and gametophyte development

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As in other eukaryotes, in plants progression through the cell cycle is controlled by cyclin dependent kinases (CDKs). The kinase activity of CDKs depends on the binding to diverse cyclins and can be further modulated by a variety of regulator proteins, such as CDK inhibitors ICK/KRPs.
In yeast and in animals, the isolation and analysis of mutants has been proven to be a powerful tool to understand cell cycle control. In Arabidopsis however, mutational analyses of core cell cycle genes are largely missing. From different T-DNA mutant collections we identified two potential CDKA;1 mutant lines. Both T-DNA insertions disrupt the protein structure and should abolish kinase activity. Whereas the heterozygous cdka;1 mutant plants showed no morphological deviations from wild type, homozygous mutants could not be recovered. The latter argues for an essential role of CDKA;1 in early Arabidopsis development. Consistent with this, arrested embryos were found in the offspring of heterozygous mutant individuals. In addition, gametophytic development seems to be affected.

T03-064

Study of the Arabidopsis ORC subunits during the cell cycle and plant development

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Meristem are the main proliferative tissues in plants, being the source of new cells for differentiation. In plants, almost all the differentiation is post-embrionic, and all the adult tissues come from the undifferentiated meristematic cells. Therefore, it is conceivable that alteration in meristematic cell division, also affects plant development.
Here we study the mechanisms invovled in controlling cell cycle progression, with a focus on the onset of S-phase. To this end, we are investigating the regulation of the pre-replicative complexes, wich are formed by a heterohexameric complex called ORC (origin recognition complex), CDC6, CDT1 and MCMs. In this work we found that in Arabidopsis cultured cells the expression of Arabidopsis ORC genes is regulated during the cell cycle. In the plant, expresion of all ORC genes is more abundant in flowers. Arabidopsis is unique in having two ORC1 genes (a and b). Interestingly these genes seem to have a different regulation at the transcriptional level. AtORC1a mRNA is more abundant in the aerial parts of dark grown seedlings while AtORC1b is equally expressed in light and dark grown seedlings. We also have obtained results of the interaction of different ORC subunits.

T03-065

Function and differentiation of endocytic organelles in Arabidopsis cells

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We have been studying mechanism, dynamics and physiological roles of endocytosis in Arabidopsis by focusing our attention on endocytic Rab/Ypt GTPases. Arabidopsis genome contains three Rab5-related GTPases. Ara6 is a plant unique Rab GTPase with several unique features in its structure, and Ara7 and Rha1 are conventional type Rab5 homologs, which are all on the subpopulation of endosomes. Using fluorescent proteins and CSLM, we examined whether these proteins are on the same endosomes or on the different population of endosomes. We also compared the subcellular localization between Rab5 homologs and some putative endosomal SNAREs, which revealed that Ara6 is localized on the later compartment in the endocytic pathway. On the other hand, Ara7 and Rha1 are on the different population of endosomes from Ara6, where GNOM-dependent recycling should occur. We also show some data suggesting transition of these endosomes from one to another.

T03-066

Genetic analysis of peroxisomal biogenesis and function in Arabidopsis

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Peroxisomes are small organelles that house enzymes required in fatty acid beta-oxidation, the glyoxylate cycle, and branched-chain amino acid catabolism. More than 20 peroxin (PEX) proteins are required for peroxisomal biogenesis and maintenance. Whereas these proteins are well studied in humans and yeast, the genetic examination of these processes and proteins in plants is just beginning.

The plant hormone auxin influences numerous aspects of growth and development, including responses to gravity and light, inhibition of root elongation, and lateral root initiation. Indole-3-butyric acid (IBA) is an endogenous auxin that efficiently induces rooting and is widely used in commercial and agricultural settings. Interestingly, IBA is converted to the more abundant auxin indole-3-acetic acid (IAA) in a mechanism similar to fatty acid beta-oxidation. We have identified 17 Arabidopsis mutants that are resistant to the inhibitory effects of IBA on root elongation, but that remain sensitive to IAA. Defects in seedling development in the absence of exogenous sucrose suggest that some of these mutants have defects in the peroxisomal beta-oxidation of seed storage lipids. We have used positional information to clone the genes defective in several distinct loci. Three mutants have defects in proteins expected to act in peroxisomal biogenesis or import. One is defective in PEX5, which encodes a receptor that binds proteins containing a peroxisomal targeting signal in the cytosol and imports them into the peroxisome. pex6 is defective in an AAA-type ATPase, which may act in either the late stages of matrix protein import or in peroxisome biogenesis. A third mutant is defective in PXA1, which encodes an ABC (ATP-binding cassette) transporter that imports both fatty acids and IBA into peroxisomes for beta-oxidation. Another mutant is defective in CHY1, which encodes an acyl-CoA hydrolase that may act in peroxisomal valine catabolism. chy1 apparently accumulates a toxic intermediate that inhibits the beta-oxidation of IBA and fatty acids. Our results indicate that IBA functions, at least in part, via its conversion to IAA in the peroxisome. Moreover, IBA resistance is a powerful tool to identify genes required for peroxisomal beta-oxidation, providing an unbiased approach for studying peroxisomal function in plants.

Zolman, B.K. and Bartel, B. (2004) PNAS 101, 1786
Zolman, B.K, Yoder, A., and Bartel, B. (2000) Genetics 156, 1323

T03-067

Study of root hair tip growth of Arabidopsis by video-enhanced light microscopy

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Non-invasive methods of light microscopy are important tools in cell biological research; they allow the study of plant morphology and structure as well as a characterisation of the phenotype of Arabidopsis roots. We used the technique of electronic light microscopy to analyse the morphology and the organelle dynamics of living root hairs. This approach takes advantage of video- and computer techniques to utilise the maximum resolution of the light microscope or even improve it; thereby we can observe sub-resolution particles in living cells.

With computer-assisted high-resolution light microscopy we analysed root hairs after treatment with chemicals that monitored and modulated the physiology and function of the plasma membrane and membrane trafficking. It is documented with the example of structural sterols and their distribution in the plasma membrane of root hairs. We observed changes in the rate of tip growth, in the cytoarchitecture of the tip and in the motility of organelles within the different developmental stages of root hairs after complexation of sterols.

Results bring basic characteristics of the cytoarchitecture of root hair tips of Arabidopsis thaliana essentially involved in tip growth.

This work was supported by a grant for the research trainings network TIPNET (HPRN-CT-2002-00265) from the European Comm

T03-068

Role of Heat Stress Granules for mRNP Storage and Decay

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The plant heat stress response is characterized by the rapid and reversible assembly of large cytoplasmic complexes (heat stress granules, HSG). They serve as storage and stabilization sites of denatured proteins and contain a considerable portion of untranslated house keeping mRNPs. The fate of these mRNPs in the recovery period remains to be determined (reinitiation into polysomes vs degradation). In animal cells it was shown that stress granules induced by heat or oxidative stress contain mRNA and associated RNA binding proteins. It was speculated that these mRNP complexes resemble defect initiation complexes and that the mRNA binding capacity of the nuclear hnRNP TIA-1 is essential for the formation of the granules. We will show that the HSG complexes contain components of the translation initiation complex like eIF4E, iso4E, 2alpha, and PABPI members. The HSG complexes are associated with mRNP granules containing polyA-RNA and Ubp the plant homolog of TIA-1. Whereas the interaction partners of Ubp Uba 1a and 2a stay nuclear as well as PABPII. By in situ hybridisation we will show the cytoplasmic behaviour of individual RNAs. However, dependent on the composition of mRNP and the cellular situation, the mRNP in the HSG can also be destined for decay. To address this question we cloned tagged versions of putative mRNA decay enzymes from Arabidopsis thaliana (AtXRN4, AtDcp1, AtRrp41, AtRrp4) and proteins that are involved in the decay pathways (AtLsm1, 2, 5, 8). Using immunofluorescence analysis, we found that AtXrn4, AtDcp1 and AtLsm1, 2, 5 colocalize in distinct cytoplasmic foci under control conditions and are comparable to the yeast P bodies and those structures observed in mammalian cells. Whereas under heat stress conditions the AtDcp1 and AtXrn4 are immediately adjacent of foci containing Hsp17-CI as indicator of HSG complexes. In contrast Lsm proteins are localized in the HSG. We assume that the HSG combine beside their function as storage sides for denatured proteins also mRNP specific functions of stress granules, P-Bodies, exosomes, and mRNP storage sides.

T03-069

Shaping plant cells using an actin mesh

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Most plant cells start up with a nearly spherical form before evolving into myriad shapes and sizes through the process of 'differential growth'. Recent molecular-genetic and cell biological studies have implicated cytoskeletal elements and their regulators in defining and fixing the site for differential growth. From these studies the following general scenario emerges: The boundaries of a cell consist of the cell wall, plasma-membrane and cytoskeletal layers. The cytoskeletal layer comprises interwoven actin micro-filaments and microtubules. In response to pertinent cues a localized loosening of the cytoskeletal mesh is achieved at the cell cortex by an alteration in actin polymerization dynamics. This possibly involves the co-ordinate activities of ROPs, Formins, the ARP2/3 complex with its immediate upstream regulatory complexes, Actin Depolymerizing Factors (ADFs), Actin-Interacting Protein1 (AIP1) and Profilins. A localized bulge is created at the weakened region of the cell as internal turgor pressure forces the cortical layers outwards. Cytoplasmic-microtubules now extend towards the actin-loosened cortical sites within the bulge to provide structural reinforcement and thereby limit the further weakening of the cortex. With both cytoskeletal elements in regional concurrence cellular polarity gets fixed and the growth machinery involving organelle and vesicle trafficking gets focused to the cytoskeletally-delineated region of the cell. The ensuing 'differential growth' is thus viewed as resulting from the cell's attempt to combat weakening of its bounding surfaces. Depending upon the extent of weakening, growth of a cell can be very localized, as in tip-growing cells. Alternatively, in diffuse growing cells, cortical weakening and consequently the growth extends over larger areas. Evidence leading to the pivotal role played by the actin-mesh in creating conditions for differential growth and its continuation during the morphogenesis of higher plant cells will be presented.

T03-070

Towards a transcript profiling of Arabidopsis trichomes

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Ultimately, the various fates and functions of different cells rely on a differential gene activity. In the course of development, the developmental potential of cells becomes more and more restricted and cell-specific gene sets are more and more pronounced, including genes regulating cell-cycle progression. However, not much is known about the dynamics of these changes in gene expression and about parallelism in gene regulation during different developmental stages. Since tissues and organs are composed of many different cell types at different developmental stages, it is necessary for a transcript profiling with a cellular resolution to analyze single cell types in the course of their development. Arabidopsis trichomes are an established model system for the study of cell differentiation. Due to their well described developmental program, and the large number of mutants and misexpression lines available, trichomes offer the unique possibility to dissect different gene sets required for defined developmental stages. Our focus is on the understanding of cell cycle regulation in a developmental context. We are currently establishing different methods for the stage-specific isolation of trichome cells, i.e. manual collection, cell sorting of protoplasted GFP-expressing trichomes, and Laser-Dissection-Microscopy of wax embedded leaf sections.

T03-071

Characterization of Arabidopsis mutants defective in the Peroxisomal Targeting Signal receptors PEX7 and PEX5

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Peroxisomes are organelles that house many vital processes and are the primary, if not exclusive, site of fatty acid beta-oxidation in plants. Using reverse genetic techniques, we isolated a mutant defective in the Peroxisomal Targeting Signal Type 2 (PTS2) receptor PEX7, and found pex7-1 to be resistant to resistant to exogenous indole-3-butyric acid (IBA), which is converted to the active auxin indole-3-acetic acid (IAA) in a peroxisomal process similar to fatty acid beta-oxidation. To examine peroxisomal matrix protein import in pex7-1, we utilized an existing PTS1-GFP and created a PTS2-tagged GFP. While PTS1-GFP fluorescence was punctate in pex7-1, PTS2-GFP was mostly cytosolic. Surprisingly, this assay revealed that the existing pex5-1 mutant, harboring a missense mutation in the PTS1 receptor, was likewise specifically defective in PTS2 protein import. Genomic analysis revealed the pex5-1 lesion to be within a region implicated in PEX7 interaction in other organisms, and the lesion is in a serine analogous to one that, when mutated in a pex5 mutant Chinese hamster ovary cell line, causes PTS2-specific defects. Further, we created a pex7-1 pex5-1 double mutant that has multiple severe defects, including an absolute requirement for exogenous sucrose for seedling establishment, reduced adult stature, and shrunken seeds with reduced viability. In addition, ~15% of pex7-1 pex5-1 seedlings display various degrees of cotyledon fusion, a phenotype characteristic of low auxin response or levels during embryogenesis. Further, auxins promote lateral root formation, and pex7-1 pex5-1 seedlings have severely reduced numbers of lateral roots. These results demonstrate that PTS2 proteins are critical for seed formation, seedling establishment, and auxin response, and implicate IBA as a critical source of auxin during embryogenesis and seedling root development. (This research is supported by the National Science Foundation.)

T03-072

Cyclic AMP signaling during the plant cell cycle: Isolation of a putative cyclic nucleotide dependent protein kinase from Arabidopsis thaliana and Nicotiana tabacum BY-2.

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Fluctuations of cyclic AMP concentrations represent an important means of signal transduction in the regulation of the eukaryotic cell cycle. Analysis of the evolution of cyclic AMP concentration during the cell cycle of synchronised tobacco BY-2 cells reveals a transient accumulation in S-phase and G1-phase. Addition of exogenous cyclic AMP to aphidicolin treated BY-2 cells affects the time of entry into M-phase. Application of indomethacin (inhibitor of adenylyl cyclase activity), Rp-cAMPS (antagonist of cAMP) or H-89 (specific inhibitor of cAMP dependent protein kinase) all result in a significant loss of mitotic activity of BY-2 cells. These results support a role for a cyclic AMP dependent protein kinase in the plant cell cycle. An in silico based strategy to isolate cAMP targets resulted in the identification of genes encoding a putative cyclic nucleotide dependent protein kinase from Arabidopsis thaliana and Nicotiana tabacum BY-2. In this communication we will report on the detection of different transcript variants, their spatio-temporal expression patterns and induction by plant growth regulators. An attempt is made to relate our findings to an involvement in cell cycle, stress and hormone signaling.

T03-073

Mitosis-specific accumulation of PORCINO reveals requirement for de novo synthesis of alpha/beta-tubulin heterodimers in elongating cells

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The PILZ genes, including PORCINO (POR), are involved in the alpha/beta-tubulin heterodimer assembly pathway. No microtubule arrays are detectable in pilz mutants. Therefore these genes can be used as tools to elucidate tubulin dynamics in development. Cell cycle-regulated expression of POR rescues the por mutant phenotype completely. Fusion of POR to the N-terminal part of CYCLIN B1;2 causes protein degradation at the metaphase-anaphase transition, which results in a severe hypocotyl elongation defect, a dwarfed plant phenotype and less-branched trichomes. Restabilization of the fusion protein by mutating the cyclin destruction box restores the wild-type plant phenotype, although dark-grown seedlings still show the hypocotyl elongation defect. These results suggest that de novo synthesis of alpha/beta-tubulin heterodimers is required for cell elongation.

T03-074

In vivo role of GNOM dimerisation

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GNOM is a member of the family of large ARF-GEFs, which are GDP/GTP exchange factors for small G-proteins of the ARF-class. By activating ARFs, ARF-GEFs regulate vesicle formation and hence vesicle trafficking. GNOM acts on endosomal compartments and is involved in the recycling of proteins to the plasma membrane. Apart from its catalytic Sec7 domain, GNOM harbours an N-terminal domain, which is highly conserved between yeast, plants and animals. This DCB-domain was first characterised by yeast-two-hybrid and in vitro studies as a dimerisation domain, which also binds cyclophilin5, a peptidyl-prolyl-cis/trans-isomerase. Even though there is genetic evidence for dimerisation of GNOM in vivo, its functional significance is still obscure. To explore the in vivo role of the DCB-domain, GNOM truncated proteins were analysed for their ability to rescue gnom mutant plants. Our results suggest that dimerisation is not absolutely required for GNOM function.

T03-075

Isolation and characterization of SIAMESE, a putative cell cycle regulator involved in endoreplication

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A recessive mutation in the SIAMESE (SIM) gene of Arabidopsis results in the production of multicellular trichomes with reduced levels of endoreplication. This phenotype is strikingly different than wild-type trichomes, which are single cells with a nuclear DNA content of approximately 20-32C. These observations suggest that SIM is required to suppress mitosis as part of the switch to endoreplication. The gene was isolated by a combination of adaptor PCR and positional cloning. The enhancer trap within the T-DNA indicates that SIM is expressed in developing trichomes and hypocotyls, consistent with the mutant defects, although the gene appears to be expressed in other tissues as well, particularly in roots. We show that SIM encodes a 14kD protein that appears to have two conserved cyclin-binding motifs as well as a possible PEST domain. Homologs of SIM are found in other plants, but no obvious animal homologs have been identified. Our current hypothesis is that SIM functions as a CDK inhibitor, repressing the G2-to-M transition when active. Models for SIM function will be discussed. We are also examining the expression pattern of SIM in more detail, as well as the role of trichome developmental regulators in the control of SIM expression in trichomes.

T03-076

Developmentally regulated nuclear-envelope targeting in plants.

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The nuclear envelope (NE) separates chromatin from the cytoplasm and is involved in organizing nuclear architecture. It also provides an anchoring surface for chromatin and plays a role in the complex dissociation and re-association of the nucleus during open mitosis. While well-researched in vertebrates, the protein composition of the NE in plants is barely understood. MAF1 is a 16 kD, NE-associated protein first identified in tomato. MAF1-like proteins are widely conserved in land plants, but no homologs are found outside the plant kingdom, consistent with a plant-specific role at the NE. Three MAF1-like proteins are encoded in the Arabidopsis genome, AtMAF1, AtMAF2 and AtMLP. AtMAF1-GFP and AtMAF2-GFP, but not AtMLP-GFP fusion proteins are targeted to the NE in transiently transformed Arabidopsis protoplasts. A core domain that is necessary and sufficient for NE targeting was mapped in AtMAF1. A point mutation that changes a conserved tryptophan-proline motif in the conserved core domain of the protein disrupts NE targeting. Transgenic Arabidopsis plants expressing AtMAF1-GFP and AtMAF2-GFP under the control of the 35S promoter show a tissue-specific sub-cellular localization pattern. Both fusion proteins are associated with the NE in undifferentiated root tip cells. In differentiated cells (mature root, leaf epidermal and hypocotyl cells), the fusion proteins are diffusely located in the nucleus and the cytoplasm. In callus induced from differentiated root segments, the fusion proteins are associated with the NE. Cumulative evidence from the above data suggests that AtMAF1 and AtMAF2 are targeted to the NE in a developmentally regulated fashion. Immunogold labeling data indicate that in callus cells AtMAF1-GFP is associated with the outer NE. RNAi lines with undetectable expression of all three genes at the RNA as well as protein level have shorter primary roots and a reduced number of lateral roots. No change is seen in cell size or number in the root elongation zone. These observations lead to the hypothesis that MAF knockdown affects the rate of cell division in root meristems. Analysis of the mitotic activity of root meristems in the RNAi lines is in progress. Together, these data indicate that AtMAF1 and AtMAF2 are involved in a NE function in undifferentiated plant cells and provide the first example for regulated NE targeting of a plant protein.

T03-077

A journey through the plant cell

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Eukaryotic cells display a dynamic organisation of membrane compartments and cytoskeletal arrays, which is fundamental to all their activities. The endomembrane system of plant cells consists of ER, Golgi stacks, vacuoles and other compartments between which proteins, lipids and polysaccharides are exchanged by membrane vesicles or other transport intermediates. Delivery of these cargo molecules to their sites of action requires molecular machineries for sorting during vesicle formation and for vesicle fusion with the target membrane. Membrane trafficking also underlies cell-cell communication, enabling secretion of soluble ligands into the extracellular space, delivery of their receptors to the plasma membrane, and endocytosis of ligand-receptor complexes for eventual degradation in the vacuole. A cytosolic network of cytoskeletal arrays consisting of microtubules and actin filaments assists in membrane trafficking and also play roles in cell division and oriented cell expansion. To illustrate the fundamental role of membrane traffic and cytoskeleton, two processes will be discussed in more detail: the formation of the cell plate, which partitions the cytoplasm of the dividing cell during cytokinesis, and the recycling of plasma-membrane proteins, which mediates polar localisation of the machinery required for polar auxin transport.

T03-078

Expression of a fungal cellulose-binding domain in Arabidopsis thaliana

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Some plant endo-1,4-b-glucanases and expansins contain a cellulose-binding domain (CBD). CBDs were first identified as part of a three-domain structure, i.e. the catalytic domain connected to the CBD by a linker peptide that is shared by most microbial cellulases. Based on sequence homology, microbial CBDs have been classified in 13 families. Familly I contains only small CBDs (from 33 to 40 amino acid residues) from fungi. All other families are made up of bacterial CBDs, much larger in size (from 90 to 250 amino acids) and sharing homologies with the CBDs from plants. CBDs have been proposed to participate in a non-hydrolytic disruption of the cellulose chains of the CMFs, thus facilitating the action of the associated catalytic domain. The present study describes the phenotype of Arabidopsis thaliana expressing a CBD from Aspergillus japonicus with affinity for both crystalline and soluble forms of cellulose (Quentin et al. 2002) under the control of an enhanced CaMV 35S promoter. The expression of the CBD in planta led to a dwarfish phenotype caused by a decrease in cell elongation. In addition, the cell radius appeared to be larger and root hair formation was affected. Microscopic analysis showed that generally the walls in these transformants exhibited a disturbed construction. FT-IR spectra with PCA analysis accordingly indicated a change in wall composition, i.e. increased levels of protein, pectin and possibly cellulose. Though the cellulose content appeared to be increased, X-ray analysis indicated a somewhat lower degree of crystallisation.

Quentin M et al. (2002) Appl Microbiol Biotechnol 58: 658-662

T03-079

Characterization of the evolutionary conserved F-Box protein FBP7 in Arabidopsis

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F-Box proteins (FBPs) are the receptor subunits of SCF-type E3 ubiquitin ligases that target proteins for degradation by the 26S proteasome. We are interested in understanding the role of the evolutionary conserved FBP7 from Arabidopsis. FBP7::GFP accumulates in the nucleus and in the mitochondria of transiently transformed protoplasts. FBP7::GUS fusions indicate that FBP7 is ubiquitously expressed showing a strong staining in floral organs and meristems.

Using the yeast two-hybrid system, we have searched for interactors of FBP7. In this screen, we identified as only interactors multiple cDNAs for three members of a plant specific transcription factor family with a role in controlling floral transition. These results suggest that FBP7 could be implicated in the control of flowering time.

The yeast FBP7 orthologue has been described to interact strongly with a component of the translation machinery. We could observe that FBP7 interacts with the corresponding Arabidopsis protein in overlay experiments and that the two proteins co-fractionate in gel filtration chromatography. These interactions point at a function for FBP7 in translational control. We are now using genetic, molecular, and biochemical approaches to understand the role of FBP7 in these interactions.

T03-080

Functional Analysis of the RING-type Ubiquitin Ligase Family of Arabidopsis

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Ubiquitin-mediated proteolysis is one of the primary means for protein turnover in eukaryotic cells. As much as 5% of the Arabidopsis proteome is predicted to be involved in ubiquitination (1). Ubiquitination involves an enzymatic cascade including an E1, E2, and E3. One type of E3 protein involved in this process is the RING domain-containing E3 ligase. Domain prediction software (Pfam, SMART, and Prosite) and subsequent manual analysis has revealed that the Arabidopsis genome contains 462 RING domain-containing proteins. The canonical RING finger domain contains an octet of cysteine and histidine residues (C3HC4 or C3H2C3) with specific spacing. These residues are responsible for the coordination of two zinc molecules in a cross-brace structure that participates in interacting with an E2. Along with these canonical RING domains, several variations have been identified called RINGv, RINGd, RINGs/t, and RINGc. These modified RING domains contain either semi-conservative amino acid substitutions or altered spacing in their RING domain. To understand the biochemical role of RING domain-containing proteins we have isolated several canonical and non-canonical RING proteins. We have used an in vitro ubiquitination assay to determine activity for putative RING E3 ligases. To date, we have shown E3 ligase activity for over 10% of the putative RING proteins as well as representatives of the non-canonical putative RING proteins. The Arabidopsis genome contains 37 UBC domain-containing proteins or potential E2s that can be categorized into 12 subgroups. The possible combination of E2-E3 interactions would provide for ubiquitination of a wide array of target proteins while maintaining spatial and temporal specificity within the plant cell. To further characterize E3 activity we are testing different E2-E3 combinations to determine E2-E3 specificity. Several of the RING proteins tested have shown activity with the UBC8 family of E2s. A more limited subset of RING proteins have shown activity with specific subgroups of E2s. According to the presence of additional domains, RING domain-containing proteins can be classified into at least 20 different subgroups. Several RING proteins have a diverse array of protein-protein interaction domains such as VWA, BRCT and WD40. A substantial number of the RING proteins also have a predicted transmembrane domain. This suggests a diverse role for RING proteins in control of many cellular functions.

Support: NSF 2010

(1) Smalle J, Vierstra RD. THE UBIQUITIN 26S PROTEASOME PROTEOLYTIC PATHWAY. Annu Rev Plant Physiol Plant Mol Biol.2004

T03-081

Regulated degradation of AUX/IAA proteins through a family of SCF F-box proteins

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Plant response to auxin is regulated by the degradation of AUX/IAA proteins through the SCF E3 ubiquitin ligase complex. The highly variable F-box protein members of this complex denote substrate specificity for the small molecular tag ubiquitin, which is a signal for processing by the 26S proteasome. SCFTIR1 consists of CUL1, ASK1/ASK2, RBX1 and the F-box protein TIR1. The weak auxin response phenotype of tir1 null mutants hints at the possibility that there may be closely related proteins that are redundant in function to TIR1. We are characterizing a family of F-box proteins related to TIR1, designated as LRF (Leucine-rich Repeat F-box) proteins. Co-immunoprecipitation of myc-tagged LRF1, LRF2 and LRF3 proteins with SCF subunits confirm that these LRFs are found in SCF complexes. Likewise, pulldown assays with GST-IAA proteins demonstrate an auxin dependent interaction, suggesting that Aux/IAA proteins are substrates of these SCFLRFs. Similar experiments are being conducted with LRF4 and LRF5. T-DNA insertion mutants have been identified for all five genes and they exhibit a range of auxin resistant phenotypes similar to tir1 mutants. Combinations of mutant lines between the family members to look at cumulative effects in auxin response are being created. Double mutant combinations of these crosses show increased aerial and root phenotypes, while triple and quadruple combinations have increasingly severe and lethal seedling phenotypes. These results suggest that this family of F-box proteins plays an important role in plant development though the regulation of auxin response via SCF complexes.

T03-082

AtNAP AND AtPIR ENCODE SUBUNITS OF A PUTATIVE WAVE

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We report here a functional analysis of two Arabidopsis genes AtNAP and AtPIR that are similar to components of the mammalian regulatory WAVE protein complex. Complementation experiments and sequencing show that the atnap-1 mutant is allelic to gnarled, one of the distorted trichome mutants.

The ARP2/3 complex is an important regulator of actin nucleation and branching in eukaryotic organisms. All seven subunits of the ARP2/3 complex have been identified in Arabidopsis, and mutation of at least three of the subunits results in defects in epidermal cell expansion, including distorted trichomes. However, the mechanisms regulating the activity of the ARP2/3 complex in plants are largely unknown. In mammalian cells, WAVE and WASP proteins are involved in activation of the ARP2/3 complex. WAVE1 activity is regulated by a protein complex containing NAP1/HEM/KETTE/GEX-3 and PIR121/Sra-1/CYFIP/GEX-2. Here, we show that the WAVE1 regulatory protein complex is partly conserved in plants. We have identified Arabidopsis genes encoding homologs of NAP1 (AtNAP), PIR121 (AtPIR) and HSPC300 (AtBRK1). AtNAP and AtPIR show a moderate, but significant similarity to human NAP1 and PIR121, respectively. Transgenic plants carrying T-DNA knockouts of AtNAP and AtPIR have a distorted trichome phenotype, indicating that the gene products are involved in organization of the actin cytoskeleton. The atnap-1 mutant is allelic to the distorted mutant gnarled (grl). The actin cytoskeleton in atnap-1 and atpir-1 mutants shows orientation defects and increased bundling compared to wild-type plants. The results presented show that activity of the ARP2/3 complex in plants is regulated through an evolutionarily conserved mechanism.

Deeks,M.J. and Hussey,P.J. (2003) Arp2/3 and 'the shape of things to come'. Curr.Opin.Plant Biol. 6:561-567.

T03-083

GENETICALLY INDUCED CELL ABLATION PRODUCE MINELESS PLANTS ¯ PLANTS WHERE MYROSIN CELLS HAVE BEEN REMOVED.

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We have been able to selectively ablate a specific cell type of a mixed tissue without damage of the neighbouring cells. Myrosinases are hydrolytic enzymes involved in degradation of glucosinolates thereby producing an array of degradation products with toxic effects. Myrosinases of Brassica napus seeds are localised in myrosin cell idioblasts - also named toxic mines. We have cloned and characterised the Myr1.Bn1 promoter and used the promoter to express the cytotoxic RNase barnase in myrosin cells thereby introducing controlled cell death of myrosin cell idioblasts. Controlled cell death (ablation) of myrosin cells produce MINELESS seeds - seeds without myrosinase containing toxic mines. MINELESS seeds produced plants characterised by increased branching (bushy phenotype), early emerging and elongated carpels, development of secondary siliques, delayed seed maturation and reduced seed production. The tissues affected co-incidence with the expression pattern directed by the Myr1.Bn1 myrosinase gene promoter used.

Myrosinase is one of several enzymes in a complex enzyme system that also includes myrosinase-binding proteins, myrosinase-associated proteins and epithiospecifier proteins (ESP). One of the isoforms of ESP is lost by the ablation of myrosin cells indicating a cellular co-localisation with myrosinase in myrosin cells. Myrosinase-binding proteins are not reduced by myrosin cell ablation supporting localisation to other cells in the seed. The glucosinolate substrates are mainly unaffected by myrosin cell ablation supporting localisation in non-myrosin cells and a cellular separation of enzyme and substrate. GC and GC-MS analysis of MINELESS seeds show a strongly reduced ability of glucosinolate hydrolysis, reducing the production of isothiocyanates and other degradation products to nearly zero. This activity can be restored by external addition of myrosinase. Taken together, these results and the phenotype associated with the MINELESS plants strongly indicate that the myrosin cells (toxic mine cells) has a role both in defence and in regulation of growth and development.

Bones and Rossiter 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. Phys. Pl 97: 194-208

T03-084

The apoplastic alpha-fucosidase of Arabidopsis thaliana (AtFXG1): Phenotypic characterization of a null mutant and of transgenic plants over-expressing AtFXG1

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The tensile strength of the cell wall of dicotyledoneus plants is provided by the xyloglucan-cellulose network at the same time that permit turgor-driven cell expansion. Molecular modelling of the binding of xyloglucan to the surface of cellulose microfibrils suggested that a binding site is initiated by the trisaccharide side-chain (i.e., fucosyl-galactosyl-xylosyl) that flattens out an adjacent region of the xyloglucan backbone. Upon contacting a cellulose microfibril this region spreads by step-wise flattening of successive segments of the backbone. So, the removal of t-fucose by an apoplastic alpha-fucosidase, may be an important point in the modulation of the xyloglucan-cellulose network. In a previous paper, the Arabidopsis gene (AtFXG1) coding for an apoplastic alpha-fucosidase has been identified and cloned.
We have isolated a knockout mutant of Arabidopsis thaliana (Atfxg1) which has not fucosidase activity, as well as transgenic Arabidopsis plants containing the AtFXG1 under the CaMV35S promoter. The phenotype of the null mutant and of the transgenic plants over-expressing the fucosidase activity will be presented.

T03-085

Towards analysis of the Arabidopsis thaliana root cellome

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We performed a pilot study to develop tools for high resolution cellome analysis. We generated at high throughput mono- and polyclonal antibodies against Arabidopsis proteins. We cloned Arabidopsis cDNAs from roots, leaves and suspension cells into pQE30NST and expressed proteins in E. coli in arrayed format as His6-tag proteins. Rearrayed cDNA expression libraries were used for preparing high density protein filters. An antibody (RGS His antibody) directed against the N-terminal tag sequence RGSHis6 of the His-tag fusion proteins was used to detect expressed proteins. Expressed genes were sequenced. 250 6xHis-Proteins were selected for large scale expression, and subsequent Ni+-NTA agarose purification under denaturing conditions. Antigens were used to immunize mice and rabbits to raise mono-and polyclonal antibodies. Antibodies are used for protein chip production and high-resolution microscopic cellome analyses.

T03-086

GFP-FABD2 construct allows in vivo visualization of the actin cytoskeleton in all cell types of Arabidopsis thaliana

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In vivo analysis of transgenic Arabidopsis thaliana seedlings, stably transformed with GFP-FABD2 construct (C-terminal actin-binding domain of Arabidopsis fimbrin), allowed us detailed scoring of F-actin arrays in diverse cell types. We document extensive arrays of longitudinal bundles in elongated cells of hypocotyl and radial F-actin arrays in stomata cells. In roots, we focus on cells which are in transition to rapid cell elongation. These cells perform extensive change of the actin cytoskeleton from perinuclear networks into axial bundles. Dramatic redistributions of F-actin networks and arrays are accomplished during formation of trichomes in hypocotyl and leaf epidermis as well as of root hairs in root epidermis. Particularly dynamic elements of F-actin are present at apices of tip-growing root hairs. Pharmacological approach reveals that this actin cytoskeleton of root hairs is essential for endosomal movements in a process which is tightly linked with actin-based tip growth of root hairs. In contrast to GFP-talin transgenic Arabidopsis roots, GFP-FABD2 plants allow visualizuation of F-actin in phragmoplasts of cytokinetic cells as well as in cells of the stele. Overall, this construct appears to be ideally suited for in vivo analysis of the actin cytoskeleton in all tissues and cell types of Arabidopsis thaliana seedlings.

T04 Interaction with the Environment 1 (Abiotic)

T04-001

Towards Understanding Changes to Arabidopsis Mitochondrial Function During Abiotic Stress

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Mitochondrial function sits at the crossroads of carbon and nitrogen metabolism in plants and provides both energy and the biosynthesis of antioxidants and essential cofactors. During environmental stress, mitochondrial function and the mechanisms of mitochondrial defense are our area of special interest (1). Through an extensive organelle proteomics programme we have an in-depth knowledge of the metabolic, regulatory and signalling protein components that are located in Arabidopsis mitochondria (2). Differential analysis of these components at the proteomic level reveals the proteins susceptible to damage and those induced as putative defense mechanisms. We have identified a dual targeted ascorbate glutathione cycle, thioredoxin dependent defense systems and ascorbate synthesis regulation, revealing a multilayer defense system in mitochondria (3-5). Differential analysis at the transcript level using whole-genome microarrays under a variety of stress conditions, allows a broader analysis of the response of the nearly 500 nuclear-gene products that are experimentally determined to be targeted to mitochondria. This reveals a variety of co-expressed genes for establishing new steady-states for the operation of respiration in plants under abiotic/oxidative stress.

1. JBC 277:42663-668; 2. Plant Cell 16:241-46; 3. JBC 278:46869-877; 4. Plant J 32:891-904; 5. Plant Phys 133:443-47.

T04-002

Functional analysis of Arabidopsis thaliana diacylglycerol kinase 2

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ABSTRACT
A variety of stimuli evoke a transient increase in cellular levels of diacylglycerol (DAG) through the hydrolysis of phosphoinositides by phospholipase C (PLC). Cellular levels of DAG are also controlled by the activity of diacylglycerol kinase (DGK), which phosphorylates DAG to produce phosphatidic acid (PA). The importance of both DAG and PA as second messengers in plants has been documented in the past. In the model plant Arabidopsis thaliana, a family of seven putative DGK genes (named AtDGK1 to AtDGK7) has been identified, but the physiological functions of these genes is not precisely known at the present stage. We have cloned the AtDGK2 cDNA and demonstrated that it encodes an enzymatically active DGK (Gómez-Merino et al., 2004). Molecular-genetic approaches are being carried out to investigate the physiological importance of AtDGK2 in plants. AtDGK2 is expressed in various tissues, including roots, leaves, flowers, siliques and seeds. Analysis of promAtDGK2::GUS reporter lines revealed that the gene is active already 1 day after seed germination. We observed that AtDGK2 transcript accumulates rapidly, within 30 minutes, after exposure to cold (4°C) and enhanced expression remained for up to 24 h. Activity of recombinant AtDGK2 expressed in E. coli was drastically reduced by the DGK inhibitor R59022. Arabidopsis wild-type seeds sown on MS medium containing different concentrations of R59022 germinate, but seedlings developed chlorosis when the concentration of the inhibitor increased; at high concentrations of R59022 seedlings died a few days after germination. To increase the activity of AtDGK2 in a constitutive manner in plants, the AtDGK2 cDNA was ligated to the CaMV 35S promoter (in vector pGreen0229) and transformed into Arabidopsis. Surprisingly, however, none of the transformants exhibited an elevated AtDGK2 transcript level, but instead AtDGK2 RNA levels were reduced in all transformants when compared with the wild-type. Further experiments trying to over-express AtDGK2 using a different plant system failed as well. Hence, we hypothesize that the protein AtDGK2 may be toxic to plant cells when expressed at an elevated level. We are currently performing an analysis of AtDGK2 antisense and T-DNA insertion lines.

Gómez-Merino, F.C., Brearley, C.A., Ornatowska, M., Abdel-Haliem, M.E., Zanor, M.I., Mueller-Roeber B. (2004) JBC 279(9)

T04-003

Cellular model for chilling tolerance activated by glycine betaine

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Glycine betaine (GB) activates expression of several stress-related genes and also leads to increased plant tolerance to the damaging effects caused by drought, frost, salt and chilling. Using microarray technology, we have identified several activated genes and have found that many of them are involved in three distinct processes; i.e. 1) Reactive oxygen species (ROS) metabolism, 2) Cell wall synthesis or 3) Regulatory signal transduction pathways. Among ROS-related genes are monodehydroascorbate reductase, NADPH oxidase, glutathione S-transferase and peroxidase. Genes for cell wall synthesis whose expression is affected by GB include AtRabA4 genes, glucuronosyl transferase, germin, xyloglucan endotransferase and arabinogalactan protein. Two GB-activated genes involved in stress tolerance (GLYB genes) have been investigated in Arabidopsis knockout mutants. One of the genes (GLYB1) codes for a bZIP transcription factor which has close homology to a rice gene (lip19) activated by cold treatments while the other gene (GLYB2) codes for a Rab protein involved in vesicle trafficking. Both knockout mutants accumulate superoxide at 4 C in the light. Based on these and related studies, we have developed a cellular model for chilling tolerance in Arabidopsis as follows: at low, non-freezing temperatures, light energy is converted in the chloroplast to strong reductant which is used to reduce oxygen to superoxide and for further ROS production. Substantial amounts of ROS are detoxified in the cell wall by peroxidases made on the ER, then transported through the Golgi system to the cell wall via vesicle trafficking to the plasma membrane which depends on Rab proteins coded by genes such as GLYB2. The bZIP gene (GLYB1) is required for activation of several genes involved in chilling tolerance.The finding that GB activates a series of genes involved in producing peroxidase in cells, then transporting it via the vesicle trafficking system to the cell wall where peroxidase can act in dissipating ROS is of interest as a new mechanism used by plants to deal with reactive oxygen species (ROS) produced when an imbalance in photosynthesis exists caused by light/temperature stress. Most other mechanisms focus on processes occuring in direct association with chloroplasts such as dissipation of PSII excitation by zeaxanthin, changes in the sizes of the Chl antenna, increased photorespiration or up-regulation of reductases associated with cyclic electron transport.

Huner, Øquist and Sarhan 1998 Trends in Plant Science 3: 224
Niyogi 1999 Ann Rev Plant Physiol Plant Mol Biol. 50: 333

T04-004

A MAP-kinase pathway for cold and salt signalling

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An Arabidopsis mitogen activated protein kinase kinase and two downstream MAPKs were isolated by functional complementation of osmosensitive yeast mutants. Yeast two-hybrid and protein kinase assays revealed a specific and direct interaction of this MKK with its substrates. The MKK in turn, was specifically activated by cold and salt stress in Arabidopsis protoplasts. Plants over-expressing this MKK exhibited enhanced constitutive activity of the two downstream MPKs, constitutive expression of stress-induced marker genes and increased freezing and salt tolerance. Knockout plants in contrast, were impaired in MPK activation and were hypersensitive to salt and cold stress. Full genome transcriptome analysis of MKK over-expressing plants demonstrated altered expression of 152 genes involved in transcriptional regulation, cellular defense, signal transduction and metabolism of cold- and salt stress responses (1, 2). Hence, our data identify a novel MAP kinase signaling cascade mediating cold and salt stress tolerance in Arabidopsis.

1. Fowler and Thomashow (2002) Plant Cell 14, 1675-1690
2. Kreps et al. (2002). Plant Physiol. 130, 2129-2141

T04-005

The Basic Helix-Loop-Helix genes involved in iron deficiency responses in Arabidopsis

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Basic-helix-loop-helix (bHLH) proteins are a family of transcriptional factors that have been characterized as important regulators in diverse biological processes. Here, we investigated 5 bHLH genes. One of them shares high similarity with Lefer which is a root-specific bHLH gene controlling iron uptake in tomato. Four other bHLH genes were up-regulated in response to iron deficiency and identified by microarray analysis. The Lefer-like gene was mainly expressed in root and inflorescence and its expression was augmented upon iron deficiency in root. The expression of promoter-GUS fusions reveal a cell-specific pattern. Gene knockouts resulted in chlorosis and blocked the physiological and molecular responses to iron starvation. Over-expression augmented plant tolerance to iron deficiency, and in accordance, the physiological and molecular responses were enhanced. Taken together, the Lefer-like gene may act as a key regulator in iron mobilization in Arabidopsis, implying that a conserved mechanism existed in tomato and Arabidopsis for iron uptake. The other four bHLH genes were expressed both in leaves and roots and induced by iron deficiency. The interactions between the bHLH genes was analyzed.

T04-006

Yellow Stripe-Like Family members may be involved in metals homeostasis.

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In low iron conditions, grasses synthesize and extrude small compounds with metal chelating properties called phytosiderophores (PS) and subsequently take up iron as a Fe3+-phytosiderophore complex (Fe-PS) via the Yellow Stripe-1 (YS1) transporter. Eight genes similar to YS1 have been identified in Arabidopsis. Although plants other than grasses do not synthesize PS, they do produce a related molecule, the nicotianamine (NA) that possesses similar chelating properties and plays an important role in iron homeostasis. NA-metal chelates represent therefore potential candidate substrates for Arabidopsis YS-Like (YSL) genes. The expression study of the YSL genes shows that they are mostly expressed in shoots. Four of the eight members are regulated by the iron status. YSL1 is induced by Fe-excess whereas YSL5 and YSL8 are induced by Fe-deficiency. YSL2 is repressed in plants grown under Fe-deprivation. Promoter-GUS transgenic lines show that YSL1, YSL2 and YSL5 promoters are active in vascular tissues, favoring their role in long distance iron transport. The absence of a clear phenotype of knock-out mutants and overexpressing lines argue in favor of a redundant function between the family members. Here we also present data of sub-cellular localization and functional analysis in yeast.

T04-007

Genome Wide RNA Expression and Metabolic Analysis of high light adaptation in wild type, tocopherol minus (vte1), and complemented (pvte-vte1) mutants of Arabidopsis thaliana.

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The vitamin E family of prenyllipid antixidants (tocopherols and tocotrienols) occur only in photosynthetic organisms and, in photosynthetic tissues, are thought to play a role in protection against oxidative stress, as these compounds are potent antioxidants. The successful elucidation of the biosynthetic pathway of alpha tocopherol biosynthesis aver the last five years using Arabidopsis thaliana reverse genetics, has also given us a number of defined tocopherol minus Arabidopsis mutants, which are ideal tools to study the biological role of tocopherols in the prevention of photo-oxidative damage. The recently identified vte1 mutant of Arabidopsis , which is deficient in tocopherol cyclase enzymatic activity, displays an apparently normal phenotype under standard growth conditions. We have utilized genome wide RNA expression analysis and comprehensive metabolic profiling of leaf samples from both wild type and vte1 mutant and vte1 mutant plants transformed with a 35S vte1 over-expression contruct (complement) Arabidopsis plants grown under both standard (90mE/m2/s) and high light (900mE/m2/s) conditions. We have compared the gene expression data obtained to that of the hpt mutant of arabidopsis which is lacking the homogentisate phytyltransferase enzyme, and is also tocopherol minus with an apparently normal phenotype under standard growth conditions.

Gene Expression results showed large changes in expression profiles between plants grown under normal light and high light. In contrast, only small changes in gene expression were seen between the wild type and vte1 plants. The metabolic profiling data conformed these conclusions, again showing only small differences between the mutant and wild type plants grown under normal growth conditions, and showing much more dramatic differences in metabolites beween plants grown under normal and high light conditions.

T04-008

Functional analysis of heat shock induced heat shock factor genes in Arabidopsis thaliana

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In Arabidopsis thaliana 21 different HSF genes have been identified. Based on amino acid homology, plant HSFs are assigned to two major classes A and B. The constitutively expressed class A-factors, HSF1 and HSF3, have been identified as transcriptional regulators required early in the stress response for transient expression of heat shock genes. A striking peculiarity of class B-HSF is the lack of AHA motifs in the carboxyterminal domain, which are crucial for activator function of class A-HSF. The heat-inducible expression of class B-factors, HSF4 and HSF7, suggests that their function is required for delayed effects in the HS response. The heat-induction of HSF7 mRNA correlates with the presence HSE-consensus sequences at three different sites in the promoter upstream region of the HSF7 gene. All three sites can bind recombinant HSF1 protein in vitro. In order to functionally characterize HSF4 and HSF7, we have isolated T-DNA single and double knock out mutants. Expression data indicate that the mutants hsf4tt1 and hsf7tt4 are knock out mutants, there is no mRNA detected without and following heat shock. The effect of hsf 4tt1/hsf 7tt4 mutants on heat shock gene expression is investigated by RT-PCR profiling.

Lohmann C, Eggers-Schumacher G, Wunderlich M, Schoffl F: Molecular genetics and genomics 271 (1): 11-21 FEB 2004

T04-009

Transcriptional Regulation of ABA-responsive Genes in Seeds, Germination Stage Plants, and Vegetative Growth Stage Plants.

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Induction of a dehydration-responsive Arabidopsis gene, rd29B is mediated mainly by ABA. The ABRE sequences and the ABRE-binding bZIP-type proteins, AREBs function as cis-acting elements and transcriptional activators, respectively, in the ABA-inducible expression of rd29B in vegetative growth stage. The expression level of rd29B is high in seeds and ABA induces the expression of rd29Bearly in germination. Expression of ABI5 and ABI3 parallel the expression of rd29B in seeds and early in germination in the presence of ABA. Expression of AREB1 and AREB2 is not observed in seeds and ABA induces the AREB1 and AREB2 expression weakly early in germination. Expression analysis of rd29B in the abi3 and abi5 mutants indicate that ABI3 and ABI5 have important roles for the expression of rd29B in seeds but other transcription factors adding to ABI3 and ABI5 might be involved in the ABA-inducible rd29B expression early in germination. Histochemical analysis using the Arabidopsis plants containing the rd29B promoter :: GUS fusion gene indicate that the ABRE sequences are necessary for the expression of rd29Bin seeds and the ABA-responsive expression of rd29Bboth in germination and vegetative growth stage. In a transient transactivation experiment using protoplasts from T87 Arabidopsis culture cells, the AREB1, ABI3 and ABI5 proteins activated transcription of a GUS reporter gene driven by ABRE, and the activation was increased by ABA. Among these proteins, ABI3 shows the strongest activity for transactivation. Co-expression of ABI5 or AREB1 with ABI3 resulted in remarkable increase for the activation. In the transgenic Arabidopsis plants containing the 35S::ABI3 fugion gene, the expression level of rd29B is high in untreated plants, and increased when we applied ABA. These results agree with the results in the transient transactivation experiment. We compared the expression of 7,000 Arabidopsis genes under ABA treatment during germination, that in vegetative growth stage, and that in the 35S::ABI3 plants using a full-length cDNA microarray. The results indicated that ABA-responsive gene expression during germination is not same to that in mature plants, and the ABI3- and/or ABA-up-regulated genes can be classified to six groups. The relationship between the expression profiles and the cis-elements on the promoter sequences will be discussed.

T04-010

Genetic and molecular characterisation of a novel Major Facilitator Superfamily protein implicated in zinc homeostasis in Arabidopsis

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Zinc (Zn) is an essential micronutrient required for metabolism in all cells. In higher eukaryotes it is the second most abundant micronutrient after iron and Zn deficiency is possibly the most wide-spread limitation on crop yields world-wide. Although candidates for uptake, storage and vascular translocation have been identified in Arabidopsis, our understanding of the mechanisms underlying Zn homeostasis remains limited. A number of protein families have been implicated in Zn transport, including Zrt- Irt-like Proteins (ZIP), Cation Diffusion Facilitators (CDF) and CPx P-type ATPases. In the current study, we have identified a novel mutant of Arabidopsis thaliana which shows sensitivity to elevated Zn. The mutation is in a gene encoding a member of the Major Facilitator Superfamily (MFS). Members of this protein family transport a wide range of substrates, but no characterised member has been implicated in Zn metabolism. The protein shows less than 50% similarity to any non-plant protein in the databases, however, two homologues exist in A. thaliana. This study involves the characterisation of mutants in these three genes, and the molecular characterisation of the gene conferring Zn sensitivity.

T04-011

Signals and signal transduction in the control of nuclear expression of chloroplast antioxidants

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Redox regulation is essential for the maintenance of the cellular homeostasis. Besides enzymes, many genes are redox regulated for feed-back control of the cellular redox status. Thereby the genes for antioxidant enzymes are of special interest as they directly can close feed-back regulatory ciruitries by increasing the antioxidant protection capacity. By analysing expressional regulation in Arabidopsis thaliana and performing mutant approaches, our study aims at identification of the signals and signal transduction elements involved in the redox regulation of nuclear gene expression.
A special focus is given to 2-Cys peroxiredoxins, which are ancient peroxidases (1) expressed earlier than many other chloroplast antioxidant enzymes during greening and which support early seedling development (2). Reporter gene analysis demonstrated that the promoter activity of the 2-Cys peroxiredoxin A (2CPA) gene is under control of photosynthetic electron transport. Signals correlating with the acceptor availability at photosystem I regulate transcription of the nuclear encoded chloroplast peroxidase. The plant hormone ABA antagonizes the oxidative redox signal. A regulatory circuitry has been deduced in which the redox signal integrates into the terminal ABA-signalling cascade.
For identification of regulators in chloroplast-to-nucleus redox signalling, a population of 2CPA:luciferase reporter gene plants was chemically mutagenized. Mutants with lower reporter gene activity were isolated from the M2 progeny. The rimb-mutants (redox-imbalanced) show lower activity of the endogenous 2CPA gene and impaired expression of genes for other nuclear encoded chloroplast antioxidant enzymes. For 6 rimb-mutants data on the regulation of gene expression of antioxidant enzymes, the photosynthetic performance, control of the cellular redox poise and stress tolerance will be presented and discussed in comparison to results on 2-Cys peroxiredoxin antisense lines (2, 3).

1. Plant Mol Biol 31: 553 ¯564; 2. Plant Physiol 119: 1407 ¯1414; 3. Plant Physiol 124: 823 - 832

T04-012

Expression in Multi-Gene Families: Analysis of Chloroplast and Mitochondrial Proteases

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Expression in Multi-Gene Families: Analysis of Chloroplast and Mitochondrial Proteases1
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The proteolytic machinery of chloroplasts and mitochondria in Arabidopsis thaliana is consisted primarily of three families of ATP-dependent proteases, Clp, Lon and FtsH and one family of ATP-independent protease, DegP. However, the functional significance of the multiplicity of their genes is not clear. To test whether expression of specific isomers could be differently affected by growth conditions, we analyzed transcript abundance following short-term exposure to different environmental stimuli, using 70-mer oligonucleotide arrays. This analysis revealed variability in the response to high light and different temperatures within members of each family. Thirty out of the 42 tested genes were up-regulated in response to high-light, whereas only six genes responded to either high or low temperature. The extent of response was variable, ranging from 2 to 20-fold increase in the steady-state levels. No preference in the response to high light was observed between chloroplast and mitochondrial isozymes. Absolute transcript levels of the tested genes, compiled from one-channel arrays, were also variable. In general, transcripts encoding mitochondrial isozymes were accumulated to a lower level than chloroplastic ones. Within the FtsH family, transcript abundance of the different genes correlated with the severity of mutant phenotypes in the relevant genes. This correlation was also evident at the protein level. Analysis of FtsH isozymes revealed that FtsH2 was the most abundant species, followed by FtsH5 and 8, with FtsH1 being accumulated to only 10% of FtsH2 level. These results suggest that, unlike previous expectations, the relative importance of different chloroplast protease isozymes, evidenced by mutant phenotypes at least in the FtsH family, is determined by their abundance, and not necessarily by different specific functions or specialized expression under certain conditions.

T04-013

Plant Modulates Its Genome Stability in Response to Stress

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The sessile plants can not escape from the ever-changing environment. They are subjected to various stress conditions including both biotic and abiotic ones, which affect many physiological aspects of the plants. In adapting to these changing surroundings, the plants have genetically evolved to fit to the niche they live. In Arabidopsis thaliana, many mutants have been characterized to be impaired in the response to various factors including pathogenic invasion, light perception, reactive oxygen species (ROS) detection, and other physical (e.g. radiation and UV) and chemical (e.g. heavy-metal ions) elements. Apparently, many genes are involved in these complicated cross-talking responses to various stresses. Recent works in our lab have discovered that a wide range of stresses affect the genome stability of plants. However, the extent of factors affecting plant genome stability is still an open question. By crossing the well-known uidA gene recombination substrates-carrying transgenic line to a series of mutants, we screened for the progenies that carry both the specific deficient gene and recombination substrates. The homologous recombination in mutants was monitored under various stress conditions.

Here we report the genome stability of Arabidopsis thaliana mutants of vtc1 (vitamin C-deficient), tt4 and tt5 (flavonoid deficient), which have deficient protection against oxidative radicals. Both the spontaneous and stress (UV and rose Bengal) induced recombination frequencies were higher in mutant plants than that in the original 651 line. Quantitative RT-PCR revealed that mRNA levels of several DNA repair genes (RAD1, RAD54-like and MSH), and radical scavenging genes (GPx1, CAT and FSD3) exhibited substantial differences in both the control and induced conditions. Evidences show that plants impaired in certain aspects of the protection against elevated levels of free radicals induced the production of scavenging enzymes earlier than that in wild type plants. The relationships between those events and expression of DNA repair genes were further discussed.

Nature 423(6941):760-2; Plant J. 7(2):203-210; Plant J. 38(1):60-9.

T04-014

Role of the zinc finger homeodomain (ZFHD) and NAC transcription factors in drought-inducible expression of the erd1 gene

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The “CACTAAATTGTCAC” cis-element and NACRS (NAC recognition sequence) play an important role in the dehydration-inducible expression of the Arabidopsis erd1 gene, which encodes a ClpA-homologous protein. Last year, we reported the isolation and functional analyses of three NACRS binding NAC transcriptional activators. Recently, we isolated a cDNA clone encoding protein that bind to the 63-bp promoter region of erd1, which contains the “CACTAAATTGTCAC” motif, by using the yeast one-hybrid system. The ZFHD1-designated protein belongs to the zinc-finger homeodomain transcription factor family. The ZFHD1 protein bound specifically to the “CACTAAATTGTCAC” motif - named ZFHD recognition sequence (ZFHDRS) - both in vitro and in vivo. The expression of ZFHD1 was induced by drought, high salinity and abscisic acid (ABA). The ZFHD1 and NAC transcriptional activators co-operatively transactivated expression of the GUS reporter gene driven by the erd1 promoter in Arabidopsis T87 protoplasts. Yeast two-hybrid system detected interaction between ZFHD1 & NAC proteins.

T04-015

Xanthine deyhdrogenase from Arabidopsis thaliana: An old fellow in purine catabolism and a new player in reactive oxygen species metabolism.

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Xanthine dehydrogenase (XDH) is involved in purine degradation where it catalyzes the two-step conversion of hypoxanthine to uric acid. While most other organisms own only one gene for XDH Arabidopsis possesses two genes in tandem orientation that we named AtXDH1 and AtXDH2. Whereas AtXDH2 mRNA is expressed constitutively alterations of AtXDH1 transcript levels were observed at various stresses like drought, salinity, cold, ABA treatment and natural senescence. Transcript alteration did not necessarily result in changes of XDH activities. While salt-treatment had no effect on XDH activities, cold stress caused a decrease, but desiccation and senescence caused a strong increase of activities in leaves. Because AtXDH1 presumably is the dominant isoenzyme in Arabidopsis it was expressed in yeast, purified and used for biochemical studies. AtXDH1 protein is a homodimer of about 300 kDa consisting of two identical subunits of 150 kDa. Like XDH from other organisms AtXDH1 uses hypoxanthine and xanthine as main substrates and is strongly inhibited by allopurinol. It is activated by purified molybdenum cofactor sulfurase ABA3 that converts inactive desulfo- into active sulfo-enzymes and regulates its activity in vivo. Finally it was found that during the conversion of hypoxanthine to uric acid and by using molecular oxygen as electron acceptor AtXDH1 produces superoxide radicals indicating that besides purine catabolism it might also be involved in response to various stresses that require reactive oxygen species.

T04-016

RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phot1

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phototropin 1 (phot1) and phot2, which are blue light receptor kinases, function in blue-light-induced hypocotyl phototropism, chloroplast relocation, and stomatal opening in Arabidopsis. Previous studies have shown that the proteins RPT2 and NPH3 transduce signals downstream of phototropins to induce the phototropic response. However, the involvement of RPT2 and NPH3 in stomatal opening and in chloroplast relocation mediated by phot1 and phot2 was unknown. Genetic analysis of the rpt2 mutant and of a series of double mutants indicates that RPT2 is involved in the phot1-induced phototropic response and stomatal opening but not in chloroplast relocation or phot2-induced movements. Biochemical analyses indicate that RPT2 is purified in the crude microsomal fraction, as well as phot1 and NPH3, and that RPT2 makes a complex with phot1 in vivo. On the other hand, NPH3 is not necessary for stomatal opening or chloroplast relocation. Thus, these results suggest that phot1 and phot2 choose different signal transducers to induce three responses: phototropic response of hypocotyl, stomatal opening, and chloroplast relocation.

Inada et al. (2004) Plant Cell 16, 887-896

T04-017

The Arabidopsis AtMYB60 transcription factor is specifically expressed in guard cells and is involved in the regulation of stomatal movements

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Stomata are microscopic pores present on the epidermis of the aerial organs of plants, playing a central role in regulating gas exchanges between plant tissues and the atmosphere.
Land plants face the need of ensuring a sufficient uptake of CO2 for photosynthesis and of minimizing water loss by transpiration. The regulation of stomatal pores aperture in response to environmental stimuli including light, humidity, CO2, and internal signals, such as the phytohormone abscissic acid (ABA), allows the plant to cope with these conflicting needs.
An increasing number of evidences indicate a critical role for mRNA metabolism in the control of guard cells activity and, more in general, in ABA-mediated plant responses.
AtMYB60 gene codes for an Arabidopsis R2-R3 MYB transcription factor. Different portions of the putative promoter of this gene were fused to reporter genes. Expression profiles indicated that this gene is specifically expressed in stomata guard cells.
We isolated from a collection of T-DNA mutagenized lines the atmyb60-1 null allele. Light-induced stomatal opening was impaired in this mutant, while the response to ABA and CO2-free air was not affected. Moreover, atmyb60-1 plants exhibited a reduction in transpirational water loss during drought. Interestingly, the levels of the AtMYB60 transcripts were down-regulated by ABA applications and in response to drought stress and up-regulated following exposure to light.
Microarray analyses revealed that the transcripts of a few ABA-regulated genes, known to be involved in the plant response to environmental stress, were altered in the null allele.
All together our findings suggest a role for the AtMYB60 product in promoting stomatal aperture, confirming the importance of the transcriptional control on the mechanisms involved in regulating guard cells movements.

T04-018

The impact of elevated boron on the development of Arabidopsis thaliana

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Boron (B) is an essential micronutrient required for plant growth and development but relatively little is known about the underlying processes of boron uptake, transport, toxicity and its role at physiological concentrations within plants. Molecular investigations of B requirement in plants could open new possibilities for improving crops tolerant to B deficiency/toxicity stress.
The aims of this work were to: (i) characterize the response of Arabidopsis thaliana to alterations in B supply, focusing on responses to elevated B, (ii) examine the interactions between B response and environmental signals (light quality and quantity), (iii) test hypotheses generated from these observations on plants with mutations in photoreceptors and cell wall structure.
Arabidopsis seedlings were grown on 0.5x Murashige and Skoog medium (which contains 0.1 mM B, a concentration typical of that of many soils) supplemented with boric acid. At very high concentrations of boron (5 mM and above) a clear toxicity effect was apparent. Seedlings were short, stunted and pale. No toxicity effects were apparent at elevated B (1-2 mM) although there was a clear stimulation of seedling hypocotyl elongation. This growth stimulation was apparent in hypocotyls and petioles but not in roots, leaves or cotyledons. Measurements of hypocotyl epidermal and cortical cell lengths and number indicated that this growth stimulation resulted from an increase in cell expansion rather than cell division. The stimulation of the elongation by elevated B was proportionally more obvious with increasing irradiance.
The stimulation of hypocotyl elongation at elevated B concentrations suggests a possible direct interaction of B with the cell wall. Thus, we have tested A. thaliana mutants with altered cell wall compositions (mur1 ¯ mur10) (Reiter et al., 1997). Elevated B stimulated hypocotyl elongation in mur1-4 but the response was much reduced in mur 5-10.

The experiments were supported by the grant of Czech Science Foundation No. 521/00/D036.

Reiter W.D., Chapple C. and Somerville C. R. (1997) Plant J. 12 : 335- 342.

T04-019

The interacting roles of light regulation and ethylene biosynthesis in modulating hypocotyl gravitropism

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Gravitropism is a plant's directional growth in response to gravity, exhibited as growth away from gravity in stems and toward the gravitational vector in roots. During stem gravitropism, the asymmetrical distribution of the auxin causes differential growth and upward curvature while the gaseous hormone ethylene plays a modulating role in regulating the kinetics of growth asymmetries. Light also modulates gravitropic curvature, potentially through phytochrome's regulation of ethylene biosynthesis. The major goal of this project was to evaluate the role of ethylene biosynthetic genes in the regulation of the gravitropic response in etiolated Arabidopsis hypocotyls. All seedlings were dark-grown and handled under dim green light. Red-treated seedlings were given a 6-minute pulse of red light 18 hours prior to experimentation using a procedure consistent with previous studies on the timeline of red-light inhibition of ethylene biosynthesis. Red-light treatment of dark-grown Arabidopsis seedlings was evaluated for its effect on ethylene production, gravitropic curvature, and gene expression changes for members of the ACS (1-aminocyclopropane-1-carboxylic acid synthase) gene family. Red light pretreatment of 3-day old wild type etiolated seedlings significantly decreased ethylene production, and also decreased gravitropic curvature 3 hours after horizontal placement. Preliminary RT-PCR studies of the expression of Arabidopsis ACS2, ACS4, ACS5, ACS6, ACS8 or ACS9 from mRNA extracts of etiolated Arabidopsis seedlings, suggest red-light inhibition of the ACS4 gene. Seedlings containing T-DNA knockouts of Arabidopsis ACS4, ACS6, or ACS8 genes all exhibited reduced ethylene production. However, gravitropic curvature was increased by 26% in the ACS4-knockout mutants 3 hours after horizontals placement, whereas the other ACS knockout mutants curved to the same extent as the wild type controls. These results suggest a role for ACS4 regulation in modulating gravitropic curvature. Also, red-light reduced ethylene level may affect the curvature 3 hours after horizontal placement, but long-term timecourse measurements for gravitropic curvature are needed to evaluate the full effects on the kinetics of curvature.

T04-020

Isolation of novel ABA-related mutants using ABA analogs

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In order to get more insight into the ABA signaling pathway, we conducted screens for Arabidopsis mutants that have an altered ABA sensitivity using ABA analogs. Using an ABA analog, PBI-51, we obtained several putative ABA hypersensitive mutants, named ahg (ABA hypersensitive germination). We selected nine mutants for further analysis. These mutants showed ABA hypersensitivity at germination and post germination seedling growth. Genetic and physiological characterization of them indicated that these are novel mutants. We identified the corresponding genes for ahg2, ahg3 and ahg11 recently. The predicted function of those gene products implies protein phosphorylation and RNA metabolism are involved in the ABA response, consistent with previous reports.

We also searched for ABA analogs that might have ability to allow us to dissect the ABA signaling pathway. We found one analog, named #18, has a growth inhibition effect like ABA but its effect is much stronger on the growth of greening portion than that of root. Based on the response of known ABA-related mutants and several ABA inducible genes to this analog, #18 seems to actually evoke the ABA response. We found that the sensitivity to this ABA analog is different among Arabidopsis ecotypes. Ler showed a decreased sensitivity to this analog compared with Col. The ABA sensitivity of Ler is slightly stronger than that of Col, indicating the sensitivity to #18 does not correlate with ABA sensitivity. It might be possible to identify novel ABA-related loci using this ABA analog. Currently we are trying to isolate mutants with altered sensitivity to#18 and to identify the locus for Ler's resistant phenotype to this ABA analog.

T04-021

Identification of a new ABA biosynthesis locus, AtABA4, in Arabidopsis thaliana

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ABA is derived from xanthoxin by the cleavage of oxygenated carotenoids, also called xanthophylls. The isolation of mutants impaired in ABA biosynthesis, has proved to be particularly effective for cloning genes involved in most steps of the pathway. The identification of mutants accumulating zeaxanthin has previously led to the cloning of the genes encoding the enzyme zeaxanthin epoxidase (ZEP) from several species. Zeaxanthin epoxidation leads to the formation of all-trans-violaxanthin. However only cis-isomers of violaxanthin and neoxanthin are cleaved into xanthoxin by a family of 9-cis-epoxycarotenoid dioxygenases. Due to the absence of mutants impaired in these catalytic steps, the genes involved in the conversion of all-trans-violaxanthin to cis-xanthophylls remained to be characterized. We recently isolated a new ABA-deficient mutant of Arabidopsis, Ataba4, which is unable to synthesize cis and trans isomers of neoxanthin. The AtABA4 gene has been identified by map-based cloning and its characterization is underway.

T04-022

Ethylene responses in Arabidopsis seedlings' roots require a local boost in auxin production.

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Exposure of dark-grown Arabidopsis seedlings to exogenous ethylene leads to characteristic morphological changes collectively known as the triple response. The phenotypic effects of ethylene in seedlings' roots require normal levels of auxin signaling and response. We are interested in understanding the mechanisms of the ethylene-mediated root shortening and, in particular, the role of ethylene-auxin crosstalk in this process. Towards this goal, we have isolated several mutants with reduced ethylene sensitivity, yet normal auxin responsiveness, of seedlings' roots.

Two complementation groups, wei2 and wei7, represented by three independent alleles each, were phenotypically indistinguishable from each other. Positional cloning of wei2 and wei7 revealed that they harbor mutations in the alpha and beta subunits of the ANTHRANILATE SYNTHASE (AS) genes ASA1 and ASB1, respectively. AS is a tryptophan (Trp) biosynthetic enzyme that catalyzes conversion of chorismate into anthranilate (Ant), the rate-limiting step of the pathway. Trp and its biosynthetic intermediates, in turn, serve as precursors to auxin. Consistent with this notion, the phenotypic defects of wei2 and wei7 in ethylene can be complemented by exogenous Ant, Trp, or auxin.

Analysis of the transcriptional fusions of the WEI2 and WEI7 promoters with GUS revealed that expression of both genes was inducible by ethylene in root tips of Arabidopsis seedlings. By increasing the levels of the rate-limiting enzymes ASA1 and ASB1 in roots, ethylene may stimulate production of tryptophan and, ultimately, auxin. In fact, expression of a synthetic auxin reporter DR5-GUS in root tips is enhanced in the presence of exogenous ethylene. Remarkably, this ethylene-mediated induction of the auxin reporter is fully dependent on functional WEI2 and WEI7. Furthermore, the wei2 and wei7 mutations can suppress the "high-auxin" phenotypes of the cyp83b1 knockout mutant, consistent with the central role of the AS genes in auxin biosynthesis in the situations of high auxin demand.

In summary, our data implicate WEI2 and WEI7 in the ethylene response. By stimulating transcription of these enzymes, ethylene enhances the rate of tryptophan and auxin biosynthesis in a tissue-specific manner. Such boost in auxin production is a prerequisite for the full-scale ethylene response of Arabidopsis seedlings' roots.

T04-023

The calcium sensor CBL1 and its interacting protein kinase CIPK1 mediate osmotic stress responses in Arabidopsis

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Calcium signals in plant cells are elicited by a variety of stimuli such as hormones, light and stress factors. We have recently described a new family of calcineurin B-like (CBL) calcium sensor proteins from Arabidopsis and identified a specific group of serine-threonine protein kinases (CIPKs, CBL-interacting protein kinases) as targets of these sensor proteins.
Our analysis of a cbl1 knock out mutant revealed that plants lacking the activity of CBL1 sensor protein displayed an increased sensitivity when exposed to NaCl and drought stress (Albrecht et al., 2003). Further phenotypical characterization indicated that this mutant is also impaired in osmotic stress responses.
CBL1 interacts specifically with six protein kinases (CIPKs) giving rise to a network involved in stress response. To characterize the function of CBL1-interacting protein kinases, we have used a reverse genetic approach to analyze loss-of-function alleles of CIPK1 and CBL1/CIPK1. Our results indicated that loss of CIPK1 function renders plants sensitive specifically to osmotic stress when compared to wild type. The phenotypic analysis of a cbl1/cipk1 double mutant showed that plants lacking the activity of both proteins were equally sensitive as the single mutants (cipk1 or cbl1) when grown on media containing manitol. This suggests, that both proteins act in the same pathway in response to osmotic stress. Taken together, our data suggest that the calcium sensor CBL1 exhibits an integrative function for several abiotic stress responses, while the interacting kinase CIPK1 contributes to an efficient channeling of plant osmotic stress signaling.

Albrecht et al., (2003) Plant J., 36, 457-470.

T04-024

Functional requirements for PIF3 in the de-etiolation process

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PIF3 is a bHLH class transcription factor that binds to a G-box DNA target site and is also capable of binding photoreversibly to phytochrome B (phyB). The pif3 mutant is compromised in a set of characteristic phytochrome responses in the photomorphogenic process. To determine whether the phyB and DNA binding capacity of PIF3 are required for its wild type function, we have undertaken pif3 phenotype rescue experiments. PIF3 variants that fail to bind to phyB, its G-box target, or both, were introduced into the pif3 mutant. Homozygous lines carrying point mutations unable to bind phyB fail to rescue PIF3 phenotypes whereas lines carrying a point mutation unable to bind to a G-box fully rescue the wild type PIF3 phenotype. Therefore, it appears phyB binding but not DNA binding, is required for full PIF3 function. Recent reports indicate that activated phytochrome induces rapid degradation of the PIF3 protein upon initial exposure of etiolated seedlings to light. We are currently assessing the role of this degradation in PIF3 function and the mechanism by which it occurs.

T04-025

DegP1 Protease in Arabidopsis - Possible Role in Degradation of Oxidatively Damaged Membrane Proteins

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DegP1 Protease in Arabidopsis - Possible Role in Degradation of Oxidatively Damaged Membrane Proteins

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DegP (HtrA) is a serine-type protease that is found in both prokaryotic and eukaryotic organisms. The Arabidopsis genome contains 14 degP genes, the products of four of these are found in chloroplasts. DegP1 was shown to be associated with the lumenal side of the thylakoid membrane, and to degrade lumenal protein in vitro. To explore the functions of DegP1 in vivo, a RNAi construct was generated and used for transforming Arabidopsis plants. All transgenic plants, resistant to the selection marker, accumulated higher levels of DegP1 transcript compared to WT, but some of these accumulated much less of the DegP1 protein. These plants were smaller than WT and their leaves thiner, although they accumulated chlorophyll to the same level as WT. When exposed to high light intensity, they demonstrated increased sensitivity to photoinhibition, as revealed by measurements of chlorophyll fluorescence. Thylakoid protein profiles of transgenic and WT plants were similar, but immunoblot analysis revealed that transgenic plants accumulated more of the D1 protein of PSII reaction center. These results suggest that DegP1 is involved in degradation of the D1 protein, a process which is essential for completion of the repair cycle of photoinhibited PSII. The possible role of DegP1 in the repair cycle, in the context of current models of the D1 protein degradation, will be discussed.

T04-026

Characteristics of GABI-Kat mutant AnnAt1 annexin (line 327B12)

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AnnAt1 is a representative of a multifunctional calcium and membrane phospholipid-binding protein family annexins in Arabidopsis thaliana1. There are some indications, that besides its role in calcium binding, it can also contribute in balancing reactive oxygen species (ROS) level during oxidative burst. Expression of AnnAt1 in A. thaliana is induced after treatments resulting in perturbation of cell redox state2. In heterologous systems AnnAt1 was able to protect cells from oxidative stress2. In this context it would be interesting to characterize its role in stress response in homologous system.
GABI-Kat mutant (Line 327B12) is functional knock-out AnnAt1 due to pAC161 transposon integration within 5' UTR fragment and because of that it is an appropriate model to compare with wild type. In our experiments we will test response of both plants on different stimulations causing changes in ROS levels. We will use panel of various stimulus leading to different biotic (for example: wounding and abscisic acid) and abiotic stresses. We were also interested in comparison of plant expressing and non-expressing AnnAt1 with A. thaliana plants over-expressing yeast catalase, which is important enzyme degrading H2O2and neutralizing effect of ROS action.

1 Plant Physiol., 2001, 126: 1072-84
2 PNAS USA 1996, 93: 11268-73

T04-027

The ABC of guard cell regulation

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ABC transporters are ATP-driven pumps for a broad spectrum of substrates and include the cystic fibrosis transmembrane channel regulator conductance (CFTR) and the sulfonylurea receptor (SUR) known to act as or to regulate ion channels. In Arabidopsis, ABC proteins constitute the largest membrane protein gene family. The multidrug transport associated protein (MRP) subfamily in plants sharing extensive homology to CFTR and SUR are involved in cellular detoxification by pumping toxic substances into the vacuole. Surprisingly, expression and localization studies revealed that AtMRP4 and AtMRP5 are strongly expressed in the plasmalemma of guard cells. Stomata of atmrp5 mutants exhibit reduced opening in the light and insensitivity to different hormones including abscisic acid. Consequently, atmrp5 plants display strongly reduced susceptibility to drought stress and increased water use efficiency. Overexpression of AtMRP5 in tobacco results in faster wilting and decreased anion channel currents as studied by patch-clamp analysis of tobacco guard cell protoplasts. Conversely, atmrp4 mutants wilt quicker. They display increased transpiration and stomatal aperture in light and dark but maintain sensitivity to ABA. AtMRP4 expressed in yeast is a functional transporter for the antifolate drug methotrexate. We will present a model introducing ABC transporters in guard cell regulation.

T04-028

Expression analysis, characterization of mutants and biochemical studies of selected osmotic stress-responsive members of the aldehyde dehydrogenase (ALDH) gene superfamily in Arabidopsis

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Aldehyde dehydrogenase (ALDH) genes have been identified in almost all organisms from prokaryotes to eukaryotes, but particularly in plants knowledge is very limited with respect to their function. The Arabidopsis thaliana genome contains 14 unique ALDH sequences encoding members of nine ALDH families, including eight previously identified families and one novel family (ALDH22) that to date is restricted to plants. To gain insight into the possible roles of aldehyde dehydrogenases from Arabidopsis, we analyzed the expression patterns of five selected ALDH genes under defined physiological conditions. Three genes (ALDH3I1, 3H1 and ALDH7B4) that belong to two different families are differentially activated by dehydration, high salinity and ABA in a tissue-specific manner. The other two genes (ALDH3F1 and ALDH22A1) are constitutively expressed at a low level. Transcript analysis of ALDH3I1 and ALDH7B4 in Arabidopsis mutants suggests that their stress response is differentially controlled by the phytohormone ABA as well as by pathways that affect sugar metabolism and fatty acid composition of membrane lipids. To learn more about the function of the ALDH genes in planta mutants with T-DNA inserts in four selected ALDH genes (ALDH3I1, 3H1, 3F1 and 7B4) were identified. Alternatively KO-mutants were generated by RNAi-technology. In addition, recombinant ALDH proteins were used to determine their enzymatic properties. Here, we will present a summary on our current understanding of the ALDH gene superfamily in A. thaliana.

T04-029

Functional characterization of RCI2A and RCI2B

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During the year, plants living in temperate regions have to survive to a variety of adverse environmental conditions, being high salinity, drought and low temperature among the most significant. Two Rare Cold Inducible genes from Arabidopsis, RCI2A and RCI2B, that encode two small and highly hydrophobic proteins were isolated in our laboratory. Their expression is induced not only by low temperature but also by salt, dehydration and exogenous ABA treatments. Interestingly RCI2A and RCI2B are able to complement the deletion of PMP3, a yeast protein involved in the maintaining of the potential of the plasma membrane. In our laboratory, we are interested on the functional characterization of RCI2A and RCI2B. We have identified rci2a and rci2b T-DNA insertion mutants, and we have generated several lines overexpressing RCI2A and RCI2B. Currently, we are undertaking the molecular and physiological characterization of both, mutants and overexpressing lines. Our preliminary results suggest that both proteins have an important role in Arabidopsis responses to abiotic stresses.

T04-030

Functional Characterisation of Arabidopsis thaliana group I GSK-3/Shaggy-like Kinases

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The ten ASK proteins (Arabidopsis thaliana GSK-3/Shaggy-like Kinases) are homologues of the mammalian GSK-3 (Glycogen Synthase Kinase 3) and the Drosophila melanogaster Shaggy kinases. The GSK-3/shaggy kinase family is implicated in a range of biological processes. In plants, GSK-3/shaggy-like kinases are important factors in plant development as well as hormone and stress signalling (Jonak and Hirt (2002) TIPS, 7:457).
We will present data on ASKalpha, gamma, and epsilon three members of group I ASKs. ASKalpha and ASKgamma have previously been shown to play a role in flower patterning, whereas no function has been assigned to ASKepsilon so far. In order to study the role of group I AKSs in more detail T-DNA insertion lines were obtained. T-DNA insertions were verified by southern analysis, PCR, and sequencing. The absence of transcript is confirmed by RT-PCR. Phenotypical characterisation of these lines is in progress. A. thaliana lines over-expressing Myc epitope-tagged group I ASKs were generated for biochemical and physiological studies. Western blot analysis of T1 plants confirmed the expression of the tagged proteins. To investigate the sub-cellular localisation of group I ASKs YFP-tagged versions of ASKalpha, gamma, and epsilon are transiently and stably transformed into A. thaliana and Nicotiana tabacum.

T04-031

Functional Analysis of Arabidopsis Group III and IV GSK-3/shaggy-like Kinases

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GSK-3/shaggy-like genes code for a group of highly conserved protein kinases that can be found in all eukaryotes and are involved in a variety of biological processes (Jonak and Hirt, 2002, TIPS, 7, 457). Arabidopsis thaliana contains ten GSK-3/shaggy-like kinases (ASKs) that can be divided into four groups according to their sequence similarity. Group I and II ASKs have been shown to function in hormone and stress signalling as well as floral and leaf development. However, to date there are no functional data available on the Arabidopsis members of groups III and IV. Therefore, we have isolated these ASKs and generated a range of tools to elucidate their biological function. Transgenic plants overexpressing epitope-tagged versions of the ASKs have been produced and will be characterised in the near future. Transient expression assays in protoplasts revealed highly different protein levels and kinase activities between closely related ASKs. Furthermore, T-DNA insertion lines have been obtained and the T-DNA insertions have been characterised by PCR and southern blotting. Functional analysis of these lines is in progress. Group III ASKs are predicted to posses a mitochondrial targeting sequence, whereas Group IV members are expected to be cytosolic. Thus, we are presently analysing the localisation by expression of YFP-tagged versions of the ASKs in Arabidopsis protoplasts and Agrobacterium infiltrated tobacco leaves.

T04-032

Genetic analysis of zig suppressor 3 suppressing AtVti11 deficiency

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To elucidate the molecular mechanism of the shoot gravitropism, we have isolated many shoot gravitropism (sgr) mutants in Arabidopsis. zig/sgr4 mutant exhibits abnormal shoot gravitropism and morphological defects in stems and leaves. ZIG encodes AtVTI11, a Qb-SNARE that is likely to function in the trans Golgi Network-Vacuole vesicle transport pathway. Actually, dysfunctional vacuoles were formed in the gravity-sensing endodermal cells, and the fragmented vacuoles or abnormal vesicles were observed in other tissues in zig-1 mutant. Thus, the vesicle transport mediated by ZIG may have pleiotropic functions in planta. To understand ZIG ¯related genetic network involving gravitropism and/or morphology, we performed a screening for the second mutation that can suppress defects of zig-1. Previously, we reported zip1, a dominant mutation in AtVTI12 which encodes a homologue of ZIG/AtVTI11, suppresses defects of zig-1 almost completely. Here we will report another suppressor mutant zip3 which can partially suppress both abnormal gravitropism and morphology of zig-1. The amyloplasts in endodermal cells were sedimented to the direction of gravity in zig-1 zip3-1, suggesting that ZIP3 functions in the vacuole formation or function. The molecular cloning of the ZIP3 gene and its functional analysis are in progress.

T04-033

The location of QTL for nutrient stress and heavy metal tolerance using Stepped Aligned Inbred Recombinant Strains (STAIRS) in Arabidopsis thaliana.

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Nutrients play an important role in plant growth and development. They are involved in many biochemical and biophysical processes but effects of heavy metals on plants result in growth inhibition, structural damage and decline of physiological and biochemical activities as well as function of plants. This study is aimed at investigating the genetic basis of tolerance to low nutrient stress and heavy metals in Arabidopsis thaliana and identifying the QTL's that regulate this tolerance. Heavy metals may enter plants through transporter channels for mineral nutrients. Identification of QTLs underlying the tolerance of low nutrient stress and heavy metals will be useful in selection for multiple traits which include low nutrient tolerance and hyper accumulation of heavy metals.
A novel form of NILS (Stepped Aligned Inbred Recombinant Strains) are being used to differentiate the regions on the chromosome which affect the stress tolerance in Arabidopsis(Koumprolglou et al, 2002).

Koumproglou et al (2002). The Plant Journal 31(3), 355 ¯ 364.

T04-034

Characterisation of Integrators of Light Perception to the Circadian Clock

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In Arabidopsis thaliana, many metabolic and developmental processes are circadian regulated. The outputs of the plant circadian system can be monitored as overt rhythms at genetic, biochemical, and physiological levels. Entrainment of the plant's endogenous clock(s) is facilitated by means of light perception. EARLY FLOWERING 3 (ELF3) and EARLY FLOWERING 4 (ELF4) are believed to function in clock entrainment by integrating light signals. ELF3 and ELF4 are members of different gene families and the biochemical activity of the encoded proteins is unknown. The phenotypes of elf3 and elf4 depend on the light environment and both mutants show photoperiod-insensitive flowering time as well circadian arrhythmicities. We isolated allelic series of both ELF3 and ELF4 to obtain a gradient of mutants, including possible subtle and gain-of-function alleles. These new mutant alleles are the basis for our characterisation of ELF3 and ELF4. Molecular and physiological screens, such as expression profiling of circadian-output genes and hypocotyl-elongation assays, are carried out under specific light regimes. In parallel, phylogenetics of ELF3 and ELF4 is applied to provide a link between amino-acid conservation and functional significance of various protein domains. We study biochemical modes-of-action of ELF3 and ELF4 using yeast two-hybrid screens, which provide candidates of protein-protein interactions. Preys were selected based on a circadian connection and/or flowering time. Protein domains involved in interaction will be studied using site-directed mutagene-sis of elf3 and elf4. Interestingly, we selected EARLY FLOWERING 20 (EF20) as an ELF4-interacting candidate. The ef20 mutant is under short-day growth a long-hypocotyl and early-flowering mutant. This implicates EF20 in ELF4 signalling. Collectively, these genetic studies should place ELF3, ELF4, and their interaction partners in appropriate signalling pathways.

T04-035

Identification of sugar-regulated genes in Arabidopsis thaliana using high-throughput RT-PCR and Affymetrix gene chips.

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We used Affymetrix ATH1 GeneChips and a real-time RT-PCR platform, comprising primer pairs of over 1400 transcription factors (Czechowski et al., 2004), to identify sugar responsive transcripts in Arabidopsis seedlings grown in liquid-culture before and in a time course after addition of sucrose and glucose. A total of 67 transcription factors were identified as sugar-regulated. Five transcription factors that were repressed after two days of sugar starvation were up-regulated after 3 hours of sucrose re-addition and nine were identified like fast-response genes (30 min after sucrose readdition).
To identify regulatory genes involved in both, sucrose- and glucose-specific sensing and signalling pathways in Arabidopsis we also carried out comparisons of global transcript expression patterns obtained after 3h of sucrose and glucose re-addition using MAPMAN software (Thimm et al., 2004). In addition, we have identified genes that specifically respond to low (15 mM D-glucose) and high sugar concentration (100 mM D-glucose) and these are involved in hormone metabolism, signalling and redox response. Transcription factors identified belong to diverse families including the AP2-EREBP, bZIP, C2C2(Zn)-CO-like, HB, MADS box, Myb, C2H2 zinc finger, C3H zinc finger, NAC domain and Triple-Helix families. Other genes that are involved in cysteine and phenylalanine synthesis, phenolpropanoids metabolism and development also showed specific response.
To study response-specificity to sugars, our interest was focused on those genes that are differentially expressed in comparison with other regimes (nitrate, phosphate and sulphate readdition) and several regulatory genes were selected. We are studying if these proteins have a main function like transacting regulators in the coordinated regulation of metabolic pathways induced or repressed by sucrose. For this purpose we have now available inducible and constitutive overexpression constructs/lines as well as T-DNA knockout mutants for some of them. It will allow us to elucidate new sucrose signalling pathways, to identify target genes involved in these processes and to study changes in the expression of these target genes. Also phenotypic analyses will be done.

Czechowski et al. (2004). Plant J. 38, 366-79.
Thimm et al. (2004). Plant J. 37, 914-39.

T04-036

Arabidopsis thaliana dehydrins ERD 14, LTI 29 and COR 47 protect thylakoid membranes during freezing

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In general dehydrins occur in plants as multi-gene families. As the name dehydrins implicates these proteins are typically expressed in response to dehydration. Dehydration can be caused by drought, osmotic stress or freezing temperatures. In the last case water is withdrawn from the cell by ice formation in the apoplast leading to a drastic increase of the solute concentration in the cell. The membranes of the cell are thought to be a primary site of freezing damage in plants. We tested four Arabidopsis dehydrins (ERD 14, RAB 18, LTI 29 and COR 47) for protection of thylakoid membranes during a freeze thaw cycle in vitro. ERD 14, LTI 29 and COR 47 had cryoprotective activity while RAB 18 did not protect the thylakoid membranes. The cryoprotective activity reached a maximum of 50% to 60 % at protein concentrations of 140-250 μg/ml in the assay. The cryoprotctive activity could not be increased further by increasing the protein concentration. This is in contrast to an other cryoprotective protein, Cryoprotectin (Hincha et al., 2001), which can protect the thylakoids up to 100%. To get a hint whether different dehydrins might protect membranes by different molecular mechanisms we tested for additive effects. The activity of dehydrins seems to be additive, but the overall cryoprotective activity could not be increased over the maximum cryoprotective activity seen for the single dehydrins. This suggests that the dehydrins protect by the same mechanism. A contribution of dehydrins to freezing tolerance in vivo is supported by the observation of Nylander et al. (2001) that LTI29 and COR 47 are cold induced on mRNA and protein level. On the other hand the non cryoprotective dehydrin RAB 18 is neither induced at mRNA level nor protein level during cold acclimation.

Hincha et al. (2001): Plant Physiol. 125 (2), 835-846.
Nylander et al. (2001): Plant Mol. Biol. 45 (3), 263-279

T04-037

Characterization of environmentally-controlled protein phosphorylation in photosynthetic membranes of plants by mass spectrometry

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Complete genome sequencing for Arabidopsis thaliana allowed for a full-power application of modern mass spectrometry to study functional proteomics of photosynthesis. It became feasible to analyze in vivo post-translational modifications for numerous proteins from plants in distinct physiological conditions. We adopted and developed dedicated mass spectrometric techniques for characterization of en masse protein phosphorylation in photosynthetic membranes. The approach exploits authentic hydrophilic nature of protein phosphorylation reactions restricted to the surface exposed segments of membrane proteins. The surface exposed protein fragments are cleaved with trypsin, removed from the membranes by centrifugation and directly analyzed by mass spectrometry or liquid chromatography-mass spectrometry. These techniques allow for identification of proteins phosphorylated in vivo and determination of the phosphorylation sites in the membrane proteins without their prior separation and fractionation. Simultaneous analyses of the ratios for phosphopeptides and corresponding nonphosphorylated peptides in the peptide mixtures from the same membrane preparations revealed the stoichiometry of in vivo protein phosphorylation for the major proteins in the photosynthetic membranes of Arabidopsis thaliana. Significant changes in the protein phosphorylation patterns in plants were found upon heat stress rather than during normal light/dark transitions. We also identified a number of previously unknown phosphoproteins in thylakoid membranes. Earlier unknown phosphorylation sites were found in N-termini of membrane proteins TMP14, PsbH and CP29 from Arabidopsis thaliana. In addition to the light-induced phosphorylation of the integral membrane proteins in plant thylakoids we found phosphorylation of two peripheral proteins: PsaD, the first phosphoprotein identified in photosystem I; and TSP9, Thylakoid Soluble Phosphoprotein of 9 kDa. TSP9 is a plant-specific protein that becomes multiply phosphorylated in thylakoids exposed to light and subsequently releases from the photosynthetic membrane. This discovery may imply involvement of TSP9 protein in cell signaling following light perception by plant photosynthetic membrane. The studies of in vivo protein phosphorylation in plants in different environmental conditions will reveal the regulatory and signaling network for adjustment and acclimation of photosynthetic machinery to stress and changes in the surroundings.

Vener et al. 2001 JBC 276, 6959
Carlberg et al. 2003 PNAS 100, 757
Hansson and Vener 2003 Mol Cell Proteomics 2, 550

T04-038

PP2C type phosphatase regulates stress-activated MAP kinase

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Protein phosphorylation is important in eukaryotic signal transduction and is mediated by concerted action of protein kinases and protein phosphatases. So far major interest was expressed for protein kinases and protein phosphatases were considered as comprising little specificity. We characterized PP2C type phosphatases from alfalfa and Arabidopsis and identified their substrate protein kinases. Arabidopsis has an unusually large family of 76 different PP2C genes in comparison to other eukaryotes. However, little is known about their functions and substrates.
We provide evidence that PP2Cs display exquisite substrate specificity for precise down-regulation of stress activated MAPKs. The data on MAPK as a substrate for PP2C and their specificity, focusing primary on the alfalfa member of PP2C family MP2C will be presented. It demonstrates the mechanism for direct MAPK inactivation through dephosphorylation of specific phospho-threonine. Mutant analysis indicated that inactivation of MAPK SIMK depends on the catalytic activity of MP2C. Comparison of MP2C with two other PP2Cs, ABI2 and HAB1, revealed that only MP2C is able to dephosphorylate and inactivate SIMK. In agreement with the data that MP2C directly interacts with SIMK in vivo and in vitro, the MAPK was identified as an interaction partner of MP2C in a yeast two-hybrid screen. Wound-induced PP2C expression correlates with the time window when SIMK is inactivated, supporting the proposed model that MP2C is involved in resetting the SIMK signalling pathway.

Schweighofer et al.2004 TPS 9(5): 236.
Meskiene et al. 2003 JBC 278, 21:18945
Meskiene et al. 1998 PNAS. USA 95: 1938

T04-039

Interaction of phosphate- and sugar-sensing in Arabidopsis thaliana

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Phosphorus is an essential plant nutrient and a key player in growth and metabolism. Although phosphorus is abundant in the rhizosphere, the bioavailability of orthophosphate (Pi) is low. Plants have evolved several strategies to obtain adequate phosphate supply under limiting conditions, including modification of carbohydrate metabolism. P-status modifies metabolic pathways by transcriptional regulation of a range of genes. However, little is known about crosstalk between sugar- and phosphorus response pathways. Here we demonstrate that P-regulated gene expression interacts with sugar-sensing. Arabidopsis thaliana plants were grown in inert media (Rockwool) supplied with a full nutrient solution except for a limiting Pi concentration (50 μM). After three weeks, half of the plants were supplied with high level Pi (4 mM Pi). Leaf sections from plants cultivated on high or low level Pi, respectively, were further incubated with combinations of Pi (5mM), sucrose (100mM) and 2-deoxyglucose (0.9 mM). The expression of selected marker genes was analyzed by RT-PCR in wildtype and glucose-insensitive mutant gin2-1.
The sugar responsive genes encoding -amylase (-AMY) and chalcone synthase (CHS) were induced by both P-deficiency and incubation of leaf segments with sucrose. In addition, several P-starvation inducible genes e.g. encoding an acid phosphatase (ACP5), a ribonuclease (RNS1), and a gene of unknown function (IPS1) were induced also by exogenously applied soluble sugars. Feeding experiments also revealed that both P-starvation induced gene expression and sugar-induced expression were reverted by Pi incubation of the leaf segments. These interactions between sugar- and P-dependent gene expression suggest a close relationship between phosphate- and sugar-sensing in Arabidopsis. Hexokinase mediated signalling was analysed by studying the influence of 2-deoxyglucose (a non-metabolised glucose analogue), and the response in the glucose-insensitive mutant gin2-1. Our data show that sugar dependent regulation of phosphate-starvation marker genes, is not dependent on hexokinase and that hexokinase-independent sugar-sensing is strongly affected by phosphate starvation.

T04-040

Salt stress in Arabidopsis: Characterisation of nhx1 ion transporter mutants

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Salt stress is one of the major agricultural drawbacks in Tunisia and the development of crops tolerant to salt stress is an urgent need. Ion transporters are key osmoregulators maintaining ion homeostasis in the cytoplasm of plant cells. We have developed a genetic and molecular approach using Arabidopsis thaliana as a model plant to understand how ion transporters mediate salt tolerance in wheat.
Sodium ions can be toxic to the plant when they accumulate in the cytoplasm. The Na+/H+ antiporter (NHX1) is one of the transporters which plays a protective role by pumping up sodium from the cytoplasm into the vacuole. A comprehensive search for T-DNA insertion mutants in the SIGNAL database (SALK Institute, USA) revealed two mutant alleles in the NHX1 gene (nhx1-1;5' UTR mutant, nhx1-2; ORF mutant). The molecular analyses of these two alleles revealed the presence of homozygous nhx1-1 T3 plants and heterozygous nhx1-2 T3 plants. Physiological experiments showed that the nhx1-1 mutants are more sensitive to 100 mM NaCl than their respective wild-type. While mutant and wild type seedlings are undistinguishable on MS plates lacking salt, the homozygous nhx1-1 seeds plated on MS plates, supplemented with 100 mM NaCl, fail to germinate. It is also noteworthy that the growth of nhx1-1 seedlings on ˝ MS plates, supplemented with 100 mM NaCl, is significantly reduced compared to wild-type plants.
Furthermore, over-expression of the NHX1 ortholog from wheat (TNHX3) gene in Arabidopsis and genetic complementation with this gene, have been undertaken. Other nhx1 mutant alleles and mutants of the vacuolar pyrophosphatase AVP1 are under current investigation.

T04-041

Expression pattern and physiological functions of the Early light-induced proteins (Elips) in Arabidosis thaliana

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Photosynthetic organisms respond to light stress by induction of Elips (Early light-induced proteins) and the accumulation of Seps (Stress-enhanced proteins) and Ohps (One-helix proteins). The three types of proteins are nuclear-encoded integral thylakoid membrane proteins, belonging to the family of Chlorophyll-binding proteins (Cabs). It is proposed that Elips participate in binding of free chlorophyll molecules released during degradation of pigment-protein complexes and thereby prevent the formation of free radicals and/or acting as sinks for excitation energy. A very limited knowledge exists for Seps and Ohps. We will present recent data on their expression pattern and will discuss their physiological functions.

T04-042

Can differences in carbon distribution within the plant explain responses of root elongation to water deficit : an analysis in Arabidopsis thaliana

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In case of water deficit, it is well known that carbon accumulate within the plant, probably due to the higher sensitivity of expansion to drought as compared to photosynthesis. To which extend this alteration triggers the response of root elongation to drought was the matter of the present study. This work was performed using Arabidopsis thaliana grown in Petri plates modified in order to allow a correct development of the shoot for 2 weeks after sowing. The carbon dependency of root elongation was quantified through positive primary and secondary root elongation rate vs local (apical) hexoses concentration relationships. These relationships accounted for the plant response to varying either incident light or external sucrose concentration. Moreover, these relationships accounted for plant to plant variability within treatments as well as root to root variability within plants. Finally, they were present in several Arabidopsis accessions although these had contrasting root elongation rates. When the plants were challenged by a moderate water deficit (-0.15 MPa), the relationships were unaltered but a higher level of water deficit induced an accumulation of hexoses together with a decrease of root elongation rate. These results strongly suggest that in this case, hexoses accumulate because elongation is slowed down and that root elongation of water stressed plants is sink rather than source limited.

T04-043

Genetic variability of leaf expansion responses to water deficit in Arabidopsis thaliana.

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Reduction in leaf area is an early response to water deficit which allows plants to reduce transpiration rate and save water for later parts of the cycle. It has essentially been studied via the change in expansion rate or cell division rate with plant water status, while the duration of leaf development was considered as unaffected by water deficit. (Granier and Tardieu, 1999, Lecoeur et al., 1995).
The genetic variability of the response of Arabidopsis thaliana leaf growth to water deficit was analysed to better understand the bases of these controls. Nine accessions were grown at contrasting soil water stati which were strictly identical for all accession during the whole plant cycle. In two independent experiments, soil water deficit was imposed after leaf 6 or leaf 10 were initiated on the meristem of the considered accession. Eight accessions reduced their total leaf area by 25 to 75% compared to the well-watered treatment. This was mainly due to a reduction in individual leaf area and to a lesser extent to a reduction in leaf number. Leaf expansion rate was decreased by water deficit but the duration of expansion was increased. A large genetic variability was observed on the responses of these two variables. Furthermore, the ninth accession did not reduce its final leaf area as neither the number of leaves per plant nor the area of individual leaves were affected, even by relatively severe deficits (-1.0 MPa). The expansion rate of each leaf was affected during the water deficit period, as in other accessions, but this reduction was fully compensated by an increase in the duration of expansion.
The results presented here show the interest of analysing the behaviour of genetically unrelated accessions of Arabidopsis thaliana which evolved in contrasting climates. Because duration of expansion had the largest genetic variability between the 9 accessions, compared with other variables, the duration of leaf development and its response to water deficit are probably essential in the response of leaf growth to water deficit. They are unlikely to be controlled by mechanisms in common with the responses of tissue expansion rate or cell cycle to water deficit, because they are related to whole-plant developmental processes. They are therefore a new source of genetic variability of plant response to drought.

Granier C and Tardieu F (1999) Plant Physiol, 119:609-619
Lecoeur J et al., (1995) J Exp Bot,290:1093-1101

T04-044

Hormonal interactions in plant abiotic stress responses

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We have isolated a series of rcd-mutants (radical-induced cell death) that display visible hypersensitive response (HR) -like lesions coupled with increased reactive oxygen species (ROS) accumulation in apoplast when exposed to ozone. However, rcd1 is more tolerant against chloroplastic ROS. The rcd1 mutant is insensitive to abscisic acid, ethylene and partially insensitive to methyl jasmonate and glucose, has constitutively more open stomata than the wild type and the expression and regulation of several ABA-regulated genes (e.g., RAB18) is compromised. In the triple response assay rcd1 behaves like Col-0, but induction of ethylene-dependent genes is compromised similar to ein2. Induction of jasmonate marker genes in rcd1 is significantly lower than in the wild type, and again, equivalent to ein2, but higher than in the jasmonate-insensitive jar1. The mutation in rcd1 disrupts an intron splice site in a gene that encodes a protein belonging to a small protein family. RCD1 protein contains a WWE-domain for protein-protein-interactions, two canonical nuclear localization sequences and poly(ADP-ribose)polymerase (PARP) core domain, which suggests that RCD1 belongs to the (ADP-ribose)transferase-subfamily of WWE-containing proteins. Yeast two-hybrid analysis identified several RCD1-interacting proteins, most of which are transcription factors or nuclear-localized proteins related to salt and osmotic stress (e.g., DREB2A); accordingly rcd1 is sensitive to salt. Cold acclimation capability and freezing tolerance of rcd1 is not, however, compromised. Moreover, ABA-induced cold acclimation is not affected in rcd1. Thus, RCD1 seems to be involved in processes that affect interplay between hormonal signaling cascades and abiotic stress responses.

T04-045

Locating Sodium Chloride Associated QTL within Arabidopsis Using STAIRS

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Salinity is one of the major limiting factors for crop growth throughout the world. Millions of acres of land are deemed as being non-productive each year, due to the accumulation of salts, primarily sodium chloride (NaCl). With the ever-growing population, NaCl accumulation represents a major limiting factor for food production. Many important agronomic traits are quantitative and multifactorial in nature. This project will locate QTL in Arabidopsis thaliana, which are associated with NaCl stress. The project will employ Stepped Aligned Inbred Recombination Strains (STAIRS), a new genetic resource developed for functional genomic studies in Arabidopsis developed at Birmingham. QTL associated to NaCl stress have been located to particular chromosomes. Current work involves mapping these QTL to more defined regions, for the identification of candidate genes.

Koumproglou, R et al., The Plant Journal (2002) 31(3), 355-364.

T04-046

Enhanced Heterosis for Biomass Production at Elevated Light Intensities

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Heterosis has been widely used in agriculture to increase yield and to broaden adaptability of hybrid varieties and is applied to an increasing number of crop species. We performed a systematic survey of the extent and degree of heterosis for dry biomass in 63 Arabidopsis thaliana accessions crossed to three reference lines (Col-0, C24, Nd). We detected a high heritability (69%) for biomass production in A. thaliana. Among the 169 crosses analysed, 29 exhibited significant mid-parent-heterosis (MPH) for shoot biomass. Furthermore, we analysed two divergent accessions, C24 and Col-0, the F1 hybrids of which were shown to exhibit hybrid vigour, in more detail. An important finding was the early onset of heterosis for biomass: in the cross Col-0 / C24, differences between parental and hybrid lines in leaf size and dry shoot mass could be detected as early as 6 days after sowing. In the combination Col-0 / C24, heterosis for biomass was enhanced at higher light intensities: we found 51 to 66% MPH at low and intermediate light intensities (60 and 120 µmol m-2 s-1), and 161% at high light intensity (240 µmol m-2 s-1). While at the low and intermediate light intensities relative growth rates (RGR) of the hybrids were higher only in the early developmental phase (0-15 DAS), at high light intensity the hybrids showed increased RGR over the entire vegetative phase (until 25 DAS). We also detected significant differences between parental and F1 hybrids in gas exchange and chlorophyll fluorescence parameters, stable isotope ratios (delta13C and delta15N), and elemental composition.
By combining morphological, physiological, transcript and metabolic analyses, we hope to elucidate which physiological and molecular mechanisms contribute to the increased biomass accumulation under high light intensity.

T04-047

Analysis of cold-induced gene expression in two Arabidopsis accessions of contrasting freezing tolerance.

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Cold has major influences on crop production, limiting geographical distribution and affecting quality and yield. Considerable effort has therefore been directed towards understanding how plants respond and adapt to low temperature. Arabidopsis, like many plants, increases its freezing tolerance when exposed to low non-freezing temperatures. This process of cold acclimation is a complex trait associated with many physiological and biochemical changes. Well-documented ones include altered lipid metabolism, accumulation of compatible solutes and the synthesis of cold-regulated proteins. Evidence for these changes includes biochemical measurements, gene cloning and characterisation and measurements of gene expression. Recently, expression profiling has revealed hundreds of changes of gene expression in response to cold. However, these studies have focused mainly on single accessions and early signalling events. In addition, it is often difficult to separate changes due to the treatment from those more directly related to the process of cold-acclimation. In order to improve this distinction, and to more thoroughly investigate cold-regulated gene expression, we have performed expression profiling for two accessions with contrasting freezing tolerance. The laboratory strains C24 and Col-0 have been shown to be genetically distinct, and to differ significantly in their acclimated and non-acclimated freezing tolerance (Rohde et al., 2004). Quantitative measurements of leaf electrolyte leakage were taken before, and after, two weeks of cold acclimation (4°C). Rosette samples taken at both times were hybridised to Affymetrix ATH1 arrays.
Ten plants were pooled for each sample and the study was replicated using independent biological experiments. Low-level normalisation and further data analyses were performed using Bioconductor, an open-source software project for analysis of expression data. Using this approach we have identified many hundreds of genes showing significant changes of expression during cold acclimation. The detection of expression changes previously reported verifies our treatment, whilst other unreported changes extend our knowledge of which genes may be cold-regulated. We find considerable overlap between the responses shown by C24 and Col but also discover differences in specificity and magnitude of gene expression changes. These findings are discussed in relation to the quantified differences in freezing tolerance observed between the accessions.

Rohde P, Hincha DK, Heyer AG. 2004. Heterosis in Arabidopsis freezing tolerance. Plant Journal, Online early 13/04/04.

T04-048

Characterization of NAC genes that are modulated by hormones that mediate stress response

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NACs are transcription factors unique to plants that are involved in different aspects of plant development and hormone signal transduction (Souer et al., 1996; Aida et al., 1997; Xie et al., 2000). They are characterized by highly conserved domains in the N-terminal region, whereas their C-terminal sequences are divergent in both length and amino acid composition.
We have isolated some NAC genes that are modulated in response to hormones involved in stress response. Two-hybrid assays using one of these NACs (NAC5) as a bait, identified two putative NAC5 interacting proteins (NAC5-ip): NAC5-ip1 showed no homology with any other known protein, whereas NAC5-ip2 was identified as an ubiquitin conjugase. In particular, the transcripts of the two NAC genes that interact with NAC-ip2 are modulated in response to wounding, methyl jasmonate (MeJa), abscisic acid (ABA) and ethylene whereas NAC-ip2 itself is expressed ubiquitously, downregulated in the dark and increased around two-fold in response to MeJa, ABA, SA and ethylene.
In order to investigate the role of NACs and NAC-ip1 and 2 in the stress processes mediated by these hormones, a reverse genetics approach was used.

Souer et al., 1996. Cell 85: 159¯170
Aida et al., 1997. Plant Cell 9: 841¯857
Xie et al., 2000. Genes & Dev 14:3024-36

T04-049

The tup5 mutant shows blue light-dependent root growth inhibition and decreased far red light inhibition of seed germination

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tup5 (tumor prone 5) is an EMS mutant which was initially found in a screen to identify tumor forming mutants responding to low phytohormone concentrations, which are not effective in wild type. tup5 plants form a very short root if grown in vitro in light. If the mutant is cultivated in the dark, the root can maintain growth. The inhibition of root growth in tup5 was shown to be light dose-dependent. Histological data show that light grown tup5 plants lack a root meristem, whereas the meristem seems to be normally developed in dark grown mutants. The site of light perception causing growth inhibition is likely to be in the root as plants grown on soil do not show the phenotype. Growth experiments with different monochromatic light qualities showed that the inhibition of root growth in tup5 is caused by blue light. Therefore we suggest that a blue light receptor signalling pathway is affected in tup5. Furthermore, the inhibiting effect of far red light on seed germination is reduced in tup5, implying that TUP5 is required to mediate red-light inhibition of seed germination. Together the data suggest altered blue and red light signalling in tup5. As far as we know, no comparable mutant has been described yet. The mutation was mapped on the lower arm of Chromosome I.

T04-050

Phytohormones maintain the circadian clock in Arabidopsis thaliana

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Phytohormones and the circadian clock are two distinct autonomous systems that regulate diverse aspect of plant development. However, the linkage between hormone signalling and the circadian system is unknown. In animal systems, the hormone melatonin is known to function in entrainment of the circadian system, and is itself regulated by the clock. To investigate whether plants have hormones that function in the circadian system, we have begun to assess this pharmacologically. The classical plant hormones were exogenously added in separate experiments to unravel whether any of these compounds influences circadian rhythms, as assayed via the promoter:luciferase reporter system. Interestingly, many hormones control various aspects of the circadian system, including period, phase, and amplitude. In particular, we found that cytokinin alters circadian phase, that auxin regulates circadian amplitude, and that gibberellin, brassinolide and abscisic acid regulate clock periodicity. Pharmacology can provide insight into physiological process, but these experiments require genetic and biochemical reinforcement to gain strong conclusions. To confirm the hormone pharmacology, we analysed circadian rhythms in a variety of hormone synthesis or perception mutants. Our interpretation is that many of these mutants affect circadian systems in predictable ways. In addition, genetically elevating endogenous hormone has the same effect as exogenous application. We are also investigating whether synthesis of plant hormones is under clock control. This is expected as many of the genes that encode hormone-synthesising enzymes are circadian regulated. As hormones regulate the plant clock, if the hormones are under clock control, this would confirm our hypothesis that plants have an input/output feedback connecting hormone signalling to the clock. The integration of hormone signalling and circadian rhythms can be viewed as a system analysis for dissecting complex signalling networks.

T04-051

PHYTOCHROME-INTERACTING FACTOR 1, a Basic Helix-Loop-Helix Transcription Factor, is a Critical Regulator of the Chlorophyll Biosynthetic Pathway

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Photosynthetic organisms must achieve a delicate balance between the light energy absorbed by chlorophyll and their capacity to channel that energy into productive photochemical reactions. Release of excess absorbed energy in the cell can cause lethal photooxidative damage. Here we identify a bHLH transcription factor, designated PIF1 (phytochrome (phy)-interacting factor 1) that negatively regulates chlorophyll biosynthesis. pif1 mutant seedlings accumulate excess free protochlorophyllide when grown in the dark, with consequent lethal bleaching upon exposure to light. PIF1 interacts specifically with the photoactivated conformer of both phytochromes A and B, suggesting a signaling pathway by which chlorophyll biosynthetic rates are tightly controlled during the critical initial emergence of seedlings from subterranean darkness into sunlight.

T04-052

Expression profiling and T-DNA knockout analysis of the Arabidopsis thaliana annexin multigene family

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Annexins are a diverse, multifunctional gene family of calcium-dependent membrane-binding proteins found in most eukaryotic cells. In plant cells, annexins are localized at the cell periphery of highly secretory cell types where they are hypothesized to play a role in Golgi-mediated secretion of new wall materials and plasma membrane. They are also implicated in imparting tolerance to various abiotic stresses. In Arabidopsis, there are eight different annexins, which range from 30% to 82% in deduced amino acid sequence identity. In this study, we have used real time PCR and T-DNA annexin knockouts to clarify the function of individual Arabidopsis annexins in cell signaling, with a focus on their expression profiles in response to exposure to salt, drought, high and low temperature conditions. Preliminary results indicate that the expression of several annexin genes is differentially regulated by these abiotic stresses. We are also assessing the function of each of the eight Arabidopsis annexins by analyzing the phenotype of mutants for each individual gene. To achieve this goal, we have obtained putative T-DNA insertions for all eight Arabidopsis annexins and are determining which of these lead to gene disruption and “knockouts” for these genes. Thus far we have confirmed three T-DNA insertion lines that have knock out alleles for the annexin genes AnnAt1, AnnAt5 and AnnAt7. Preliminary results indicate that these annexin mutant lines have decreased germination rates under high salt conditions. Phenotypic data for annexin mutants available and expression data for all eight annexin genes will be presented. (Supported by NASA: NAGW 1519).

T04-053

Transcriptional regulation of mitochondrial alternative respiratory pathway genes in response to stress

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In addition to the major complexes of the mitochondrial respiratory chain common to most eukaryotes, plants possess additional components including several external and internal NAD(P)H dehydrogenases (NDH), an alternative oxidase (AOX) and uncoupling proteins (UCP). These components form the alternative respiratory chain which has been postulated to play a key role in plant stress responses. In Arabidopsis each of these alternative pathway components are encoded by genes belonging to multi-gene families.

Gene expression analysis was performed to explore the role of the alternative pathway components in response to a range of stresses, and to examine the specific roles of the individual gene family members. Using quantitative real-time PCR, transcript response profiles of three members of the Aox gene family, six members of the alternative NDH gene family, and two members of the UCP gene family were examined. An additional eight genes encoding non-alternative components of the mETC and TCA cycle enzymes were investigated to place the response profiles of the alternative components in a wider mitochondrial context.

The resulting expression data revealed clear differences in the expression profiles within gene families, suggesting different members of the gene family are differentially regulated, with not all members being stress responsive. Interestingly, whilst members of the Aox and NDH gene families display large fold changes in expression under specific treatments, the expression levels of the UCP genes remained largely steady under all conditions examined. Predictably, most of the genes encoding components of the alternative respiratory pathway display similar transcript expression patterns in response to treatments that are predicted to have similar metabolic effects. Additionally we present evidence for coordinated expression of a mitochondrial and a nuclear encoded subunit of ATP synthase.

T04-054

Characterization of Different Sensitive Mutants to UV-B Radiation from Activation Tagging Lines

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Activation tagging is a method for gain-of-function mutagenesis. We have created about 50,000 independent Arabidopsis activation tagging lines. We isolated mutants of different sensitivity to UV-B radiation from this Arabidopsis activation tagging lines.

Fourteen days after germination each line was irradiated with white light (6 W/m2) with UV-B (0.25 W/m2) for 35 hours and searched for lines that showed altered UV damage response compared to wild type. As a result of screening of approximately 4,000 lines, we isolated several sensitive and resistant mutants. One of UV-resistant mutants showed remarkable tolerance to UV-B radiation on the condition that causes complete bleaching of leaves in wild type. Increased accumulation of UV-absorbing compounds and also reduction of DNA damage were observed in this mutant.

T04-055

Arabidopsis MYC(bHLH) and MYB proteins function as transcriptional activators in abscisic acid signaling

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In Arabidopsis, the induction of a dehydration-responsive gene, rd22, is mediated by abscisic acid (ABA). We reported previously that MYC and MYB recognition sites in the rd22 promoter region function as cis-acting elements in the drought- and ABA-induced gene expression of rd22. bHLH- and MYB-related transcription factors, rd22BP1/AtMYC2 and AtMYB2, interact specifically with the MYC and MYB recognition sites, respectively, in vitro and activate the transcription of the b-glucuronidase reporter gene driven by the MYC and MYB recognition sites in Arabidopsis leaf protoplasts. Moreover, we show that transgenic plants overexpressing rd22BP1/AtMYC2 and/or AtMYB2 cDNAs have higher sensitivity to ABA. The ABA-induced gene expression of rd22 and AtADH1 was enhanced in these transgenic plants. Microarray analysis of the transgenic plants overexpressing both rd22BP1/AtMYC2 and AtMYB2 cDNAs revealed that several ABA-inducible genes also are upregulated in the transgenic plants. By contrast, a knockout mutant of the rd22BP1/AtMYC2 gene was less sensitive to ABA and showed significantly Furthermore, knockout mutant of AtMYB2 gene was less sensitivity to ABA. These results indicate that both rd22BP1/AtMYC2 and AtMYB2 proteins function as transcriptional activators in ABA-inducible gene expression under drought stress in plants.

Abe et al. (2003) The Plant Cell. 15(1), 63-78
Abe et al. (1997) The Plant Cell. 9(10), 1859-68

T04-056

Dynamics of root to shoot signaling of ABA revealed by in vivo imaging of water-stressed Arabidopsis

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Plant development and responses to environmental cues are mediated by endogenous signals. Those signals include phytohormones that act specifically in time and space of multicellular organisms to allow intercellular as well as interorgan communication. A major challenge for comprehensive analysis of the process is to address hormone action in vivo in a threedimensional, non-invasive and cell-autonomous manner. We examined a non-invasive system to analyze ABA action at the whole plant level with the aim of single cell resolution. The system recruits ABA-controlled expression of firefly luciferase in Arabidopsis plants to monitor physiologically active ABA. The analyses which employed two ABA-regulated genes in combination with ABA-deficient (aba2) and response (abi1) mutants clearly support ABA-specific in vivo imaging. Parallel analysis of plants in which the luciferase reporter was replaced by ß-glucuronidase yielded comparable expression patterns. Furthermore, ABA levels of plant organs perfectly correlated with luciferase activity. The results strongly indicate the suitability of the reporter system to reveal the presence and abundance of physiologically active pools of ABA.
We used the system to study physiologically active ABA pools in non-stressed seedlings and their generation during water stress. Well-watered seedlings revealed ABA-indicative reporter expression in columella cells of the root cap and in the root cells of the quiescent zone. In cotelydons, activity was apparent at vascular tissues and stomates. In the ABA-deficient mutant aba2 these signals were strongly reduced, however, not abolished. ABA exogenously applied to roots triggered a generalized response of luciferase activation within the organ, while root-applied water stress resulted in a localized ABA response along the vascular veins. In contrast to the limited response in roots, shoot tissues of root-stressed seedlings strongly activated ABA-mediated gene expression. The increased ABA response of the shoot was paralled by approximately tenfold higher ABA levels in shoot versus root tissues. Thus, water stress recognized by the root system results in the generation of a major physiological active ABA pool in the shoot. Within the leaf tissue, cells associated with vascular veins and guard cells were identified as primary sites of ABA action by in planta imaging.

T04-057

The SPA1 family: WD-repeat proteins with a central role in suppression of photomorphogenesis

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The four-member SPA1 gene family encodes WD-repeat proteins that also contain a coiled-coil domain and a kinase-like region. We have shown previously that SPA1, SPA3 and SPA4 function as repressors of photomorphogenesis in light-grown seedlings. Here, we demonstrate the function of SPA2. We show that SPA2 is a potent repressor of photomorphogenesis in the dark, but not in the light. The SPA2 protein is constitutively nuclear-localized in planta and can physically interact with the repressor COP1. Epistasis analysis between spa2 and cop1 mutations provides strong genetic support for a biological significance of a COP1-SPA2 interaction in the plant. Taken together, we have identified a family of proteins that functions in concert with COP1 in suppression of photomorphogenesis.

T04-058

Overexpression of AtMYB90 gene confers the enhancement of salt tolerance

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Sodium toxicity represents the major ionic stress associated with high salinity. Soil salinity significantly limits plant productivity on agricultural lands. The low osmotic potential of saline solutions hampers plant water uptake, resulting in “physiological drought”. It is believed that in the past soil salinity has contributed to the decline of several ancient civilizations.
Plants are constantly bombarded with environmental signals, in response to the stress signals, plants activate a number of defense responses that increase tolerance to the stress conditions. This inducible adaptation or acclimation process has evolved throughout the plant kingdom and is critical for the survival of all plants. Many drought-responsive genes also are responsive to salt or cold (Shinozaki et al., 2000; Xiong et al., 2002). The gene delta1-pyrroline-5-carboxylate synthase (P5CS) is an example of such a common stress-inducible gene (Abraham et al., 2003).
AtMYB90 gene expression are induced by salt stress. Transgenic plants overexpressing AtMYB90 showed enhanced salt tolerance and reduced tolerance to freezing. By contrast, AtMYB90 antisense plants displayed less tolerance to salt. These studies suggest that AtMYB90 functions as a positive regulator of the response to high salinity.

T04-059

Localisation of light stress proteins in photosynthetic complexes of Arabidopsis thaliana

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Eukaryotic photosynthetic organisms and cyanobacteria respond to light stress by transient accumulation of light stress proteins from the Elip (early light induced protein) family. This family consists of three types of proteins: Elips (early light induced proteins), Seps (stress enhanced proteins) and Ohps (one-helix proteins) that are nuclear encoded in plants. Elip family members are integral thylakoid membrane proteins related to the family of chlorophyll a/b ¯ binding proteins from the photosystem I (Lhcpl) and II (Lhcpll). It is assumed that Elips participate in binding of free chlorophyll molecules released during the degradation of pigment ¯ protein complexes under stress conditions. Thereby they are preventing the formation of free radicals and / or are acting as sinks for excess excitation energy. We will present recent data on the detailed localisation of Elip family members in the photosynthetic apparatus of A. thaliana.

T04-060

Functional analysis of two members of the CHX family of putative sodium transporters in Arabidopsis

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The functional roles of two members of the previously uncharacterised Arabidopsis CHX cation transporter family have been investigated in plants growing under 'ideal' conditions and in the presence of elevated NaCl levels. In public data bases, AtCHX21 and AtCHX23 are annotated as putative Na+/H+ antiporters. In this study, genotypes homozygous for knock-outs in both genes were developed. In the absence of salt stress, knock-outs in both AtCHX21 and AtCHX23 showed significant quantitative differences from wild type genotypes in their development with respect to characters such as rosette width and flowering time. In the presence of 50mM NaCl, a) roots of the AtCHX21 mutant, but not the AtCHX23 mutant, elongated more slowly than wild type, b) the leaf sap Na+ concentration was significantly lower in the AtCHX21 mutant (but not the AtCHX23 mutant) than in wild type, and c) for the AtCHX21 mutant (AtCHX23 not tested) the concentration of Na+ in the xylem was lower compared to wild type genotypes. The concentration of Na+ exported from the leaf in the phloem was unchanged. Thus, loading of Na+ into the root xylem could explain changes in leaf concentration of Na+. This hypothesis was supported by immunolocalisation which demonstrated that the AtCHX21 transporter could only be detected in root endodermal cells. The double knock-out condition (at AtCHX21 and AtCHX23) was lethal even in the absence of salt stress, strongly suggesting that the genes play a role other than in Na+ transport. Taken together, the data demonstrate that a) there is overlap in function between these two genes, b) that this function is critical for survival, c) that the function is not provided by other members of this gene family, and hence, d) that there has clearly been functional diversification within this transporter family.

T04-061

LOW-LIGHT- AND ETHYLENE-INDUCED HYPONASTIC GROWTH IN ARABIDOPSIS THALIANA

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Hyponastic growth is upward bending of leaves in response to low-light, a low red/far-red light ratio and ethylene. Using a computer controlled digital camera setup the kinetics of hyponastic growth is measured in great detail. Natural accessions of Arabidopsis thaliana (Be0, Col, Cvi, Kas, Ler, Nd, Rld, Shah and Ws) showed considerable genetic variation in hyponastic growth upon exposure to ethylene (Col strongest, Ler no effect) and low light (both Col and Ler show effect). In order to unravel the signal transduction chain, mutants in the ethylene and light perception were exposed to 5ppm ethylene and/or low-light (LL). Ethylene signal transduction mutants, which not responded to ethylene, did show hyponastic growth after transfer to low-light. Also some light perception mutants that did not respond to LL, showed hyponastic growth upon ethylene exposure. Therefore, we concluded that ethylene- and LL-induced hyponastic growth are regulated by, at least partly, parallel operating pathways. Since differential growth often is associated with auxin, mutants in auxin ubiquitination, transport and transcription were studied during ethylene addition and/or LL. All these mutants are characterized by a wild-type hyponastic growth, only a few auxin transport or transcription mutants did not respond to LL and ethylene. Micro-array analysis did not show an up-regulation of ethylene responsive genes in LL.

T04-062

Functional analysis and subcellular localization of STO in planta

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Calcium is involved in the response of plants and microorganisms to various stimuli, among them salt stress. We developed a yeast complementation system using a knock out mutant of the CCH1 gene of Saccharomyces cerevisiae that encodes a putative calcium channel. This KO strain is growth sensitive to high salt concentrations. Complementation analysis of the cch1 mutant with an A. thaliana cDNA library resulted in the isolation of 16 clones that could rescue the salt sensitive growth phenotype of the mutant. One of this clones contained the cDNA of STO, a gene that previously has been shown to complement other salt defective growth yeast mutants (Lippuner et al., 1996). STO encodes a Constans-like B-box Zn-finger protein. It has been shown that STO expression does not change in response to high salt concentrations, therefore we focused our studies on the pattern expression and the subcellular localization of the protein in planta, and investigate the possible functions of STO using a knock out line of Arabidopsis and transgenic plants overexpressing the protein.

The putative promoter of STO was cloned upstream the GFP and the expression pattern of the gene was analyzed by epifluorescence microscopy in transgenic plants. STO becomes expressed in various tissues including cotyledons, hypocotyls, and roots in dark etiolated seedlings. Similar results were obtained using GUS as reporter gene. To get experimental evidence of the subcellular localization in planta, STO was fused to the GFP protein driven by the constitutive 35S promoter. Transient expression of the GFP::STO fusion protein in onion cells lead to an accumulation of the protein preferentially in the nucleus. Similar results were obtained in roots of transgenic dark etiolated seedlings. Deletion constructs of the STO protein to investigate for the nuclear localization signal will be analyzed.

We verified an Arabidopsis KO line containing the T-DNA in the first intron of STO. First results indicate that the KO mutant is in general affected in root growth. Further investigations on possible phenotypes using the KO and overexpressor lines will be carried out.

Lippuner V, Cyert MS, and Gasser CS. (1996), J Biol Chem., 271,12859-66.

T04-063

Nutritional regulation of root architecture by ANR1, a MADS-box transcription factor

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ANR1, a root-specific NO3--inducible of the MADS-box family of transcription factor has been shown to be involved in lateral root elongation in response to nitrate (1). To further investigate the function of ANR1 in root development we generated transgenic lines that carry a transgene encoding a fusion between the ANR1 protein and the rat glucocorticoid receptor (ANR1-rGR) under the control of the CaMV 35S promoter. This is to enable the expression of ANR1 to be post-translationally activated by dexamethasone (DEX) (2). In these lines, only lateral root growth is stimulated by 1 mM DEX, confirming that ANR1 can act as an effective modulator of lateral root elongation rates. We were able to distinguish two separate populations of lateral roots, those that continued elongating and those that stopped growing when they were 1 to 5 mm long. Preliminary experiments show that DEX induction of ANR1 in the ANR1-rGR lines reduces the number of early growth-arrested laterals and increases the number of non-arrested laterals. This effect is in addition to stimulating the elongation rate of the laterals.
For a better understanding of ANR1 function, we are using the ANR1-rGR transgenic lines to identify the downstream target genes of this transcription factor. With this aim we are conducting microarray experiments using the full genome Affymetrix chip. We will present the latest results.

(1) Zhang H and Forde BG (1998) Science 279: 407-409.
(2) Sablowski RWM and Meyerowitz EM (1998) Cell 92: 93-103.

T04-064

Differential effect of modifications on polyamine metabolism in salt stress responses.

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Plants are exposed to continually and rapidly changing environmental factors being polyamine metabolism responsive to external conditions. Major shifts in polyamine metabolism can occur when plants are starved for nutrients, or exposed to osmotic shock or atmospheric pollutans. Exposure to low temperatures has been reported to induce putrescine accumulation in several species, other plants respond to low temperature acclimation with an uniform and substantial increase in spermidine levels. On the other hand, changes in SAMdC expression levels have been observed as a response to environmental factors. Rice plants showed a differental increase of SAMdC levels during saline, drought stress or external ABA application.
Arabidopsis plants overexpressing the arginine decarboxylase 2 (ADC2) or the S-adenosyl-L-methionine decarboxylase 1 (SAMdC1) genes, accumulate putrescine or spermine respectively. Several transgenic lines have been exposed to salt stress by transferring 4 week old plants to a medium containing 250 mM NaCl. Plant injury is characterised by chlorosis, appearance of necrotic spots in leaves, and general weakness.
Wild type ecotype Col0 and ADC2 overexpressing plants showed more severe stress symptoms than SAMDC1 overexpressing plants. This observation suggests that espermine, but not putrescine, accumulation may confer tolerance to salt stress.
Real-time RT-PCR has been used to determine mRNA levels of several genes involved in abiotic stress responses. Expression levels of genes cor15A, NCED3, rd22BP1, rd26 and rd29a were similar in all Col0, and ADC2 or SAMdC1 overexpressing plants after salt treatment. However, unstressed SAMdC1 overexpressing lines showed significantly higher mRNA levels of those genes. This results suggest that improved tolerance to salt stress of spermine accumulating plants could be due to the induction of a number of stress specific genes even under non stressing conditions.

T04-065

"Genetical genomics" of petiole movement

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Hyponastic growth, the upward movement of petioles and leaf blades, enables plants to respond to changing variables in their environment. Raised temperatures, increased ethylene concentration caused by physical entrapment and decreased light quantity as well as quality are amongst the conditions that can induce this response in Arabidopsis.

For QTL mapping two RIL populations are used, Columbia x Landsberg erecta (Col x Ler) and Landsberg erecta x Cape Verde Islands (Ler x Cvi). In both populations considerable variation was found in starting angle and amplitude of the response, of both leaf blades and petioles. Several (mostly co-locating) QTLs have been identified for the traits involved in this response. Some of the QTLs are already confirmed by NILs. Microscopy showed a distinct structure at the base of the petiole that might be involved in the response.
The expression profile study of the whole Ler x Cvi population using microarrays makes the calculation of QTL(s) for each differentially expressed gene possible. By linking these expression QTLs with the QTLs of the hyponastic response correlations can be found. Constructing the molecular pathway(s) that underlie this response then may come in to reach.
On this poster we show and discuss the different phenotypes used in QTL mapping as a response to low light (neutral shade), increased ethylene concentration and reduced blue light and a strategy to unravel the genetics of a complex trait like hyponastic growth.

T04-066

Analysis of PTEN-like gene homologues from Arabidopsis thaliana.

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In animals, the phosphatidylinositol 3-kinase (PI3K) signalling pathway has emerged as a very important process which controls cell number, cell and organ growth. This pathway is a complex network, activated by insulin or growth factors. It does involve different kinases such as the PI3K which is producing the second messenger phosphatidylinositol (3,4,5) triphosphate (PIP3), and downstream the kinases TOR and S6K which regulate the biogenesis and the efficiency of the translational machinery. The pathway is down regulated by the lipid phosphatase PTEN which catalyses the dephosphorylation of PIP3.
In Arabidopsis thaliana, putative gene homologues for almost all members of the PI3K pathway are found. Recent work [Anthony et al., 2004; Turck et al., 2004] suggest that this signalling pathway is at least partially conserved throughout plant and animal kingdom and is regulated by phytohormones. We are focusing our study on the PTEN-like multigenic family because PTEN is a key negative regulator of the PI3K pathway in animals and his loss of function is responsible for many cancers in human. Among the three AtPTEN like gene members, AtPTEN1 is essential for pollen development [Gupta et al., 2002]. We started a study on the two other AtPTEN genes. Gene expression analysis revealed that contrary to AtPTEN1, AtPTEN2 and AtPTEN3 show a wide expression which is more abundant in the plantlet and in the fast-growing leaves and siliques. We aim to analyze the substrate specificities of the recombinant proteins and use RNAi mutants in order to unravel the respective biological roles of AtPTEN2 and AtPTEN3.

Gupta R et al., 2002,Plant Cell 14:2495-2507
Anthony RG et al.,2004,EMBO J. 23:572-581
Turck F et al.,2004,Plant Phys

T04-067

The UV-B response in Arabidopsis involves the bZIP transcription factor HY5

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The light environment is a key factor that governs a multitude of developmental processes during the entire life cycle of plants. Part of the incident sunlight encompasses a segment of the UV-B region (280-320 nm) that, in contrast to solar UV-C (<280 nm), is not entirely absorbed by the ozone layer in the stratosphere of the earth. This fraction of the solar radiation that inevitably reaches the sessile plants is not merely an environmental stress but can also cause morphogenic effects through molecularly yet unidentified UV-B photoreceptor(s). UV-B mediated physiological changes include hypocotyl growth inhibition and upregulation of phenylpropanoid biosynthesis. Non-damage-mediated UV-B effects pose three major, mostly unanswered questions at present: (i) what are the photoreceptors that mediate UV-B action, (ii) what are the signaling components that transduce the photomorphogenic UV-B signal and (iii) what is the interplay of the UV-B responses with other environmental cues that results in an optimal adaptation of the organism to the complex natural environment.
To address these questions, we have performed global transcriptional profiling using high-density oligonucleotide microarrays comprising almost the full Arabidopsis genome (>24,000 genes) to compare responses of Arabidopsis to different UV-B wavelength ranges. In all cases analysed, UV-B induction was found to be independent of known photoreceptors. However, a subset of genes required the bZIP transcription factor HY5, a main player of the transition from dark- to light-growth. These results will be presented, together with our recent progress in further elucidating the UV-B response.

T04-068

VITAMIN C IS IMORTANT FOR ACCLIMATION TO AND GROWTH IN HIGH LIGHT

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To understand the importance of vitamin C and zeaxanthin as photoprotective mechanisms, several ascorbate-deficient Arabidopsis mutants were subjected to growth in high light (HL) or to a transfer from low light (LL) to HL. The vtc2 mutant has only 10 ¯ 30% ascorbate compared to the wild type, while the vtc2npq1 mutant in addition lacks zeaxanthin, which is necessary for nonphotochemical quenching (NPQ), mechanisms that dissipate excess light energy harmlessly as heat. Lastly, the vtc2npq4 mutant is also impaired in nonphotochemical quenching, but has normal xanthophyll content.
All mutants were obviously impaired when grown in HL compared to the wild type. They had lower electron transport, lower oxygen evolution rates and lower quantum efficiency of PSII compared with the wild type, implying that they experienced chronic photo-oxidative stress. The mutants lacking NPQ in addition to ascorbate were only slightly more affected than vtc2. All three mutants had higher glutathione levels than the wild type in HL, suggesting a possible compensation for the lower ascorbate content.
In addition, when transferring vtc2 and vtc2npq1 plants from LL to HL, the mature rosette leaves bleached partially or fully, respectively. The bleaching in vtc2npq1 and vtc2 was paralleled by an increase in lipid peroxidation and a drastic decrease in Fv/Fm, a fluorescence parameter used to estimate photoinhibition. These results demonstrate the importance of ascorbate for acclimation to HL and growth in HL. The more drastic phenotype of vtc2npq1 plants after transfer to HL also supports the proposed role of zeaxanthin as an antioxidant.

T04-069

Comparative micro-array analysis of zinc deficiency and zinc excess response of Arabidopsis thaliana and the zinc hyper-accumulator Thlaspi caerulescens

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Thlaspi caerulescens is a natural Zn, Cd and Ni hyperaccumulator species belonging to the Brassicaceae family. It is a self-fertilizing species, closely related to Arabidopsis, with about 85-90% DNA identity in coding regions. While the physiology of metal (hyper)accumulation in plants has received increasing attention in previous years, the molecular genetics is still largely unexplored. We address this topic by comparing global and individual gene expression of Arabidopsis and T. caerulescens (Tc) exposed to deficient, sufficient and excess zinc concentrations using micro-array hybridisation. To establish a reference gene expression set, we hybridised whole-genome Agilent oligo DNA micro-arrays with cDNA from Arabidopsis roots exposed to deficient (0), sufficient (2) and excess (25 µM) zinc. As comparison we also hybrised these microarrays with cDNA from Tc roots exposed to deficient, sufficient and excess zinc. Since Tc requires much more zinc the respective concentrations were 0, 100 and 1000 µM zinc. This comparative analysis revealed many genes, including several previously implicated in metal homeostasis, which are significantly higher expressed in Tc than in Arabidopsis. There are also many genes which are constitutively expressed in Tc, while induced or reduced in Arabidopsis upon increased zinc concentration. The biological signficance of these genes, b oth in Arabidopsis and in Thlaspi caerulescens, and the results of hybridising new Agilent arrays containing a large set of probes for non-coding RNAs will be discussed.

T04-070

Genetic analysis of suppressor mutants of shoot gravitropism 2.

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To clarify the molecular mechanism of the gravitropism in higher plants, we have isolated many mutants from Arabidopsis with abnomal shoot gravitropism. The shoot gravitropism 2 (sgr2) recessive mutant exhibits abnormal gravitropic response in hypocotyls and inflorescence stems. We have demonstrated that the endodermal cells containing sedimentable amyloplasts are the site of gravity perception in Arabidopsis shoots. However, amyloplasts did not sediment in sgr2 and their movement was severely restricted. The SGR2 encodes a protein homologous to bovine phosphatidic acid-preferring phospholipase A1, which localizes mainly to vacuolar membrane and is an integral membrane protein. To investigate the molecular function of SGR2 in the gravitropism, we performed a genetic screen for the second mutation suppressing the sgr2-1 phenotype. The kiritsu171 (krt171) mutation suppressed abnormal gravitropic response semidominantly. In addition, amyloplasts sedimented normaly in the sgr2-1 krt171 double mutant and amyloplast movement was comparable to that of wild-type. Our previous study has suggested that biogenesis and/or function of vacuoles in the endodermal cells is closely related to the amyloplast movement. Thus, KRT171 gene may be involved in the function of vacuoles. Based on the map position, we found a mutation in a gene encoding a putative acyltransferase containing an EF-hand motif and a transmembrane domain. Taken together, KRT171 and SGR2 may be involved in the modulation of vacuolar membrane by acylation and deacylation of phospholipids.

T04-071

Hormonal regulation on molecular level in Arabidopsis thaliana seedlings under sulphur starvation

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Arabidopsis thaliana seedlings were grown under sulphur deficiency conditions to different developmental stages. 140 metabolites in the pooled samples were determined using different analytical methods to cover a broad range of metabolites. Number of metabolites were complemented by the measurements of the phytohormones 1-amino-cyclopropanoic acid (ACC), salicylic acid (SA), jasmonic acid (JA), indole-3-acetic acid (IAA), abscisic acid (ABA) and zeatin (Z). Among them, ACC and IAA levels were decreasing, while JA accumulated under sulphur starvation.

From the responses of metabolic profiles a correlation matrix was calculated and metabolites co-regulated to the levels of the measured hormones were selected. The list of all co-regulated metabolites was introduced to the software "PaVESy" to find pathways possibly affected by or as a result of hormone signalling under sulphur starvation conditions. This software allows generating new hypothetical paths as well. These new paths may pioneer for new ways of information processing in plants beside already known information flow.

By comparison of all sulphur-affected pathways to hormone-co-regulated pathways the influence of hormone regulation to sulphur starvation and stand-alone hormonal effects is discussed with respect to current knowledge and particular attention to hormone-hormone-interactions. To examine changes seeming independent from known hormonal regulation (if no other effects) may be a promising way to point out and discuss novel candidates for their signalling potential.

T04-072

Raffinose is dispensable in cold acclimation of Arabidopsis thaliana

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In many species raffinose family oligosaccharides (RFO) are specifically accumulated during cold acclimation. The molecular mechanisms by which raffinose influences freezing tolerance is not clear, but we have shown previously that raffinose can stabilize model membranes in the dry state (1). Here we investigated whether synthesis of raffinose is an essential component for acquiring frost tolerance. The synthesis of galactinol from myo-inositol and UDP-galactose is considered a key regulatory step in RFO synthesis. Therefore, we created transgenic lines of A. thaliana accessions Columbia-0 (Col-0) and Cap Verde Islands (Cvi) overexpressing a galactinol synthase (GS) gene from cucumber under a constitutive promoter. Furthermore, we used a mutant of Col-0 carrying a knock-out of the endogenous raffinose synthase (RS) gene. Raffinose was completely absent in the mutant, while galactinol content was strongly increased, especially in acclimated leaves. GS overexpressing lines contained up to 4 times as much raffinose as the wild type under non-acclimated conditions, and up to 2.4 times more after 14 days of cold acclimation. To assess freeze-induced cell damage of leaf tissue we measured electrolyte leakage from rosette leaves. Neither the basic freezing tolerance of non-acclimated leaves, nor their ability to cold acclimate was influenced in the RS mutant or in the GS overexpressing lines. GS overexpression did also not lead to differences in freezing tolerance either in Col-0 with a good ability to cold acclimate or Cvi with a low acclimation ability. A comparison of the levels of other soluble sugars and proline gave no indication of a compensatory increase in concentrations of alternative compatible solutes in the raffinose synthase mutant, except for an increase in galactinol content. We therefore conclude that raffinose is neither essential for basic freezing tolerance nor for cold acclimation of Arabidopsis.

(1) Hincha DK, Zuther E, Heyer AG (2003) Biochim Biophys Acta 1612:172-177

T04-073

CHARACTERIZATION OF COL3, A POSITIVE REGULATOR OF LIGHT SIGNALING.

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Arabidopsis COP1 acts to repress photomorphogenesis in the absence of light.
It has previously been shown that COP1 directly interacts with the bZIP transcription factors HY5, HYH and LAF1 in the dark, and promotes the proteasome-mediated degradation of these positive regulators of photomorphogenesis,. Here we identify a physical and genetic interaction between COP1 and COL3. COL3 was identified in a yeast two hybrid screen for COP1 interacting proteins and we have mapped the interaction to the WD40 domain in COP1 and a conserved valine-proline pair in the C-terminus of COL3. GFP fused COL3 protein gives a diffuse nuclear fluorescence in onion cells. However, when expressed together with COP1, COL3 co-localizes with COP1 in distinct nuclear speckles suggesting that the two proteins interact also in living plant cells. We identified a T-DNA insertion in the COL3 gene that results in loss of detectable COL3 mRNA. Plants carrying insertions in the COL3 gene display decreased blue light sensitivity and the col3 mutation can suppress cop1 mutations. These findings suggest that COL3 act as a positive regulator of light signaling and provides genetic support for an interaction between COL3 and COP1. col3 plants flower early, opposite to co but similar to hy5 and hyh.

T04-074

Characterization of a novel, RCD1-related gene family

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Stress-induced plant hormones and reactive oxygen species (ROS) interact in a complicated manner during the regulation of programmed cell death (PCD). The rcd1 (radical-induced cell death1) mutant has been shown to be defective in the containment of PCD and in the signalling of ethylene, jasmonic acid and abscisic acid. The rcd1-1 mutation results in mis-spliced transcripts of At1g32230. Here we show that T-DNA interrupting RCD1 yields rcd1 phenotype, which further suggests that rcd1 is a lack-of-function mutant. The RCD1 function is yet unknown, but according to a yeast two-hybrid analysis, it may include interactions with several stress-related transcription factors. RCD1 belongs to a putative novel gene family with 5 unknown genes encoding proteins distinctively similar to RCD1 (SRO1-SRO5; SIMILAR TO RCD-ONE 1-5). A genome duplication event connects RCD1 to SRO1, SRO2 to SRO3 and SRO4 to SRO5, after which all except SRO3 have remained as expressed genes. Both RCD1 and SRO1 have nuclear localization signals and a WWE-protein-protein interaction domain implicated to ubiquitination and ADP-ribose conjugation systems. These conserved domains are lacking from SRO2-SRO5. However, RCD1 and all SROs possess ADP-ribosylation domains, which assigns them to the same superfamily as for instance poly(ADP-ribose)polymerases (PARP)s. The promoter regions of RCD1 and SRO genes contain several transcription factor-binding sites, for instance cis-acting elements involved in abscisic acid, salicylic acid, defence and stress responses as well as light responsive elements. However, the promoter structures within the RCD1-SRO gene family vary indicating that they may response differently to environmental stimuli. The putative function of the RCD1-SRO proteins is discussed in respect to the latest results obtained from a system biology approach chosen for their characterization.

T04-075

Salicylic acid accumulation interferes with excess light acclimation.

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When Arabidopsis plants are cultivated in different light conditions, the level of salicylic acid (SA) is positively correlated to light intensity. Screens for mutants with increased pathogen resistance in low light have identified several mutants with constitutive accumulation of SA. We wondered if accumulation of SA is compatible with acclimation to stressful light conditions. For this purpose we compared excess light tolerance of wt, SA deficient sid2-2 and SA overproducing cpr6-1.
In LL, H2O2 levels paralleled the levels of SA and DAB staining showed that cpr6-1 accumulated H2O2 mainly in the vascular tissue. Surprisingly, catalase activity was lowest in sid2-2 and highest in cpr6-1. Excess light treatment reduced catalase activity and led to strongly increased H2O2 production.
cpr6-1 was more susceptible to photoinhibition by excess light with strongest inhibition being observed in the vascular tissue. Additionally, cpr6-1 was retarded in fresh weight accumulation and secondary growth of the hypocotyl.
We conclude that SA is interfering with the plants' capacity to acclimate to challenging light conditions and that genetic engineering of stress tolerant plants requires a holistic approach considering both biotic and abiotic stress factors.

T04-076

Phytochelatin synthase catalyzes key step in turnover of glutathione conjugates

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Conjugation of xenobiotic compounds and endogenous metabolites to glutathione is an ubiquitous process in eukaryotes. In animals, the first and rate-limiting step of glutathione-S-conjugate metabolism is characterized by the removal of the aminoterminal glutamic acid residue of glutathione. In plants, however, glutathione-S-conjugates are generally metabolized by removal of the carboxylterminal glycine residue of the tripeptide glutathione to give rise to the S-glutamylcysteinyl-derivative. Purification of the glutathione-conjugate catabolizing activity from cell suspension cultures of the plant Silene cucubalus indicated that phytochelatin synthase catalyzes the first step of the pathway. Heterologously expressed phytochelatin synthase from Arabidopsis efficiently converted S-bimane glutathione to S-bimane-glutamylcysteinyl, the formation of which was unequivocally identified by mass spectrometry. Several different glutathione-S-conjugates served as substrates for the enzyme and were processed to the corresponding glutamylcysteinyl-adducts. Affinity-purified phytochelatin synthase preparations required divalent heavy metal ions such as Cd2+, Zn2+ or Cu2+ for detectable turnover of glutathione-S-conjugates. Characterization of the enzymatic properties of phytochelatin synthase argues for both cellular functions of the g-glutamylcysteinyl-dipeptidyltransferase, the formation of heavy-metal binding peptides and the degradation of glutathione-S-conjugates. Mechanistically, the former role is the result of g-glutamylcysteinyl transpeptidation onto glutathione or derivatives thereof, while the catabolic function reflects transpeptidation of S-glutamylcysteinyl-adducts onto the acceptor molecule water. Thus, phytochelatin synthase seems to fulfil a second crucial role in glutathione metabolism.

T04-077

P-regulated transcription factors in Arabidopsis revealed by comprehensive real-time RT-PCR

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Evolution has endowed plants with an array of adaptive responses to phosphorous limitation, which are manifest at different levels: morphological; physiological; and biochemical (Raghothama, 1999). Changes in gene expression underpin many of these responses, which imply the involvement of transcription factors (TFs). We are using a reverse-genetics approach to identify TF genes that orchestrate plant responses to P-stress.

To begin, we developed a real-time RT-PCR resource to profile transcripts of all known transcription factor (TF) genes in Arabidopsis (Czechowski et al., 2004). This resource was then used to identify TF genes that are regulated by changes in phosphate supply. RNA was isolated from ecotype Columbia plants grown in axenic culture under different P-regimes: Full phosphate nutrition (1); 48 hours of P-deprivation (2); 30 minutes of phosphate re-supply to deprived plants (3), and 3 hours of phosphate re-supply (4). Transcript levels for approximately 1400 TF genes were measured from whole plants exposed to the four P-treatments, using real-time RT-PCR. Ubiquitin and ß-tubulin transcript levels were used to normalize the data. Expression of 180 TF genes changed in response to altered P nutrition (>5-fold changes). Biological replicates confirmed changes for about half of these. Approximately one-third of all P-regulated TF genes responded rapidly to phosphate re-addition, by reversal of their P-deprivation response. Comparisons between RT-PCR and Affymetrix chip data obtained from the same RNA indicated greater sensitivity and precision for the former method. Nine P-regulated TF genes have been selected for further investigation. These will be expressed ectopically in Arabidopsis, under the control of an alcohol-inducible promoter (Roslan et al., 2001), before physiological and molecular analysis of the resulting plants.

1, Ann. Rev. Plant Physiol. Plant Mol. Bio. 50, 665-693; 2, Plant J. 28, 225-235;3, Plant J. 38, 366-379.

T04-078

LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy

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The lsd1 mutant of Arabidopsis thaliana fails to limit the boundaries of hypersensitive cell death response (HR) during avirulent pathogen infection and initiates unchecked lesions in long day photoperiod giving rise to the runaway cell death (rcd) phenotype. We link here the initiation and propagation of the rcd phenotype to the activity of photosystem II (PSII), stomata conductance and ultimately to photorespiratory H2O2. The cross of lsd1 with the chlorophyll a/b binding harvesting-organelle specific (designated gene CAO) mutant, which has a reduced PSII antenna, led to a reduced lesion formation in the lsd1/cao double mutant. The mutant had also reduced stomatal conductance and catalase activity in short-day permissive conditions and induced H2O2 accumulation followed by rcd when stomatal gas exchange was further impeded. These traits depended on the defense regulators EDS1 and PAD4. Furthermore, non-photorespiratory conditions retarded propagation of lesions in lsd1. In accordance to these observations, we consider that lsd1 failed to acclimate to light conditions that promote excess excitation energy (EEE) and that LSD1 function was required for optimal catalase activity. Through this regulation LSD1 can control the effectiveness of photorespiration in dissipating EEE and consequently be a key determinant of acclimatory processes. Salicylic acid, which induces stomatal closure, inhibits catalase activity and triggers the rcd phenotype in lsd1, also impaired acclimation of wild type plants to conditions that promote EEE. We propose that the roles of LSD1 in light acclimation and in restricting pathogen-induced cell death are functionally linked.

Rusterucci et al. (2001) Plant Cell, 13:2211-24.
Willekens et al. (1997) EMBO J, 16: 4806-16

T04-079

CHARACTERIZATION OF THE PHOSPHATE SIGNAL TRANSDUCTION PATHWAY IN ARABIDOPSIS THALIANA

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Phosphate availability is one of the major limiting factors for plant development. Plants respond to the level of phosphate available by activating specific mechanisms to improve phosphate uptake and utilization. These include molecular and phenotypic changes : numerous genes are regulated by phosphate (transporters, phosphatases, …) and the root architecture is drastically modified by phosphate starvation (shorter primary root). Nevertheless, most of the elements of the phosphate signal transduction pathway are unknown. We develop in the lab different approaches in order to characterize the response of the plant Arabidopsis thaliana (WS ecotype) to phosphate deficiency and to identify the elements of the regulation involved in the phosphate signal transduction pathway.
The plant response to Pi strarvation was characterized at different levels : phenotypic (root development, anthocyanin accumulation), biochemical (potential Pi absorption, lipid composition) and molecular (gene transcripts). Microarray experiments (Affymetrix microarray) were performed with the whole genome of Arabidopsis thaliana and allowed the identification of elements of the metabolic pathways modified in Pi-starved plants and of the transcriptional regulation of the genes.
Moreover, we used a T-DNA insertion line of Arabidopsis thaliana, a mutant for the high affinity phosphate transporter AtPT2. The insertion prevents the transcription of the gene and contains a GUS reporter gene driven by the native AtPT2 promoter, which permits localization of the gene expression (time and spatial localization). Mutagenesis of this line will permit identification of the regulations involved in the phosphate signal transduction pathway.

T04-080

Poly(ADP-ribose) Polymerases (PARPs) in Arabidopsis

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The ability to maintain genomic integrity is essential to the survival of all organisms. Single-strand breaks in DNA are produced by various endogenous and exogenous factors, including water, reactive metabolites, ionising radiation and UV light. One of the responses to many DNA-damaging stresses is the synthesis of poly(ADP-ribose). This is catalysed by poly(ADP-ribose) polymerases (PARPs), eukaryotic enzymes usually found in the nucleus. Most of our knowledge of PARPs is derived from experiments with human PARP-1 (hPARP-1). hPARP-1 has a low basal catalytic activity, but this is greatly increased (~500-fold) in the presence of single-strand breaks in DNA. hPARP-1 has three functional domains, an N-terminal DNA binding domain, a central automodification domain, and a C-terminal 'PARP homology' region, which contains the catalytic region. Seven PARP genes have been characterised from humans. hPARP-1 and hPARP-2 have been shown to be involved in the base excision repair (BER) DNA repair pathway and hPARP-1 has a demonstrated role in the resistance to genotoxic stresses. I have identified two mutants in AtPARP-1, the Arabidopsis homologue of human PARP-1. One mutant has a nonsense mutation about two-thirds into the protein. It is probable that this is a loss of function mutant as the truncated protein produced would lack the catalytic domain. Recent results show that this mutant is more sensitive than wild-type to ionising radiation. These data thus indicate that AtPARP-1 is required for the repair of damage induced by this genotoxic stress. The second AtPARP-1 mutant has a missense mutation located in the PARP homology domain. In contrast to the nonsense mutant, mutant plants carrying this lesion are more resistant than wild-type both to ionising radiation and to UV-C. It is thus possible that these missense mutant plants may have an increased capacity to repair DNA. Interestingly, both mutant lines exhibit enhanced anthocyanin accumulation. At this time, it is unclear as to why the two mutations, which have contrasting DNA damage phenotypes, have the same effect on anthocyanin accumulation, but the observations clearly indicate a possible link between DNA damage and repair and anthocyanin biosynthesis.

T04-081

AN ARABIDOPSIS THALIANA T-DNA TAGGED MUTANT DEFECTIVE FOR A PEPTIDE TRANSPORTER GENE INDUCED BY WOUNDING AND NaCl

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Wounding not only damages plant tissues, but also provides the signalling for pest and pathogen invasion. Abiotic stresses like salt and osmotic stress is important to understand the plant's osmo-balance and ion homeostasis. We have produced T-DNA tagged lines of Arabidopsis thaliana using a promoter trap vector pMHA2, which contains a promoterless gus (uidA) as a reporter gene fused to a synthetic right border sequence. We identified a transgenic line that exhibited an induced expression of the gus reporter gene in response to mechanical wounding. In this line GUS activity was also induced by salt (NaCl). Expression of GUS activity was induced also by the plant defence signalling compounds jasmonic acid, ACC (ethylene precursor) and ABA as well as amino acids histidine, phenylalanine and leucine. With the help of Southern analysis, we identified and cloned a DNA fragment containing vector sequences coupled with Arabidopsis genomic DNA. The other end of the T-DNA insert with the flanking chromosomal DNA was cloned with PCR. Homology search with the cloned Arabidopsis sequences revealed that the T-DNA had knocked out an Arabidopsis peptide transporter gene. The multiple alignment, phylogeny and prediction of the transmembrane domains by hydropathy profiling strongly suggest its role as a putative peptide transporter. The promoter sequences and transcription factors binding sites of the gene are recognized with the help of AGRIS (http://arabidopsis.med.ohio-state.edu/). The knock out of the peptide transporter gene in the mutant line and wound inducible expression of the same gene in wild type plants were confirmed with RT-PCR. This report is so far the first that describes the involvement of an A. thaliana peptide transporter in wound signalling and plant defence as well as in osmotic stress. Recent completion of the Arabidopsis genome revealed that this organism has ten times more peptide transporters than any other sequenced organism (prokaryote or eukaryote). The abundance of these transporters suggests that they play diverse and important roles in plant growth and development (Stacey et al. 2002. Trends in Plant Science).

T04-082

Roles of the Pseudo Response Regulator genes in the Arabidopsis circadian clock

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Loss of function mutations of each of the five Arabidopsis Pseudo Response Regulator (PRR) genes affect either circadian period or phase. Mutations in TOC1 (Timing of CAB Expression 1) results in a strong period shortening in white light, and arrhythmicity in red light and in the dark. However, toc1 mutants are entrained to temperature cycles, and retain clock function in blue light. In an attempt to define roles of the PRRs in the Arabidopsis clock, we are characterizing the circadian defects of prr mutants under a number of entraining and free-running conditions. For cotyledon movement during temperature entrainment, prr3 and prr5 mutants show no phenotype, although they display a slight short period after entrainment to light-dark or temperature cycles, implicating them in a light input pathway. In contrast, prr7 and prr9 mutants show a lagging phase in cotyledon movement during temperature entrainment, consistent with their phenotypes after entrainment of long period and lagging phase, respectively. This indicates possible roles in both light and temperature input pathways for PRR7 and PRR9, or directly in oscillator function. These analyses are being extended to examine effects of the prr mutations on the clock itself through analysis of luciferase fusions driven by promoters of the clock component genes CCA1, LHY and TOC1/PRR1. Double mutants are also being generated to test the degree of redundancy among PRRs.

This work is supported by the NSF.

T04-083

Identification of a new root-specific ethylene-insensitive mutant potentially involved in auxin biosynthesis

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Using the ethylene induced triple response phenotype of Arabidopsis seedlings, a mutant, <I>wei8</I> was identified which shows insensitivity to ethylene in the root, but has a wild-type phenotype in the apical hook and hypocotyl. Additionally, this phenotype can be complemented with exogenous auxin, and is partially complemented by the auxin precursor, tryptophan. <I>WEI8</I> was cloned and shows a strong similarity at the amino acid level to C-S lyase and aminotransferase. Although many pathways involve C-S lyase/aminotransferase activity, the phenotypic and molecular analysis of <I>wei8</I> suggests a role for this C-S lyase in auxin metabolism. WEI8 may be involved in crosstalk between ethylene and auxin. Currently, the genes involved in auxin biosynthesis in plants are highly unresolved, and regulation by ethylene and other hormones remain largely unknown. Further functional characterization of <I>wei8</I> will reveal the extent of its role in auxin biosynthesis and may suggest new regulatory mechanisms for this hormone biosynthesis pathway.

T04-084

SGR6, a novel protein, is involved in a signaling process of the shoot gravitropism

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The gravitropic response is composed of four sequential steps: gravity perception, signal transduction in the gravi-perceptive cells, intercellular signal transmission, and differential growth of the upper and lower tissues of the responding organ. We have previously isolated many shoot gravitropism (sgr) mutants with abnormal gavitropism in shoots and shown by analyzing some of them that amyloplasts sedimentation in the endodermal cells of inflorescence stems is important for gravity perception.
The sgr6 mutant is one of the recessive mutants which performed the normal phototoropism. Interestingly, the sgr6-1 has sedimented amyloplasts in the endodermal cells as well as those of wild-type. The SGR6 encodes a novel protein with 1703 amino acid-length. The SGR6 protein has no well-conserved domain, except for two HEAT repeats which is thought to be related to protein-protein interaction. The SGR6 promoter analysis revealed that the SGR6 was expressed in several tissues of inflorescence stems including the endodermis. Endodermis-specific expression of the SGR6 could by SCR promoter complement the abnormal shoot gravitropism of sgr6-1. These results suggest that SGR6 is involved in the signaling process after the amyloplasts sedimentation in the endodermal cells. We expect that further investigation of SGR6 would provide us a clue to understand the molecular mechanism of the signaling process of the gravi-stimulation.

Fukaki et al., 1998; Kato et al., 2002; Morita et al., 2002; Yano et al., 2003

T04-085

Functional Genomics of Abscisic Acid-Insensitive-1-, -3- and -5-Like Gene Families

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Abscisic acid (ABA) is a plant hormone that mediates myriad physiological responses to the environment such as drought, chilling, salinity, etc., and plays fundamental roles in seed and vegetative development. However, knowledge of the molecular mechanisms that mediate ABA signaling and its complex interactions with other enviromental and hormonal signaling pathways is fragmentary. The Abscisic Acid-Insensitive genes (ABI) were identified genetically based on altered seed germination. ABI1 and 2 are negative regulators of ABA signaling and encode protein phosphatases type 2C, of which there are 20 closely related homologues (of > 70 total) in Arabidopsis. ABI3 is the orthologue of maize VIVIPAROUS1 (VP1), a B3 domain transcription factor (TF) that comprises about 30 homologues in Arabidopsis, including FUSCA3 and LEAFY COTYLEDON-2 genes and some Auxin Response TFs. ABI5 is a basic leucine zipper (bZIP) TF that binds with ABI3 and is the cognate of a family of 9 closely related bZIPs (out of > 80).

We hypothesize that the ABI1-, -3-, and 5-Like genes carry out redundant, tissue-specific, and/or developmentally controlled functions in stress and hormonal responses. With a view to rapidly test this hypothesis and facilitate generation of "functional gene space" for uncharacterized Arabidopsis genes, we have engineered cre-lox "acceptor" vectors for transient expression in protoplasts of pUNI Arabidopsis cDNAs (>12,000 available from the SSP/RAFL Consortium). Preliminary results demonstrate the B3 domain RAV2 protein is an ABA agonist in maize protoplasts, as are ABI1-Like P2C15 and most ABI5-Like bZIPs, several of the latter which functionally interact with VP1. Many of the tested ABI1-Like PP2Cs have antagonist effects on ABA-inducible Em::GUS reporter gene expression in maize protoplasts. Further, a TILLING-induced mutant allele of ABI5-Like ABF3 has a root-specific ABA-insensitive phenotype. Our results demonstrate the functional conservation of ABA signaling between monocots and dicots and the utility of transient gene expression assays for functional genomics.

Physiological and molecular characterization of T-DNA knockout mutants of these and other ABA-related genes, combined with transient/stable overexpression and genetic studies in Arabidopsis and in situ hybridization will provide critical information on the function of ABI1-, -3- and -5-Like gene families in ABA signaling, hormonal crosstalk, stress responses, and growth and development.

T04-086

Analyses of knockout mutants for the cell wall associated receptor like kinase genes reveal their important roles in Arabidopsis heavy metal responses.

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A family of genes with sequence similarity to the cell wall-associated kinase (WAK) genes in Arabidopsis has been identified (Verica and He, Plant Physiol 129:455-459, 2002; Verica et al., Plant Physiol 133:1732-1746, 2003). The WAK and the WAK-like kinase (WAKL) genes encode receptor protein kinases that may serve as signaling molecules physically linking the cell wall to the cytoplasm of a plant cell. WAK/WAKL proteins thus may play important roles in cell-cell and cell-environment communications. Our previous molecular and genetic studies have shown that WAK/WAKL genes are required for various cellular processes including pathogenesis, cell elongation and heavy metal responses (Lally et al., Plant Cell 13:1317-1331, 2001; Sivaguru et al., Plant Physiol 132:2256-2266, 2003). In this study, the functional roles for two of the newly discovered WAKL members, WAKL4 and WAKL14, were examined. WAKL14 gene was found to encode a protein that has auto-phosphorylation activities indicating WAKL14, as predicted, encodes an active kinase. The WAKL14 antibody localized WAKL14 protein to the cell surface suggesting, like other WAK proteins, WAKL14 is also a plasma membrane localized receptor kinase. WAKL14 promoter-GUS analyses revealed that WAKL14 was highly expressed in roots and its expression are responding to various abiotic stresses. Three alleles of T-DNA knockout mutant for WAKL14 gene have been isolated. Southern and northern analyses confirmed that the isolated wakl14 mutants are null mutants. The wakl14 growth analyses on media containing various heavy metals have revealed that wakl14 mutant is hypersensitive to heavy metals including zinc and nickel. The hypersensitive response phenotypes are complemented when a wild type copy of WAKL14 gene is placed back into the knockout mutant. Over-expression of WAKL14 genes renders significant more tolerances for zinc and nickel when compared to WT. Knockout mutants for another member, WAKL4, have also been obtained and characterized. Based on root length and growth assays, WAKL4 knockout mutants are hypersensitive to a number of minerals including potassium, sodium, lithium, and zinc. Interestingly WAKL4 knockout mutants are more tolerant to copper and nickel. Our biochemical, molecular and genetic analyses for the two WAKL members strongly suggest that members of the WAK/WAKL gene family may play important roles in plant heavy metal responses.

T04-087

A rice calcium binding protein OsCBL1 activates two reversely-regulated protein kinases and affects stress-responsive gene expression in transgenic Arabidopsis

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Calcium is a critical signaling component in plant responses to environmental stresses. The plant calcineurin B-like (CBL) protein family is a unique group of calcium binding protein which specifically interacts with the SNF1-related protein kinase (SnRK3). We previously isolated two rice SnRK3 kinase whose gene expression was reversely regulated by cold and salt signals. Here, we report a rice CBL protein OsCBL1 which interacts with and activates those kinases. OsCBL1 contains EF-hand motifs with Ca2+ binding activity, as confirmed by in vitro 45Ca2+ overlay assay. Expression of OsCBL1 gene was not inducible under various stress conditions. Results of in vivo targeting experiment using a green fluorescent protein (GFP) showed that the GFP-OsCBL1 fusion protein was localized primarily to the endomembrane in onion epidermal cells. In vitro kinase assay using GST-fused recombinant protein showed interesting results that OsCBL1 could activate the two kinases and, at the same time, strongly phosphorylated by those kinases. When OsCBL1 was overexpressed in transgenic Arabidopsis, it affects expression of stress-responsive genes in response to cold and salt stress. Together, our data implicates complex interplay between OsCBL1 and its interacting protein kinases and suggests that OsCBL1 may constitute a calcium signaling node involved in diverse stress responses of rice. This work is supported by NIAB and ARPC grant to Dr. In-Sun Yoon.

Kyung-Nam Kim, Jung-Sook Lee, Hee Han, Seung Ah Choi, Seung Joo Go and In-Sun Yoon. 2003. Plant Molecular Biology 52:1191-1202

T04-088

Arabidopsis pdr2 Reveals a Phosphate-sensitive Checkpoint in Root Development

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Environmental phosphate (Pi) availability directly impacts plant performance via photosynthesis and intermediary metabolism. To cope with Pi limitation, a common situation in many ecosystems, plants have evolved complex strategies to maintain Pi homeostasis and to maximize Pi acquisition, which include reprogramming of metabolism and remodeling of root system architecture via changes in meristem initiation and activity. However, the sensory mechanisms that monitor external Pi status and interpret the nutritional signal remain to be elucidated. Here, we present evidence that the Pi deficiency response mutation, pdr2, disrupts local Pi sensing. The sensitivity and amplitude of metabolic Pi starvation responses, such as Pi-responsive gene expression or accumulation of anthocyanins and starch, are enhanced in pdr2 seedlings. The most conspicuous alteration of pdr2 is a conditional short root phenotype that is specific for Pi-deficiency and caused by selective inhibition of root cell division, followed by cell death, below a threshold concentration of about 0.1 mM external Pi. Measurements of general Pi uptake and of total phosphorus in root tips exclude a defect in high-affinity Pi acquisition. Rescue of root meristem activity in Pi-starved pdr2 by phosphite, a non-metabolizable Pi analog, and divided-root experiments suggest that pdr2 disrupts local Pi sensing. None of the major plant hormones rescues or mimics the pdr2 phenotype. We therefore propose that PDR2 functions at a Pi-sensitive checkpoint in late root development, which monitors environmental Pi status, maintains and fine-tunes meristematic activity, and finally adjusts root system architecture to maximize Pi acquisition.
Acknowledgement: We thank B. Lahner and D. Salt (Purdue University) for ICP-MS analysis of total phosphorus. This work is supported by the US Department of Energy.

Ticconi et al. (2004) Plant J. 37:801-814.

T04-089

Molecular genetic characterization of SGR5 encoding a zinc-finger protein required for gravitropism of Arabidopsis.

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Plants can sense the direction of gravity and change the growth orientation of their organs. To elucidate molecular mechanisms of the gravity perception and the signal transduction of gravitropism, we have characterized a number of shoot gravitropism (sgr) mutants of Arabidopsis. They exhibit little or reduced gravitropic response in their inflorescence stems. We have shown that the endodermal cells containing sedimentable amyloplasts are the gravity-sensing cells of inflorescence stems. In some of sgr mutants (sgr2, sgr3, zig/sgr4, sgr8), many amyloplasts did not sediment in the endodermal cells. Most of the responsible genes are likely to be involved in function or biogenesis of vacuoles.
sgr5-1 mutant shows reduced gravitropism in the inflorescence stem but its root and hypocotyl have normal gravitropism. In contrast to sgr2 etc, amyloplasts in the endodermal cells sedimented the direction of gravity and their movement appeared to be normal in sgr5-1. The SGR5 encodes a zinc-finger protein with a coiled-coil motif. The SGR5-GFP fusion protein is localized in the nucleus in the BY-2 protoplasts, suggesting that SGR5 may act as a transcription factor. Analysis of GUS expression driven by SGR5 promoter revealed that SGR5 is expressed in several tissues of the inflorecence stem. However, endodermis-specific expression of the genes by using SCR promoter could complement the abnormal shoot gravitropism in sgr5-1 mutant. Taken together, SGR5 may be involved in the gravity perception and/or signaling process subsequent to amyloplast sedimentation as a putative transcription factor in the gravity-perceptive cells.

Yamauchi, Y., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997) Plant Cell Physiol. 38, 530-535.

T04-090

Identification and Molecular Characterization of the Arabidopsis Mutants Showing Low Acid Phosphatase Activities under Phosphate-Deficient and Phosphate-Sufficient Conditions

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Phosphorus (P) is an essential macronutrient required for various basic biological functions. Activation-tagged (Weigel) lines of Arabidopsis were exploited in a large-scale gain-of-function (or loss-of function) screen to study phosphate (Pi) deficiency responses in higher plants. A total of 11,199 lines (114 pools) were screened on MS media containing reduced phosphate concentration (10 μM), which were supplemented with 0.008% 5-bromo-4-chloro-3-indolyl phosphate (BCIP) for colorimetric assay of acid phosphatase (APase) activity. Three mutant lines, showing lower APase activity compared with wild type plants under both Pi-deficient and Pi-sufficient conditions, were obtained. Abnormally-low APase activities of these mutants (lap) were confirmed by quantitative enzyme assays in vitro using p-nitrophenyl phosphate as the substrate. In lap2, activation-tagging vector was found to be inserted into approximately 400-bp upstream region from the start codon of At3g19600 locus and approximately 2.5-kb downstream region from the start codon of At3g19610 locus based on sequencing of the T-DNA flanking regions. Knockout lines for At3g19600 locus showed reduced growth and lower APase activity compared with wild type plants, suggesting that At3g19600 expression was suppressed in lap2. The function of At3g19600 gene product is under investigation.

T04-091

Comparative analysis of ten new alleles of the circadian clock gene ZEITLUPE

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In order to identify new components of the Arabidopsis circadian clock we initiated a large-scale genetic screen to isolate mutants with aberrant circadian phenotypes. Besides previously uncharacterised clock components, the screen resulted in ten new alleles of the already described clock gene ZTL (ZEITLUPE). The ZTL protein contains three predicted functional/structural domains: LOV/PAS, F-box and kelch-repeat. It has been shown that ZTL interacts with TOC1 through the LOV/PAS domain and directs it for ubiquitin-mediated degradation. The F-box and kelch motifs of the protein may play a role in establishing and stabilizing the interaction of the TOC1-ZTL complex with the corresponding E3 ubiquitin ligase SCF complexes. TOC1 is an essential clock component functioning in the central oscillatory negative feedback loop together with the transcription factors CCA1/LHY, thus, providing a link of ZTL function to the clock-mechanism.
Among the ten newly isolated alleles of ZTL, mutations in each domain were identified, which provided an opportunity to obtain more detailed information on the function of the molecule. Western-analysis showed that all the mutants express the ZTL protein. All the alleles showed long period phenotype for different circadian-regulated processes (expression of CAB2, CCA1, CCR2 genes, leaf movement) under all free-running conditions tested, indicating that all the three domains are important for proper ZTL function. The long period phenotype was most strongly expressed at low fluences of red light (similarly to the previously described alleles), except for the LOV/PAS mutant, which displayed a fluence rate independent phenotype. This suggests that proper light-regulation of the remaining ZTL activity is retained in this mutant. Consistent with this, the LOV/PAS mutant showed no alterations in the light-inhibited hypocotyl elongation, while all the other alleles were hypersensitive specifically to red light. Yeast two-hybrid experiments with known interactors (TOC1, ASK1, PHYB) suggest that light regulation could operate by affecting the effectivity of ZTL to forward target proteins for ubiquitin-mediated degradation. Mutations in ZTL affect period length in warm/cold entrained etiolated seedlings and also alter temperature compensation; indicating an integrated role for the protein in the plant circadian system.

T04-092

May Na+ and Cl- accumulation in rosette leaves be compatible with normal growth of Arabidopsis thaliana (accession COL) ?

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The sensitivity to NaCl in relation to leaf ionic content has been explored in nine accessions of A. thaliana. The effect of NaCl treatment on dry weight rosette leaves after two weeks was used to establish a scale of salt sensitivity, on which COL is the most sensitive (Labidi et al., 2002). In this accession, growth inhibition by salt was associated to an important accumulation of Na+ and Cl- in rosette leaves, and to a depletion of these organs in K+ and Ca2+. It is interesting to know whether the salt inhibiting effect is due to NaCl of medium, or to Na+ and Cl- accumulated in the tissues. To assess the degree of responsibility of each of the two phenomena, respectively Na+ and Cl- accumulation and nutrient deficiency, two experiences authorizing in the same time accumulation of salt in leaves and adequate supply with essential nutrients of these organs, were achieved.
In the first experience, plants were submitted during 15 days to alternate treatment without and with NaCl, 50 mM. In the second one, plants were grown on hydroponics medium with split-root systems: one part of the plant's roots was in control medium (without NaCl), and the other in the same medium, but added with NaCl, 50 mM.
In spite of very large accumulation of Na+ and Cl-, in both experiences, growth rate and nutrients (K+, Ca2+) concentrations in treated plants rosette (alternate and split-root) were similar to the control plants. These results prove that plant sensitivity is due to the inhibition by salt of K+ and Ca2+ uptake, rather than to NaCl accumulation in leaves.

Labidi N. et al., 2002. Variability of the response to NaCl of eight ecotypes of A. thaliana. J. P. Nutr. 25, 2627-2638.

T04-093

The model system Arabidopsis halleri: towards an understanding of plant metal homeostasis and metal accumulation

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A regulated network of metal transport, chelation, trafficking and sequestration functions to provide the uptake, distribution and detoxification of metal ions1. Our work is aiming at elucidating the mechanisms of metal homeostasis and metal hyperaccumulation.

We are studying Arabidopsis thaliana and its relative, the Zn- and Cd-hyper-accumulating metallophyte Arabidopsis halleri, as plant model systems. Fission yeast serves as our cellular model. Hyperaccumulation is a constitutive property of the metallophyte Arabidopsis halleri. In the past two years we have taken advantage of Arabidopsis GeneChips for comparative transcriptome studies with A. thaliana and A. halleri roots. One finding that emerged from these experiments is, that several genes involved in metal homeostasis were found to be dramatically more active in A. halleri. Many of these are known in non-hyperaccumulating plants to be activated by micronutrient deficiency and down-regulated under replete conditions, leading to the hypothesis that a de-regulation of deficiency responses might be underlying the metal hyperaccumulation phenomenon. The identified genes encode, for instance, metal transporters (ZIP transporters) and enzymes involved in chelator synthesis (nicotianamine synthase)2. These data and the functional characterization of potential metal hyperaccumulation factors using a variety of molecular, biochemical and analytical techniques will be presented. Our main objectives now are to understand the role of nicotianamine for metal hyperaccumulation and to elucidate the molecular basis behind the constitutively high expression of metal homeostasis genes in A. halleri. This might in the future lead to an understanding of hyperaccumulation and to insights into the mechanisms involved in plant responses to micronutrient deficiency.

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2. Weber M, et al. (2004) Plant Journal 37, 269-281.

T04-094

salt-induced expression of peroxisoem-associated genes requires components of the ethylene, jasmonate and ABA signalling pathways

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Peroxisomes are organelles that participate in many biological functions including detoxification of reactive oxygen species and biosynthesis of some auxin and oxylipin signalling molecules. Peroxisome protein and enzyme activities have been reported to change in response to salt stress, and over expression of a peroxisomal-targeted betaine aldehyde dehydrogenase increased salt resistance in rice. To investigate the relationship between peroxisomes and salt stress we analysed the level of expression of 3 peroxisome associated genes. Thiolase (PED1) catalyses the final step of -oxidation in peroxisomes, PEX10 is a peroxisome protein essential for embryo viability and PEX1 is an AAA ATPase that is homologous to mammalian and yeast proteins required for peroxisome assembly. The steady state transcript level of all 3 genes was increased by salt and ABA treatment, and was blocked in the abi1-1 mutant. Salt dependent increase of transcript for all 3 genes also required the JAR1 gene as it was prevented in the jar1-1 mutant. Expression of thiolase, and to a lesser extent PEX1, was increased in the jar1-1 mutant in the absence of salt, suggesting JA may negatively regulate these genes in the absence of salt stress. PEX1 was up-regulated in the ethylene over producer eto1-1 and salt had an additive effect on PEX1 transcript level, but salt induction may be inhibited in the ethylene insensitive line etr1-1. PEX1 was up-regulated in transgenic plants over-expressing the ethylene response element binding factors AtERF1 and AtERF5.

T04-095

Molecular Analysis of Phytochelatin Synthesis: AtPCS2 from Arabidopsis thaliana and the metallophyte Arabidopsis halleri

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We are using Arabidopsis thaliana and its relative, the Zn- and Cd-hyper-accumulating metallophyte Arabidopsis halleri, as plant model systems to understand metal tolerance and accumulation mechanism. Fission yeast serves as our cellular model.
The synthesis of phytochelatins (PCs), small metal-binding peptides derived from glutathione, represents one of the main metal detoxification mechanisms in plants, fungi, marine diatoms and also certain animals. PC formation, catalyzed by the enzyme PC synthase (PCS), is induced by exposure to a variety of different metal ions. Some metal ions, most notably Cd2+, are bound by PCs. The resulting low-molecular-weight PC-metal complexes are then transported into the vacuole. PC deficiency due to mutations in AtPCS1 causes a loss of cadmium and arsenic tolerance.
Not understood at all is the function of a second PCS gene in Arabidopsis. The initial characterization of AtPCS2 had shown that it is capable of rescuing the Cd2+-sensitivity of PC-deficient S. pombe pcs- cells. However, the efficiency was far lower than that of AtPCS1 expressed in mutant cells. We initiated a more thorough investigation of possible differences between AtPCS1 and AtPCS2 to help understand the function of AtPCS2. To date the existence of more than one functional phytochelatin synthase has not been reported from any species other than A. thaliana even though divergence of the intron sequences of AtPCS1 and 2 and positions in the A. thaliana genome clearly suggest that these two genes are not the result of a recent duplication event. In order to obtain an additional pair of phytochelatin synthases for functional studies, we searched for PCS genes in the Zn/ Cd hyperaccumulator A. halleri and cloned its two PCS genes. We will report experiments designed to elucidate the function of AtPCS2 and comparative biochemical studies on the activity and post-translational regulation of the PCS synthases from A. thaliana and its Cd-hypertolerant relative A. halleri.

T04-096

Genetic complementation of phytochrome chromophore-deficient hy2 mutant by expression of phycocyanobilin:ferredoxin oxdoreductase in Arabidopsis

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The recognision of light is essential for photomorphogenesis in vascular plants. The plant phytochromes all possess the chromophores, phytochromobilin, that is thioether-linked to a highly conserved chromodomain of phytochromes. The diverse photoregulatory activities of phytochromes is incomplete without the association of phytochromobilin in plants, while algal phytochromes utilize phycocyanobilin (PCB) as chromophore. Previously, we reported the generation of PCYA1 in which the phytochrome chromophore was modified from phytochromobilin to PCB by expression of pcyA in the chromophore-deficient hy2 mutant. In this study, we analyzed the action spectra of phytochrome A in PCYA1 by using Okazaki large spectrograph. In physiological analysis, PCYA1 showed blue-shifted phytochrome A-mediated far-red high irradiance response. In contrast, PCYA1 showed fully rescued phytochrome-mediated red high irradiance responses, low fluence R/FR photoreversible responses and very low fluence responses. These results indicate that PCB can complement function as chromophore for phytochrome-mediated responses.

T04-097

Using Arabidopsis thaliana to progress in modelling plant transpiration under fluctuating environments.

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Preventing excessive transpirational loss of water while maintaining photosynthesis is the main challenge for most plants facing water deficits. This leads to conflicting needs on plant leaf area and stomatal aperture that should be minimal for low transpiration, but maximal for high photosynthesis. A finely tuned combination of regulatory and adaptive processes enables plants to permanently cope with this conflict by modulating stomatal aperture and leaf growth as environmental conditions fluctuate. Additional traits conspire at different time-scales to determine genetic variation in whole plant transpiration (stomatal density, leaf cuticule, shoot architecture that influences coupling with atmospheric evaporative demand).
Our laboratory is committed in several approaches that aim to identify which traits account for the major part of the genetic variation in whole plant transpiration, and how they vary with the environment. Our approaches combine the phenotypic analysis of various accessions and mutants of Arabidopsis with biophysically based equations for water vapour, heat and radiation transfer. Works in progress focus on 3 main axes.
(i) Quantifying to what extent plant transpiration is influenced by the activity of K+ channels in the membrane of guard cells (K+ transfer in these cells induces changes in turgor that controls stomatal aperture). This first axis relies on reverse genetics approaches as illustrated by gork-1, a mutant disrupted in a gene encoding the major outwardly rectifying K+ channel of the guard cell membrane [1]. gork-1 exhibits higher transpiration rate than wild-type in both light and dark conditions and also a delayed stomatal closure in response to a light-dark transition.
(ii) Elucidating what causes plant transpiration to vary in response to air humidity, with contrasting sensitivities among and within species. We have identified the plant hormone abscisic acid as a putative mediator of stomatal response to high evaporative demand when conductance for water in the soil is low [2]. In this second axis, genetic modifications of Arabidopsis allows processes to be uncoupled for dissecting their respective impacts.
(iii) Last axis draws the bases of a QTL approach by prospecting for ecotypes with graduated responses of transpiration to water deficit.
All the results are integrated into whole plant models of plant transpiration to be tested in cultivated plants [3].

[1]Hosy et al (2003) PNAS 100 :5549-54
[2]Borel et al (2001) PCE 24:477-89
[3]Gutschick & Simonneau (2002) PCE 25:142-

T04-098

Genetic interaction of growth in leaves regulated by light environment -Light signals oppositely control growth between leaf blade and petiole-

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Leaf photomorphogenesis is a complex developmental event, and growth of leaves is regulated so that expansion of leaf-blade is inhibited, but petiole elongation is enhanced in a shade condition. In this study, we analyzed the differential photomorphogenesis between leaf blade and petiole separately. In our assay condition, cell expansion plays a major role to control the leaf growth. Enhanced cell expansion in leaf blade is associated with an increase in the ploidy level while stimulation of cell elongation in petiole by a dark condition occurred without an increase in ploidy level. As the analyses of light signals, phyB mutant in the red light and cry1cry2 double mutant in the blue light showed defect both in enhanced leaf-blade expansion and in inhibited petiole elongation, suggesting that the signals from phytochromes and cryptochromes play a major role to regulate the photomorphogenesis of leaf blades and petioles. On the growth of leaf blade and petiole, we also examined effects of Suc that is accumulated in the leaves by photosynthesis. Our data showed that promotive effect of the Suc on the growth in leaf blades and petioles were significantly affected by the light signals.

We also examined the role of phytohormones in leaf photomorphogenesis. ABA deficient mutants showed defect in blue-light dependent expansion of leaf blades, but normally expanded in the red light. On the other hand, we found that treatments with NPA, an inhibitor of polar auxin transport, decreased the growth of leaf petiole in the dark. Moreover, we analyzed the role of the ROT3/CYP90C1 and ROT3 homolog/CYP90D1, brassinosteroid biosynthesis genes in the photomorphogenesis of leaves. The leaf petioles of rot3 mutant showed defect in elongation in the dark, red and blue light, but leaf blades did not. In addition, the expression of CYP90D1 was induced in the leaf petioles in the dark, but not in the white light. Our results indicate that these phytohormones play a role in organ-specific regulation of leaf blade or leaf petiole in response to light environments. Taken together, interaction among photoreceptors, sugar and phytohormones are important for the control of the growth of the leaf blade and petiole in response to shade.

T04-099

Effect of NaCl on photosynthesis of two accessions of Arabidopsis thaliana

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Salt stress is one of the major environmental factors that limits growth and productivity of plants. Growth reduction under salt stress occurring in these plants has been currently attributed to excessive ion accumulation in the plant tissues, or/ and to osmotic stress. Photosynthesis is often affected by stress salt. The reduction of photosynthesis could be attributed to stomatal limitation or / and to the altering of the biochemical efficiency.
Two accessions of Arabidopsis thaliana, namely NOK2 and Columbia were cultivated on solid substrate for 30 days, and subjected to salt stress (50 mM NaCl) up to 21 days. The young and old leaves from the bottom are separated, every 7 days, up to 21 days. The dry weight, the area and the amount of ions Na+ and Cl- are determined. The stomatal conductance, the capacity of ribulose bisphosphate carboxylase-oxygenase are measured in the young and old leaves.
Results show that NaCl treatment resulted in growth reduction for both of accessions of Arabidopsis but less for NOK2 than for Columbia. Both accessions have the glycophitic behaviour but NOK2 seems less sensitive to salt stress because it accumulates more Na+ and Cl- than Col, and has a selective behaviour in favour of K+ (data not shown). However, the stomata conductance is reduced in the two kinds of salt treated leaves more in NOK2 than in Columbia. The capacity of ribulose-bisphosphate carboxylase is more affected by salt treatment in the young leaves than in the old ones of the two accessions. Although, the capacity of rubilose bis-phosphate carboxylase of the young leaves of Columbia is more affected by salt than those of NOK2, we can say that NOK2 is less sensitive than Columbia, because it closes its stomata, accumulates more Na+ and Cl-, has a good hydration and has a less changed capacity of ribulose bisphosphate carboxylase.

T04-100

Glycine betaine accumulating Arabidopsis thaliana survives strong salt treatment ¯ a cDNA microarray study

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Glycine betaine (GB) is a compound known to help organisms to cope with osmotic stress. The weed A. thaliana does not have the enzymes required to produce GB. We show that Arabidopsis accumulated GB from agar MS media supplemented with 10 mM GB. Furthermore, 50% of the Arabidopsis seedlings that accumulated GB survived a 24 hour 450 mM NaCl treatment, while 100% of the Arabidopsis seedlings that germinated and grew on media without GB died after the same treatment. To further understand why the GB treated Arabidopsis survived, global gene expression was studied using DNA microarray technology. After a 3 hours NaCl treatment, seedlings were immersed in liquid N2. The seedlings gene expression levels were analysed on Affymetrix Arabidopsis ATH1 Genome Arrays. When comparing untreated and GB treated samples, 65 genes were down regulated and 141 genes were up regulated. Comparing untreated and NaCl treated Arabidopsis 1961 genes were down regulated and 1726 genes were up regulated. In addition, the number of regulated genes were similar when comparing Arabidopsis that accumulated GB and then was treated and untreated with NaCl. 282 genes were down regulated and 426 up regulated comparing NaCl treated Arabidopsis that either had or not had accumulated GB. 1205 genes were up regulated in both GB treated and untreated Arabidopsis when treating with NaCl. The corresponding number for down regulated was 1277. Between 500 and 1000 genes were thus differently regulated due to GB accumulation when treating with NaCl. Some aspects of differences in gene regulation are currently being investigated.

T04-101

Characterization of QTL underlying whole-plant physiology in Arabidopsis: delta C13, stomatal conductance, and transpiration efficiency

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Water limitation is one of the most important factors limiting crop productivity worldwide and has likely been an important selective regime influencing the evolution of plant morphology, development, and physiology. Understanding the genetic and physiological basis of drought adaptation is therefore important for improving crops as well as for understanding the ecology and evolution of wild species. Here, we present results from quantitative trait loci (QTL) mapping of carbon stable isotope ratio (delta 13C) (a drought avoidance mechanism) in Arabidopsis thaliana. We performed whole-genome scans using multiple-QTL models for both additive and epistatic QTL effects. We mapped a total of five QTL affecting delta 13C. We captured two delta 13C QTL in nearly-isogenic lines to further characterize their physiological basis. These experiments revealed allelic effects on delta 13C through the upstream trait of stomatal conductance with subsequent consequences for whole plant transpiration efficiency and water loss. Our findings document considerable genetic variation among natural accessions in whole-plant physiology and highlight the value of quantitative genetic approaches for exploring the functional relationships regulating physiology. Furthermore, they provide a first step in the positional cloning of genes underlying natural variation in whole-plant physiology in A. thaliana.

T04-102

A SEMIDOMINANT MUTATION IN A PHOSPHATE TRANSPORTER INCREASED ARSENIC ACCUMULATION IN ARABIDOPSIS THALIANA.

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The presence of arsenic (As) in the soils and water is a major public concern of environmental impact. In plants, arsenate (AsV), the most bioavailable form of As, is assimilated through phosphate transporters. Previously, we had identified a semidominant mutation at the AtPT1 high affinity phosphate transporter in Arabidopsis. In this study we have further characterised pht1-1 and evaluated its performance in the presence of arsenate. pht1-1 shows reduced phosphate accumulation and as expected displays increased arsenic tolerance. We have conducted an experiment to establish the arsenate accumulation behaviour of the mutant. In the early stages after arsenate supply (8-24 hours), pht1-1 accumulates arsenate to a somewhat lower level than that in the wild type. Noteworthy, at later stages (2-12 days), the situation is completely shifted, so that accumulation in the mutant grealy exceeded that in the wild type (1.87 µg As/mgr dry weight in mutant versus 0.65 µg/mg dry weight in wt). These results can be interpreted as if reducing the uptake capacity, the plant acclimates more efficiently to arsenate, leading to increased accumulation. Therefore pht1-1 allele could provide a positive molecular tool for arsenic phytorremediation.
To identify markers of the response to arsenate and potentially relevant regulators, we used a gene trap transposon mutagenesis approximation to identify genes induced by arsenate. We analyzed approximately 7000 lines and found 26 lines with differential expression in arsenate. One of the homozygous lines, which displayed significant increased sensitivity to Arsenate, is currently under study. In addition, the results of a transcriptomic analysis of the arsenate response in wild type plants will be presented.

T04-103

Characterization of sas1: a novel salt, ABA and sugar hypersensitive Arabidopsis mutant.

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Functional analysis of plant genes most often use knock-out or gain-of function mutants. Insertion mutants offer the advantage, that besides creating a mutation, the genes are marked by a DNA tag, which facilitates their identification. We have created and partially characterized a T-DNA-tagged mutant collection in Arabidopsis thaliana Col-0 ecotype (1,2). By screening for altered stress responses, we have isolated an insertion mutant, which is characterized by salt, ABA and sugar hypersensitivity. The recessive sas1 mutant is semidwarf, but has no other distinct phenotypic alteration in normal growth conditions. Germination and root growth however was reduced by osmotic stress or ABA. T-DNA insert disrupted the first exon of a gene encoding an unknown protein, containing 12 pentatricopeptide (PPR) repeats. PPR domain proteins are greatly expanded, but not well characterized in plants. Although their exact function is not known, members of this family can be involved in RNA stabilization and processing. Transcriptional activity of the disrupted gene could not be detected in the mutant by RT-PCR. Quantitative RT-PCR analysis revealed, that expression of stress-induced genes, such as RD22 or P5CS1, was upregulated in the mutant. While germination efficiency of the sas1 mutant was negatively affected by high salt, ABA or sugar, it could be complemented with the full length cDNA, driven by the constitutive CaMV35S promoter. Moreover, overexpression of the cDNA in wild type Col-0 background increased germination frequency in such stress conditions. Localization experiments suggested, that the HA epitope-tagged SAS1 protein is transported into the mitochondria.
This research was supported by OTKA Grant no.T046552, and EU FP5 project ROST, no. QLRT-2001-00841.

1) Szabados L et al., 2002, Plant J. 32:233-242,
2) Alvarado M et al., 2004, Plant Physiol. 134:18-27

T04-104

Identification of Potential Substrates of AtCPK11, a Calcium-Dependent Protein Kinase Induced by Drought and Salt Stress in Arabidopsis thaliana

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Calcium is an important second messenger in plant signal transduction pathways mediating a wide variety of environmental stimuli. Calcium-dependent protein kinases (CDPKs) function as an important group of sensor-transducers to decode calcium signals leading to protein phosphorylation events, which change enzyme activities and protein conformations. In this study, we have used a yeast two-hybrid (YTH) approach to identify potential substrates of AtCPK11, a calcium-dependent protein kinase induced by water deficit and salt stress in Arabidopsis. Six independent YTH screens using three catalytically impaired AtCPK11 mutants as baits and two different prey libraries were performed using a high throughput procedure. In vitro kinase assays using a recombinant His-AtCPK11 showed that H2A.Z, a histone variant involved in transcription regulation in yeast and Di19 (a ring finger drought-inducible protein) were phosphorylated by AtCPK11. In addition, a PB1 domain-containing protein and AtTOC33 (a GTP-binding protein involved in chloroplast protein import) were weakly phosphorylated in vitro by AtCPK11. To provide further evidence of in vivo interactions, the subcellular localization of AtCPK11 and substrates was studied using a transient expression system. AtCPK11-GFP fusion protein was present in the nucleus and cytosol of transformed cells. Interestingly, the Di19-DsRed2 fusion targeted to the nucleus where it showed a speckled pattern, in contrast to the even nuclear distribution of H2A.Z-DsRed2. AtToc33-DsRed2 was targeted to the chloroplasts, consistent with published data, whereas the PB1-DsRed2 fusion was present in an unknown organelle. Currently, additional studies are in progress to confirm the presence of AtCPK11 in the nucleus. We are also investigating the substrate specificity of this protein kinase by comparison to three other Arabidopsis CDPKs. Finally, expression of AtCPK11 and its substrates will also be analyzed in plants exposed to stress conditions. The identification and characterization of AtCPK11 substrates will provide greater insight into the role of CDPKs in the perception and response to water deficit and salt stress stimuli. This work was support by a grant from the National Science Foundation (MCB0114769).

T04-105

Crosstalk and differential response to abiotic and biotic stressors reflected at the transcriptional level of effector genes from secondary metabolism

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Plant secondary metabolism significantly contributes to defense against adverse abiotic and biotic cues. Therefore, A. thaliana effector genes involved in consecutive steps of plant secondary metabolism had been chosen for a combined analysis of transcriptional responses to abiotic and biotic stressors. In order to distinguish between highly homologous members gene-specific probes derived from 3´-regions were designed. A thematic array (MetArray) comprised complete sets of genes encoding 116 secondary product glycosyltransferases and 63 glutathione-utilizing enzymes along with 62 cytochrome P450 monooxygenases and 26 ABC transporters. Their transcriptome was monitored in response to herbicides, UV-B radiation, endogenous stress hormones, and pathogen infection. A principal component analysis defined three distinct responses indicating that the transcriptome of this small set of effector gene families appeared to monitor and differentiate plant responses to various environmental cues.
Methyl jasmonate and ethylene treatments were separated from a group combining reactions towards two sulfonylurea herbicides, salicylate and an avirulent strain of Pseudomonas syringae pv. tomato. The latter group indicated an important overlap between abiotic and biotic stress responses. The responses to the herbicide bromoxynil and UV-B radiation were distinct from both former groups.
In addition, these analyses pinpointed individual effector genes indicating their role in these stress responses. A small group of genes was diagnostic in differentiating the response to two herbicide classes used. This probably reflects the different modes of action and potential side effects of these compounds. Interestingly, a subset of genes induced by P. syringae was not responsive to the applied stress hormones. Small groups of comprehensively induced effector genes indicate common defense strategies. Homologous members within branches of these effector gene families displayed differential expression patterns either in both organs or during stress responses arguing for their non-redundant functions.
In conclusion, this study highlights the potential of transcriptome analysis based on a focused collection of effector genes for a fast and straightforward analysis of new active compounds and stress responses.

T04-106

new insights in the ascorbate glutathione cycle from studies of the dehydroascorbate reductase in Arabidopsis thaliana

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Sulfate is one of the major plant nutrients. In this study we use sulfur starvation as a tool to investigate the complex response of Arabidopsis thaliana to a nutrient stress. Glutathione is one of the prominent sulfur containing metabolites in plants and its decrease is one of the first plant reactions to sulfate starvation. Glutathione is believed to be an important compound in stress metabolism. Thus, we are interested to study the behavior of the genes and metabolites involved in the glutathione ascorbate cycle as response to sulfur deprivation.
In our studies we used metabolomic and transcriptomic tools for the analysis of Arabidopsis cultivated under sulfate deficient conditions. A co response analysis of the genes of the glutathione ascorbate cycle was performed using publicly available microarray data.
One of the results from this analysis was, that the family of dehydroascorbate reductases shows a specific reaction to sulfate deprivation. This reaction differs in various compartments of the plant cell and seems to be distinct from main stress response. Knock out plants from different dehydroascorbate reductase isoforms were created and studied together with plants, which are altered in ascorbate and glutathione levels under different environmental conditions. We developed a technique, which allows to simultaneous measure of ascorbate and glutathione from plant extracts. Our results show the strong influence of dehydroascorbate reductase activity on ascorbate and glutathione homeostasis.

Plant Journal 33: 633-650 (2003)

T04-107

Positional cloning and characterization of the Arabidopsis pho2 mutant

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The Arabidopsis pho2 mutant accumulates excessive amounts of phosphate (2 to 5-fold wild-type level) into shoots, whereas the phosphate concentration in the mutant root is indistinguishable from wild-type root. We have identified the PHO2 gene using a positional cloning approach, and confirmed the identity by molecular functional complementation. The PHO2 gene encodes a component in the ubiquitin-dependent protein degradation pathway, which is involved in selective protein degradation via the 26S proteasome. Real-time reverse transcription PCR revealed PHO2 expression in all major plant organs, including roots, leaves, stems, flowers and siliques. Analysis of the PHO2 promoter activity using promoter-GUS fusions revealed a predominant expression in the vascular tissues of roots, leaves, stems, and flowers. For global expression analyses, RNA samples from both pho2 and Col-0 seedlings were hybridised to Affymetrix ATH1 arrays. Preliminary analysis of the Affymetrix results indicates that the PHO2 gene itself is repressed in the pho2 mutant (which carries an early stop codon in the PHO2 gene), and several genes with potential functions in phosphate uptake, -metabolism and -sensing are also differently expressed in the pho2 mutant. Transcript profiling of over 1,400 transcriptional regulator genes (TF) shows differential expression of several TFs in pho2 compared to wild-type plants. One or several of these TF genes might be targets of PHO2-regulated protein degradation. Yeast-two hybrid studies and immunopulldown / mass spectrometry approaches are currently being used to identify the PHO2 interacting partners, i.e. the target proteins. The yeast two-hybrid screening has already resulted in the identification of 4 potential PHO2 interacting partners. The results from global expression analyses of pho2 mutant and wild type in different Pi regimes will also be presented.

T04-108

Potential role of a member of the PHO1 gene family in Pi re-distribution in Arabidopsis

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PHO1 has been identified as a protein involved in the loading of inorganic phosphate into the xylem of roots in Arabidopsis. The genome of Arabidopsis contains 11 members of the PHO1 gene family. The cDNAs of all PHO1 homologs have been cloned and sequenced. All proteins have the same topology and harbour a SPX tripartite domain in the N-terminal hydrophilic portion and an EXS domain in the C-terminal hydrophobic portion. The SPX and EXS domains have been identified in yeast (Saccharomyces cerevisiae) proteins involved in either phosphate transport or sensing, or in sorting proteins to endomembranes. Phylogenetic analysis indicated that the PHO1 family is subdivided into at least three clusters. PHO1;H1 is the closest homolog to PHO1 having 46% of amino acid identity and 61% of amino acid similarity. PHO1;H1 is able to complement the pho1 mutant when expressed under the control of the PHO1 promoter. Reverse transcription-PCR revealed an expression of PHO1;H1 in leaves, roots and stems. Analysis of the activity of the promoter of PHO1;H1 homolog using promoter-glucuronidase fusions showed its predominant expression in the vascular tissues of roots, leaves and stems. Upregulation of PHO1;H1 transcription upon Pi stress, its pattern of expression in the phosphate response mutants backgrounds (phr1 and pdr1) and response to cytokinin suggest that the expression of PHO1;H1 is closely linked to the Pi status of the plant. RT-PCR and Northern analysis revealed increased expression of PHO1;H1 in older and senescing leaves compared to the young leaves. Taking into account the complementation of pho1 mutant by PHO1;H1, as well as the expression profile of the gene in leaves and its regulation by Pi, we hypothesise the role of PHO1;H1 in partioning of Pi in-between source and sink tissues under Pi stress and senescence.

Structure and Expression Profile of the Arabidopsis PHO1 Gene Family Indicates a Broad Role in Inorganic Phosphate Homeostasis.
Plant Physiol. 2004 May;135(1):400-11. Epub 2004 Apr 30.

T04-109

SRR1, a gene involved in phyB signalling and circadian clock function.

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The srr1 mutant has been identified in a screen for new components required for normal photomorphogenesis. The srr1 mutant exhibits typical phenotypes of a defective phyB pathway such as elongated hypocotyl in white and red light, reduced end-of-day far-red response and early flowering time in long and short days. In addition to the phyB phenotypes srr1 is affected in multiple circadian outputs. Luciferase reporter genes driven by circadian regulated promoters allowed us to show circadian phenotypes in continuous light and in continuous darkness, thus inferring a role of SRR1 in the central oscillator rather than in the light input pathway. However, the SRR1 transcript is light induced but not under circadian control. Sucrose-gradient fractionation of cell extracts revealed that SRR1 is part of a complex of about 150 kDa. SRR1 homologues are present in numerous eukaryotes, including mammals and yeast. We use complementation of the Arabidopsis srr1 mutant to test for functional conservation of the different homologues.

T04-110

The Role of GRAS proteins in Phytochrome Signal Transduction

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Phytochrome A (PHYA) plays a major role in morphological responses of seedlings to light such as cotyledon unfolding and expansion, hypocotyl growth inhibition and cell differentiation. In order to identify regulatory intermediates in light signal transduction, we have been investigating the role of Arabidopsis GRAS proteins in photomorphogenesis. This plant-specific protein family consists of 33 members in Arabidopsis and is characterized by a variable N-terminus and a conserved C-terminus with two leucine-rich domains.

PAT1 has been described as an intermediate of phytochrome A signal transduction. Physiological and molecular data indicate that this mutant is disrupted at an early step of phytochrome A signal transduction. pat1-1 is a semi-dominant mutant and the phenotype is caused by an expression of a C-terminally truncated mRNA leading to a dominant negative effect. Knock-out lines have a less severe, but still far-red light specific phenotype. Together with four other proteins (SCL1, 5,13, and 21) PAT1 constitutes a subgroup of the GRAS proteins found in Arabidopsis. We could demonstrate that its closest homolog, SCL21, is also impaired in far-red light signal transduction. On the other hand, antisense lines of SCL13 are impaired in a distinct subset of red light responses. Recent results of the functional analysis of these proteins will be presented.

T04-111

Abiotic stress signaling and tolerance

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Detecting and responding to environmental perturbations are important for all living organisms. Our lab is interested in the molecular mechanisms underlying plant responses to harsh environments such as soil salinity, drought and cold temperatures. In addition, we are interested in the mechanisms of transcriptional gene silencing and in the role of epigenetic gene regulation in stress adaptation. We use a combination of genetic, biochemical, genomic and proteomic approaches to analyze various levels of gene regulation (chromatin level/epigenetic, transcriptional, posttranscriptional, and protein activity) and to understand stress signaling and stress tolerance. Our long-term goals are to elucidate the signaling pathways used by plants in responding to environmental stresses and to identify key genes for modifying the responses of crops to environmental stresses which ultimately will lead to major contributions to agriculture and the environment. In this presentation, I will give an overview of recent progress in the area of abiotic stress responses, and describe some recent results from my lab on salt, drought and cold stress signal transduction in Arabidopsis. Potential involvement of microRNAs and other small regulatory RNAs in abiotic stress responses will be discussed.

T04-112

Role of membrane fluidity in cold perception in Arabidopsis thaliana suspension cells.

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Perception and transduction mechanisms of cold signal begin to be elucidated in plant cells. Signalisation pathways implying phospholipases are part of the transduction (Ruelland et al., (2002) Plant Physiol.130: 999-1007). We wanted to characterize some of their properties in Arabidopsis thaliana suspension cells. The existence of a temperature threshold for phosphatidic acid production by phospholipase C and D in response to cold was demonstrated. Besides, we have shown that this temperature triggering of phospholipase C and D activation was shifted in systems where membrane fluidity was modified artificially (pretreating cells with fluidifying or rigidifying agents as benzyl alcohol or dimethylsulfoxyde, respectively), physiologically (acclimated cells) or genetically (mutants of desaturation pathways). Results have evidenced that membrane rigidification plays a central role in signal perception leading to phospholipase C and D activation when plant cells are exposed to cold

T05 Interaction with the Environment 2 (Biotic)

T05-001

Immunolocalization of a Fusarium-induced stress associated protein in wheat (Triticum aestivum) root.

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A protein with an apparant molecular mass of 51kDa cross-reacted with Fusarium induced stress-associated protein(FISAP)-antiserum was detected in seven days old germinated wheat(Triticum aestivum) seedlings when infected with Fusarium oxysporum. The overexpression of such 51kDa protein band was observed in case of infected seedlings.Polyclonal antibody raised against the FISAP was used to check the specificity of the same protein by western-blot method.The cellular localization of FISAP was probed by immuno-gold localization using anti-FISAP-antiserum in wheat root tissues.The number of labeled gold particles in case of Fusarium infected seedlings were found to be 105.75±26.69 where as,the labeled gold particles observed in case of untreated (control) seedlings to be 6.15±1.62. Interrestingly,anti-FISAP-antibody did not label to freshly prepared F.oxysporum tissues at all. Hence, we suggest this FISAP is associated with pathogen-related (PR) tolerance in wheat.Further characterization of such protein is on progress.

Nicholas C.Collins et al;
SNARE-Protein --------at the plant cell wall.
Letters to nature:2003, 425, 973-977.

T05-002

Analysis of PMR6: linking an altered cell wall composition with powdery mildew resistance

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Plant cell walls have a very complex structure. Despite years of work, we do not fully understand the molecular arrangement of their components and even less do we understand the necessity and function of this complexity. Since the late 1970s, it is known that plant cell wall fragments can elicit defense responses. Recently, several genetic studies have provided new lines of evidence implicating cell wall polysaccharides as factors of host-pathogen interactions (1). In this context we analyzed the powdery mildew resistant mutant pmr6, which no longer expresses a functional GPI-anchored pectate lyase. The resistance phenotype is not dependent on salicylic acid, jasmonic acid or ethylene and thus reveals a novel resistance pathway (2).
Fourier Transform Infrared spectroscopy analyses have shown that the cell wall of the mutant is enriched in pectin, the most complex component among the wall polymers. PMR6 might normally be required to modify a pectin molecule during some stage of pectin biosynthesis. Perhaps PMR6 processes a precursor to a final form and loss of this activity results in a novel pectin structure in the wall. It was hypothesized that this altered cell wall of the mutant might contain a latent, very potent signaling molecule that is released during the infection process and elicits a stronger and/or faster response than the molecules present in the wild type cell wall. Here, we present recent results from Affymetrix gene chip analyses that indicate that this hypothesis has to be revised and we will describe a new model explaining the resistance phenotype of pmr6.

(1) Vorwerk et al. (2004), TIPS 9:203-9 (2) Vogel et al. (2002), Plant Cell 14: 2095-2106

T05-003

Understanding the molecular mechanisms of the glucosinolate-myrosinase system in plant-aphid interactions

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Plants have evolved a variety of mechanisms including physical and chemical barriers (repellents, toxins) to protect themselves from herbivory. In Arabidopsis and other crucifers, the glucosinolate-myrosinase system acts as a chemical defense against herbivore attack. Glucosinolates, a class of thioglucosides, and the enzyme myrosinase (b-thioglucoside glucohydrolase, TGG) are compartmentalized in different plant cells. Tissue disruption, e.g. wounding caused by insect herbivores, allows myrosinase to cleave glucosinolates and results in the release of toxic products such as isothiocyanates, nitriles and thiocyanates. Although glucosinolate breakdown products repel most insects, they are also involved in host plant recognition by some crucifer-feeding specialist herbivores.

The goal of this work is to understand the role of the Arabidopsis myrosinase enzyme in glucosinolate turnover and insect defense. T-DNA insertion lines with defects in TGG1 and TGG2, the two known functional myrosinase genes in Arabidopsis, are being studied. Complete knockout mutations of the respective TGG genes were verified by RT-PCR. The mutants do not differ morphologically from the wild-type plants. However, at different developmental stages and in various tissues, myrosinase activity, determined spectrophotometrically as degradation of the glucosinolate sinigrin, is approximately 5% of wild-type activity in tgg1 mutants, but is not significantly different from wild type in tgg2 mutants. Initial analysis of the glucosinolate content of tgg mutants showed no significant quantitative or qualitative changes from wild-type levels.

The mutant lines were investigated to determine whether altered myrosinase activity is involved in defense against the generalist herbivore Myzus persicae (green peach aphid) and the specialist herbivore Brevicoryne brassicae (cabbage aphid). Reproduction of M. persicae was not significantly different on wild type and mutant plants. In contrast, the specialist B. brassicae reproduced two to three times better on the tgg1 mutants than on the wild type and the tgg2 mutant plants. Feeding strategies and the role of the glucosinolate-myrosinase system in defense responses to M. persicae and B. brassicae will be discussed.

T05-004

DETERMINATION IN ARABIDOPSIS THALIANA OF PGPR EFFECT, ISR ACTIVITY AND THE POSSIBLE ISR-RESPONDING-WAY IN HIZOBACTERIAS ISOLATED FROM THE ROOTS OF NICOTIANA GLAUCA.

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According to experiments made before with 56 strains of Nicotiana glauca, which include siderophore production, chitinolytic activity and PGPR effect on tomato, (in preparation), six rhizobacteria were selected: N5.18, N6.8, N11.37, N17.35, N19.27, N21.4. In these work PGPR effect will be tested on Arabidopsis thaliana Col-0. On the other hand, ISR bioassays were also carried out to determine if these PGPR strains, induce protection against the pathogen Xanthomonas campestris CECT 95. Finally, the strains that induces systemic resistance were tested in transformed NahG plants and etr-1 and jar-1 mutants, to determined if these strains follows the SA-dependent or independent way.

Biometrical parameter used to determined the PGPR effect were: perimeter and area of the plants. Strains that produce best results in perimeter compared with the control are N11.37, N17.35 and N19.27. When plant area was analysed best results were obtained only with the strain N11.37. It is interesting to indicate that although these bacteria produce significant differences in PGPR effect on tomato, it does not happen in Arabidopsis. A possible explanation might be that these bacteria were isolated from the rhizosphere of Nicotiana glauca which are evolutionary much nearer to tomato than to Arabidopsis. However, N11.37 gave good results also in tomato and in Arabidopsis.

For ISR bioassays Xanthomonas campestris CECT 95 was selected as foliar pathogen, according with experiments carried out in our lab, on tomato. The six before cited strains were tested. Only N11.37 and N6.8 gave significant differences compared with the control with only 20,6 and 21,7 % respectivesly of disease incidence.

Finally, bioassays were carried out using NahG transplants, and ert-1 and jar-1 mutants with N11.37 and N6.8. These experiment indicate that the possible ISR-route followed by these bacteria are dependent of SA.

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T05-005

Genomewide transcriptional analysis specifies the Fusarium toxin Zearalenone to interfere with stress responses and cell wall modification in Arabidopsis thaliana

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Zearalenone (ZON) also known as F-2 toxin is a nonsteroidal estrogenic mycotoxin produced by plant pathogenic Fusarium species responsible for ear blight disease in cereal crops. ZON is one of the most potent myco/phyto-estrogens and therefore of particular interest to human and domestic animal health.
Although ZON may serve as potential virulence factor its phytotoxic effect is not well characterized todate. Here we present our attempts to reveal the molecular mechanism of ZONs action on plant development and defense using Arabidopsis.
The long-term effect of ZON was evaluated by a root growth assay on nutrient agar plates with increasing ZON concentrations and revealed a dose dependent inhibition of root growth of Arabidopsis. To study short-term genome wide transcriptional responses to ZON we used the ATH1 GeneChips (Affymetrix) of the NASC facility with RNA of seedlings treated with 50 µM ZON or solvent. Expression of individual transcripts were evaluated with real-time RT-PCR and mirrored the microarray results. Most transcriptional changes were observed after two hours where 227 genes were either induced (108) or repressed (119). The highest induction was observed with members of the cytosolic sHSP class and genes involved in detoxification as transporters, gluthathione transferase and cytochrome P450. After 24 hours the expression of these genes reaches background levels and few defense related genes (i.e. PDF1.2a, b) exhibited an elevated expression. The pattern of repressed genes included a large fraction of cell wall modifying genes. Similar patterns were detected upon abiotic stress treatments as heat, drought, cold, H2O2 and ozone.

T05-006

Identification of membrane-associated and infection-related transcripts of Arabidopsis by microarray analysis of polysomal fractions

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The obligate biotroph Peronospora parasitica, the causal agent of downy mildew on Arabidopsis, is completely dependent on the living host for growth and reproduction. Like many other plant pathogens it interacts with host cells via the formation of feeding structures, called haustoria. For haustorium formation, the pathogen breaches through the cell wall but remains surrounded by a membrane of the plant called the extrahaustorial membrane which is continuous with the plasma membrane. The haustorium plays an important role in nutrient uptake and signaling between pathogen and plant. However, the processes that occur at the host-pathogen interface are poorly understood. To investigate Arabidopsis gene expression associated with the development and functioning of the haustorium, we have isolated membrane-associated polysomes that are enriched for transcripts encoding integral membrane and secreted proteins. By using a combination of differential centrifugation and optimal buffer systems we have isolated high quality mRNA from 'membrane-associated' and 'free' polysomes. Differential hybridisation of labeled cDNA from polysomal mRNA on Arabidopsis microarrays (CATMA) resulted in the identification of transcripts that are enriched in the 'membrane-associated' fraction. We will report on the reproducibility of the method and the different classes of transcripts that we have identified. By comparing 'membrane-associated' transcripts from pathogen- and mock-inoculated plants we have selected a group of Arabidopsis genes that are potentially involved in the infection process. Their functional characterization will reveal their role at the host-pathogen interface and in disease susceptibility.

T05-007

Observations of new colonies on the root surface of Arabidopsis thaliana by Azorhizobium caulinodans

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N2-fixing bacteria colonize plant root and promote its growth by transfering fixed-N2, but sometimes they affect negatively as the result of their over-growth in plant tissue. To use N2-fixing bacteria efficiently in agriculture, it is important to elucidate the mechanism of their root colonization.
Azorhizobium caulinodans is a N2-fixing bacterium which fixes N2 both as a symbiont in the nodule induced on the tropical legume Sesbania rostrata, and as a free-living microorganism. It has previously shown that the N2-fixation of A.caulinodans was deeply activated by supplementation with an extra C source, but the growth of inoculated plant(rice) was not promoted(C. V.Niewenhove et al.2000,Biol Fertil Soils 31:14-149). Since this experiment was carried out in field trial, the cause of this effect on rice has not proved. To apply N2-fixing organism including A.caulinodans for promoting plant growth, we should investigate what happened in the rhizosphere. Therefore we conducted an experiment in labolatory scale. Arabidopsis thaliana grown on the surface of sterlile agar medium was inoculated with A.caulinodans, then we have observed the root colonization in detail.
Light microscopy analysis showed that A.caulinodans formed new type colonies on the root surface when C source was supplied. Our observation differs from the recent report that A.caulinodans colonizes mainly on lateral root crack(LRC) on root surface and comes into the intercellular space and xylem(P.J.Stone et al.2001,MPMI 14:93-97). These new colonies formed oval shape, and developed on the root surface except for the root base where the plant epidermal cells had already matured. In our experiment, the growth of A.thaliana was inhibited with the root tip curving and forming a club shape. The indole acetic acid(IAA) excreted from A.caulinodans could negatively affect the root growth. We are now investigating why A.caulinodans can not colonize on the base of the root, and whether these colonies fix N2 efficiently.

T05-008

Identification of genetic suppressors and enhancers of rar1 in Arabidopsis

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RAR1 is required for a diverse subset of Resistance (R) proteins. Recent findings by a number of groups have shown RAR1 is required for the proper accumulation of R proteins. This, together with data showing in planta interaction of RAR1 with HSP90, suggests RAR1 may have a co-chaperone function. Co-chaperones assist chaperones to select and process proteins for conformational maturation, complex formation and/or activation. HSP90 has already been implicated in the resistance response, and RAR1 may function with it to act on the R protein itself or upon one or more components of an R protein complex. As a complementary strategy to biochemical approaches to characterize R protein complexes we took a genetic approach to identify enhancers and suppressors of a partially defective rar1 mutant, rar1-15, that we isolated in earlier work. Characterization of these mutations may reveal positively or negatively acting intermediates of RAR1 signalling, some of which may themselves be important for R protein assembly and/or activation. Following re-mutagenesis of rar1-15, we have screened approximately 3000 M2 families and identified multiple rar1 enhancer and suppressor loci. Progress will be presented on initial steps to characterize our candidate locis.

T05-009

Is Annexin 1 involved in cellular defense against oxidative stress in Arabidopsis?

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Oxidative stress is a health-threatening phenomenon resulting from accumulation of partially reduced oxygen species (reactive oxygen species, ROS) that are both by-products of normal metabolism and are generated in a consequence of stress. Annexin 1 (AnnAt1) has been recently identified as a new player contributing to balance ROS level during oxidative burst (1). Induction of AnnAt1 expression in Arabidopsis was reported after treatments resulting in perturbation of cell redox state, i.e. H2O2 and salicylic acid (SA) treatments (2). Moreover, in heterologous systems, AnnAt1 was able to protect yeast and mammalian cells from oxidative stress (2, 3). In this context it is interesting to characterize AnnAt1 role in stress response in homologous system.
In preliminary experiments we found, that expression of AnnAt1 is elevated after different stimuli leading to elevation of intracellular calcium level. In contrast to PR1-a gene expression, induction of annexin 1, on the mRNA level, was limited not only to the infection. Elevated expression of AnnAt1 gene was also observed after wounding or abscisic acid treatment. So, it seems that annexin 1 can participate either upstream of jasmonic acid (JA) and salicylic acid (SA) in signaling pathways as a calcium sensor and/or downstream of them as a ROS scavenging enzyme. To verify this hypothesis, transgenic plants over-expressing AnnAt1 gene and plants with mutated AnnAt1 gene were generated, and their reaction on various stressors is in the progress.

1. Plant Phys. 2001, 126: 1072-84;
2. PNAS 1996, 93: 11268-73
3. Int. J. Bioch. Cell, 2001, 33: 591-602

T05-010

Testing the infectivity and RNA recombination of brome mosaic bromovirus on Arabidopsis gene-knockout lines related to RNA interference/PTGS.

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Recently we have reported that the expression of a suppressor of RNA interference/post-transcriptional gene silencing (RNAi/PTGS) facilitated the infection of brome mosaic bromovirus (BMV) in Arabidopsis thaliana (Dzianott and Bujarski, Virology 318,482-492, 2004). To further test this phenomenon, several groups of Arabidopsis lines carrying T-DNA insertional knockouts in genes related to the RNAi/PTGS pathway were infected with BMV and the levels of virus accumulation determined. This revealed that most of the knockouts in RNA polymerase (RdRp) genes as well as in Argonaute-like and in SGS3 (suppressor of gene silencing) genes increased the levels of BMV infection, as compared to unmutated Arabidopsis (Col-0). These results confirmed that interfering with various stages of the RNAi/PTGS pathway facilitated BMV infection. Currently we are testing other groups of Arabidopsis lines that are partially or totally deficient in RNAi/PTGS. In contrast, an unrelated Arabidopsis line carrying a knockout within the gene for eukaryotic translation initiation factor 3b abolished BMV infection, demonstrating the importance of this factor for BMV life cycle. We in the process of determination the extent to which RNAi/PTGS mediates genetic BMV RNA recombination. The implications of these findings for our understanding of the role of host factors in virus replication will be discussed.

T05-011

Transcriptome analysis of Arabidopsis clubroots and disease resistance of CKX gene overexpressing plants indicate a key role for cytokinin in disease development

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The clubroot disease of Brassicaceae is caused by the obligate biotrophic protist, Plasmodiophora brassicae. Infected roots undergo a developmental switch that results in the formation of aberrant roots (clubs). To investigate host gene expression during the development of the disease we have used Affymetrix chip (22k chip). Two time points were chosen, an early time point, at which the pathogen has colonised the root but has induced only very limited change of host cell and root morphology. At a later time point, more than 60% of the host root cells were colonized and root morphology was drastically altered. At both time points more than 1000 genes were differentially expressed in infected versus control roots. These included genes associated with growth and cell cycle, sugar phosphate metabolism and defense. The involvement of plant hormones in club development was further supported; genes involved in auxin homeostasis, such as nitrilases and members of the GH3 family, were up-regulated, whereas genes involved in cytokinin homeostasis (cytokinin synthases and cytokinin-oxidases/dehydrogenases) were strongly down-regulated already at a very early time point. Cytokinin oxidase/dehydrogenase overexpressing lines were disease resistant, clearly indicating the importance of (protist-derived) cytokinin as a key factor in clubroot disease development

T05-012

The jasmonate-insensitive mutant jin1 shows increased resistance to biotrophic as well as necrotrophic pathogens

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Jasmonic acid and related oxylipin compounds are plant signaling molecules that are involved in the response to pathogens, insects, wounding and ozone. To get further inside the role of jasmonates in stress signal transduction the response of two jasmonate-signaling mutants, jin1 and jin4, to pathogens and ozone was analyzed in this study. Upon treatment with the biotrophic bacterial pathogen Pseudomonas syringae, endogenous jasmonate levels increased in jin1 and jin4 similar to wild-type demonstrating that these mutants are not defective in jasmonate biosynthesis. Jin1 but not jin4 is more resistant to P. syringae and this higher resistance is accompanied by higher levels of salicylic acid. Jin1 is also more resistant to the the necrotrophic fungal pathogen Botrytis cinerea and shows wild-type sensitivity to ozone while jin4 is more susceptible to B. cinerea and ozone. These results indicate that the mutations in jin1 and jin4 affect different branches of the jasmonate signaling pathway and provide support for a crosstalk between the jasmonate and salicylate pathways. Additionally, in this combination of phenotypes, jin1 is unique among all other jasmonate-related mutants described so far.

T05-013

Virulent bacterial pathogens induce a pathogen-mediated hypersensitive cell death in pflp-trangenic Arabidopsis

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Plant ferredoxin-like protein (PFLP) isolated from sweet pepper influences pathogen- mediated hypersensitive response (HR). We have reported that PFLP can change the redox state of the cell upon harpin inoculation to increase active oxygen species (AOS) generation and hypersensitive cell death in tobacco suspension cell (ref.1). Transgenic studies indicated that over-expressing of pflp in transgenic plants could increase disease resistance against many bacterial pathogens, including Erwinia, Pseudomonas, Ralstonia, Xanthomonas spp.(ref.2,3) and even some tested fungal pathogens, such as Botrytis cinerea, phytophthora infestans and Fusarium oxysporum. In this communication we present how does the pflp-transgenic Arabidopsis system become resistance to a virulent pathogen, Erwinia carotovora subsp. carotovora (Ecc). Our results indicated that the disease resistance does not originate from a constitutive HR, because some HR hallmark, including endogenous levels of AOS, ion leakage and activation of HR marker genes (Athsr2 and Athsr3) mRNA, showed no significant difference in transgenic and wild type Arabidopsis under no infected conditions. However, following Ecc infection in transgenic Arabidopsis, both AOS and ion leakage generation were enhanced and the activation of HR marker genes was stronger than that of wild type. In other hands, harpinEcc was also selected to challenge transgenic Arabidopsis. Compared with wilt type of Arabidopsis, the same amount of harpinEcc can induce stronger activation of Athsr3 and generation of AOS in pflp- transgenic plant. In addition, when transgenic Arabidopsis challenged with hrpN- (a harpinEcc defective) mutant of Ecc, the disease resistance activity was not observed. We may conclude that the disease resistance of pflp-transgenic plants results from the induction of harpin-mediated HR by virulent pathogens.

1. Plant Mol. Biol. 2003, 51:913.
2. Plant Science 2001, 160:1035
3. Transgenic Res. 2003, 112:329.

T05-014

The Arabidopsis gene CAD1 controls programmed cell death in the plant innate immune system and encodes a protein containing a MACPF domain.

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Plants respond to pathogen infection by activating a defence mechanism known as innate immunity. To clarify the processes involved in plant innate immunity, here we have isolated and characterized a single recessive Arabidopsis mutant, cad1 (constitutively activated cell death), which shows a phenotype that mimics the lesions seen in the hypersensitive response (HR). This mutant shows spontaneously activated expression of pathogenesis-related (PR) genes, leading to a 32-fold increase in salicylic acid (SA), indicating that activated SA signalling leads to the HR in cad1. Infection with a Clover yellow vein virus (ClYVV) modified with GFP showed that the cad1 mutation results in the restriction of long-distance viral movement between leaves. Cloning of CAD1 revealed that this gene encodes a protein containing a domain with significant homology to the MACPF (membrane attack complex and perforin) domain of complement components and perforin, which are involved in animal innate immunity. Furthermore, cell death was suppressed in transgenic cad1 plants expressing nahG, which encodes SA-degrading enzyme. We therefore conclude that the CAD1 protein negatively controls the SA-mediated pathway of programmed cell death.

T05-015

The interaction between AtbZIP10 and LSD1 ¯ a new mechanism for the regulation of pathogen response?

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In plants bZIP transcription factors play crucial roles in the regulation of many processes including pathogen defense, light or stress signalling. The activity of bZIP transcription factors is triggered mostly on posttranslational levels. Particularly, we are interested in bZIP10, which is localized not only in the nucleus but also in the cytoplasm. In a retention screen in yeast we found that LSD1 (lesions simulating disease 1) retains AtbZIP10 in the cytoplasm by interaction with its C-terminus. Using split YFP analyses we further demonstrated LSD1 and AtbZIP10 interaction in planta. Our data suggest that LSD1 masks the NLS (nuclear localization sequence) which also represents the DNA binding domain of AtbZIP10. In Arabidopsis LSD1 is an essential negative regulator of plant cell death, therefore the interaction with LSD1 may serve as posttranslational control of bZIP10 in regulation of target genes involved in pathogen response. To determine the role of bZIP10 in pathogen response we determined the expression of marker genes for hypersensitive response and found that in atbzip10 mutants the induction of the cytosolic Cu/Zn SOD and the ascorbate peroxidase is affected. We demonstrate a connection between AtbZIP10 and the hypersensitive response and propose a function of AtbZIP10 in the regulation of pathogen defense.

T05-016

Da(1)-12 x Ei-2 Recombinant Inbred Lines: A Tool for Mapping Genes that Control Resistance to Specialist Insect Herbivores

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Arabidopsis recombinant inbred lines (RILs) are an excellent tool to map QTL for a multitude of traits, including traits that mediate the interaction with a plant´s biotic environment. Previously, resistance to insect herbivory has been investigated in the Col-0 x Ler-0 and Ler-0 x Cvi-0 RILs, and a variety of QTL have been mapped. The majority of these QTL control resistance against generalist insect herbivores like Spodoptera exigua or Trichoplusia ni, which are able to utilize a broad range of non-cruciferous host plants as their food source. Moreover, QTL for resistance against generalist insects usually co-localize with QTL that either affect accumulation of glucosinolates ('mustard oil glycosides'), plant secondary compounds from crucifers, or determine glucosinolate breakdown into toxic, bioactive molecules. Currently, only minor QTL have been identified for resistance against crucifer specialist insects.
To determine the genetic basis for resistance against Plutella xylostella, a world-wide insect pest on crucifers, we developed a new set of RILs from a cross between the Arabidopsis ecotypes Da(1)-12 and Ei-2. These ecotypes represented the most resistant and susceptible extremes in a Plutella herbivory screen on 40 Arabidopsis accessions. We genotyped 204 Da(1)-12 x Ei-2 RILs with more than 70 agarose gel-based markers, and mapped QTL for glucosinolate quantity, breakdown, and plant damage due to Plutella herbivory. In addition to a multitude of glucosinolate QTL (some of which were previously unknown), we detected one major Plutella resistance QTL that does not co-localize with any of the QTL controlling glucosinolate accumulation.

T05-017

Regulation of Cell Death in Arabidopsis by the LSD1-Gene Family

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LSD1, a negative regulator of oxidative stress-induced cell death, is a member of a small gene family of zinc finger proteins. It shares a novel consensus motif (CxxCRxxLMYxxGASxVxCxxC) with three other proteins designated LOL1 (LSD One Like 1, At1g32540), LOL2 (At4g21610) and LOL3 (At1g02170). Analysis of a lol1-mutant demonstrated that LOL1 function is required for lsd1 runaway cell death (rcd). Conversely, conditional over-expression of LOL1 triggers cell death in Ws-0 and lsd1 backgrounds. Yeast two-hybrid analysis demonstrated that LOL1 can interact with LSD1 and with additional LSD1 interactors. We are now analyzing the in vivo interaction between LSD1 and LOL1. Conditional over-expression of LOL2 induces cell death in Ws-0 and lsd1 backgrounds as well. Although LOL2 doesn't interact with LSD1 or LOL1, it does interact in yeast two-hybrid assays with AtTip49a, which itself interacts with LSD1, but not LOL1. Thus regulation of cell death in Arabidopsis might be dependent on complexes that contain LSD1 and it's different interactors. We are now analyzing these complexes in vitro and in vivo.

Dietrich et al. (1997) Cell 99, 685-694
Holt et al. (2002) Dev. Cell 2,807-817
Epple et al. (2003) PNAS 100, 6831-6836

T05-018

Structure / Function Analyses of Pseudomonas syringae Type III Effectors

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Many gram-negative bacterial pathogens of both plants and animals use an evolutionarily conserved type-III secretion system (TTSS) to deliver virulence proteins termed type III effectors directly into host cells. Once inside the cell, these type III effectors manipulate signaling pathways in order to inhibit host defense mechanisms and aid pathogen colonization. Importantly, recent findings suggest that the plant immune system, mediated by plant disease resistance (R) genes, recognizes the virulence activity of type III effectors, supporting a general model suggesting that indirect recognition of the action of pathogen virulence factors is the initiator of successful plant immune responses. Recent genome-wide analyses for proteins secreted in a type III system-dependent manner by phytopathogenic Pseudomonas syringae pathovar tomato DC3000 have revealed ~50 confirmed or predicted type III effectors. Furthermore, several type III effectors of P. syringae increase virulence on genetically susceptible hosts. However, despite increasing efforts, biochemical functions have been assigned to a paltry few P. syringae type III effectors. Consequently, understanding the function of type III effector proteins in virulence and resistance is currently a major goal in the study of plant pathology. In response to this dearth of functional data, we have initiated a focused structural proteomics approach to complement biochemical and genetic techniques in order to further characterize P. syringae type III effectors and to gain insight into their mechanisms of action.

T05-019

Characterization of AvrPpiB, a P. Syringae type III effector protein that enhances bacterial virulence on Arabidopsis.

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Many bacteria pathogenic on plants and animals introduce proteins into host cells using the type III secretion system. There, these type III effectors work in concert to enhance the environment for bacterial growth, possibly by suppressing host immune responses and extracting nutrients to create a comfortable niche. The molecular function of most of these proteins remains a mystery. Most are unrelated in sequence to proteins of known function. Our goal is to investigate the function of one of the P. syringae type III effectors AvrPpiB. AvrPpiB was first identified because it stimulates an R gene mediated resistance response in some pea varieties. We have shown that the delivery of AvrPpiB from a weak P. syringae pathogen leads to a 10-fold enhancement in bacterial growth on Arabidopsis. This virulence function is only seen in plants grown under mild drought stress. Delivery of AvrPpiB also causes an unusual spreading chlorosis phenotype in which the meristem and new leaves of the plant become chlorotic after 4-5 days of bacterial inoculation in older leaves. Importantly, some Arabidopsis ecotypes do not support either enhanced growth or systemic chlorosis when infected with the weak pathogen delivering AvrPpiB. We are mapping the genetic differences between ecotypes to determine how many Arabidopsis genes are potentially involved in AvrPpiB response. Expression of AvrPpiB in yeast is lethal. In order to identify eukaryotic proteins that would suppress AvrPpiB mediated lethality, we performed a multicopy suppression assay in yeast. We identified three candidate genes that, when over-expressed in yeast, allowed growth in the presence of AvrPpiB. Two of these genes have homologues in Arabidopsis. We are now testing the effect of T-DNA insertions in these genes in Arabidopsis to determine if they play a role in the virulence effects of AvrPpiB. We have also generated transgenic Arabidopsis that conditionally express AvrPpiB. We will use these for genetic screens aimed at identification of AvrPpiB targets. We have also performed a transcriptome analysis of leaves infected with bacteria delivering AvrPpiB, compared with isogenic bacteria that lack AvrPpiB. These results will be discussed.

T05-020

Dissecting the role of WRKY transcription factors by comparative protein profiling

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WRKY transcription factors are encoded by a large multigene family which is characterized by their DNA-binding WRKY domain, a peptide stretch of 60 amino acids containing the highly conserved W, R, K, Y amino acids and a Zn-finger motif. Present data indicate that WRKY proteins are translocated into the nucleus via a nuclear localisation sequence (NLS) where, upon binding to a cis regulatory element designated the W box (C/T TGAC T/C), they modulate the expression of target genes.
As transcripition factors of the WRKY-type are prevalent in the plant kingdom it is not surprising that they appear to play important regulatory roles in plant specific processes linked to plant development (e.g. senescence and trichome formation) and to the integration of environmental cues (e.g. pathogen/stress induced signaling pathways). In Arabidopsis thaliana 74 members were identified, 71 of which have been found to be expressed. Over 70% are rapidly induced upon biotic and abiotic stresses.
To complement the data coming from the application of genomic tools we will employ proteomic methods to study the WRKY family. Comparative protein profiling experiments will be carried out to identify WRKY-controlled targets and downstream pathways. We will analyse a range of Arabidopsis knockout and inducible WRKY lines under several defined stress-related conditions in comparison with wild type plants. In addition, several epitope-tagged recombinant WRKY factors have been immobilised on a chromatography resin with which we plan to identify WRKY interacting partners via affinity capture.

T05-021

Mutations in Arabidopsis RIN4 that affect the virulence of AvrRpm1, AvrB, and AvrRpt2 and R-gene mediated HR.

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RPM1-interacting protein 4 or RIN4 of Arabidopsis thaliana, is targeted by three different Pseudomonas syringae Type III effector proteins, AvrB, AvrRpm1, and AvrRpt2. Upon infection with P. syringae carrying either avrB or avrRpm1of susceptible hosts (rpm1), RIN4 becomes phosphorylated and plant disease occurs. During infection of susceptible hosts lacking RPS2 with P. syringae carrying avrRpt2, RIN4, however, is thought to be degraded-proteolytically, perhaps contributing to pathogen growth. In RPS2 plants, the AvrRpt2-driven disappearance of RIN4 is required to activate RPS2 function. To determine whether or not RIN4 phosphorylation or proteolytic cleavage of RIN4 is required for the virulence function of AvrB and AvrRpm1, or AvrRpt2, we have made mutations in putative phosphorylation sites in RIN4 as well as two highly conserved cysteine protease cleavage sites at the N- and C-terminal ends of RIN4. We present data on transgenic Arabidopsis plants transformed with either the RIN4 phosphorylation mutants or the N- and C-terminal cysteine protease cleavage site mutants. We show the effect of the RIN4 mutations on the ability of AvrB or AvrRpm1 and AvrRpt2 to cause disease on susceptible hosts. In addition, the region of RIN4 responsible for the interaction with AvrB contains one of the conserved cysteine protease cleavage sites. We therefore also show what affect the RIN4 mutations has on the ability of AvrRpt2 to interfere with the avirulence function of AvrB or AvrRpm1. Since modifications of RIN4 are detected by RPM1 and RPS2, we also show the effect of the RIN4 mutations on R-gene mediated hypersensitive response (HR).

T05-022

Searching For Novel Components Involved In Plant Nonhost Disease Resistance In Arabidopsis

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Nonhost disease resistance is the major form of disease resistance by plants which is effective against the majority of parasitic microorganisms such as bacteria, fungi and oomycetes. Plants have evolved several defence mechanisms to protect themselves against invading pathogens. These include preformed physical barriers like plant cytoskeleton as well as inducible mechanisms like the hypersensitive response with cell death.
Here, we describe the characterization of a nonhost-pathosystem involving the model plant Arabidopsis and the economically important oomycete pathogen Phytophthora infestans. Cytological analysis of Arabidopsis wildtype plants challenged with Phytophthora infestans spores revealed that the cell wall penetration attempts are usually aborted and only few spores can trigger a hypersensitive response with cell death.
To find novel components of the nonhost disease resistance we started an EMS-mutagenesis approach. A microscope-based screen for altered resistance phenotypes upon P. infestans challenge conducted with more than 70.000 M2 plants allowed the isolation of several mutant plants with an enhanced penetration frequency phenotype.

T05-023

An Arabidopsis mutant with decreased non-host penetration resistance has increased resistance to a host pathogen.

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Plants are constantly exposed to a wide variety of pathogens. However, a given plant species is host to only a subset of these pathogens. Resistance to pathogens outside this subset is termed non-host resistance. Non-host resistance is thought to be multigenic, non-specific, and durable. In contrast, the resistance of certain genotypes of an otherwise susceptible species to a pathogen is termed host resistance. This type of resistance often follows gene for gene interactions and is typically short lived in the field. Isolating plant factors that affect non-host resistance may lead to a better understanding of non-host resistance.
Arabidopsis is a host to the powdery mildew Erisyphe cichoracearum, and a non-host to Blumeria graminis f.sp. hordei, a pathogen of barley. 12,000 EMS-mutagenized plants were screened for increased penetration of the non-host mildew on Arabidopsis. Four different loci were identified. Two of these were allelic to pen1 and pen2, penetration mutants isolated simultaneously in other labs (1)(Hans Thordal-Christensen and Paul Shulze-Lefert, personal communication). A third locus, pen3, is unique in that it permits both increased penetration and increased hyphal growth by the non-host fungus. In contrast to pen2 and the fourth locus pen4, which are hyper-susceptible to the host powdery mildew, pen3 is resistant to the host fungus and this resistance is salycilic acid-dependent. The pen3 phenotype is caused by mutations in an ABC transporter. Microarray analysis, metabolite profiling, and localization experiments are underway.

1. Thordal-Christensen et al., Nature (2003)

T05-024

Putative plant molecular target molecules of the pathogenicity protein effector POPP1 secreted by Ralstonia solanacearum.

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Analysis of the genomic sequence of the plant pathogen R. solanacearum permitted the identification of numerous open reading frames that may encode molecules effecting pathogenicity. PopP1 is such a protein showing homology to YopP of Yersinia, which is injected into animal cells by the conserved type III secretion system. A R. solanacearum strain carrying a mutation in PopP1 was unexpectedly more aggressive on susceptible tomato and Arabidopsis lines, but became virulent on normally resistant genotypes of Petunia, the latter observation thus showing that PopP1 can behave as an avirulence gene. We show that, in contrast to YopP, PopP1 does not interact with map kinase kinases in the yeast two-hybrid system. We have identified three possible target molecules of PopP1 by screening a cDNA library of the model host plant Arabidopsis thaliana using the yeast two-hybrid system. One of these proteins (AtSNX1) may be involved in protein sorting, since in mammalian systems its homologues are implicated in the internalisation of an important class of membrane receptor molecules. Preliminary results obtained using transient expression of GFP-fused hybrid proteins in transfected protoplasts suggest that both PopP1 and AtSNX1 are cytoplasmically located. Currently, we are attempting to verify the interactions observed in yeast by co-immunoprecipitation in vitro.

Salanoubat, M., et al. (2002). Nature 415, 497-502.
Lavie et al. 2002. Mol. Plant-Microbe Interact 15 :1058-1068.

T05-025

Reactive Oxygen species (ROS) mediate the IAA-induced ethylene production

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Row concentration of ROS has been considered as a second messenger mediating various intercellular network for hormone singnaling as well as plant defense responses in plants. We previously reported that ROS generation was increase by auxin treatment mimic to the gravistimulus in maize roots. And auxin is well known to induce the ethylene production through the activation of ethylene biosynthetic enzymes. To investigate whether the ROS are involved in auxin-induced ethylene synthesis, we examined the ethylene production in presence of H2O2 or ROS scavenger, N-acethyl-Cys (NAC), in the level of transcription and enzymatic activity of ACC synthase and ACC oxidase in mungbean hypocotyls and Arabidopsis seedlings. While treatment of ethylene biosynthetic inhibitor, AVG, had little effect on the ROS generation induced by auxin, ethylene production was reduced with the treatment of NAC. Furthermore, H2O2-treated mungbean hypocotyls showed higher ethylene accumulation compared with that of untreated samples, resulting from the activation of biosynthetic gene expressions for ACC synthase (ACS1,ACS7) and ACC oxidase (ACO1,ACO2). Also in Arabidopsis seedling, the transcription of AtACS4 was increased in response to H2O2. These results indicate ROS can act in upstream to promote the ethylene production. We also identified a protein that belongs to the auxin-responsive GH3 family protein in auxin-treated Arabidopsis seedlings as a target molecule of ROS using BIAM labeling and MALDI-TOF MS analysis. T-DNA insertional gh3 null mutant plants contained higher level of endogenous ROS and more ethylene production compared with those of wild type, indicating AtGH3 could be a fine tuning point for ethylene production via inhibiting ROS generation.

T05-026

Characterization of two Arabidopsis-Erwinia pathosystems

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We report here the initial characterization of two pathosytems involving Arabidopsis thaliana and two necrotrophic phytopathogenic bacteria: Erwinia chrysanthemi (Ech) and Erwinia amylovora (Ea). Both phytopathogens attack agronomic plants and thus cause important economic losses.
Ech is a broad-host range pathogen that causes soft rot disease by maceration of host tissues. Virulence of Ech depends strongly on the secretion of several pectinases by a type II secretion system. These enzymes degrade the host cell wall and allow the pathogen to invade the host tissue. Ea causes fire blight on pomoideae. Ea depends mostly on the type three secretion system (TTSS) which injects virulence factors into the plant cell.
A number of genes involved in plant defence reactions against pathogens have been identified in Arabidopsis, leading to the description of three main defence signal transduction pathways (SA, ET, JA). Resistance of Arabidopsis to the biotrophic phytopathogenic bacterium Pseudomonas syringae involves mainly the SA pathway, whereas resistance to necrotrophs such as B. cinerea and E. carotovora seem to involve the JA pathway.
In this work, we analyze the set of defences raised by Arabidopsis to counter the attack by the two phytopathogenic necrotrophs Ech and Ea. Namely: is there an oxidative burst upon infection? Which defence pathways are activated by infection? What role does the cell wall play?

T05-027

SYNTAXIN REQUIRED FOR PENETRATION RESISTANCE

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The molecular mechanism of penetration resistance (PER) remains one of the major unknown in the study of plant-pathogen interactions. We used a genetic approach to study PER in Arabidopsis. Mutants, easily penetrated by the non-host barley powdery mildew fungus (Blumeria graminis f.sp. hordei, Bgh), have been identified. Four recessive mutations occur in the PEN1 gene. This gene was isolated by map-based cloning and found to encode a syntaxin (AtSYP121) (Collins et al., 2003). Syntaxins belong to the eukaryotic t-SNAREs that are part of protein complexes driving vesicle and target membranes to merge during vesicle trafficking. The fusion protein GFP-PEN1 complements the mutations, and reveals that PEN1 is located at the plasma membrane. Furthermore, the fusion protein accumulates around papillae, which are formed at the sites of penetration attempts. A mutation in the PEN1 gene also results in increased penetration by the host powdery mildew fungus (Erysiphe cichoracearum), suggesting the existing of a basic resistance mechanism common to hosts and non-hosts. Knock-out of PEN1 cause a delay in papilla formation, suggesting that the vesicle trafficking, involved in building up this cell wall structure, is hampered. More evidence for the importance of vesicle trafficking in PER, come from tests with Brefeldin A (a vesicle trafficking inhibitor) and cytochalasin E (an actin polymerization inhibitor). Both can phenocopy the pen1 mutations in wild-type plants. Phenotypes related to SAR and ABA signalling are uncovered in pen1 mutants. We speculate what additional function these may suggest for SYP121 in plants.

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Hückelhoven R, Stein M (2003) Nature 425, 973-977

T05-028

Isolation and Identification of Phosphatidic Acid Targets from Plants

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Phosphatidic acid (PA) is emerging as an important lipid second messenger. In plants, it is implicated in various abiotic stress-signalling pathways including wounding, osmotic- and cold stress. Recently, PA has been shown to play a role during the plant's defence against pathogens (de Jong et al., 2004; Laxalt and Munnik, 2002; Van der Luit et al., 2000).
How PA exerts its effects is essentially unknown, mainly due to the lack of characterised PA targets. In an approach to isolate such targets we have used PA-affinity chromatography. Several PA-binding proteins were present in the soluble fraction of tomato and Arabidopsis cells, some of which were identified using mass spectrometric analysis. These included PEPC, Hsp90, 14-3-3, a SnRK2 serine/threonine protein kinase and RCN1, a PP2A regulatory subunit.
As an example, the binding of one major PA-binding protein, phosphoenolpyruvate carboxylase (PEPC), was characterised further. Competition experiments with different phospholipids confirmed specificity for PA, validating that PA-affinity chromatography/mass spectrometry is an effective way to isolate and identify PA-binding proteins from plants (Testerink et al., 2004).
We are currently focussing on the role of PA in regulation of Hsp90 and RCN1. Hsp90 is a chaperone that directly interacts with several disease resistance proteins. RCN1 was found to be involved in auxin, ABA and ethylene signalling. For both proteins, their lipid binding characteristics and cellular localisation are being studied.

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Testerink, C., Dekker, H.L., Lim, Z-Y., Johns, M.K., Holmes, A.B., de Koster, C.G., Ktistakis, N.T. and Munnik, T. (2004). Isolation and Identification of Phosphatidic Acid Targets from Plants. Plant J., accepted for publication.
Van der Luit, A.H., Piatti, T., van Doorn, A., Musgrave, A., Felix, G., Boller, T. and Munnik, T. (2000) Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol., 123, 1507-1516.

T05-029

An Arabidopsis Mlo knock-out mutant phenocopies the barley mlo broad spectrum powdery mildew resistance phenotype

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Powdery mildew is a common disease of plants. A range of powdery mildew species colonize almost all plant species. In temperate climate, powdery mildew infections cause severe yield losses in a wide range of crops. Recessively inherited loss-of-function alleles (mlo) of the barley Mlo gene confer resistance that is effective against all known isolates of the barley powdery mildew fungus, Blumeria graminis f. sp. hordei. In susceptible Mlo wild type plants, the fungus potentially manipulates the protein encoded by this gene for plant cell invasion. Until recently, it was unclear whether mlo resistance represents a species-specific phenomenon restricted to barley. Although Mlo homologs are found in all flowering plants examined to date it was uncertain whether powdery mildews target MLO proteins in other plant species for plant cell invasion. Our results demonstrate that mlo resistance can be induced in the model plant Arabidopsis by inactivation of a particular of the 15 Mlo homologs. The respective Arabidopsis insertion mutants were found to be highly resistant against the virulent powdery mildew fungus, Golovinomyces orontii, while infection phenotypes to the bacterial pathogen Pseudomonas syringae, and the oomycete Peronospora parasitica appeared unaltered. Similar to barley mlo resistant plants, the powdery mildew sporelings fail to switch from surface to invasive growth in the Arabidopsis mutants. No recognizable pleiotropic effects are detectable in the mutants. These data demonstrate that mlo resistance is effective in both major clades of flowering plants, suggesting that the role of MLO proteins for colonization by powdery mildews is ancient and evolutionarily conserved.

Panstruga, R. & Schulze-Lefert, P.; Microbes and Infection 5, 429-437 (2003).

T05-030

Genetic variation of powdery mildew resistance in Arabidopsis thaliana as a resource for the identification of novel host “compatibility factors”

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In a compatible microbe-plant-interaction, host “compatibility factors” are required for the establishment and maintenance of the infection. Consequently, loss of function of such host genes is predicted to result in incompatibility and therefore resistance against the pathogen. This recessively inherited resistance functions without constitutive activation of defense responses and can be effective for single or closely related pathogen species. There are already several single recessive loci known in a range of plant species that confer resistance to several types of pathogens.

However, genetic analysis in a single genotype is likely to unravel only a subset of potential host compatibility factors because of genetic redundancy or functional specialization. We therefore intend to exploit natural genetic variation within Arabidopsis thaliana as an additional resource for the identification of novel genes conferring powdery mildew resistance, preferably due to absence of specific host compatibility factors.

We determined resistance phenotypes of selected accessions with the powdery mildew fungus Golovinomyces orontii, crossed the resistant accessions with the susceptible accession Col-0 and selected candidate genes after analysis of the respective F1 and F2 generation for putative single and recessive loci.

In first two candidate accessions conferring resistance, Shadara and Sorbo (Tadjikistan), the “compatibility” locus was mapped to the lower arm of chromosome III by SSLP markers in Sorbo and with a Bay-0 x Shadara RIL population in Shadara. It remains to be determined how resistance is achieved and if it is due to the same locus in these accessions.

T05-031

Arabidopsis, a model plant to study the molecular bases of biological control

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Bacterial wilt caused by the phytopathogenic bacterium, Ralstonia solanacearum is one of the most important plant diseases world-wide. Methods to control the disease are limited. Biological control, based upon the preinoculation of potato or tomato by an hrp- strain followed by the inoculation of a virulent strain leads to a significant reduction of wilt symptoms under controlled conditions. Molecular mechanisms are however poorly understood. We have shown that Arabidopsis can be used as a model plant to study these mechanisms. Our results indicate that the increased resistance of hrp- -protected plants is not caused by physical competition between the virulent and avirulent strains and that living bacteria are required to obtain full protection. Several lines of evidence that will be presented suggest that the protection caused by the preinoculation with an hrp- mutant is related neither to systemic acquired resistance, nor to induced systemic resistance. The understanding of mechanisms controlling hrp--induced protection may render possible a rational development of biological control for agriculture.

T05-032

Sulfate-based performance of Arabidopsis thaliana in response to fungal infection

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The link of sulfur metabolism with resistance towards biotic and abiotic stress factors has first been observed in agricultural sciences. An optimal support of crop plants like rape with sulfate leads to an increase of resistance especially towards compatible fungi. This form of resistance is a quantitative effect due to nutritional conditions which is clearly separated from the gene-for-gene model, systemic acquired or induced resistance by rhizospheric bacteria. Here we analysed the effects of variable sulfate supply in an accurately defined modell pathosystem of A. thaliana and A. brassicicola on the molecular level. To quantificate the proliferation of the fungus, measurement of S-metabolites and multiparallel gene analysis were performed during the infection process. Cellular signal mechanisms that mediate between mineral sulfur support and activation of defense reactions will be identified by using mutants that are affected in pathogen-relevant signal transduction pathways and transgenic lines of Arabidopsis with modified steps of sulfur metabolism. The phenomenon of sulfate-triggered defense is an important new approach in phytopathology which has to be analysed with regard to known resistance genes and expression of marker genes for use in basic science and agrobiotechnology.

T05-033

RepA protein from geminivirus alters cell proliferation in Arabidopsis thaliana.

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Geminiviruses are single stranded DNA viruses able to replicate their genetic material in non-proliferating cells where DNA replication proteins are absent or inactive. To overcome this barrier, they induce cellular S phase reentry by reprogramming host gene expression. It has been shown that the viral protein RepA interacts with the retinoblastoma protein (RBR) and it is believed that the induction of a permissive environment for viral replication take place through the RBR/E2F pathway.

We are currently investigating the role of the wheat dwarf virus (WDV) RepA protein in such processes. For this purpose, transgenic plants expressing RepA under the control of an inducible promoter have been generated. In these plants, we showed that the proliferative activity of the host cells is modified and that the presence of the viral protein increases the amount of free E2F able to bind DNA. We also demonstrated that E2F target cell cycle genes such as CDC6, PCNA, ORC2 and ORC3 are induced. Moreover, the expression level of these genes are not modified in plants expressing in an inducible manner a RepA mutant unable to bind RBR.

These data indicate that RepA alone is able to interfere with the RBR/E2F pathway in vivo inducing G1/S transition and therefore facilitating viral replication in differentiated tissues.

T05-034

The Arabidopsis csb3 mutant shows enhanced resistance to biotrophic pathogens

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The defense-related P69C gene was previously identified in tomato plants and shown to be induced during the course of an incompatible and compatible plant pathogen interaction (1).To determine which components of the plant defense response make important contributions to limiting pathogen attack, and M2 mutagenized populations of a transgenic Arabidopsis line was screened for mutants showing constitutive expression of glucoronidase activity driven by the promoter region of the P69C gene. This screening render the isolation of a recessive mutant named as constitutive subtilisin3 (csb3). csb3 shows enhanced resistance to the biotrophic pathogens Pseudomonas syringae and Peronospora parasitica, and exhibits normal susceptibility to Plectospharella and the necrotrophic pathogen Botrytis cinerea. This csb3 resistance to biotrophic pathogen is associated with synthesis and accumulation of SA and constitutive expression of some PR genes. Moreover, csb3 shows spontaneous formation of microHR-like lesions in the absence of pathogen. We generated double mutants between csb3 and previous described SA-dependent signalling mutants to analyse the implication of the SA pathway in csb3 phenotype. These double mutants suggest that the characteristic resistance of csb3 to biotrophic pathogens is channel through the salycilic-acid and NPR1 pathway. Furthermore, we show evidences suggesting that CSB3 may link SA not only to defense but also to development

(1) Jordá, L. y Vera, P. (2000) Plant Physiol.124, 1049-1058

T05-035

Indirect activation of RPS5-mediated resistance by AvrPphB

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The induction of plant disease resistance requires the simultaneous presence of a resistance (R) gene in the host and a matching avirulence or effector gene in the pathogen. Whereas this genetic correspondence remains unchallenged, several mechanistic models have been proposed for the molecular events that activate the resistance response. Two models are supported by experimental evidence in different pathosystems. In some cases, activation of the R gene product appears to be triggered by direct physical interaction with the pathogen effector, whereas in others, activation occurs indirectly via the modification of a distinct virulence target by the pathogen effector. In Arabidopsis, resistance to Pseudomonas syringae strains expressing AvrPphB requires the RPS5 R gene product and the PBS1 protein kinase. AvrPphB encodes a cysteine protease that is exported directly into plant cells via the type III secretion system. Consistent with the indirect model, we have previously shown that AvrPphB targets and cleaves PBS1, not RPS5, and that PBS1 cleavage is required to induce RPS5-mediated resistance (1). New data indicate that RPS5 and PBS1 exist in a complex prior to pathogen exposure. Upon elicitation with AvrPphB, RPS5 becomes undetectable, suggesting that PBS1 cleavage by AvrPphB triggers RPS5 degradation. I will report on our ongoing progress in elucidating the molecular steps that lead to the activation and apparent degradation of RPS5.

(1) Shao et al., (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector, Science. 301:1230-3

T05-036

Analysis of mechanism underlying the inhibition of AvrRpm1/RPM1 functions by AvrRpt2 in the Arabidopsis thaliana

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Recent progress in the individual virulence factors phytopathogens contributing to parasitism and disease development have revealed the molecular interaction underlying pathogenesis and plant response. By using isogenic P. syringae strains and Arabidopsis mutants that have difference only in specific avirulence genes and resistance genes, respectively, we investigated the molecular mechanisms by which AvrRpt2 not only interfered with AvrRpm1/RPM1-mediated resistance signal but also induced degradation of RPM1 protein. Disappearance of RPM1 was not a direct cause for interference of AvrRpm1/RPM1-mediated resistance signal by AvrRpt2, because RPM1 protein was not completely degraded at the time point when AvrRpt2 interferes with AvrRpm1/RPM1-mediated resistance signal. In consistent with recent reports, AvrRpt2 targeted and eliminated RIN4 prior to phosphorylation of RIN4 by avrRpm1 that is required for activation and stability of RPM1. As a result, AvrRpt2 preventing from action of AvrRpm1 equally delivered to plant cell and induced disappearance of RPM1. Furthermore, AvrRpt2 also eliminated RIN4 that is already phosphorylated by AvrRpm1. These results clearly demonstrated that only AvrRpt2 can interfere with AvrRpm1 function, but not in the reverse. To overcome this one-side competition, at least two times more AvrRpm1 was required. We also developed screening method and identified mutants that are deficient in AvrRpt2-mediated RPM1 degradation. We found two kinds of ner (no elimination of RPM1 by AvrRpt2) mutants. RIN4 was eliminated by AvrRpt2 but not RPM1 in the ner1 mutant. However, AvrRpt2 can eliminate neither RIN4 nor RPM1 in the ner2 mutant. At present, the possible role of these genetic loci in the RPM1 and RPS2-mediated resistance is under investigation.

T05-037

Aquaporin genes regulated by dark adaptation and far-red light illumination in roots of Arabidopsis thaliana

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Plants have a light-conducting mechanism where light passes directly into the stem and is then conducted (particularly through vascular tissue) to the roots. Previous research in this laboratory has revealed that far-red (FR) light from the environment is the dominant wavelength inside stems and roots under the natural conditions and it has been proposed that this internal FR light is transduced near the root tips (Sun et al., 2003). However, roots in the soil are in complete darkness at night, although FR light was shown to pass through plant tissues in the daytime. In order to investigate whether or not gene expression of roots is affected by a dark-FR light cycle, gene expression profiles for dark adapted versus light-grown plants and for FR light illuminated versus dark-adapted plants were analyzed using a RIKEN Arabidopsis full-length cDNA microarray system. Eleven dark-inducible and five dark-repressed genes were reconfirmed by Northern analysis. Six aquaporin genes were found to be regulated by FR light in root tissues. The expression profiles of the genes were analyzed following different periods of FR illumination. TIP2;2 mRNA declined markedly within 1 h after FR illumination and increased within 4 h. The photoreceptor phyA exists in the root tissues and this repression of the aquaporin gene was not observed in phyA mutant. Therefore, it was indicated that, the phytochrome A signaling pathway is involved in the regulation of root gene expression by FR light.

T05-038

UTA1 locus encoding AtVDAC1 regulates the competency of Arabidopsis to Agrobacterium-mediated transformation

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Agrobacterium tumefaciens is a soil born plant pathogenic bacterium that elicits the crown gall disease in many plant species. Unlike other pathogenic bacteria which affect the host plant physiology by secreting or translocating the pathogenicity factors, Agrobacterium tumefaciens directly modify the genetic material of host plants. This genetic modification results from the transfer and integration of a T-DNA from bacterial Ti plasmid into the plant genome. To extend the range of application which Agrobacterium-mediated plant transformation technology can be applied, researches on the mechanism by which T-DNA is introduced into plant cells and stably integrated into the plant genome are indispensable. To identify plant factors that required for successful Agrobacterium-mediated plant transformation, we screened T-DNA insertion mutant lines of Arabidopsis thaliana for resistance in response to Agrobacterium infection using an in-vitro root inoculation assay. uta mutants (untransformed by Agrobacterium) resistant to infection by Agrobacterium were identified. First of all, we characterized uta1 mutant in which T-DNA insertion disrupted a voltage dependent anion channel (AtVDAC) gene known to function as a mitochondrial outer membrane ion channel. uta1 mutant exhibited much lower efficiency of transient GUS gene expression than wild-type did, indicating that uta1 mutant has a deficiency at the early steps of Agrobacterium infection process. Arabidopsis genome has four AtVDAC isoform genes. Three including UTA1 of them restored yeast vdac mutant phenotype and only one AtVDAC isoform gene besides UTA1 partially complemented uta1 mutant phenotype. These results indicated that there are differential functions in the AtVDAC gene family. AtVDAC1/UTA1 and AtVDAC2 are required for successful Agrobacterium-mediated plant transformation. More interestingly, overexpression of VDAC1 in uta1 plants increased Agrobacterium-mediated transformation efficiency of transient T-DNA gene expression rather than wild-type did. Our results demonstrate that the expression of the AtVDAC1 gene regulates the competency of Arabidopsis to Agrobacterium-mediated transformation.

T05-039

Expression profiling of the constitutive allene oxide synthase mutant cas1

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The enzyme allene oxide synthase (AOS) catalizes the first specific step in the biosynthesis of jasmonates, cyclic fatty acid derivatives, which serve as important signal compounds during plant defense mechanisms and plant reproduction. Coded by a single gene in Arabidopsis, the expression of AOS is highly regulated on the transcriptional level by both developmental and exogenous stimuli (e.g. wounding) (Kubigsteltig et al. (1999) Planta 208, 463-471). In order to identify components involved in the signal transduction pathway, we performed a chemical mutagenesis expriment based on transgenic Arabidopsis plants expressing the uidA-reportergene (coding for b-glucuronidase) under the control of the inducible AOS-promoter (Kubigsteltig and Weiler (2003) Planta 217, 748-757).
The mutant constitutive allene oxide synthase 1, cas1, has been selected for its higher AOS-promoter activity in the control situation (untreated, sterile-grown plants) and is recently subjected to PCR- and BAC-based positional cloning of the recessive mutation locus (chromosome 4, near CIW7 marker). The cas1 phenotype includes stunted root and shoot growth, curled leaves, production of additional rosettes at the inflorescence axis and short anthers which avoid normal fertitlity. Due to the elevated levels of jasmonic acid (JA) and 12-oxo-phytodienoic acid (OPDA) detected in cas1, we started to analyze the expression of 150 defense-related genes by microarray technique (Reymond et al. (2000) Plant Cell 12, 707-720). In contrast to our expectations only very few members of this group of genes have shown misregulation in cas1. But further expression profiling of the whole cas1 transcriptome using Affymetrix gene chips (ATH1) revealed a total of 388 genes to be overexpressed while 208 genes seem to be repressed in the mutant compared to the wildtype. Although these results still need to be confirmed, it is getting clearer, that not only the expected jasmonate-dependent, but also other processes are influenced by CAS1.

T05-040

TRANSCRIPTION FACTOR Dof15 AS A CANDIDATE GENE REGULATING AUXIN/INDOLE GLUCOSINOLATE HOMEOSTASIS IN ARABIDOPSIS

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Glucosinolates a group of secondary metabolites function as defence substances against herbivores and micro-organisms in the plant order Capparales. Indole-3-acetaldoxime (IAOx) serves as a precursor of indole glucosinolates (IGS) and is also an intermediate in the biosynthetic pathway of indole acetic acid (IAA), while IGS can serve as a source for the IAA precursor 3-indole-acetonitrile (IAN). During our studies on Arabidopsis Dof transcription factors we found that Dof15 is a candidate gene for IAA/IGS regulation under normal growth conditions. Dof15 expression is elevated upon several stresses and itself apparently induces the expression of genes related to IGS metabolism. Transgenic Arabidopsis plants over-expressing Dof15 (Dof15 OE) exhibited distinct phenotypic and developmental changes that included a significant reduction in plant size due to reduced cell expansion, shorter hypocotyls, small and curly leaves, reduction in lateral root formation, reduced flower number and a delay in main inflorescence formation. Genome-wide analysis of transcript levels in Dof15 OE lines revealed an induction of genes involved in biotic defence reactions, including genes of the glucosinolate/myrosinase system, which represents an essential component of resistance against herbivores and pathogens in Brassicaceae. Genes encoding enzymes of the tryptophan biosynthesis pathway are elevated as well. The branching enzyme CYP83B1 converts IAOx to IGS and the CYP83B1 gene is induced in Dof15 OE lines, consistent with an elevated content of indole and aliphatic glucosinolates. In contrast RNAi-Dof15 plants are characterized by reduced expression of CYP83B1. IAA levels were highly increased in Dof15 OE and slightly increased in RNAi-Dof15 plants supporting the dual role of IGs for IAA biosynthesis not only as a sink for IAOx but also as a source of the IAA precursor IAN. In wild-type plants, jasmonic acid and wounding cause an increase of Dof15 transcript level within hours, hence it is conceivable that Dof15 might play a role further downstream in the jasmonic acid signalling pathway. Promoter-GUS studies revealed that Dof15 is active in vascular tissues of all Arabidopsis organs examined, suggesting that it is involved in the regulation of glucosinolate homeostasis during normal growth conditions. Since glucosinolates can serve as auxin sink besides having a role in defence responses, they might also have an important function during plant growth and development.

Wittstock, U., Halkier, B.A. (2002) Glucosinolate research in the Arabidopsis era. Trends Plant Sci 7, 263˙270

T05-041

Systematic analysis of cytochromes P450 biotic stress signaling response in A. thaliana

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With a total number of 272 genes, cytochromes P450 represent the largest multigene family involved in secondary metabolism and hormone synthesis in A. thaliana. P450s catalyze key steps in numerous biosynthetic and degradative pathways participating to development, structure and defense of the plant. However, the function of most of these genes is still unknown. A P450 probe library has been constructed, using carefully selected specific 3' end segments of each gene in A. thaliana. It was spotted on glass slides together with probes for markers of metabolic pathways. These dedicated microarrays were used to investigate P450 expression profiles in plants exposed to biotic stress in order to (i) identify defense-specific from generic P450s, (ii) isolate P450s activated in early or late defense response (iii) characterize co-regulated genes sharing common expression patterns over the different conditions, and potentially implicated in the same signaling or biosynthetic pathway.
As a first part of the project, we monitored P450s expression in response to chemical effectors participating to described signaling pathways and/or known as potential or confirmed resistance elicitors. Treatment were performed on 4 weeks old soil grown plants, and experimental designs were optimized considering technical limitations (biological replicates, total number of hybridizations, amount of total RNA...). Microarray data were analyzed using the TIGR programm, and the expression profile of a subset of the induced genes was confirmed with quantitave PCR.
Cluster analyses identifies several groups of genes clearly involved in defense responses, several of them with already known function. This analysis will be pursued to determine and compare the plant responses to pathogen (bacteria, virus, fungi, oomycetes...) and insect attacks.

With the support of Génoplante

T05-042

Characterization of antifungal compounds purified from transgenic Arabidopsis plants expressing a bacterial non-heme haloperoxidase gene

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The natural function of bacterial so-called 'non-heme haloperoxidases' remains to be elucidated. It has been proposed that these enzymes, which belong to the large family of α/β hydrolases, may be involved in the metabolism of plant-derived compounds (1) or in defense against oxidative stress (2). Additionally, a number of studies attributed antimicrobial activity to the Burkholderia pyrrocinia enzyme or its metabolites upon expression in transgenic tobacco plants (3).
In the present study, transgenic Arabidopsis thaliana plants producing non-heme haloperoxidases, encoded by a gene either from Rhodococcus erythropolis NI86/21 or Streptomyces coelicolor A3(2), were obtained by Agrobacterium-mediated floral dip transformation. First generation transformants were analyzed for gene integration and expression by PCR and RT-PCR, respectively. Immuno-detection revealed the production of both enzymes. Leaf extracts from both wild-type and transgenic plants were found to inhibit fungal growth in vitro. However, extracts from transgenic plants showed a significantly stronger inhibitory activity. Partial purification of these extracts indicated that the active compounds in both wild-type and transgenic fractions are heat-stable and protease-insensitive. Active fractions are currently being purified and will be used for structural characterization of the compound(s) present. In addition, transgenic plants will be assessed for enhanced in vivo fungal resistance. An update of the recent biochemical and biological results will be discussed.

1 De Mot 2003 FEMS Microbiol Lett 224:197
2 Honda 2003 Eur J Biochem 270:486
3 Jacks 2002 J Agric Food Chem 50:706

T05-043

Gene Expression Profiling to elucidate EDS1 and PAD4 functions in Arabidopsis

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EDS1 (Enhanced Disease Susceptibility 1) and PAD4 (Phytoalexin Deficient 4) encode lipase-like proteins that are essential components of plant basal resistance to virulent pathogens. Additionally, EDS1 and PAD4 are recruited by resistance (R) genes of the TIR-NB-LRR but not the CC-NB-LRR type in R-gene mediated resistance. The precise biochemical function of EDS1 and PAD4 remains elusive. We reasoned that gene expression profiling of wild-type and mutant responses may help to identify signalling components of EDS1/PAD4-regulated pathways. The experimental design includes 21 samples derived from leaves of wild-type Wassilewskija (Ws-0) and the corresponding null mutants eds1-1 and pad4-5. Leaves were untreated, treated with MgCl2 solution (mock) or with Pseudomonas syringae strains expressing either avrRps4 (triggering EDS1/PAD4 dependent responses) or avrRpm1 (conditioning EDS1/PAD4 independent defence). Leaves were harvested at 3 and 6 hours post inoculation and analysed using oligonucleotide chips (ATH-1, Affymetrix). A number of gene clusters have been identified in this analysis and we have focused particularly on the characterisation of these two:
1. Genes whose expression is strongly induced upon DC3000 avrRps4 in wildtype but not in eds1 and pad4. These genes are also induced by DC3000 avrRpm1 but in an EDS1/PAD4 independent manner. These genes may be signalling components functioning downstream of EDS1/PAD4.
2. Genes whose expression is strongly induced by both bacterial strains in an EDS1/PAD4 dependent manner. These genes are of particular interest as the local defence responses (oxidative burst, hypersensitive response, salicylic acid increase) upon DC3000 avrRpm1 in eds1 and pad4 are undistinguishable from those in wild-type. We hypothesise that these genes are closely regulated by EDS1 and PAD4 and their characterisation might help elucidate EDS1/PAD4 functions in signal potentiation.

Hammond-Kosack KE, Parker JE; Curr Opin Biotechnol. 2003 Apr;14(2):177-93

T05-044

Characterization of two novel Arabidopsis mutants demonstrates the diversity of defence pathways involved in BABA induced resistance.

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The non-protein amino acid β-aminobutyric acid (BABA) is a potent inducer of resistance against a wide range of biotic and abiotic stresses. Applied as a soil drench or in foliar spray, BABA has been shown to protect against viruses, bacteria, oomycetes, fungi, nematodes and abiotic stresses. This broad-spectrum protection by BABA is based on priming for different basal defence responses, i.e. a faster and stronger basal defence response once the induced plant is exposed to stress. The signalling pathway controlling BABA-induced resistance (BABA-IR) depends on the stress applied. BABA-IR against the bacterium P. syringae depends on the salicylic acid (SA)- and NPR1-dependent defence pathway, whereas BABA-IR against necrotrophic fungi functions independently of SA but requires callose accumulation that is controlled by an abscisic acid (ABA)-dependent defence pathway. To gain more insight into the molecular mechanisms behind BABA-IR, mutants with an impaired BABA-induced sterility (ibs) phenotype were selected from a population of T-DNA mutagenized Arabidopsis. Previously, we have shown that three of these ibs mutants (ibs1, ibs2 and ibs3) were impaired in different priming responses. In the present work, we have characterized two additional ibs mutants: ibs5 and ibs6. Southern blot analyses revealed that ibs5 and ibs6 have five and two T-DNA inserts, respectively. The gemonic positions of the mutations were determined by TAIL-PCR.

To elucidate the defence phenotype of ibs5 and ibs6, the mutants have been tested for BABA-IR against the bacterium P. syringae, the necrotophyc fungus A. brassicicola, the oomycete H. parasitica and salt stress. Mutant ibs5 showed partial loss of BABA-IR, while ibs6 was completely blocked in BABA-IR against the bacterium and the oomycete. Interestingly, the level of basal resistance against Pst was enhanced in the ibs6 mutant. Both mutants were impaired in BABA-IR against A. brassicicola, which correlates with reduced levels of callose deposition. Upon exposure to salt stress, both ibs5 and ibs6 were capable of expressing BABA-induced tolerance to salinity, although ibs5 mutant was compromised in its basal tolerance to the salt.

T05-045

Investigating the basis for differential functions between Arabidopsis SGT1a and SGT1b

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SGT1, which has molecular features of a co-chaperone, is a positive regulator of R (Resistance) protein-mediated defence in diverse plant species. Recent studies indicate that SGT1 together with RAR1 and HSP90 may be required for R protein accumulation. SGT1 is an essential gene, and in Arabidopsis there are two recently duplicated highly sequence-related copies, AtSGT1a and AtSGT1b. AtSGT1b, but not AtSGT1a, is genetically required for resistance mediated by a subset of R genes and phytohormone signaling controlled by at least two plant SCF E3 ligases (SCFTIR1 and SCFCOI1). However, a double sgt1a/sgt1b is embryo lethal, indicating that AtSGT1a and AtSGT1b have overlapping essential functions early in development. To understand further the molecular basis for differential activities between AtSGT1a and AtSGT1b, we are analyzing their mode of expression at the protein level and their transcriptional activities using AtSGT1a or AtSGT1b promoter GUS fusions in a wild type background. AtSGT1a/AtSGT1b-promoter-swap and AtSGT1a over expressing constructs were also made to assess their potential to complement sgt1b-3 phenotype in R gene-mediated resistance and phytohormone signaling. Our analysis will determine if the different functions of AtSGT1a and AtSGT1b lie at the level of their mode of expression or the respective protein activities. Current progress in this study will be presented.

T05-046

Cloning of Arabidopsis homologues of IAA-amidohydrolases from Chinese cabbage and expression during the development of clubroot disease

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Clubroot disease is caused by an obligate biotroph organism called Plasmodiophora brassicae, who infects roots of all members of the Brassicaceae. In infested soil the resting spores of the pathogen can survive for over ten years and the infection includes a primary cycle in the root hairs followed by the secondary cycle during which the secondary zoospores enter the root itself. Each zoospore develops into a plasmodium which spreads through the host cells. The pathogen development proceeds from young plasmodia over vegetative plasmodia to a mass of resting spores. Correlated with the development of vegetative plasmodia an abnormal growth of the root is observed, induced by enhanced cell enlargement and cell proliferation. One interesting point in the life cycle of Plasmodiophora is the hormonal control of these processes in the host cells. It has been reported that they correlate with an increase in cytokinins and auxins, especially IAA. One source of free IAA is the release from conjugates with amino acids. The catalyzing enzymes for these reactions are the IAA-amidohydrolases. Genes for these enzymes have first been characterized in Arabidopsis thaliana but since clubroot disease is economically important in crops and the time course of infection as well as the localization of the plasmodia seems to vary between Arabidopsis thaliana and Brassica rapa we started to isolate IAA-amidohydrolases from Chinese cabbage. We were able to isolate cDNA fragments with homology to IAR3, ILL2, ILL6, ILL3 and ILR1 out of infected roots of Brassica rapa at different time points of infection, as well as of uninfected seedlings and leaves and stems of uninfected plants. Using a RACE approach we have so far isolated full length cDNAs from the following amidohydrolases like genes: IAR3, were we found 2 different clones varying in the 5'-region of the genes, ILL2, closely related to IAR3, and ILL6 which is placed in a separate group in the dendrogram of the IAA-amidohydrolases. Expression studies of the corresponding genes in infected and uninfected roots using Real Time RT-PCR revealed a differentially and very stage specific regulation. How far the observed up-regulation can account for an increased auxin level has to be further investigated. Heterologous expression studies in E. coli to run in-vitro enzyme activity assays are under way.

T05-047

Structure-function studies of Arabidopsis thaliana TGG4 Myrosinase

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Brassicaceae plants protect themselves against insect herbivory via a two-component activated defence system, the myrosinase-glucosinolate system or the “mustard oil bomb”. Myrosinase (EC3.2.3.1) is a beta-glucosidase specific to hydrolyse sulphur ethers and belong to the glycosidase super family (http://www.afmb.cnrs-mrs.fr/~pedro/ca24/db.html). Multiple sequence alignment of beta¯glucosidases show many conserved amino acid residues implied to be important for structure and function. Myrosinase catalyze a two-step reaction where the enzyme attacks the S-ether and a polarised water molecule cleaves the enzyme-glycone intermediate to restore the enzyme and the reaction is activated by low concentrations of ascorbate. The crystal structure is available for a mustard myrosinase (1MYR). Myrosinases have several functional sites that include binding sites for ascorbate, glucosinolate, water, Zn and myrosinase-binding protein. Arabidopsis thaliana seems to contain six potential myrosinase genes, which we are studying. We use site-directed mutagenesis to study the role of different amino acid residues on enzyme structure and function. For that purpose we cloned and over-expressed the Arabidopsis myrosinase TGG4 cDNA using intracellular expression in Pichia pastoris. The His-tagged protein is purified using IMAC and the recombinant protein characterised. Mutations are introduced using long primers with a mismatch in the middle and plasmid rolling circle PCR using the Phusion DNA polymerase. A glutamine postulated to direct specificity to S-glucosides was mutated into glutamate that is postulated to support O-glucosidase cleavage. The mutation was indeed found to increase Km and lower Vmax for the glucosinolate sinigrin but decreased Km for O-glucosidase activity. Increased Km and Vmax was obtained using desulfosinigrin as substrate. Other mutations address glucosinolate binding site, ascorbate activation and potentially stabilising residues. This study was supported by grants from FORMAS and Persons stiftelse.

T05-048

Bacillus as beneficial bacteria for plant protection

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Use of microorganisms for protection of plants can support a more environmental friendly agriculture. Plant Growth Promoting Rhizobacteria (PGPR) have beneficial effects on growth and ideally also provide protection to pests. Bacterial protection can be due to activation of defence mechanisms in the plant e.g. induced resistance (IR), competition of available nutrients and growth space and/or production of antibiotics. Bacillus subtilis is a naturally occurring soil organism but many strains are also known to colonize plants and some has been shown to function as PGPR. Bacillus produce spores that are much more tolerant than vegetative cells to different kinds of stress in the environment. These are important traits to support a persistent and effective establishment of the bacteria under field conditions.

Bacillus strains were isolated from different sources and grouped after genotyping. Several Bacillus strains that represented a broad genetic variation were chosen for experiments with plant protection to fungal phytopathogens. Bacillus strains were inoculated on seeds of oilseed rape (Brassica napus) and Arabidopsis thaliana and challenged with pathogens in controlled environment. The strains provided varying degrees of protection if any. However, certain Bacillus strains gave protection against the ascomycete Leptosphaeria maculans in two different experimental set-ups. The protective effect varied between oilseed rape and Arabidopsis showing that there is a specific interaction. The effects on plant tissue by Bacillus will be studied by DNA microarrays, metabolomics and mutants to indicate if IR (jasmonate dependent but salicylate-independent) is stimulated. The Bacillus strains were also tested for production of antibiotics against the pathogen under in vitro conditions, which showed that some strains indeed caused direct growth inhibition. Preliminary results from phenological analysis of plants after seed treatment do in general not support PGPR abilities. Effects of the seed treatment on herbivory by Brassica insect specialists will also be studied in the future. This study was supported by grants from IMOP/SLU and Lamms stiftelse.

T05-049

FERMENTING OVER A COMPLEX MATTER: Heterologous expression of Brassica napus Myrosinase Binding Proteins in Pichia pastoris.

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Myrosinases (MYRs) are enzymes that catalyse the hydrolysis of glucosinolates present in Brassicaceae species. Due to their toxicity, the released end products (nitriles, isothiocyanates…) are parts of the plant defense system against generalist pests. Three different MYR classes (MA, MB and MC) are present in Brassica napus. Each of these classes comprises several MYR isoenzymes. In seed protein extracts, the MA exist as free, disulfide-linked dimers. In contrast, MB and MC have been found in large (200-800 kDa) complexes with several non-MYR proteins e.g. Myrosinase-Binding Proteins (MBPs) [1]. The first MBPs reported were of 50 and 52 kDa molecular mass but were later shown to belong to a family of proteins ranging from 30 to 110 kDa, encoded by a large and repetitive transcript. MBP transcripts are inducible in vegetative tissues by wounding but the function of MBP is not known although they seem to be lectins. Though MBPs are required for MYRs complex formation in B. napus seeds, they do not seem to influence the catalytic activity of MYR.
As a prerequisite to elucidate the physiological role of the MBP family, we needed to produce functional proteins. Expression into a eukaryotic system was preferred and we designed a strategy to overexpress MBPs in the methylotrophic yeast Pichia pastoris. Sequences coding for a long (110 kDa) and for a short (52 kDa) MBP were PCR-amplified from the full-length cDNA clone of the repetitive MBP transcript using specific primers located either at the end borders of the CDS or encompassing only its three major internal repeats. These sequences were cloned into the pPIC3.5K vector, under the control of the alcohol oxidase 1 promoter, allowing intracellular methanol-induced expression in P. pastoris. All constructs were transformed into two yeast strains and small-scale expression studies were performed to determine the optimal conditions for expression of the recombinant proteins. The presence at their N- or C-terminus of an enterokinase-cleavable Histidine tag allow purification by Ni-NTA chromatography.
Once purified in large amounts, these proteins will be used (i) in crystallographic studies to resolve their three-dimensional structure, (ii) in complex reconstitution experiments to test their ability to bind different MYR isoenzymes in vitro, (iii) as baits to identify other proteins in MYR complexes and (iv) in studies of physiological function.
This study was supported by EU (HPRN-CT-2002-00262) and FORMAS.

[1] Rask et al., Plant Mol Biol (2000) 42(1):93-113

T05-050

SAG101, a novel EDS1 interactor, is involved in plant disease resistance

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In Arabidopsis, EDS1 and PAD4 are essential components of disease resistance conditioned by certain Resistance (R)-Proteins against bacterial and fungal pathogens. Phenotypic analysis of eds1 (enhanced disease susceptibility) and pad4 (phytoalexin deficient) null mutant plants has shown that EDS1 is necessary for expression of R protein-triggered hypersensitive cell death (HR) and both EDS1 and PAD4 are required for defence potentiation around infection sites. Consistent with the combined role of EDS1 and PAD4 in defence signal amplification we find that both proteins are essential components of basal resistance against virulent pathogens. The predicted amino acid sequences of EDS1 and PAD4 show similarities to eukaryotic triacylglycerol lipases and reveal a novel conserved domain in their C-terminal parts (EP domain, for EDS1 and PAD4). The only other Arabidopsis gene containing the EP domain is SAG101 that was previously characterised as a Senescence Associated Gene (He & Gan, 2002). SAG101 encodes an acyl hydrolase. Using affinity purification of tagged EDS1 coupled with MALDI- and Q-TOF mass spectrometry we have identified SAG101 as a new EDS1 interactor in planta. Phenotypic analysis of single null sag101 mutant alleles revealed no effect on plant disease resistance. However, when sag101 was combined with a null pad4 mutation, it strongly enhanced suppression of both R gene-mediated and basal resistance to a level at least equivalent to that found in the single eds1 mutant. Levels of EDS1 protein are also severely depleted in pad4/sag101. These data show that SAG101 is a necessary component of EDS1-directed defence signalling only in the absence of PAD4 and is thus partially redundant with PAD4. For further analysis we are examining the expression, cellular localisations and dynamics of EDS1, PAD4 and SAG101 association in healthy and pathogen-challenged tissues using a combination of epitope- and fluorescent protein-tagged variants, both in transient assays and stable transgenic plants.

T05-051

Analysis of Atapy1 promoter activity in clubroot disease caused by P. brassicae

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The obligate biotrophic protist Plasmodiophora brassicae leads to uncontrolled cell growth in roots in most Brassicaceae. The resulting clubroot disease is characterized by destruction of root tissue, cutting off the plant from nutrients and, most importantly water. In a microarray experiment, P. brassicae infected and non-infected A. thaliana plants were compared. 10d after inoculation with P. brassicae spores, the amount of Atapy1 mRNA decreased by 27.4% compared to the control. 24d after inoculation, the amount of Atapy1 mRNA even dropped to 50.5% of the control value. Apyrases hydrolyze nucleoside tri- and diphosphates. Proposed functions of apyrases include phosphate transport, conferral of toxin resistance and involvement in catabolism and anabolism of the cytoskeleton.
The GUS experiment presented here was based on results from this microarray experiment. The objective was to find out if the decrease in the amount of Atapy1 mRNA correlated with the activity of the Atapy1 promoter. Also, the histochemical GUS-staining was used to locate the site of Atapy1 expression in roots infected by P. brassicae. In addition, the course of infection was surveyed in more detail by increasing the number of time points studied after inoculation with P. brassicae.
Our results showed that the expression of ß-glucuronidase which was regulated by the Atapy1 promoter was very different in infected versus control plants. Generally, it was confirmed that Atapy1 expression was downregulated in infected plants. Root tips of infected plants, for example, showed no GUS staining while those of healthy plants were stained dark blue. However, in root clubs higher Atapy1 promoter activity was observed.
The different GUS staining pattern of root systems of infected and healthy plants suggests a role of AtAPY1 in the progression of the disease.

T05-052

Characterization of the transcriptional changes that result from infection with Pseudomonas syringae

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In response to pathogen attack, the plant activates several different signaling pathways and initiates changes in gene expression. While changes in the expression level of some genes have been well characterized and are commonly used as markers for the defense response, questions still remain about the number and timing of the transcriptional activation and repression events induced by different types of infection. Using microarray technology, we sought to characterize the changes in gene expression that resulted from infection with the bacterial pathogen Pseudomonas syringae. At five different time points, 4, 8, 16, 24 and 48 hours, we infected half of an Arabidopsis leaf with either virulent P. syringae, avirulent P. syringae/avrRpt2, or 10mM MgCl2, as a control. The uninfected half of the leaves were then collected and prepared for microarray analysis using the Affymetrix GeneChip Arabidopsis ATH1 Genome Array, which was designed to detect the expression level of about 24,000 genes. For genes that showed significant changes in transcript level, we searched for SALK T-DNA insertion lines that are potential knockout mutants. These lines are currently being tested for disease resistance phenotypes and will be discussed.

T05-053

Genetic dissection of non-host disease resistance to fungal pathogens in Arabidopsis

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Wild-type Arabidopsis is a non-host to the biotrophic barley powdery mildew fungus, Blumeria graminis f.sp. hordei (Bgh). We identified several Arabidopsis mutants that are partially compromised in non-host resistance to this inappropriate powdery mildew species. On these pen (penetration) mutants (pen1 and pen2), the entry success rate into attacked epidermal cells is dramatically increased and combined with efficient haustorium formation. Further fungal growth is however terminated coincident with a cell death response of haustorium containing cells. Interestingly, pen2 mutant plants show aberrant interaction phenotypes with multiple different plant pathogen species (e.g. Phytophthora infestans, Plectospherella cucumerina, Colletotrichum lagenarium) whereas impaired penetration resistance of pen1 mutants appears to be restricted to powdery mildew species.
We recently documented the identification of PEN1, which encodes a syntaxin potentially directing exocytotic vesicle traffic towards sites of attempted fungal penetration (Collins et al., 2003). Here, we present the identification of PEN2 which exhibits significant sequence similarity to family 1 ß-glycosyl hydrolases.
In plants, ß-glycosyl hydrolase activity is involved in processes such as activation of phytohormones, floral development and pigmentation, defense mechanisms, lignification and cell wall decomposition. Transgenic complementation analysis with wt PEN2-cDNA as well as catalytically inactive variants revealed that catalytic activity is required for PEN2 function in non-host resistance. First results from comparative metabolic profiling experiments aiming at the identification of the in planta substrate(s) of PEN2 will be presented.
To assess the contribution of already described defence components to Arabidopsis non-host resistance we generated a collection of pen2 double/triple mutant combinations and analyzed their interaction phenotypes with Bgh and another inappropriate fungal pathogen, the pea powdery mildew Erysiphe pisi (Ep). Despite a significant increase of secondary hyphal growth on particular double mutant lines, Bgh was still unable to complete its asexual life-cycle by sporulation. In marked contrast, the same double mutant combinations supported growth and sporulation of Ep, suggesting that inactivation of least two defence layers is sufficient to make Arabidopsis a host for Ep but not for Bgh. The conceptual implications of these findings will be discussed.

Collins et al. (2003) SNARE protein mediated disease resistance at the plant cell wall. Nature 425: 973-977

T05-054

A new Myrosinase gene family in Arabidopsis thaliana

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Myrosinases (EC 3.2.3.1), catalyse hydrolysis of the secondary metabolites glucosinolates into various toxic products to prevent pest damage. This binary system is present in Capparales and different species have evolved to contain a unique blend of different glucosinolates and several myrosinases that provides a chemical barrier to most pests as well as to serve in plant development. We are studying myrosinase genes in Brassicas in comparison with Arabidopsis thaliana. Gene scanning of the Arabidopsis genome using conserved features of known myrosinase genes in combination with preliminary biochemical studies suggested the presence of six myrosinase genes TGG1 - TGG6 in Arabidopsis. These genes are organized into two subgroups consisting of TGG1-TGG3 (on chromosome 1) and TGG4-TGG6 (on chromosome 5), respectively. While TGG4 and TGG5 are almost identical and >60% sequence identities occur between earlier known myrosinase genes, the sequence identities between the Arabidopsis two myrosinase gene groups are <60 %. The TGG4 - TGG6 genes also have a slightly different exon-intron organization and signal peptide sequences, and the unusual GC intron splice sites are used in different positions compared to the TGG1-TGG3. The two subfamilies also have different reactivity with different myrosinase antibodes. We have overexpressed TGG4 and TGG5 cDNAs as His-tagged recombinant protein in Pichia pastoris. Kinetic studies have been performed on the purified proteins and they both show significant myrosinase activity to sinigrin, which is stimulated by low concentrations of ascorbate. The effects of the His-tag on kinetics was investigated since the substrate has a negative charge but was not found to affect the catalytic properties. Specific RT-PCR analysis of Arabidopsis tissues for expression of different myrosinases could only detect TGG4 and TGG5 transcripts in the root tissue. Roles for myrosinases in root tissue may be protection to e.g. nematodes and as growth stimulation for ectomycorrhiza, which need to be further investigated. This study was supported by grants from FORMAS and Persons stiftelse.

T05-055

The role of RIN13 (RPM1 Interacting protein 13) in RPM1 mediated disease resistance in Arabidopsis

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The Arabidopsis RPM1 is a membrane associated disease resistance gene containing both a nucleotide-binding domain and leucine-rich repeats (NBS-LRR).

In the presence of bacterial effector, avrRpm1, RPM1 triggers hypersensitive cell death within 5 h and restriction of bacterial growth as disease resistance responses. We have identified an interactor of RPM1, RIN13 (RPM1 Interacting protein 13), from a 2-hybrid screen using an expanded region of the evolutionary conserved apoptotic ATPase domain of RPM1 as a bait.

RIN13 expression levels are not discernible on Gene Chips nor does it have any homology on motifs indicative of function.

To analyze RIN13 function, we generated RIN13 antisense and overexpressing transgenic lines. All sense and antisense lines of RIN13 are phenotypically normal compared to a wild type RPM1 response.

Conversely RIN13 antisense or knock-out plants underwent a normal hypersensitive response (HR). In the RIN13 overexpression lines, leaf collapse was concomitant with the virulent carrier isolate DC3000. Although RIN13 plants were hyper-restrictive, no increases in cytosolic calcium level nor H2O2 were detected and the timing of induction of RPM1 specific molecular markers was modified.

In contrast to the avrRPM1/RPM1 interaction, the avrRpt2/RPS2 interaction in all transgenic lines was the same in wild type plants, indicating RIN13 functions as a RPM1 specific positive regulator.

Our data suggest that RIN13 positively regulates resistance specified by RPM1 and negatively regulates cell death.

Currently the modes of interaction between RIN13 and RPM1 are being tested by identifying RIN13 and RPM1 associated protein complex in plants.

T05-056

Host Factors Controlling Potato Virus X Movement in Arabidopsis thaliana

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Movement of plant viruses is a complex process facilitated by the virus' ability to use host-encoded proteins. Viruses act as intercellular parasites with small genomes strongly dependent on plant functions to complete their life cycles. Hence, for a virus to successfully establish a systemic infection, numerous molecular interactions between the virus-encoded proteins and host factors must occur. It is not yet clear how the virus makes use of plant host factors to move throughout the plant. We have exploited the natural genetic variation in Arabidopsis thaliana to identify host factors controlling Potato virus X (PVX) movement. This approach has been successfully used previously to identify host factors involved in the long-distance movement of viruses. For example, the Carrington lab have shown that RTM1 on the top arm of chromosome I restricts the long-distance movement of Tobacco etch virus.

We screened 300 ecotypes and found that unlike La-er and Col-0, some ecotypes do not support long-distance movement of PVX. This screen utilised a method of agro-infecting the plant with PVX tagged with the B-glucuronidase (GUS) reporter gene, allowing easy visualisation of viral movement. Genetic analysis revealed that a single dominant gene restricts PVX movement. Mapping has shown that the locus lies on the top arm of chromosome I, but is not RTM1, and is therefore a novel gene. We have cloned the gene by complementation, and comparative sequencing has revealed four amino acid changes, three of which would have a dramatic effect on protein structure.

T05-057

ARF-GTPases in plant pathogen interactions

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Cell polarization induced by developmental stimuli is mechanistically based on cytoskeleton rearrangements, targeted vesicle transport and secretion. The same processes have recently also been demonstrated to be crucial for plant defence against fungal intruders (Schmelzer, 2002; Collins et al., 2003). Small GTPases of the ADP RYBOSYLATION FACTOR (ARF) family are believed to be involved in vesicle coatomer formation, cargo loading, transport and uncoating in many organisms, including plants. The Arabidopsis genome harbours 19 ARF or ARF-like genes that have not been systematically characterized so far. In this project we aim to assess the role of ARF controlled vesicle traffic in plant pathogen interactions. To do so, a collection of arf T-DNA insertion lines will be analyzed for aberrant pathogen interaction phenotypes. In addition, transgenic Arabidopsis lines expressing ARF-GFP fusion proteins were generated that allow tracing pathogen induced vesicle dynamics in planta.

Schmelzer (2002) Trends in Plant Science 7(9): 411-415
Collins et al. (2003) Nature 425: 973-97

T05-058

Identifying pathogen-induced changes in the plant defense signaling network

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Three compounds play a central role in the transduction of signals generated during pathogen attack: salicylic acid (SA), ethylene (ET), and jasmonic acid (JA). We performed a global analysis of gene expression in Arabidopsis submitted to exogenous treatment with SA, ET, and JA, as well as their combinations (SA+JA, SA+ET, JA+ET, SA+JA+ET). Genes have been grouped in regulons according to their expression profile. The resulting classes were used to build a Boolean model of the signaling network activated by the three hormones. The model has been inferred as minimal digital circuit and is represented in the form of a single network for computer simulation. Data mining was conducted to find transcription factors that undergo changes in expression during interactions with virulent but not with avirulent pathogens. A thorough analysis of these data reveals that some virulent bacteria manipulate the expression of genes controlling morphological traits and/or genes involved in salt and drought resistance. A new molecular strategy has been implemented to counteract the modifications of the signaling/genetic network induced by such pathogens.

T05-059

Identification and Characterisation of a Novel Pathogen Regulated Gene by Enhancer Trapping

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Tobacco rattle virus (TRV) tagged with the green fluorescent protein (GFP) was used to screen Arabidopsis thaliana lines containing a T-DNA based enhancer trap consisting of the beta-glucuronidase (GUS) reporter fused to a minimal cauliflower mosaic virus 35S promoter. One line showed induction of GUS activity by TRV-GFP inoculation, indicating that the T-DNA had inserted close to a virus responsive element. The gene controlled by this element was identified and named TRI (Tobacco Rattle virus Induced gene). The gene is alternatively spliced, and is predicted to encode two small proteins. TRI has significant similarity to a hypothetical open reading frame in Arabidopsis and to ACRE169, a Nicotiana tabacum gene rapidly induced in the Avr9/Cf-9 incompatible interaction.

We have shown that TRI is also induced by other viruses, Pseudomonas syringae, Perenospora parasitica, salicylic acid and jasmonic acid, but not by wounding, ethylene, excess light, abscisic acid or gibberellic acid.

Analysis of the DNA flanking the T-DNA insertion confirmed the presence of a pathogen responsive regulatory sequence. Potential stress and pathogen responsive cis-acting elements in this region are being characterised. We are also currently investigating the role of TRI in plant defence.

T05-060

Functional characterisation of LRR-type receptor-like kinases implicated in pathogen defense

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Members of the large gene family of LRR-receptor-like kinases have been shown to be both, products of plant R-genes, such as rice Xa21 as well as PAMP-receptors such as the flagellin sensing protein FLS2. These proteins show in one molecule high homology to receptors of the innate immune response of animals such as the TLR-receptors, and to the TLR-associated kinases. As only very few members of this gene family have been analyzed so far, we focus on the identification and functional characterization of novel LRR-RLKs implicated in plant pathogen response. By microarray hybridizations we identified a number of RLK-genes with increased transcript levels after treatment of the plants with either pathogens or elicitor molecules. T-DNA insertion lines of selected RLK-genes were tested for phenotypic alterations and altered stress responsiveness. So far, T-DNA knockouts in three of a total of 39 pathogen-responsive RLK-genes were analysed in more detail and revealed interesting pathogenesis-related phenotypes. One is more susceptible to infection with the virulent bacterium Pseudomonas syringae pv tomato DC3000 as well as to the necrotrophic fungus Alternaria brassicicola. Another one shows necrosis formation upon infiltration with type III-secretion system deficient bacteria which normally do not cause any macroscopically visible symptoms on wild type plants, and a third one is more sensitive to osmotic stress. The localization, biochemical activity and functional analysis of the encoded proteins as well as a screening for their interacting partners is currently in progress.

T05-061

Elongation factor Tu ¯ a novel PAMP involved in plant defence

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As all multi-cellular organisms plants have evolved sensitive perception systems to detect microbial invasion. Similar to the innate immune system in animals and insects, some of these detection systems are targeted against epitopes characteristic for fungi or bacteria in general ¯ so called PAMPs (Pathogen-Associated Molecular Patterns). In earlier work, we identified flagellin, the major building block of the bacterial flagellum, as a PAMP recognized by many plant species. In this study, by using a bacteria strain lacking a flagellin gene, we demonstrate that the most abundant and conserved bacterial protein, elongation factor Tu (EF-Tu), functions as a PAMP in Arabidopsis thaliana. By using a combined approach of chromatography, enzymatic digestions and MALDI-MS we could restrict elicitor-activity to an epitope of 18 amino acids. Elf18, a peptide representing this domain, is able to induce oxidative burst, increased ethylene biosynthesis, massive seedling growth inhibition, and expression changes in a high number of genes at subnanomolar concentrations as potent as flagellin (flg22). Moreover, elf18 signalling acts through a MAP kinase cascade, and pre-treatment of Arabidopsis leaves with elf18 induces increased resistance to subsequent infection with pathogenic Pseudomonas syringae pv. tomato. Clearly, Arabidopsis contains a high affinity binding-site for elf18 that cannot be competed by flg22. This demonstrates that elf18 perception involves a different receptor than FLS2. Currently we are identifying potential receptor candidates by reverse and forward genetic screens. Our experiments indicate that Arabidopsis thaliana has a highly specific recognition system for bacterial EF-Tu, which elicits a broad array of defence responses.

T05-062

Functional Genomics of Arabidopsis Heat Stress Transcription Factors (Hsfs)

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Heat stress transcription factors (Hsfs) are the major regulators of the plant heat stress response. Sequencing of the Arabidopsis genome revealed the existence of 21 ORFs encoding putative Hsfs assigned to classes A, B and C. Here we present results of a functional genomics approach to the Arabidopsis Hsf family focused on the analysis of their C-terminal domains harboring conserved modules for their function as transcription factors and their intracellular localization. Using reporter assays in tobacco protoplasts and yeast as well as GST pull-down assays, we demonstrate that short peptide motifs enriched in aromatic and large hydrophobic amino acid residues embedded in an acidic surrounding (AHA motifs) are essential for transcriptional activity of class A Hsfs. In contrast to this, class B and class C Hsfs lack AHA motifs and have no activator function on their own. We provide also evidence for the function of a leucine-rich region at the very C-terminus as a nuclear export signal (NES) of class A Hsfs. Sequence comparison indicates that the combination of a C-terminal AHA motif with the consensus sequence FWxxF/L,F/I/L as well as the adjacent NES represent a signature domain for plant class A Hsfs, which allowed to identify more than 60 new Hsfs from the EST data base.

Kotak et al. (2004) Plant J. 38, in press
Nover et al. (2001) Cell Stress Chap 6: 177-189

T05-063

Dissecting the oxylipin signature using a Gene Specific Tag (GST) microarray.

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The jasmonate family of regulators includes a variety of molecules all derived from the same source (tri-unsaturated fatty acids) but with different structures and different roles at the molecular level. In Arabidopsis leaves, 12-oxo-phytodienoic acid (OPDA) and the 16 carbon dinor-oxo-phytodienoic (dinor-OPDA) act together as essential signals in resistance to some insects, fungi and bacteria. Jasmonic acid (JA) is dispensible for this resistance but it, or one or more of its derivatives, helps modulate defense transcript abundance and is thus essential for a wild-type gene expression pattern during attack. Based on these and related results we predicted that some genes will be regulated exclusively by JA (or JA-derivatives) while others will be regulated in vivo by OPDA/dinor-OPDA or by a combination of JA and these cyclopentenone signals. A combination of mutant analyses and microarrays is being used to identify target genes for different jasmonates. We show here that dinor-OPDA can function as a signal similar to OPDA and that these cyclopentenone signals can preferentially (with respect to JA) activate the transcription of some target genes. Differentially expressed genes were displayed on a dedicated GST (Gene Specific Tag) microarray. Using various mutants including opr3 we found genes specifically up- and down-regulated by JA and a putative lectin gene that may be OPDA-regulated in vivo. The GST array can be used to compare gene expression in WT and mutant plants and can help dissect the relative importance of the cyclopentanone and cyclopentenone components of the jasmonate pathway in tissues distal to the wound site.

Stintzi, A. et al.,(2001) PNAS 98, 12837.
Liechti, R. and Farmer, E.E. (2002) Science, 296, 1649.
Farmer, E.E. et al.,(2003) Curr. Op. Plant Biol. 6, 372.
Hilson, P. et al.,.(2004) Genome Research, in press.

T05-064

Identification of CPR5-interacting factors using split-ubiquitin system

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Plants are constantly attacked by a variety of bacterial, fungal, and viral pathogens. They are often able to resist infection through a plant defense mechanism known as the hypersensitive response (HR). The HR causes localized cell death upon avirulent pathogen infection. Consequently, pathogens are restricted to a few infected cells, unable to spread to other parts of the plant. The HR releases a systemic signal that results in systemic acquired resistance (SAR). Mutation of the CPR5 gene in Arabidopsis thaliana mimics the HR, initiates SAR. Previous studies have shown that the CPR5 protein negatively regulates cell death. However, the molecular mechanism or downstream pathway remains unclear. CPR5 has two distinctive features: five transmembrane domains and a nuclear localization signal (NLS) region. Since these features do not tell how CPR5 regulates the cell death, we looked for interacting proteins. We used the split-ubiquitin system as it is designed for transmembrane proteins. Full length as well as several different truncated forms of CPR5 have been cloned into a bait vector in order to identify all possible interactors. Six putative candidates have been identified so far. Once the interacting proteins of CPR5 are characterized, molecular and genetic studies will be performed to better understand CPR5-mediated programmed cell death.

T05-065

Physiological plasticity of inducible defence responses in Arabidopsis

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Despite the identification of several key mechanism underlying disease resistance, comparatively little is known about the way environmental and developmental factors influence the capability of plants to defend themselves against microbial pathogens. We are currently investigating the physiological plasticity of individual defence responses in Arabidopsis plants challenged with avirulent strains of the bacterial pathogen Pseudomonas syringae. The approach seeks to characterize physiological situations strengthening or attenuating defence responses and aims to get insight into underlying cross-talk events. Moreover, comparison of the plants defence behaviour in different physiological conditions has the potential to provide new insights into the molecular mechanism underlying disease resistance.
For instance, we showed recently that the presence of light during pathogen infection is an essential requirement for a specific subset of inducible defence responses including local defences associated with salicylic acid signaling and the development of hypersensitive cell death. Light is also required for the development of systemic acquired resistance (SAR) in unchallenged leaves. Interestingly, plants exposed to high photon flux densities during infection exhibited a SAR response without systemic accumulation of salicylic acid (SA) and transcripts of the pathogenesis-related protein 1 (PR-1) suggesting that the SAR response can be separated from these 'characteristic' SAR markers. Comparing the local defence behaviour of rosette leaves differing in age we found that younger leaves generally had to invest in faster or more pronounced inducible defences than older (non-senescent) leaves to achieve a similar degree of resistance. Systemic resistance developed most effectively in younger leaves, and this was associated with strong accumulation of SA and PR-1. However, older leaves still exhibited SAR to a certain degree, and this resistance developed without systemic SA or PR-1 accumulation.

Zeier J, Pink B, Mueller MJ, Berger, S (2004). Planta (in press, published online April 20).

T05-066

Disease Resistance Signalling in snc1, a constitutively active TIR-NB-LRR Resistance-gene

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Plants have evolved a range of sophisticated mechanisms to defend themselves against all kinds of attacking pathogens. In gene-for-gene resistance plants are able to recognize specific pathogens by products of resistance (R)-genes, culminating in the onset of a variety of host responses that ultimately limit pathogen spread. Work in our group is focusing on unraveling the signal transduction pathways downstream of an R-gene in the TIR-NB-LRR class. In snc1 (suppressor of npr1-1, constitutive 1) a single amino acid change in the NL-linker region of an RPP5-homolog renders the R-gene and its downstream signalling pathways constitutively active. As a consequence, snc1 exhibits high endogenous levels of salicylic acid, constitutive expression of several pathogenesis-related (PR)-genes, and increased resistance to the bacterial pathogen Pseudomonas syringae maculicola ES4326 as well as the oomycete Peronospora parasitica Noco2 (Li et al., 2001; Zhang et al., 2003). snc1 also displays distinct developmental phenotypes including stunted growth and curly leaves.
In a screen for suppressors of the constitutive defence responses in snc1, a number of mutants were identified. So far 15 complementation groups of mos (modifier of snc1) mutants were compiled, including several alleles of the previously described pad4 (Jirage et al., 1999). 6 mos-mutants have recently been cloned, and all encode novel components of the snc1 signalling pathway. The mutants restore wild-type morphology either completely or partially and abolish the constitutive PR-gene expression in snc1. Our results indicate a complex signalling network downstream of SNC1 that may involve RNA processing and protein degradation among other things.
Here, I am presenting the story of mos5, a mutant that exhibits no constitutive PR-gene expression, lost the constitutive resistance to pathogens, has reduced endogenous levels of salicylic acid and suppresses the morphological phenotype of snc1 partially. MOS5 was cloned using a map-based approach and the mos5-1 mutant contains a 5 amino acid deletion. The cloning and analysis of the protein function of mos5 will be presented.

Jirage et al. (1999) PNAS 96:13583-88. Li et al. (2001) MPMI 14(10):1131-39. Zhang et al. (2003) Plant Cell 15:2636-46.

T05-067

NPR1 modulates salicylate- and jasmonate-dependent defense responses in plants

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Plants are continuously exposed to very different attackers in their natural environment. Immunity against these attackers depends on various signaling pathways in which salicylic acid (SA) and jasmonic acid (JA) play key roles. SA-dependent signaling is often responsible for resistance to biotrophic pathogens, whereas JA-dependent signaling is mainly effective against necrotrophic pathogens as well as insects. Cross-communication between signaling pathways provides the plant with the opportunity to efficiently fine-tune its defense response. Evidently, disrupting the optimal balance between endogenous SA and JA levels caused loss of resistance to the necrotrophic pathogen Alternaria brassicicola. SA exerts an inhibitory effect on JA signaling through activation of the regulatory protein NPR1. Nuclear translocation of NPR1 is essential for SA-mediated expression of pathogenesis-related genes. However, by controlling the nucleocytoplasmic localization of NPR1 we show that nuclear movement is not required for SA-induced suppression of the JA signal. Accordingly, SA-induced nuclear accumulation of an NPR1-GFP fusion protein was reduced in the presence of JA, suggesting that part of the NPR1 pool remained in the cytoplasm to suppress the JA signal. Cytosolic NPR1 may facilitate the delivery of negative regulators of JA signaling to the nucleus or inhibit positive regulators of JA signaling by physical interaction. We are currently investigating the signals that regulate distinct NPR1 functions and attempt to identify novel NPR1-interacting proteins involved in cross talk between SA and JA signaling pathways.

T05-068

EDM2: a novel regulator of disease resistance in Arabidopsis thaliana

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Resistance of plants to pathogenic microorganisms is often mediated by R-genes that allow specific molecular recognition of distinct pathogens and trigger signaling cascades activating defense reactions. The R-gene RPP7 mediates strong salicylate-independent resistance of Arabidopsis to the Oomycete Peronospora parasitica. A screen for mutants compromised in RPP7 mediated resistance led to the identification of the edm2-1 mutant. The edm2-1 mutation is recessive and blocks RPP7 mediated signaling upstream of the oxidative burst, one of the earliest known defense responses. We mapped the edm2-1 mutation to the gene At5g55390 on the lower arm of chromosome 5. Three independent T-DNA insertions in Atg55390 also resulted in complete loss of RPP7 function confirming that At5g55390 is EDM2. The predicted EDM2 protein shares no similarity with other known proteins required for R function and has several features typical for transcription factors, such as putative nuclear localization signals, transcriptional activation domains and two repeats similar to PhD-finger type zinc-finger domains. Experiments to confirm its role as a transcription factor and micro-array analyses are currently being performed.

T05-069

Cell-specific Gene Activation by Salicylic Acid

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Cell-specific Gene Activation by Salicylic Acid.

Induction of systemic acquired resistance (SAR) to plant pathogens (fungi, bacteria and viruses) requires activation of a signal transduction pathway that includes salicylic acid (SA) as a key component. The signal transduction pathway branches downstream of SA. One branch leads to induction of pathogenesis-related (PR) protein genes and resistance to bacteria and fungi. The other branch activates resistance to virus replication and movement (Sing et al., 2004, MPP 5: 57-64). SA induced changes in host gene expression may affect viruses by: a) induction of host genes encoding inhibitors of virus replication or movement, or b) repression of genes encoding proteins required to support virus replication or movement. These changes are likely to occur in a cell/tissue-specific manner because findings in our laboratory showed that in the leaf mesophyll cells of SA-treated tobacco plants the replication of TMV is greatly decreased (Murphy & Carr, 2002, Plant Phys. 28: 552-563). In contrast, SA did not decrease TMV replication in the initially inoculated epidermal cells. Instead, it induced resistance to movement between the epidermal cells. To identify cell-specific SA-induced changes in gene expression, more than 2000 transgenic lines of Arabidopsis which carry random insertions of the enhancer trap T-DNA (Haseloff, 1999, Methods in Cell Biology, Vol. 58) have been generated. If the enhancer trap T-DNA integrates close to a SA-activated promoter/enhancer, the synthesis of GFP will be activated. Approximately 700 of these lines have been screened for SA-induced cell-specific GFP fluorescence using epifluorescent microscopy. Eight lines, showing SA-induced alterations in tissue-specific GFP fluorescence in the root, have been rescued to date. These lines are being further characterized with the aim of isolating new genes regulated by the viral defense pathway.

T05-070

dsRNAi ¯ A reverse genetic tool to discover gene function in plant nonhost resistance

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Post-transcriptional gene silencing triggered by self-complementary hairpin RNA is widely used to knockdown transcripts of genes to elucidate their function. Compared to conventional mutant screens, dsRNAi technology has the potential to overcome the problem of genetic redundancy by targeting highly sequence related members of a gene family. When used in combination with an inducible expression system, dsRNAi might facilitate the analysis of genes whose heritable inactivation lead to severe developmental defects or lethality. To identify new components of nonhost disease resistance, hundreds of dsRNAi lines will be generated to target genes whose expression is shown to be up-regulated upon pathogen infection. The dsRNAi lines will be screened for altered infection phenotypes upon challenge with inappropriate foliar pathogens. To streamline the cloning procedure we developed GATEWAY compatible dsRNAi plant transformation vectors for constitutive and ethanol(EtOH)-inducible gene silencing. In order to evaluate the efficiency and effectiveness of these vectors we generated dsRNAi lines for GLABROUS1 (GL1) and the phytoene desaturase gene (PDS) as there knock-out phenotypes are readily discernable. In addition, dsRNAi constructs for silencing of EDS1, PAD4 and PEN1 were generated to optimize the timing of inducer (EtOH) and pathogen challenge. All tested dsRNAi lines were found to phenocopy heritable mutants of the respective genes. Because phenotypic stability of gene silencing over several generations is of crucial importance for our long-term goal, we are currently analyzing T3 and T4 generations of the dsRNAi lines mentioned above.

T05-071

OsEREBP BINDS TO TATA-BINDING PROTEIN, THEREBY REPRESSING THE BASAL TRANSCRIPTION OF GENES

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Many transcription factors such as WRKY, bZIP and ethylene-responsive element binding protein (EREBP) factors are known to be involved in plant defence responses. It is clear by now that control of gene expression in eukaryotes involves repression as well as activation of transcription. OsEREBP has previously shown to function as transcription activator upon pathogen infections. However, based on the result of transcription repression assay in yeast, OsEREBP represses GAL4 enhanced activation. The deletion analysis of OsEREBP indicates that its repressor domain is located at the N-Terminal of OsEREBP. There are number of ways in which transcriptional repressors can function. OsEREBP interacts to basal transcription factors such as TATA-binding protein and TFIIB, indicating that the mechanistic function of repression by OsEREBP is through the binding of basal transcription factors such as an human oncogene, p53. T33A and S66A mutants of OsEREBP did not interact with the TATA-binding protein and TFIIB, indicating that the N-terminal domain of OsEREBP is important for its repression activity. To investigate what kinds of genes can be repressed, microarray experiment with an OsEREBP RNAi transgenic line was carried out. Interestingly genes involved in abiotic stress tolerance is upregulated in the OsEREBP knock-out lines generated by RNAi vector. OsEREBP appears to repress genes involved in the abiotic stress tolerance. Taken together, the OsEREBP is a novel type of transcription factor reported so far in plants. This work is partly supported by the NIAB and ARPC grant to Dr. Duk-Ju Hwang in Korea

T05-072

Analysis of CIR1-mediated disease resistance in Arabidopsis

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Disease resistance in plants is controlled by a complex signal transduction network dependent on salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). To identify further components of this network, the cir1 mutant was previously identified in Arabidopsis thaliana (Murray et al. 2002). cir1 exhibits constitutive expression of SA- and JA/ET-dependent genes and constitutive resistance to the bacterial pathogen Pseudomonas syringae pv. tomato (Pst) and the oomycete pathogen Peronospora parasitica. The CIR1 gene appears to be a regulator of disease resistance and we are using global gene profiling to investigate the specific defence genes conferring resistance to these two different pathogens.

Mutants exist that disrupt the SA (npr1, nahG), JA (jar1) and ET (ein2) signalling pathways. Double mutants were constructed containing cir1 and each of these signalling mutants. cir1:NahG double mutants lost cir1-mediated resistance to both Pst and P. parasitica. However, cir1:jar1 and cir1:ein2 lost resistance to Pst but maintained resistance to P. parasitica... These double mutant phenotypes provide us with an avenue to identify defence genes required for bacterial resistance versus those required for resistance against the oomycete pathogen. Presumably, cir1 plants express a range of genes required for resistance against both pathogens. cir1:NahG plants have lost essential defence genes, whereas cir1:jar1 and cir1:ein2 plants have lost genes essential for resistance to Pst but not those essential for resistance to P. parasitica. We aim to identify these genes by microarray analysis of cir1 and cir1 double mutants.

Initially, Affymetrix Genechip assays (carried out at the GARNet facility) were used to determine expression of 8000 genes in cir1, cir1:ein2, ein2 and wild-type plants. These experiments identified 45 genes with a >2-fold increase in expression in cir1 compared to wild-type. Out of these, two subsets could be identified: 22 genes up-regulated in cir1 and cir1:ein2 and 23 genes up-regulated in cir1 only. These subsets represent possible disease resistance genes for P. parasitica and Pst respectively. Interestingly, approximately half of the genes up-regulated in cir1 encode unknown proteins. Verification of gene expression patterns via independent reverse Northern blot analysis has confirmed expression of 7 of these genes. We obtained T-DNA insertion lines in four of these genes from SALK and are currently investigating disease resistance in these plants. We are also extending the gene expression studies of the double mutants using full-genome oligo arrays printed by the University of Arizona.

T05-073

The PEN1 syntaxin defines a novel compartment upon fungal attack and is required for the timely assembly of papilla

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Attack by the host powdery mildew Erysiphe cichoracearum usually results in successful penetration and rapid proliferation of the fungus on Arabidopsis. By contrast, the non-host barley powdery mildew Blumeria graminis f. sp. hordei (Bgh) typically fails to penetrate Arabidopsis epidermal cells. In both instances the plant secretes cell wall appositions or papillae beneath the penetration peg of the fungus. Genetic screens for mutations that result in increased penetration of Bgh on Arabidopsis have recently identified the PEN1 syntaxin (Collins et al., 2003). Here we examine the role of PEN1's closest homologue, SYP122, identified as a syntaxin whose expression is responsive to disease. pen1 syp122 double mutants are both dwarfed and necrotic, suggesting that the two syntaxins have overlapping functions. Although syp122-1 and mur1 mutants have considerably more pronounced primary cell wall defects than pen1 mutants, SYP122 and MUR1 have only a very subtle, yet measurable, effect on penetration resistance. Upon fungal attack, PEN1 appears to be actively recruited to papillae, and there is a 2-hour delay in papillae formation in the pen1-1 mutant. We conclude that, while SYP122 may be involved in diffuse secretion, PEN1 is involved in the polarized secretion events that give rise to papilla formation.

Collins, N.C., Thordal-Christensen, H., Lipka, et al., 2003. Nature 425, 973-7

T05-074

Enhanced resistance to Cucumber mosaic virus in the Arabidopsis thaliana ssi2 mutant is mediated via an SA-independent mechanism

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The Arabidopsis thaliana SSI2 gene encodes a plastid-localized stearoyl-ACP desaturase. The recessive ssi2 mutant allele confers constitutive accumulation of the pathogenesis-related-1 (PR-1) gene transcript and salicylic acid (SA), and enhanced resistance to bacterial and oomycete pathogens. In addition, the ssi2 mutant is a dwarf and spontaneously develops lesions containing dead cells (Shah et al., 2001). Here, we show that the ssi2 mutant also confers enhanced resistance to Cucumber mosaic virus (CMV). Compared with the wild type plant, viral multiplication and systemic spread were diminished in the ssi2 mutant plant. However, unlike the ssi2-conferred resistance to bacterial and oomycete pathogens, the ssi2-conferred enhanced resistance to CMV was retained in the SA-deficient ssi2 nahG plant. In addition, SA application was not effective in limiting CMV multiplication and systemic spread in the CMV-susceptible wild type plant. The acd1, acd2 and cpr5 mutants which, like the ssi2 mutant, accumulate elevated SA levels, constitutively express the PR-1 gene, spontaneously develop lesions containing dead cells and are dwarfs, are, however, fully susceptible to CMV. Our results suggest that the basal resistance to CMV is enhanced specifically in the ssi2 mutant plant and that dwarfing, cell death and constitutive activation of SA signaling are not important for the ssi2-conferred enhanced resistance to CMV. However, the sfd1 and sfd4 mutations which, in addition to disturbing the ssi2-confers phenotypes containing dwarfing, spontaneously developing lesions and constitutively accumulating elevated SA levels, also affect lipid metabolism, suppress the ssi2-conferred enhanced resistance to CMV, thus implicating a lipid or lipids in the ssi2-conferred resistance to CMV. Interestingly, the ssi2-conferred resistance to CMV was compromised in the ssi2 eds5 plant, suggesting the involvement of a SA-independent, EDS5-dependent mechanism in the ssi2-conferred resistance to CMV (Sekine et al., 2004).

Shah et al., Plant J., 25:563-574. (2001)
Sekine et al., Mol. Plant-Microbe Interact., 17:623-632. (2004)

T05-075

Identification of General and Isolate-Specific Botrytis cinerea resistance mechanisms in Arabidopsis

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Botrytis cinerea is a major pathogen of fruit and vegetable crops throughout the world, causing both pre- and post-harvest grey mould. Plant resistance mechanisms used to defend against this pathogen, and their associated signaling pathways, are poorly understood. We used the Ler x Col recombinant inbred population to identify QTL governing susceptibility to this pathogen. Interestingly, most QTL were specific to individual B. cinerea isolates and not broad resistance loci. Additionally, there was significant epistasis among the resistance QTL. We are expanding the initial mapping population to help fine map the defined QTL, as well as including additional isolates of B. cinerea from a wide variety of plant hosts.

Camalexin is a major determinant of resistance to this pathogen as seen in both
Arabidopsis mutants and ecotypes. B. cinerea has variation for resistance to a number of plant produced toxins and we are screening numerous isolates for camalexin-insensitivity. This will allow us to test the ability of small metabolites to generate isolate-specifc resistance as well as investigate chemical warfare in plant-pathogen interactions.

In addition, we have uncovered a novel mutant, cir3, that constitutively expresses SA-, JA/ET- and ROI-dependent genes and has increased B. cinerea resistance. This resistance requires SA accumulation, as well as a functional EIN2 protein placing the cir3 locus upstream of both SA and JA/ET in the
plant/pathogen signaling network. Additional double mutant analysis is underway to investigate this phenotype further.

Denby KJ, Kumar P, Kliebenstein DJ (2004). Plant Journal 38, 473-486.

T05-076

Cell death induced by AtMEK5 activation is different with pathogen induced HR-cell death

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Mitogen-activated protein kinase (MAPK) pathways play important roles in the transduction of extracellular signals to intracellular targets in all eukaryotes. A specific set of three functionally interlinked protein kinases (MAPKKK or MEKK-MAPKK or MEK-MAPK) forms the basic module of a MAPK pathway. Expression of AtMEK5DD, an active mutant of the Arabidopsis AtMEK5, leads to the activation of AtMAPK3 and AtMAPK6 and promote a rapidly cell death (Ren, et al. (2002) J Biol Chem, 276:559-565). To compare the cell death induced by activation of AtMEK5DD with HR-cell death, we analyzed the activation of downstream MAP Kinase and induction of PR genes expression in permanent transgenic Arabidopsis plants. In gel kinase assay results show that the infection of Pseudomonas syringae DC3000 harboring Avr Rpt2 gene, also lead to activation of AtMAPK3 and AtMAPK6. Both of the PR1 and PR5 were strongly induced in plants undergoing HR-cell death caused by the infection of P. syringae DC3000, while only the expression of PR5 was strongly induced in plants expressing AtMEK5DD protein, and the PR1 was undetectable. We further analyzed the AtMEK5DD x NahG Arabidopsis plants. The expression of NahG protein can’t suppress the AtMEK5DD induced cell death phenotype. The results suggest that the AtMEK5 induced cell death and pathogen induced HR-cell death is controlled by the different pathways.

This work was supported by Fok Ying Tung Education Foundation (Grant No.91022), the National Natural Science Foundation of China (Grant Nos.30270664,30370140), the Excellent Young Teacher Program of MOE. P.R.China and the Scientific Research Foundation for the Returned overseas Chinese Scholar, State Education Ministry.

T05-077

RPM1-interacting protein RIN12 is a positive regulator of defense responses

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Early events in plant-pathogen interaction are mediated by highly specific molecular recognition processes. We are exploiting the model Arabidopsis thaliana-Pseudomonas syringae pathosystem to advance our understanding of the mechanisms of plant disease resistance.
Arabidopsis RPM1 is a membrane-associated protein that confers resistance against Pseudomonas syringae pv. tomato (Pst) strain DC3000 expressing either of two effector proteins, AvrRpm1 or AvrB. We have screened an Arabidopsis yeast two-hybrid (Y2H) library to identify proteins interacting with the apoptotic ATPase domain of RPM1. Several putative interactors were recovered, including a 72aa protein named RIN12 (RPM1-Interacting Protein 12).
Transgenic plants expressing sense or antisense RIN12 transcripts under the control of the strong viral promoter 35S are phenotypically normal. However, RIN12 overexpressers (RIN12S) challenged with DC3000(avrRpm1) show a dramatically delayed hypersensitive response (HR) and restriction of bacterial growth relative to wild-type. Conversely, RIN12 underexpressers (RIN12AS) challenged in the same way exhibit an accelerated HR and significantly increased bacterial growth. RIN12 transgenics also show altered timing of a number of physiological responses including H2O2 accumulation, cell viability and biophoton emission following challenge with DC3000(avrRpm1).
RIN12 encodes a protein with striking similarity to serine protease inhibitors of the potato inhibitor I class. RIN12 inhibits the proteolytic activity of subtilisin but does not inhibit trypsin. Point mutations in the predicted combining loop of RIN12 can abrogate inhibitory function. Detailed biochemical characterisation of the potential role(s) of this protein are ongoing.

T05-078

IDENTIFICATION OF KEY ARABIDOPSIS GENES REQUIRED FOR RESISTANCE AGAINST BOTRYTIS CINEREA

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We are interested in the molecular basis of disease resistance against necrotrophic plant pathogens, which is an important but relatively unexplored research area. To address this problem we are utilising the interaction of Botrytis cinerea with Arabidopsis thaliana as a model pathosystem. We have generated and screened a large population of T-DNA tagged Arabidopsis lines for either increased resistance or enhanced susceptibility to B. cinerea. Currently, we are focussing on four distinct mutations, two of which convey increased B. cinerea resistance and two that result in enhanced susceptibility towards this pathogen. The detailed characterisation of these mutations and the isolation of the corresponding genes are currently in progress. Recessive mutations in the Increased Botrytis Resistance (IBR1) gene were found to result in significant resistance against B. cinerea. The molecular basis of this phenotype was strongly potentiated expression of jasmonate-dependent genes, following attempted B. cinerea infection. The IBR1 gene was identified and found to encode a product possessing a MYB domain, which may function as a transcriptional activator or repressor. Interestingly, ibr1 plants exhibited enhanced susceptibility to the non-host pathogens, Pseudomonas fluorescens and Pseudomonas phaseolicola. Thus, IBR1 negatively regulates resistance against Botrytis cinerea and positively regulates non-host resistance against at least two bacterial pathogens. While the analysis of a substantial series of ibr1 double mutants is still in progress, early results suggest that IBR1 may function as a terminal node which integrates cross-talk from distinct disease resistance signalling pathways.

T05-079

Antagonistic interactions between the SA- and JA- signaling pathways in Arabidopsis modulate expression of defense genes and gene-for-gene resistance to Cucumber mosaic virus

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Gene-for-gene resistance to a yellow strain of Cucumber mosaic virus [CMV(Y)] is conferred by the dominant RCY1 allele in the Arabidopsis thaliana ecotype C24. An extensive network of cross-talk between the SA, JA and ethylene signal transduction pathways fine-tunes plant defense response to pathogens (1). While resistance to CMV(Y) was partly compromised in both, eds5 single mutant (eds5 COI1 RCY1) and eds5 coi1 double mutant (eds5 coi1 RCY1) plants that block accumulation of SA, direct comparison of the extent of viral growth indicated that the eds5 coi1 double mutant was less susceptible to CMV(Y) than the eds5 single mutant. Because the JA-insensitive coi1 single mutant plant (EDS5 coi1 RCY1) retains complete resistance to CMV(Y), our result suggests that the coi1 mutant partially restores resistance to CMV(Y) in the double mutant plants. Thus COI1, and hence probably JA signaling negatively regulate the SA-dependent signaling pathway in RCY1-conferred resistance to CMV(Y). To further confirm the negative regulatory effect of COI1 on SA-dependent signaling pathway in the CMV(Y)-infected C24 plant, the induction of four defense-related genes, PR-1, PR-5, PDF1.2 and HEL, was analyzed. The expression of PR-1 and PR-5 is controlled by SA-dependent signaling pathway, whereas the expression of PDF1.2 and HEL is mediated by the JA-dependent signaling pathway. As expected, the expression of PR-1 and PR-5 was induced in the CMV(Y)-inoculated leaves of C24. CMV(Y) inoculation-activated accumulation of the PR-1 and PR-5 gene transcripts was suppressed in CMV(Y)-inoculated leaves of the eds5 single mutant. In striking contrast, CMV(Y) inoculation-activated PR-1 and PR-5 expression was restored in the leaves of the eds5 coi1 double mutant. The levels of PDF1.2 and HEL transcripts were very low in the CMV(Y)-inoculated leaves of C24. However, the expression of PDF1.2 and HEL was strongly induced by CMV(Y) inoculation of the eds5 single plant, suggesting that EDS5 negatively regulates PDF1-2 and HEL gene expression in the CMV(Y)-inoculated leaves of a RCY1-bearing plant. In comparison to the eds5 single mutant, presence of the coi1 mutation in the eds5 coi1 double mutant depressed the CMV(Y)-induced expression of PDF1.2 and HEL genes. These results suggest that COI1 and EDS5 antagonize SA- and JA-signaling, respectively, in the CMV(Y)-inoculated RCY1-bearing tissue. Moreover, this cross-talk has important implications for the RCY1-conferred resistance to CMV(Y)(2).

(1) Glazebrook, J. (2001) Cur. Opin. Plant Biol. 4:301-308 (2)Takahashi, H. et al. (2004) Plant Cell Physiol. (in press)

T05-080

Arabidopsis basal immunity to the food-borne human pathogen Escherichia coli O157:H7

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Plants are normally immune to most bacteria including human pathogens. However, certain environmental and physiological conditions may effect plant basal resistance to bacteria and allow human pathogenic bacteria to multiply within the plant tissue. Enhanced survival and multiplication of human pathogenic bacteria such as Escherichia coli O157:H7 in fresh vegetables could lead to food poisoning. The molecular basis of plant immunity to human pathogens is unknown, but is likely to be mediated by the same or similar defense mechanisms that prevent the multiplication of other nonphytopathogenic bacteria. Aniline blue staining of Arabidopsis leaves inoculated with E. coli O157:H7 to detect callose revealed that Arabidopsis responds with a cell wall-based immunity similar to the response activated by nonpathogenic mutants of the plant pathogen Pseudomonas syringae. Suppression of host cell wall-based immunity by transgenic expression of the P. syringae type III effector AvrPto resulted in significantly enhanced multiplication of E. coli O157:H7. Transgenic expression of the P. syringae type III effector HopPtoD2 also resulted in enhanced multiplication of E. coli O157:H7. We are currently investigating the potential role of HopPtoD2 in suppressing host cell wall-based immunity. We are also conducting microarray analysis comparing Arabidopsis leaf tissue inoculated with E. coli O157:H7 or a flagellar mutant of this strain to tissue inoculated with the P. syringae hrpA mutant or a hrpA flagellar double mutant to identify genes whose products may be involved in cell wall-based immunity and genes that are regulated specifically by flagellin perception.

T05-081

The BIK1 gene of Arabidopsis encodes a protein kinase required for resistance to Botrytis cinerea.

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Plants activate a wide array of defense mechanisms against potential pathogens. These defense responses are regulated through different signaling cascades that can interact negatively or positively. In Arabidopsis, infection by the necrotrophic fungal pathogen Botrytis cinerea triggers the expression of the defense-related genes PR-1 and PDF1.2 that are molecular markers for SA- and ET/JA-dependent signal transduction pathways, respectively. The role of SA-dependent defense mechanisms in mediating resistance to Botrytis is not clear both in Arabidopsis and other plant species. In contrast, resistance to the pathogen is strongly impaired by changes in ET/JA accumulation or responsiveness in Arabidopsis and tomato. Here, we present data identifying the Botrytis-induced serine-threonine protein kinase gene BIK1 as a novel component of the Arabidopsis defense-signaling pathways. The BIK1 transcript is not induced by exogenous application of SA, MeJA or ET and its transcriptional activation upon Botrytis infection does not require the accumulation and signaling of these hormones. Loss of BIK1 results in severe disease susceptibility to Botrytis but increased resistance to the virulent strain of the bacterial pathogen Pseudomonas syringae pv. tomato. These altered disease phenotypes are not associated with a constitutive expression of defense-related genes and the presence of spontaneous cell death lesions typical of lesion mimic mutants but rather to hyper accumulation of PR-1 and down-regulation of PDF1.2 gene transcripts upon pathogen attack. Our data implicates BIK1 as a modulator of antagonistic interactions between defense signaling pathways.

T05-082

The Hyaloperonospora parasitica avirulence gene ATR13 reveals an intense “arms race” in progress with the Arabidopsis resistance gene RPP13.

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The resistance gene RPP13 is a classic resistance gene of the coiled-coil: nucleotide binding site: leucine rich repeat class. The gene is present as a functional allelic series where different alleles recognise different pathogen isolates. It is the most variable plant gene cloned to date and exhibits extreme variation within the leucine rich repeat domain. This suggests it is under positive environmental selection for change. A key candidate that could be driving this change is the complementary avirulence gene ATR13. Hence, we have applied map-based cloning techniques to define a mapping interval for ATR13. We used the SSH technique to identify in planta expressed genes and one of these co-segregated with ATR13. In planta assays were used to confirm that this gene caused an RPP13 specific response. ATR13 is a highly variable protein suggesting that there is indeed an ‘arms race’ in progress between plant and pathogen. We will describe the structure of ATR13 and the relationship between RPP13 alleles and recognition of ATR13 alleles.

T05-083

scv1 is a suppressor of cpr5-mediated disease resistance

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Plants are often able to resist disease by inducing the Hypersensitive Response (HR), a process characterized by rapid cell death at the site of infection. The HR not only restricts the primary infection, but also initiates Systemic Acquired Resistance (SAR), which protects against subsequent secondary infection. In the absence of pathogens, the Arabidopsis cpr5 (constitutive expresser of pathogenesis related genes) mutant develops lesions that mimic the HR. Consequently, cpr5 induces SAR, resulting in elevated salicylic acid (SA) levels and enhanced resistance to virulent strains of the bacterial pathogen Pseudomonas syringae and the oomycete pathogen Peronospora parasitica. Conversely, the constitutive HR in the cpr5 mutant renders it more susceptible to several necrotrophs, such as Botrytis cinerea. The cpr5 mutant also shows developmental phenotypes, including early senescence and reduced cell expansion. To better understand the role of cpr5 in disease resistance signaling, 100,000 mutagenized cpr5 seedlings were screened for suppressors with restored susceptibility to P. parasitica NOCO2. No second site mutation could completely rescue the cpr5 mutant morphology. One partial suppressor, however, restored all of the cpr5 disease resistance phenotypes to wild-type. The cpr5scv1 (suppressor of cprV) double mutant has wild-type susceptibility to P. parasitica, slightly enhanced susceptibility to P. syringae and resistance to B. cinerea. Additional characterization and positional cloning data for scv1 will be discussed.

T05-084

Global transcription profile changes controlled by the Arabidopsis gene Botrytis susceptible 1 (AtMYB108).

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A genetic screen for altered disease phenotypes in response to the necrotrophic fungal pathogen Botrytis cinerea resulted in the isolation of the Arabidopsis bos1 mutant characterized by enhanced pathogen susceptibility. The BOTRYTIS SUSEPTIBLE1 gene encodes an R2R3MYB transcription factor protein involved in the control of Botrytis growth and disease symptom expression. Analyses of the spectrum of bos1 susceptibility to other pathogens revealed that BOS1 mediates resistance also to another necrotrophic pathogen Alternaria brassicicola. Interestingly, compared to wild type, bos1 showed more symptoms but comparable extent of microbial growth upon challenge with Pseudomonas syringae pv. tomato and Peronospora parasitica. The bos1 plants were more sensitive to several abiotic stresses and to treatments with reactive oxygen species (ROS)-generating compounds. To identify downstream components of the BOS1-mediated signal transduction pathway and to elucidate its role in controlling plant tolerance to multiple environmental stimuli, we compared the global expression profile of 35S::BOS1 and wild type plants. The expression of a total of 126 genes was significantly (more than 2-fold) changed by the constitutive expression of the transcription factor. Among 48 up-regulated transcripts we found: several genes encoding defensins, small cysteine-rich antimicrobial peptides associated with the induction of ET/JA-dependent defense responses, and one gene each encoding a member of family 1, 4, and 5 of the pathogenesis-related proteins; genes encoding enzymes involved in detoxification of ROS; genes with regulatory function in the activation of tolerance mechanisms to different abiotic stresses. The data indicate that BOS1 is responsible for the activation of a subset of defense pathways that are explicitly active in controlling the spread of necrotrophic pathogens. BOS1 plays a role in conferring tolerance to multiple stresses most likely by activating recovery mechanisms to oxidative stress.

T05-085

Prediction of multiple Arabidopsis targets for the Pseudomonas effector protease AvrRpt2

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During infection, the bacterium Pseudomonas syringae secretes effector proteins such as AvrRpt2 into Arabidopsis thaliana cells. Following delivery into the plant cell, AvrRpt2 is N-terminally processed to its mature form, which has cysteine protease activity that causes elimination of the Arabidopsis RIN4 protein. Elimination of RIN4 activates the disease resistance protein RPS2, resulting in localized cell death, systemic resistance and decreased pathogen proliferation. Mutation of predicted catalytic triad residues of AvrRpt2 prevents AvrRpt2 processing, RIN4 elimination and RPS2-mediated pathogen recognition.

In plants lacking RPS2, AvrRpt2 contributes to pathogen virulence. Interestingly, AvrRpt2 increases virulence even in the absence of RIN4, suggesting that elimination of RIN4 is not the sole function of AvrRpt2 and that additional Arabidopsis proteins are targets of its protease activity. To facilitate identification of AvrRpt2 targets, the cleavage recognition sequence for AvrRpt2 was determined. Variants of this seven amino acid sequence span the AvrRpt2 processing site and occur twice in RIN4. Mutation of these sites in AvrRpt2 and RIN4 prevent processing and elimination, respectively. Furthermore, a synthetic substrate containing these sequences is cleaved by AvrRpt2, demonstrating that seven amino acids are sufficient to elicit AvrRpt2-dependent cleavage. Based on these sequences, hidden Markov models were developed to identify Arabidopsis proteins containing putative AvrRpt2 cleavage sites. The potential of these proteins to serve as substrates for AvrRpt2 protease activity will be presented.

T05-086

Functional analysis and expression studies of the flagellin receptor FLS2

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Innate immunity is based on the recognition of so-called pathogen-associated molecular patterns (PAMPs). One prominent PAMP in plants and animals is flagellin, the major component of the bacterial motility organ. Flagellin signalling in Arabidopsis is initiated by the perception of its most conserved domain, represented by the flg22 peptide. This leads to the activation of a MAP kinase cascade, the induction of numerous defence-related genes, and induced resistance to virulent bacteria. The FLS2 gene, encoding the receptor-like kinase FLS2, is essential for flagellin signalling, since fls2 mutants are insensitive to flg22.

Here, we demonstrate that FLS2 functions as the flagellin receptor. Like the previously identified flg22 binding site, FLS2 is found to be a glycosylated protein. Cross-linking experiments with radioactive labelled flg22 reveal a band of ~160 kDa, which co-migrates with a band detected by antibodies against FLS2. Moreover, in plants expressing a FLS2-GFP fusion protein, both the cross-linked and the immuno-detected bands are shifted to ~190 kDa.

In previous work we have shown that the protein kinase activity of FLS2 is not only required for signal transmission, but also for flg22 binding. Here, we focus on several conserved serine and threonine residues within the protein kinase domain of FLS2. We have transformed fls2 mutant plants with FLS2 constructs, in which these residues are substituted. All three FLS2 mutations in one of the three conserved threonines are able to restore binding of flg22, but not the early and late responses to flg22. Thus, phosphorylation of these residues appears to be critical for signal transmission but not for binding.

We have generated transgenic fls2 mutant plants expressing an FLS2-GFP fusion under the control of its native promoter, which re-gain full responsiveness to flg22. Using confocal microscopy, we show that FLS2-GFP expression in leaves occurs in mesophyll and epidermal cells including stomata, the possible entry sites for invading bacteria. Furthermore, the fluorescence signal is also present in roots, stems and flowers, and it is always clearly localized to the plasma membrane. Ongoing studies focus on the localization of the fluorescence signal after treatment of the transgenic plants with flg22.

T05-087

The Dead Zone: Exploration of the Host Pathogen Interface of the Alternaria brassicicola-Brassicaceae Pathosystem

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A. brassicicola causes black spot disease of plants found within the Brassicaceae plant family and has been used as a model necrotrophic fungal pathogen of Arabidopsis for over a decade. We are undertaking a multi-faceted approach at dissecting the interaction between select members found within the Brassicaceae (including Arabidopsis) and this pathogen. To this effect we have sequenced and characterized approximately 10,000 expressed sequence tags (ESTs) from subtracted and full-length cDNA libraries derived from RNA isolated from 1) compatible interactions with cabbage and canola (Brassica oleracea, B. napus), 2) A. brassicicola spore germination on the Arabidopsis leaf, and 3) A. brassicicola during nitrogen starvation. A blastable database containing these ESTs was created. Annotation/ putative ID of the ESTs derived from cultivated Brassicas and the fungus has relied heavily upon the utility of the Arabidopsis and publicly available fungal genome sequences. Several fungal genes (secondary metabolism/signal transduction) have been selected from ESTs for functional analysis using KO mutagenesis. A related project undertaken is a traditional biochemical and proteomics approach to characterize putative virulence factors in the form of secreted phytotoxic proteins from A. brassicicola. Secreted phytotoxic proteins in culture filtrates and spore germination fluids were initially identified by fractionation using HPLC coupled with in vivo bioassays. 1-D PAGE was used to separate select mixtures. Gel plugs were excised from bands visualized using a BioRad ProteomeWorks Spot Cutter and trypsin digested. Resulting peptides were then subjected to nLC-microESI-MS/MS analysis (Finnigan LTQ). Peptide sequences were blasted against our EST databases in order to identify putative fungal genes encoding phytotoxic proteins. Interestingly one of the thus far confirmed toxic proteins (Altb1) has homology to a major human allergen suggesting it may be an ancient eukaryotic virulence factor (see Cramer and Lawrence, 2003). Functional analysis of a subset of these genes is currently underway. Finally, in order to further elucidate mechanisms of host incompatibility, we have been using recombinant inbred lines derived from Col x Ler to map susceptibility loci in Arabidopsis. We have identified two regions (Chr2 and Chr3) that appear to significantly contribute to susceptibility in Ler. A map-based cloning approach is being used to isolate these genes.

Cramer, R and Lawrence, CB. 2003. Applied and Environmental Microbiology 69: 2361-2364.

T05-088

HPH, a Repressor of Phototropic Responsiveness in Arabidopsis

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Phototropism, or the bending of a plant organ towards or away from directional light, represents a rapid and visually obvious response of plants to changes in their light environment. A number of proteins involved in the phototropic perception, transduction, and response have been identified, most via loss-of-function mutational screens. While robust, screens for loss-of-function mutations fail to identify either redundant genes or negative regulators of the process being examined. Thus, we have developed a new screen to identify physiological gain-of-function mutants to allow a broader coverage of the possible recoverable mutations. Briefly, dark-grown mutagenized seedlings are exposed to a unidirectional blue light treatment that normally fails to induce a response in wild-type seedlings and positively phototropic seedlings are selected. Using this approach we have identified three mutants that respond both more quickly and stronger than their wild-type background. The recessive nature of each of these mutations indicates that the associated gain-of-function phenotypes result from genetic loss-of-function lesions in a negative regulator. One of these mutants, hph/ (h/yper-p/ hototropic h/ypocotyl), is being characterized in detail. Physiological analyses indicate that HPH is involved specifically in phototropism as the mutant exhibits a wild-type gravitropic response. A large F2 population has been generated from a cross of hph with a polymorphic wild-type background and hph mutants subsequently selected for mapping purposes. HPH represents the first repressor molecule identified in the phototropic signal-response pathway.

T05-89

Compositional changes in the plant defence compounds of Arabidopsis thaliana alter the feeding behaviour of the generalist herbivore Spodoptera littoralis

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Plants have devised a wide array of defensive mechanisms against herbivores and pathogens. Secondary metabolites appear to function in chemical defence as feeding deterrents or toxins as exemplified by the glucosinolate-myrosinase system commonly found in plants of the order Brassicales. Glucosinolates are sulphur-rich _-thioglucosides, while myrosinases are thioglucosidases capable of hydrolyzing glucosinolates. Upon cell breakage the glucosinolates and myrosinases come into contact with each other. The glucosinolates are subsequently hydrolyzed to a suite of compounds some of which have cytotoxic effects. Over 20 different glucosinolates have been identified in the Columbia accession. The most abundant glucosinolates and those showing the highest structural diversity are the16 derived from methionine. To produce these glucosinolates, methionine is modified by a stepwise addition of one to six carbon molecules. After each addition the modified methionine can be converted to a glucosinolate or undergo a further carbon addition. The chemical properties of the hydrolysis products of methionine-derived glucosinolates such as volatility will differ with their carbon chain length and these characteristics will be important when considering the biological function and possible toxic effects of the glucosinolate-myrosinase system. The main subject of inquiry has been to determine if there is a measurable difference in the performance of generalist herbivores due to the chain length of methionine-derived glucosinolates in Arabidopsis thaliana. We have used four different mutants providing a spectrum of plants varying in the profiles of methionine derived glucosinolates and assayed them for differences in feeding deterrence and toxicity using choice and null choice trials with the generalist herbivore Spodoptera littoralis. In choice experiments, caterpillars were allowed to choose freely in randomized populations of the plant lines. The amount of plant tissue consumed for each line was determined. In null choice experiments, caterpillars at the neonate stage were placed on trays containing a single plant line. Caterpillars were reared throughout their development on this particular line. Several parameters of development including caterpillar weight gain were measured. Together, the results indicate an effect of chain length differences of the methionine-derived glucosinolates on the preference of food choice and caterpillar performance.

T05-090

A Synthesis for Understanding Disease and Disease Resistance

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Our understanding of the molecular control of plant disease resistance has increased dramatically in the last 15 years, and the Arabidopsis community has had a large role in this progress. We now know the limited classes of proteins that are specifically “recognizing ” pathogen encoded molecules. We continue to ascribe function to more individual members from each of these protein classes. Forward genetic screens for loss of resistance phenotypes have led to the construction of a network of inter-digitated signaling pathways involved in successful disease resistance responses. A variety of rapid signaling events, ion and calcium fluxes, an oxidative burst, and activation of MAP kinase cascades control the output of recognition, and generate small signaling hormones like Salicylic Acid, Jasmonic Acid and ethylene. These small molecules play important roles in controlling the ultimate level of disease resistance in the infected tissue, and in systemic tissue of both apical and basal plant organs. Their differential induction appears to correlate with their life history of the pathogen in question. Massive transcriptional and metabolic re-programming are part of the defense response, and the tools of genomics are being applied to analysis these phenomena. Yet, I have the sense that this field is beginning to drown in details as the dissection of disease resistance become more and more refined while still lacking an overall governing paradigm. As requested by the organizers for overview talks, I will review what I consider to be the current high points of the field and I will present what I think is an increasingly defensible model for how pathogen recognition in plants is controlled and how pathogens have evolved means to manipulate steps in the recognition pathway(s).

T06 Natural Variation and Comparative Genomics Including Genome

T06-001

Transposon Activation in Arabidopsis Neopolyploids

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Polyploids are common and arise frequently by genome duplication (autoployploids) or interspecific hybridization (allopolyploids). In spite of their frequency in nature, early generations of human-made allopolyploids display sterility, inviability, phenotypic instability, gene silencing and epigenetic changes. Little is known about the molecular basis of these problems and of their resolution upon the stabilization that occurs with progressing generations. We present evidence that instability of Arabidopsis auto- and allopolyploids is associated with activation of some but not all transposons, which become transcriptionally and transpositionally activated. One of the activated transposons represents a new family of conditionally active En/Spm-like transposons in Arabidopsis.

T06-002

An inducible targeted tagging system for localized saturation mutagenesis in Arabidopsis thaliana.

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We describe a system of inducible insertional mutagenesis based on the Ac-Ds family of transposons for targeted tagging in Arabidopsis. In this system, the T-DNA construct has both Ac and Ds elements placed on the same construct.

The approach incorporates inducible gene tagging where the levels of transposase gene expression can be controlled to generate transpositions that are subsequently stabilized without requiring crossing and segregation. We have mapped 41 single copy lines by TAIL-PCR, which can be used as potential launch pads for heat shock mutagenesis. Using a starter line selected for detailed analysis, the efficiency of tagging over a 50Kb region in the genome was examined.

Hits were obtained in the targeted genes with multiple alleles for most of the genes including one relatively small gene for which there are no knockouts available in other publicly available large databases. There were approximately equal numbers of hits detected in genes on either side of the T-DNA with no preference of insertions observed in the region targeted.

These results establish the feasibility of our approach, which could be applied to plants for which T-DNA tagging is not simple.

T06-003

Diversity and redundancy within the Arabidopsis formin family

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Formins (FH proteins) are a family of actin-nucleating proteins conserved from yeast to mammals, playing important roles in cytokinesis, cell polarity, vesicle trafficking and motile cell migration. Their hallmark actin-binding domain, FH2, is capable of dimerization and is usually located C-terminally, while N-terminal domains serve as regulatory regions. Formin homologues have been recently identified also in higher plants [1,2].
We have performed a thorough bioinformatic analysis of available angiosperm formin sequences, with main focus on the Arabidopsis and rice genomes. This led to major revision in the annotation of the 21 Arabidopsis formin-related genes, supported by cDNA sequencing in selected cases. We confirmed a good conservation of the previously described two subfamilies of plant formins (Class I and Class II), both exhibiting N-terminal plant-specific features. Class I contains mostly proteins with putative membrane insertion signals, suggesting a role in cytoskeleton-membrane connections, while many Class II formins share a conserved domain, related to human Pten domain PDB 1d5r that may also participate in formin localization [Cvrckova et al., BMC Genomics, submitted].
The large size of the plant formin family, effectively increased by protein heterodimerization and alternative splicing, is in a sharp contrast with the other conserved actin nucleator - the Arp2/3 complex - whose inactivation is, surprisingly, non-lethal in Arabidopsis. We have demonstrated differential regulation of some of the Arabidopsis formin paralogues by analysis of public microarray data; however, functional redundancy can be expected in genes whose expression overlaps. Indeed, neither single nor several double T-DNA insertions in formin genes characterized so far in our laboratory show obvious phenotypes, and crosses of single mutants with stable GFP-talin transformants did not reveal obvious abnormalities in actin organization. However, we could not obtain AtFH12 homozygous mutants expressing GFP-talin, which itself is moderately toxic, suggesting possible synthetic lethality.
This work has been supported by the GACR204/02/1461 grant and by Uppsala University and the Linnaeus Centre for Bioinformatics funds to M.N.

[1] Cvrckova, Genome Biol. 1:research 001, 2000
[2] Deeks et al., TiPS 7:492, 2002

T06-004

Comparative Biochemical Genomics of Glucosinolate Chain Elongation in Arabidopsis & Friends

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Glucosinolates (‚mustard oil glycosides') are a diverse group of amino acid-derived plant secondary compounds found in Arabidopsis and other cruciferous plants. They contribute to resistance against herbivorous insects and other pests, but also influence the quality of agriculturally important crops. Glucosinolate biosynthesis occurs in three independent stages, side chain elongation of a precursor amino acid, formation of the glucosinolate core structure, and side chain modification. Precursor amino acid, side chain elongation and side chain modification contribute to variation of glucosinolate structures: More than 30 different glucosinolates are known from Arabidopsis, and 120 different glucosinolate structures have been identified in crucifers and related plant orders.
Methylthioalkylmalate synthase (MAM) enzymes encoded at the GS-Elong locus are central to diversity of methionine-derived glucosinolate structures as they are responsible for the committed step in glucosinolate side chain elongation, the condensation of a 2-oxo acid derived from methionine homologues with acetyl-CoA. Due to recurrent cycles of glucosinolate side-chain elongation, MAM synthases predetermine the outcome of subsequent modification steps. In Arabidopsis thaliana, MAM gene composition at GS-Elong is highly variable, and frequent independent gene deletion and gene conversion events indicate extreme evolutionary dynamics.
To understand the evolution of GS-Elong at a broader phylogenetic scale, we have sequenced the corresponding locus from other, closely related crucifer species. MAM genes were heterologously expressed and their biochemical properties were investigated. Our findings suggest on one hand that tandemly linked MAM genes at GS-Elong in general evolve according to a birth-and-death model including gene conversion, but, on the other, highlight exceptionally high evolutionary dynamics in A. thaliana.

T06-005

Quantitative trait locus analysis of the phase of the Arabidopsis circadian clock

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The plant clock is the least well characterised of circadian systems. Mutant screens have identified a number of putative components, but an alternative approach using forward genetics takes advantage of natural allelic variation in Arabidopsis ecotypes to identify areas of genome responsible for variation in clock traits. This means that intact, viable systems are the basis of analysis. We use a transgenic approach to monitor circadian output, measuring the activity of the cab2::luc+ transgene in Arabidopsis seedlings. The circadian parameter we are primarily interested in is 'phase', i.e. the timing of a point of the circadian cycle relative to an external timing cue. We chose the timing of the peak of cab2::luc+ expression after the last light-based timing cue (i.e. dawn) as our phase marker. Phase was measured in transgenic Arabidopsis seedlings from a population of recombinant inbred lines (RILs), whose genomes are homozygous mosaics derived from two inbred parental genotypes (Ler x Cvi). Quantitative trait locus ('QTL') analysis attempts to correlate phenotypic variation with genetic variation within a population; both types of variation arise from the natural allelic variation present in the parents. The analysis was repeated on phase data obtained after entrainment to different photoperiods. We found loci in which Cvi alleles either advanced or delayed the phase relative to Ler. We are investigating whether these phase loci are the same as those previously identified for period as this will help clarify the relationship between parameters of the circadian clock. Current research is therefore focused on characterising these loci.

T06-006

Genomics of the Arabidopsis and rice CBL-CIPK signaling networks

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Plant responses to many abiotic stimuli involve calcium signals. Calcium-binding proteins are involved in sensing and relaying these signals to downstream signaling and adaptation responses. Calcineurin B-like proteins (CBLs) represent a novel group of calcium sensor proteins likely to function in deciphering calcium signals. CBLs exclusively interact with a group of serine-threonine kinases designated as CBL-interacting protein kinases (CIPKs). Here, we present a comparative genomics analysis of the full complement of CBLs and CIPKs in Arabidopsis and rice (Oryza sativa). We confirm the expression and transcript composition of the 10 CBLs and 25 CIPKs encoded in the Arabidopsis genome. Our identification of 10 CBLs and 30 CIPKs from rice indicates a similar complexity of this signaling network in both species. An analysis of the genomic evolution suggests that the extant number of gene family members largely results from segmental duplications. A phylogenetic comparison of protein sequences and intron positions indicates an early diversification of separate branches within both gene families. These branches may represent proteins with different functions. Protein interaction analyses and expression studies of closely related family members suggest that even recently duplicated representatives may fulfill different functions. This work provides a basis for a defined further functional dissection of this important plant-specific signaling system.

Kolukisaoglu Ü., S. Weinl, D. Blazevic, O. Batistic, J. Kudla (2004), Plant Physiol., 134, 43-58.

T06-007

Comparative transciptomics analyses of abiotic stress responses in Arabidopsis

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Plants respond to environmental challenges in part by altering their gene expression profiles, which ultimately leads to various adaptive responses at the cell and whole-plant levels. Low temperature, drought, high salinity and osmotic stress are common stress conditions that adversely affect plant growth and crop production. These factors are very complex stimuli that possess many different yet related attributes, each of which may provide the plant cell with quite different information. Understanding the mechanisms by which plants perceive environmental signals and transmit the signals to cellular machinery to activate adaptive responses is of fundamental importance to biology.
Here we present a comparative full-genome expression analysis of Arabidopsis in response to drought, cold, osmotic and NaCl stress. After the respective stress treatments of hydroponically grown two-week-old plants, RNAs were extracted from root and shoot tissues. The kinetics of stress responses covered seven time points ranging from 15 min to 24 hours. The respective cRNAs were hybridized to Affymetrix Arabidopsis 25k-Chips. Here will describe the details of experimental design and a summary of our data analyses.

This work is part of the AtGeneExpress initiative funded by the DFG.

T06-008

Geographical variation in Arabidopsis thaliana arises from recombination

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Abstract for the poster:
A representative sample of 93 accessions of Arabidopsis thaliana was chosen covering the entire natural Eurasian range of the species. A gene (~1500 bp) and a non coding region (~500 bp) were sequenced, which showed correlation between genetic variation of single nucleotide polymorphisms (SNPs) and geographical distribution. Both genomic regions were located on the short arm of chromosome 2, 300 kbp apart. In summary, 106 SNPs were identified in the two genomic fragments.
Minimum spanning trees and split composition analysis indicated recombination between two distinct ("old") haplotypes in addition to ("young") mutations as the basis of haplotype evolution in each of the two fragments. A. thaliana seems to have survived the ice ages in two widely separated (South-western European and Middle Asian) refugial populations. These populations meet and recombine during their ongoing (and anthropogenic) post-glacial range expansion.

T06-009

Natural Variation of Flowering Time of Arabidopsis thaliana Wild Strains with Focus on FRIGIDA and FLOWERING LOCUS C

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Wild strains of Arabidopsis thaliana grow and reproduce in a wide range of environments on the Northern hemisphere. These wild strains show extensive genetically-controlled phenotypic variation, which is a valuable resource both for mechanistic studies and for understanding the basis of genomic and genetic variation.
In order to explore the natural variation of flowering time, a systematic flowering time screen was performed. Flowering time is influenced mostly by light, ambient growth temperature and vernalization. Therefore, 165 A. thaliana wild strains and several flowering time mutants were examined under four growth conditions, differing in light, ambient growth temperature and vernalization (16°C long day, 23°C long day, 23°C short day, 16°C long day with vernalization). These studies revealed that there is substantial variation in the flowering behaviour of A. thaliana wild strains. The large number of investigated wild strains confirmed FRIGIDA (FRI) and FLOWERING LOCUS C (FLC) as major determinants of flowering time, conferring the winter annual habit. But even within the groups of winter annuals and rapidly cycling wild strains there remains extensive continuous variation that cannot be explained by FRI/FLC. In addition, much of the variation that remains after vernalization is likely to be independent of FRI/FLC. Several interesting wild strains with an attenuated response to ambient temperature, photoperiod or vernalization respectively, could be identified.

Further analyses of FRIGIDA (FRI) and FLOWERING LOCUS C (FLC) were undertaken. Non-functionality of FRI or FLC leads to an early flowering response. Although the two known deletions in FRI (Col- and Ler-type deletions) were present in almost half of the surveyed wild strains, we also found early flowering strains that lacked these FRI deletions. Thirteen early flowering strains without these prevalent deletions in FRI were further analyzed by complementation crosses to FRI flc, fri FLC, fri flc and FRI FLC, as well as sequence analysis. The early flowering phenotype of these strains could be explained by new weak or non-functional alleles of FRI and FLC.

T06-010

Natural Genetic Variation Within and Between Species

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Why do we find functionally important genetic variation within and between plant species? What evolutionary and historical forces influence these polymorphisms, and how do they enable us to better understand function and mechanism in plant biology? I will summarize recent advances in studies of natural genetic variation and comparative functional genomics, with emphasis on biological hypotheses and conceptual background. We will consider association studies, evolutionary history, and the evolution of gene regulation. Finally, I will show parallels with the genetics of human complex disease, and show how advances in related fields have important implications for Arabidopsis biology and plant comparative genomics.

T06-011

Mapping Quantitative Trait Loci governing flowering time in Arabidopsis using Stepped Aligned Recombinant Inbred Strains(STAIRS)

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Mapping Quantitative Trait Loci governing flowering time in Arabidopsis thaliana using Stepped Aligned Recombinant Inbred Strains (STAIRS).

The majority of genetic variation in plants and animals is quantitative and multifactorial in nature with individual traits being under the control of a number of genes. Mapping such quantitative trait loci (QTL) is extremely important for trait manipulation in plant breeding programs. A major problem in QTL mapping is that the confidence intervals associated with their location are frequently large (10-30 cM) making it difficult to identify candidate genes. The objective of our current research is to test the feasibility of a novel approach based on 'chromosome engineering' for identifying the source and underlying nature of allelic variation at individual QTL. We are using a specially generated resource collection of Arabidopsis thaliana lines, designed for fine gene mapping across the whole genome.
This resource consists of individual Chromosome Substitution lines (CSS) and the associated Stepped Aligned Inbred Recombinant Strains (STAIRS) (Koumproglou et.al, 2002). QTL analysis studies using STAIRS have indicated the presence of significant flower QTL in the top 20 cM of chromosome 3. In order to further define the region we used a marker assisted backcross breeding program to generate additional STAIRS, which differ in introgression size by 1 cM within the region of interest. We have started plant house trials with the refined STAIRS for the quantitative genetic analysis, which is expected to reveal the location of the relevant QTL with greater precision. Gene expression profiling and allele sequence analysis are planned at the moment to study the nature and action of the genes involved.

Koumproglou et. al. (2002) High-resolution QTL analysis using STAIRS in Arabidopsis. Plant Journal 31(3) 355-364

T06-012

Evolutionary potential of the chalcone synthase cis-regulatory region in Arabidopsis thaliana

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Cis-regulatory domains can evolve rapidly because the multiple transcription factor binding sites they harbour are often short and redundant. However, little is known about the adaptive evolution of these sequences over short evolutionary time scales. The cis-regulatory region of chalcone synthase (CHS), a key enzyme in the flavonoid pathway, offers a good opportunity to examine this question, because it is one of the best known promoters in plants. Phylogenetic footprinting across Brassicacae species has highlighted the presence of several conserved DNA regions which confer light-responsive expression.
Using pyrosequencing, we have developed an allele specific assay to examine cis-regulatory variation for the CHS gene in A. thaliana. We correlate the observed functional variation with within-species nucleotide polymorphisms in the promoter region of the gene. The distribution of nucleotide variation in the promoter does not depart from neutral expectations indicating that selection has not affected diversity in this region. Nonetheless we have identified three groups of promoter alleles that are functionnaly differentiated with respect to light response. These allelic groups could provide different selective advantages in certain environmental conditions. Thus, our results indicate that the CHS promoter has a potential for functional evolution. Furthermore, the sequence survey suggests that this potential can result from a limited number of mutations and further supports the hypothesis that cis-regulatory variation can significantly contribute to rapid adaptation.

T06-013

Trichome evolution in tetraploid Arabidopsis

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Populations of the tetraploid Arabidopsis lyrata subsp. kamchatica are polymorphic for the presence or absence of trichomes or hair cells. In an effort to examine the nature of this trichome-less (glabrous) polymorphism, we collected wild lines without trichomes and crossed them to A. thaliana. The crosses yielded hybrid F1 plants and enabled the application of the 'interspecific allelism test'. Hybrids between the A. thaliana gl1 hairless mutant and the wild hairless lines never produced trichomes. Sequence analysis revealed that frameshift mutations disrupt the two GLABROUS1 genes in the wild trichome-less lines. These results strongly suggest that these mutations are the causes of the evolutionary loss of trichomes.
GL1 belongs to a large MYB gene family, and has a sibling MYB gene WEREWOLF (WER), which is responsible for the specification of root hairs. It is known that GL1 and WER genes were generated by gene duplication and subsequent subfunctionalization through the partitioning of promoter functions. In contrast, GL2, GL3 and TTG1 genes are required not only for trichome but also for other epidermis.
We propose that the dynamics of gene duplication and gene loss generates morphological diversity. First, gene loss is a major mechanism of phenotypic evolution. Second, the gene duplication does not necessarily inhibit the evolution by gene loss, since two GL1 genes had disruptive mutations. Moreove, the trichome-specific regulatory gene GL1 was generated by gene duplication and subsequent subfunctionalization, facilitating the differential regulation of the trichome and other epidermal features. This was a prerequisite for phenotypic evolution by gene loss, allowing the constraints inherent in pleiotropy to be avoided.
The Arabidopsis trichome is an ecologically important and well-studied morphological character. It prevents herbivory, but there is fitness cost to make it. Evolutionary forces underlining the morphological evolution will be discussed based on the molecular population genetic analysis of GL1 genes in the tetraploid population.

T06-014

Elucidating the Molecular Basis of Heterosis in Arabidopsis thaliana

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The term heterosis describes increased size and yield in crossbred as compared to the corresponding inbred lines. It has also been applied to the expression of adaptive traits such as increased fertility and resistance to biotic and abiotic stress. Heterosis has been widely used in agriculture to increase yield and to broaden adaptability of hybrid varieties and is applied to an increasing number of crop species. We observed heterosis for biomass production in Arabidopsis thaliana. This allows us to apply the vast array of genetic and genomic tools available for this model plant to the analysis of the molecular basis of heterosis.
We are analysing two divergent Arabidopsis thaliana accessions, C24 and Col-0, the F1 hybrids of which were shown to exhibit hybrid vigour. An important finding was the early onset of heterosis for biomass: in the cross Col-0 / C24, differences between parental and hybrid lines in leaf size and dry shoot mass could be detected as early as 6 days after sowing. Through the analysis of the two parent lines, their F1 hybrids, ca. 400 derived genotyped recombinant inbred lines (RILs) and their testcross hybrids, heterotic quantitative trait loci (hQTL) will be identified that affect growth (biomass, leaf area) at early stages of plant development. High-throughput estimation of total leaf area at early developmental stages was achieved through a purpose-built imaging system using a high-resolution digital video camera mounted on computer controlled tables.
Parallel gene expression and metabolic profiling experiments are being performed using Arabidopsis ATH1 gene chips and GC-MS to identify genes and metabolites potentially responsible for the manifestation of heterosis. These profiles are obtained for three time points: prior / during / after manifestation of seedling size differences.
Candidates genes to investigate the genetic basis of the heterosis phenomenon will be identified by combining expression, metabolic and QTL data analyses.

T06-015

QTLs for an important growth trait (Specific Leaf Area).

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Specific Leaf Area is a parameter that indicates how much leaf area a plant makes for a given investment in leaf biomass (SLA, m2 leaf area kg-1 leaf biomass). It is an important factor in determining variation in growth rate between species. It also plays a major role in the acclimation of plants to high or low light.

To investigate the genetic regulation of SLA in Arabidopsis, we did a QTL analysis on a RIL population of Landsberg erecta x Cape Verdian Islands. SLA as well as its underlying components (leaf thickness and leaf density) were determined. There were relatively strong QTLs found at the top of chromosome 1 and 3, the middle of chromosome 5 and the bottom of chromosome 2. Strangely enough these were not always co-located by one of the factors that underlies SLA (density or thickness). Light intensity during growth only had a small effect on the location of the QTLs.

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T06-016

Genetic analysis of natural variation for mineral composition and phytate levels in Arabidopsis thaliana seeds

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Plants require more than fifteen mineral elements. These include macronutrient elements like P, K, Ca etc. and a variety of micronutrient elements, such as Zn, Fe, Mn and Mg. Plants provide these elements as dietary nutrients for humans and animals. The presence of phytate, which can form a strong complex with cationic minerals, severely reduces the bioavailability of these minerals.
At the moment little is known about the genes controlling cation mineral uptake and distribution in plants and phytate biosynthesis and storage. Identification of these genes will increase our understanding of the mineral uptake and distribution process and may facilitate the improvement of plant nutrient content with potentially beneficial effects on human and/or animal health as well as on crop yield and quality.
We examined the natural variation for mineral content in several Arabidopsis accessions and used QTL approach to identify and unveil the genetic loci controlling seed content of P, K, Ca, Mg, Mn, Fe and Zn as well as phytate in Recombinant Imbred Line populations based on Ler x Cvi, Ler x Eri and Ler x Kond crosses. For each mineral several QTL were identified. Co-localization of some QTL suggested single loci to be involved in the accumulation of multiple minerals. In many cases, genes (putatively) encoding mineral transporters co-localized to QTL, suggesting several candidate genes for the identified QTL.

T06-017

Investigation of the molecular basis of heterosis using a combined genomic and metabolomic approach

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Heterosis, or hybrid vigour, refers to the phenomenon that progeny of crosses between inbred varieties exhibit greater biomass, speed of development, and fertility than the two parents. Although the effect of hybrid vigour has been widely exploited in agriculture and breeding, very little is known about the molecular mechanisms underlying heterosis. The widespread occurrence of heterosis in the model plant Arabidopsis thaliana opens the possibility to investigate the genetic basis of this phenomenon using the tools of genetical genomics.
We established a Recombinant Inbred Line (RIL) population consisting of ca. 400 F8 lines of the reciprocal combination C24 /Col-0, the F1 hybrids of which exhibit significant heterosis for biomass production. The RILs are being genotyped with 110 SNP markers to create a genetic linkage map. QTL analysis is being performed using a modified design III with ca. 400 RIL and their 800 test-crosses to each parent. The phenotypic characterisation included the main heterotic trait biomass production, as well as traits potentially contributing to the heterotic effect such as leaf size and morphology, gas exchange parameters, and photosynthetic capacity. In soil grown plants, size differences between parents and F1 were observed as early as 6 days after sowing. To investigate possible differences in metabolic status between parents and hybrids at the earliest developmental stages, we have began implementing metabolite profiling using GC-MS (Gas Chromatography-Mass Spectrometry) and LC-MS (Liquid Chromatography-Mass Spectrometry). The metabolic analysis will be complemented by microscopic analysis of germinating seeds from hybrid and parental lines. This will allow scrutiny of differences in cell organisation, and/or numbers, which may contribute to heterosis.

T06-018

Comparative analysis of the FRIGIDA genomic region reveals a recent transposition event in Arabidopsis thaliana

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Comparative genetic mapping analysis has been used extensively to study and understand genome organization and evolution at the macro level, however, due to limited marker density, exceptions to colinearity at the microlevel will be overlooked. This limitation can be overcome by large scale sequencing efforts. We are interested in patterns of sequence conservation and divergence at loci controlling flowering time. Here, we present initial data focusing on the region containing the FRIGIDA gene between Arabidopsis thaliana, Arabidopsis halleri, Boechera (syn. Arabis) drummondii and Capsella rubella. We shot-gun sequenced BACs containing the FRIGIDA gene from all three species. An initial analysis reveals conservation of synteny between the species that are being compared. This is in agreement with what has been reported for other regions of A. thaliana and C. rubella. However, the study also reveals that the present location of the FRIGIDA gene on top of chromosome 4 in the Arabidopsis thaliana genome is a very recent event. We are carrying out further analysis to map this transposition event on an evolutionary time scale.

T06-019

High diversity genes in the Arabidopsis genome

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We used Arabidopsis thaliana as a model for the study of highly variable genes by exploring DNA sequence variation among ecotypes of this species. A comparison of DNA sequence fragments from the Landsberg erecta ecotype with the Columbia ecotype full genome sequence has revealed several highly polymorphic genes. We investigated the molecular population genetics of three of these genes in 14-20 ecotypes. These genes encode a soluble starch synthase, a BEL1-like homeobox-leucine zipper family protein, and a myb family transcription factor. Gene regions approximately 1 kb in length were also sequenced from several genes located directly upstream and downstream of our target gene. Results indicate varying levels and patterns of nucleotide diversity across linked genomic regions, with at least one gene in each data set showing extremely elevated levels of nucleotide polymorphism. Tests of selection suggest balanced polymorphisms might be maintained at several of these loci. These results suggest genome wide scans for targets of natural selection will likely be an effective tool for identifying adaptive genes in the future.

T06-020

Ecological genomics of naturally occurring flowering time variation among Arabidopsis accessions

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A latitudinal cline in flowering time in accessions of Arabidopsis thaliana has been widely predicted because the environmental cues that promote flowering vary systematically with latitude, but evidence for such clines has been lacking. Here, we report evidence of a significant latitudinal cline in flowering time among 70 Northern European and Mediterranean ecotypes when grown under ecologically realistic conditions in a common garden environment. The detected cline, however, is found only in ecotypes with alleles of the flowering time gene FRIGIDA (FRI) that lack major deletions that would disrupt protein function, whereas there is no relationship between flowering time and latitude of origin among accessions with FRI alleles containing such deletions. Analysis of climatological data suggests that late flowering in accessions with putatively functional FRI was associated with reduced January precipitation at the site of origin, consistent with previous reports of a positive genetic correlation between water use efficiency and flowering time in Arabidopsis, and the pleiotropic effects of FRI of increasing water use efficiency. In accessions collected from Southern latitudes, we detected that putatively functional FRI alleles were associated with accelerated flowering relative to accessions with nonfunctional FRI under the winter conditions of our experiment. These data suggest that that Southern ecotypes with putatively functional FRI are more sensitive to vernalization cues, and results of a follow-up experiment specifically designed to test this hypothesis will be presented. More generally, our results and approach indicate that by combining ecological and molecular genetic data, it is possible to understand the forces acting on life history transitions at the level of specific loci.

T06-021

Gene Subfunctionalization and Neofunctionalization in Arabidopsis Gene Families studied using Gene Expression and Sequence Data

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The process of subfunctionalization (a gene acquiring different expression patterns) and/or neofunctionalization (a gene acquiring a new biochemical function) is thought to promote the preservation of duplicated genes (Lynch and Force, 2000). Several papers have been published recently which provide a theoretical framework for this process (Lynch and Force, 2000; Ohta, 2003).

Collections of gene expression data represent a rich resource for many aspects of biology. In the case of Arabidopsis, one collection recently made available is that from NASCArrays (Craigon et al., 2004), in which expression levels for more than 22 000 genes in >124 samples have been measured using the Affymetrix GeneChip technology platform. We have used the data in an unbiased way, as proxies for promoter composition: the data were used to calculate the similarity of expression patterns of all possible pairwise combinations of genes in each of 3377 gene families in Arabidopsis. At the same time, we performed pairwise global sequence alignments using ClustalW for the same gene pairs, and calculated the similarity for each pair. These pairwise comparision values for expression correlation and sequence similarity were plotted against each other, and the process was repeated for all gene families. Binning averages were generated in order to simplify the resultant data sets. In general, even for gene which exhibit very high levels of sequence similarity, expression levels did not correlate by more than 10-20%. Furthermore, when we examined the pairwise sequence alignments and used them to calculate Kn/Ks ratios, we found, as have others (Kondrashov et al., 2002), that most pairs had a Kn/Ks ratio << 1, indicating that subfunctionalization, and not neofunctionalization, is the driving force resulting in the fixation of duplicated genes. We will also present results on the functional classification of the gene families members in the context of expression and nucleotide changes, as well as whether pairs of genes represent recent or old duplications (Vision et al., 2000).

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T06-022

A START: Putative lipid/sterol binding proteins in plant genomes

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StAR-related lipid transfer (START) domains are conserved lipid/sterol-binding modules implicated in lipid/sterol transport, metabolism, and signal transduction. We present a survey of START domains from Arabidopsis and rice in comparison to sequenced genomes from animals, bacteria and protists. Strikingly, START domains are amplified in plant genomes: The majority are found in a novel class of plant-specific transcription factors, the homeodomain-START family that is conserved across the plant kingdom. In the largest subfamily, the homeodomain DNA-binding motif is N-terminal to a plant-specific leucine zipper with an internal loop, while in a smaller subfamily the homeodomain precedes a classic leucine zipper. START domains in homeodomain-START transcription factors are not closely related to animal START domains, implying that protein-lipid/sterol binding partners evolved collaterally with the synthesis and uptake of endogenous lipids/sterols. However, plant homeodomain-START and animal nuclear receptor transcription factors, although they are unrelated in sequence, seem to share a common mechanism to regulate gene transcription by binding to lipid/steroid signals. In addition we show that as in animals, a subset of START domains from plants is associated with N-terminal pleckstrin homology domains, although sequence comparison reveals a non-orthologous relationship. Plant START proteins having pleckstrin homology domains also contain a plant-specific C-terminal domain of unknown function, DUF1336. Although most START domain proteins appear to have evolved in the plant lineage, one subset shows similarity to the mammalian phosphatidylcholine transfer protein. Using crystal structure data from mammals, we predict 3D protein models and suggest phosphatidylcholine lipid ligands for this conserved subset of plant proteins. We postulate that molecular mechanisms underlying plant growth and development are likely to involve lipid/sterol signaling mediated by START domain-containing proteins.

T06-023

Progenitor-dependent gene expression and evolution of transcriptome in Arabidopsis allopolyploids

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Polyploidy is recognized as evolutionarily innovation for every eukaryote, yet the immediate consequence of polyploidy is unknown. In early stages, sudden reunification of the evolutionarily diverged species in the allopolyploids creates genome instability and regulatory incompatibility that must be reconciled. Here we show that >8% of transcriptome in various biological pathways is expressed significantly different between the new allotetraploids and their actual progenitors, A. thaliana and A. arenosa. Remarkably, the genes differentially expressed between the progenitors are susceptible to changes in the allotetraploids and >94% of the genes highly expressed in A. thaliana are repressed, consistent with the overall suppression of A. thaliana phenotypes in the new and natural Arabidopsis allotetraploids. Developmental and progenitor-dependent repression of regulatory pathways including phytohormone biosynthesis and signaling is a mode of regulation for homoeologous genes, which may create de novo variation that contributes to the adaptation of new allopolyploid species.

T06-024

Natural variation in root system architecture

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Root system architecture plays a key role in the plant's ability to access water and nutrients from the soil. There is considerable natural variation in the root system architecture in many plant species, including Arabidopsis;. These differences may reflect adaptation to distinct soil conditions, or may be a secondary effect of other selective pressures. In either case, understanding how to manipulate development of the plant root system would be of tremendous importance to crop improvement efforts. To understand the molecular mechanism that underlies root system architecture, we have focused on the Columbia (Col) and Landsberg erecta (Ler) ecotypes. Seedlings were grown on agar plates with mannitol to simulate mild drought stress. Clear differences were observed between the ecotypes: Ler has many lateral roots while the Col root system is almost completely unbranched. Further analysis revealed that the difference in lateral root formation is caused by two phenomema: 1) Ler initiates more lateral roots/cm under all conditions tested; and 2) Development of the Ler lateral root primordia into lateral roots is less repressed by drought stress. QTL mapping of the root branching trait revealed two robust, major effect loci, termed Elicitors of Drought Growth (EDG)1 and 2. The Col allele of EDG1 is associated with a reduction in root branching, while the Col allele of EDG2 is associated with increased root branching. Interestingly, the Col allele of EDG1 completely masks the effect of EDG2, such that this loci would never have been identified in standard screens. A novel mapping technique using regression analysis has allowed us to predict whether each QTL is involved in drought response or in the drought-independent regulation of root system architecture. Finally, progress will be presented in the identification of the genes associated with EDG1 and EDG2 QTL.

T06-025

A floral homeotic polymorphism in Capsella: studying a hopeful monster

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The molecular basis of evolutionary novelties is a highly contentious issue. While homeotic mutants and genes have been of tremendous value for a better understanding of animal as well as plant development and form, their role in evolution is highly controversial (1,2). It is often maintained that homeotic mutants have such a drastic effect on the phenotype that fitness is always heavily reduced, so that evolution generally proceeds in a gradualistic and never in a saltational way. On the other hand there is both molecular and morphological evidence that homeotic, or heterotopic, i.e. saltational changes occurred during plant evolution and resulted in the establishment of new lineages with novel morphological features (1,2). To better understand the evolutionary significance of homeotic mutants we are studying a floral homeotic variety of Capsella bursa-pastoris (shepherd's purse) in which all petals are transformed into stamens. In contrast to most other homeotic mutants this "Staminoid petals" (Spe) variety occurs on several locations in larger populations in the wild. It thus qualifies as a drastic morphological variant that might have the potential to establish a new evolutionary lineage (a 'hopeful monster' in Richard Goldschmidt's provocative terminology). Due to its close relationship to the model plant Arabidopsis, the Spe variant of Capsella can be rigorously studied, from the molecular genetic basis of the phenotype to its consequences on the ecology in the field. Respective investigations are underway and may help to answer the long-standing question as to whether non-gradualistic changes at the phenotypic level, such as homeotic transformations, have the potential to contribute to macroevolution.

1. Theissen G (2000) Bioessays 22, 209-213
2. Ronse De Craene LP (2003) Int J Plant Sci 164 (5 Suppl.), S225-S230

T06-026

The genomic pattern of polymorphism in Arabidopsis thaliana

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Its wealth of naturally occurring variation, high level of inbreeding, and resulting extensive linkage disequilibrium make Arabidopsis thaliana an attractive model plant for carrying out genome-wide association mapping studies. The genome-wide surveys necessary for association mapping will also lead to a more comprehensive model of variation for a species and ultimately a better understanding of the genetic process of adaptation to diverse environments.

As part of a genome-wide survey of polymorphism in A. thaliana, we have re-sequenced and processed data from 876 short (500-700 bp) fragments distributed throughout the genome in a sample of 95 accessions and identified thousands of SNPs.

Here we present the results of a population genetics analysis of these data. We discuss how well the genome-wide pattern of variation fits standard population genetics models, and what we have learned about Arabidopsis population structure. We focus in particular on the influence that the duplicated nature of the genome has on levels of polymorphism. We also investigate the relationship between estimators of recombination rate and levels of polymorphism across the genome. Finally, we show that despite the limited value of tests that assume the standard population genetics model, analyzing loci in light of the genome-wide empirical distribution of several population genetics statistics can uncover possible traces of selection.

T06-027

Mining publicly available sequence information to detect SNP markers

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In order to reveal the molecular cause of observed phenotypic variation, geneticists map the responsible locus in segregating populations using genetic markers. The mapping of laboratory induced mutations has usually been done between standard laboratory strains like Columbia and Landsberg erecta and by now there are many markers between these two strains available. Studies of natural variation, however, aim at finding different alleles at one locus (or many loci at once) between different accessions (ecotypes), which are usually not very well characterized. Thus, mapping the genetic cause of phenotypic differences between any two accessions first requires the development of markers.
We were in the particular need to develop markers to genotype 5 sets of Recombinant Imbred Lines (RILs) our laboratory has constructed (http://naturalvariation.org). For all parents of our RIL-sets sequencing information is publicly available (1) and we developed software to detect SNP-markers using that information. Our software requires the input of DNA sequences of the same locus from (many) different accessions and it will return SNP markers between any subsets of accessions the user specifies. The output is organized such, that it can directly be used to design the SNP detection assay. Our programs are written in Perl and make use of the emboss-package (2).
As of now, we mined 854 fragments (600-700 bp in length) and detected 173 SNP markers, that can theoretically be used to genotype our 5 RIL sets. 54 of these markers have already been tested by genotyping 192 lines of our est-1 x col-0 RIL-set and 92 lines of our nd-1 x col-0 RIL-set and all markers worked.

(1) "A genomic survey of polymorphism and linkage disequilibrium" (http://walnut.usc.edu/2010.html)
(2) http://www.hgmp.mrc.ac.uk/Software/EMBOSS/

T06-028

The genetic architecture of trichome density in A. thaliana: results from multiple mapping populations

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The genetic and molecular bases of complex traits are poorly understood. A common approach to this problem is the use of whole-genome scans to identify polygenes, or quantitative trait loci (QTL). The results of such analyses provide estimates of several genetic parameters that may underlie phenotypic variation, including the number of loci, the type and magnitude of their effects, interactions between genes (epistasis), and gene-by-environment interactions. This collection of parameters is often referred to as the “genetic architecture” of a trait. Because these parameters are largely population specific, however, we investigated the potential for variation in genetic architecture by mapping QTL for trichome density in four experimental populations of Arabidopsis thaliana. Our mapping results identify many new QTL with significant effects on trichome density. We shall provide mapping results, comparative analyses, and interpretations of these findings.

T06-029

Adaptive trait genes in Arabidopsis thaliana: Discerning the role of selection and demography in patterns of genetic variation.

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Numerous traits of A. thaliana show naturally occurring diversity and in several cases, the genes underlying phenotypic variation were identified. Patterns of genetic variation suggest that these genes evolved under balancing or directional selection and were involved in adaptive processes such as host-pathogen coevolution or local adaptation. To identify novel candidate adaptive trait genes that may contribute to adaptive phenotypic divergence, we conducted a genome-wide screen for genes with footprints of selection in patterns of intra- and interspecific genetic variation. Several genes exhibit a strong signal of selection-driven evolution and we have begun to characterize a small set of candidate adaptive trait genes by evolutionary and functional approaches. One candidate encodes a leucine rich repeat (LRR)-receptor protein kinase of as yet unknown function, but may be involved in pathogen resistance. This gene exhibits extremely high levels of inter- and intraspecific variation that are not consistent with neutral evolution and rather suggest that it evolves under balancing selection as indicated by the presence of two strongly divergent haplotypes, which was also observed at other LRR disease resistance genes. However, the geographic distribution of genetic variation and comparisons to multiple other loci of A. thaliana suggest that past demographic events such as changes in population size and population structure, or genetic processes such as increased mutation rates or recombination suppression in repetitive genes may create patterns of genetic variation that are very similar to those expected under selection. We conclude that comparisons across multiple loci and individuals are necessary to infer the role of selection in phenotypic diversity of A. thaliana.

T06-030

FRI haplotype structure: implications for detecting selection and for genome-wide association mapping

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The vernalization response locus FRIGIDA (FRI) delays flowering in Arabidopsis thaliana by positively regulating Flowering Locus C (FLC) expression. A substantial fraction of early-flowering accessions carry one of two known FRI loss-of-function alleles, friCol and friLer, characteristic of the early-flowing Col and Ler, respectively.

We have sequenced FRI and several surrounding loci evenly distributed in a 100 kbp window in 192 accessions that represent a wide range of flowering responses. We use these data in combination with the results of genome wide polymorphism survey to address two questions about FRI: 1) Given the major phenotypic effects of the two FRI loss-of-function alleles, can we detect evidence of selection in that pattern of variation at FRI? 2) Given that FRI is a major determinant of flowering time, can it be detected using linkage disequilibrium in a genome-wide screen?

T06-031

Two Arabidopsis halleri MTP1 genes co-segregate with naturally selected zinc tolerance and account for high MTP1 transcript levels

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Arabidopsis halleri ssp. halleri (acc. Langelsheim) is able to accumulate about 100-fold higher leaf Zn concentrations when compared to the closely related A. thaliana, without showing any toxicity symptoms. Heterologous screening of an A. halleri cDNA library in the yeast Saccharomyces cerevisiae, as well as comparative transcript profiling in A. halleri and A. thaliana using A. thaliana GeneChips, identified a candidate gene encoding a member of the so-called cation diffusion facilitator metal transporter family, termed AhMTP1-3 (metal transport protein 1). Expression of A. halleri MTP1-3 and the homologous A. thaliana ZAT / MTP1 proteins complemented zinc hypersensitivity of a yeast zrc1 cot1 mutant strain. A GFP fusion of the AhMTP1-3 protein localized to the vacuolar membrane of transfected A. thaliana protoplasts. A genomic DNA gel blot indicated that the genome of A. halleri hosts several MTP1 gene copies, whereas only a single MTP1 gene is present in the genomes of the zinc sensitive plant species A. lyrata and A. thaliana, respectively. When compared to A. lyrata and A. thaliana, total MTP1 transcript levels were substantially higher in the leaves of A. halleri. High MTP1 transcript levels in A. halleri were primarily attributed to two genetically unlinked genomic AhMTP1 gene copies. The two corresponding loci were observed to co-segregate with Zn tolerance in the back-cross 1 generation of a cross between the zinc-tolerant species A. halleri and the zinc-sensitive species A. lyrata. In contrast, a third MTP1 gene in the genome of A. halleri was found not to co-segregate with Zn tolerance and was expressed at low levels in BC1 individuals. Our data suggests that zinc tolerance in A. halleri involves an expanded copy number of an ancestral MTP1 gene, encoding functional proteins that mediate the detoxification of zinc in the cell vacuole.

T06-032

Natural variation, genomic imprinting and modifiers of mea seed abortion in the Arabidopsis species genepool.

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Studies of the medea (mea) mutant of Arabidopsis have demonstrated that the MEA gene is regulated by genomic imprinting. The paternal MEA allele is not expressed during early embryogenesis whereas the maternally inherited allele is. Little is known regarding the extent of variation in the genetic or epigenetic regulation of imprinted loci across species genepools. In mammalians systems, some imprinted genes (such as Igf2R) are polymorphic whereby the gene is imprinted in some individuals but not in others. Such polymorphism between biallelic and monoallelic expression patterns could be due to the existence of a non-imprintable allele, or the presence of genetic modifiers of imprinting, in the population. One component of our overall strategy to identify modifiers of imprinting is to screen existing ecotypes/accessions of Arabidopsis thaliana for any naturally occurring variant genotypes, which alter the mea seed abortion characteristics. We have screened over 50 Arabidopsis ecotypes in order to identify ecotypes which act as suppressors or enhancers of mea seed abortion. Such modifier ecotypes may contain cis- or trans-acting factors affecting genomic imprinting or imprinting at the MEA locus itself may be affected.

T06-033

Heterosis and transcriptome remodelling in Arabidopsis thaliana

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The term hybrid vigour or heterosis (Shull, Genetics 33, 439-446, 1948) is used to describe an increase in the vigour, yield, and development of a crossbred (hybrid) organism over its inbred parents. Initially used to improve maize yields in the US in the late 1920s (Somerville, Cell 100, 13-25, 2000), heterosis continues to have enormous economic importance by increasing yields of a large number of crops.
In China alone, more than 55% of the rice growing area has been given over to hybrid plants which produce yields 15-20% above the best inbred varieties (Xiao et al., Genetics 140, 745-754, 1995). An increased understanding of the molecular basis of heterosis, therefore, would greatly impact on our appreciation of the fundamental mechanisms regulating an organism’s growth and development, facilitating improvements in a wide variety of crop species.

It has recently become clear that Arabidopsis thaliana provides an excellent model for the study of heterosis (Barth et al., Heredity 91 36-42,2003; Meyer et al., Plant Physiology 134, 1813-1823, 2003). We have observed significant heterosis (155 – 167% of the mid-parent fresh weight) for the hybrid of the A. thaliana accessions Kondara and Br-0, recorded differences in transcriptome between the parent accessions, and significant transcriptome remodelling, between the parents and hybrid. In addition we have also generated a diverse range of hybrid combinations, displaying a variety of heterosis strengths, by crossing 13 ecotypes against male-sterile Landsberg erecta (Wilson, Z.A., et al., Plant Journal 28, 27-39, 2001). These hybrid combinations provide an excellent opportunity to associate specific transcriptomes, and potentially candidate genes, with levels of heterosis, and hence to progress our understanding of the molecular mechanisms underlying heterosis in A. thaliana.

T07 Metabolism

(Primary, Secondary, Cross-Talk
and Short Distance Metabolite Transport)

T07-001

The At4g12720 gene encoding a homologue of the human GFG protein is active on ADP-ribose and flavine adenine dinucleotide (FAD).

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Human GFG, a 35-kDa protein, is encoded by antisense fibroblast growth factor (FGF-2) mRNA. The translation product of sense FGF-2 RNA is a member of a growth factor family with mitogenic and hormone-regulatory functions. Human antisense FGF-2 RNA, complementary to the 3'- untranslated region of its sense partner, is involved in FGF-2 mRNA editing and stability. However, apart from this function the antisense RNA encodes the GFG protein which has homology to the MutT family of antimutator nucleotide hydrolases and can partially complement this function in a MutT- deficient E. coli strain. In the human pituitary the GFG protein enhances prolactine expression and inhibits cell proliferation, independent of FGF-2 expression (1).
In the genome of Arabidopsis thaliana seven genes coding for proteins with substantial homology to human GFG are indicated in the TIGR database. Similarly to the human protein, they all possess, within their primary amino acid sequences, conserved MutT/Nudix domains GX5EX7REUXEEXGU (U =Ile, Leu or Val). This motif is characteristic for a widespread family of Nudix proteins which catalyze mostly the hydrolysis of nucleoside diphosphates linked to certain moieties X. The list of substrates of the Nudix enzymes includes: nucleotide triphosphates NTP, nucleotide sugars, NADH, NAD, FAD, coenzymeA, m7GTP-mRNA cap, dinucleoside polyphosphates and a non nucleoside compounds - diphosphoinositol polyphosphates (2).
Three cDNA clones, coding for different Arabidopsis thaliana GFG proteins (AtGFG), obtained from the Arabidopsis Biological Resource Center, Ohio, USA, were subcloned into expression vectors. The first clone investigated, At4g12720* (AtGFG1), was used for complementation tests in a E.coli mutT mutator strain. We did not observe any complementation of MutT function nor hydrolysis of mutagenic 8-oxoGTP, a main substrate of the MutT protein. We have overexpressed AtGFG1 in E.coli and purified the recombinant AtGFG1 protein. We confirmed the protein identity and integrity by Western hybridization and mass spectrometry. The enzymatic activity was tested with purified recombinant protein and a number of different substrates. At reaction conditions typical for Nudix enzymes, at pH 8. 5 and in the presence of Mg2+ ions, AtGFG1 was active mainly on ADP-ribose and FAD.
* the gene marked according to its position on chromosome, follow the TIGR database

1.Asa, S.L. at al (2001) Molecular Endocrinology, 4, 589-599
2.Bessman, M.J. at al (1996) J.Biol.Chem. 271, 25059-2506

T07-002

Characterisation of the transparent testa 10 mutant affected in Arabidopsis seed coat flavonoid metabolism

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Flavonoids found in seeds have important impacts on agronomic seed qualities. Particularly, they increase seed dormancy and longevity. Genetic and molecular analysis of flavonoid metabolism in Arabidopsis thaliana can be performed using mutant lines affected in seed coat pigmentation (transparent testa mutants, « tt mutants»). To date, 22 loci involved in flavonoid biosynthesis have been identified in Arabidopsis.
Here, we present the characterisation of the tt10 mutant. Browning of the tt10 seed coat is delayed compared to that of the wild-type seed coat, suggesting that an enzyme involved in tannin metabolism may be affected in this mutant. We identified five new independent mutants that appeared to be allelic to the tt10-1 mutant previously isolated. The six tt10 alleles were used to identify the TT10 gene. Preliminary analysis by semi-quantitative RT-PCR showed that TT10 is essentially expressed in developping siliques. TT10 promoter activity has been studied using promoter : reporter (GUS and GFP) fusions in transgenic plants. Promoter activity is detected first in the endothelium at early stages of embryo morphogenesis and, later on, the expression spreads to the outer cell layers of the integuments. LC-ESI-MS-MS analysis of flavonoids in tt10 and wild-type seeds is currently in process. Details on gene and mutant characterisation will be presented and discussed.

T07-003

A conserved uORF mediates sucrose-induced translational control on bZIP transcription factors

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Plants are autotrophic organisms and synthesize sugars for growth and storage de novo. These sugars also function as hormone-like signaling molecules that adjust metabolism, growth and development of plants. Most sugar signaling effects appear to be mediated through transcriptional control; changes in the sugar concentration cause induction or repression of gene-transcription. Sugars also exert control post-transcriptionally, by changing mRNA stability, translation or protein stability. Several sugar sensing pathways have been identified or predicted, plants clearly distinguish between the dominant transport form of sugar sucrose and the hexoses. Sucrose specifically controls translation of the group S bZIP-type transcription factor ATB2/AtbZIP11 (Rook et al., Plant J. 1998 Jul;15(2):253-63). This control mechanism requires the unusually long 5'UTR of the gene and is independent of the endogenous 3`UTR. Point mutations and deletions of the 5'UTR have uncovered the sequences involved. A highly conserved uORF coding for 42 amino acids is essential for the repression mechanism. It is conserved in 5'UTRs of other Arabidopsis S-class bZIP-transcription factors as well as in bZIP genes of other mono- and dicotyledonous plants.
Expression of the ATB2/AtbZIP11 gene is specifically associated with vascular tissues. Ectopic expression of a 5'UTR marker gene construct shows that the sucrose repression system is functional in all tissues. Other Arabidopsis bZIP-transcription factor gene harboring the conserved uORF like AtbZIP2, are regulated similarly via sucrose-induced repression of translation. This suggests a general function of the conserved uORF in sucrose-controlled regulation of expression.
Our findings imply the operation of a sucrose-sensing pathway that controls translation of several plant bZIP transcription factor genes harbouring the conserved uORF in their 5'UTRs. Target genes of such transcription factors will then be regulated in a sucrose-dependent way.
Expression of the ATB2/AtbZIP11 transcription factor gene, lacking the sucrose control element in the 5`UTR will allow the characterization of the physiological importance of this control mechanism on the gene expression and the growth and development of the plant.
Mutants lacking the sucrose control of translation have been identified and are under further characterization.

Wiese and Elzinga et al. A conserved uORF mediates sucrose-induced repression of translation. Plant Cell, 2004 in press

T07-004

Different roles of branched-chain aminotransferases (BCAT) in the metabolism of aliphatic amino acid in plants

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Valine (Val), leucine (Leu) and isoleucine (Ile) are essential amino acids that are synthesized in plastids of plants. In recent years evidence has accumulated that these apliphatic amino acids can also be degraded in mitochondria of plants. We have now analyzed the function and expression of the six transcribed members of the branched-chain aminotransferase gene family in Arabidopsis thaliana (AtBCAT-1 to –6). In vivo GFP tagging by N-terminal sequences revealed three plastid (AtBCAT-2, -3 and 5) and a single mitochondrial-located BCAT (AtBCAT-1), while two enzymes seem to remain in the cytoplasm (AtBCAT-4 and –6). Expression studies applying quantitative RT-PCR and promoter GUS fusions in transgenic plants indicate highly divergent expression patterns of these genes. This suggests the individual members to fulfill their functions in distinct tissues at different developmental stages. Studies of the enzyme activities by yeast complementation corroborated the branched-chain aminotransferase activity of five members of this protein family, while no activity was found with AtBCAT-4, at least in yeast. Kinetic studies with the mitochondrially located branched-chain aminotransferase AtBCAT-1 and the aliphatic amino acids and their respective ketoacids suggest that Ile and Leu are preferred substrates in the deamination reaction. Ketoisovalerate, the 2-oxoacid of Val, is the preferred substrate in the reverse amination reaction, indicating Val to be degraded only at higher concentrations. Substrate specificity assays further suggest a function of branched-chain aminotransferases in other metabolic pathways.

T07-005

Roles of BOR1 homologs in B transport

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Arabidopsis thaliana BOR1 was identified from the bor1-1 mutant that exhibits reduced growth under the limited B supply. B concentrations in the aerial portion of the mutant plants are lower than the wild type plants and the mutant is defective in active xylem loading of B. BOR1 is the first boron transporter identified in the living systems. Six BOR1-like genes are present in the genome of Arabidopsis thaliana and at least five of them are expressed. An Arabidopsis thaliana mutant in which one of the BOR1-like gene, At3g62270, was knocked out, exhibited growth retardation only under limited B supply. The phenotype of this KO plants were distinct from the bor1-1 mutant plants in that growth of the roots were more inhibited than the aerial portions of the plants. B concentrations in the aerial portion of the KO plants were significantly reduced compared to the wild type plants but to a lesser extent compared to the bor1-1 mutant plants. The double KO mutant plants for the BOR1 and the homolog exhibited much severer growth defects than the single KO mutants only under the limited B supply. These results suggest that the BOR1 and At3g62270 have distinct roles in B transport in Arabidopsis thaliana. Accumulation of the transcripts for BOR1 or At3g62270 was not strongly affected by the levels of B supply. In rice, there are three BOR1-like genes in the genome. One of the cDNA encoding the rice homologs was expressed in yeast and was found to reduce B concentrations in the cells, suggesting that the gene encodes an efflux-type B transporter. In yeast, YNL275w is homologous to BOR1 and is an efflux-type B transporter. Overexpression of YNL275w reduced B concentrations in the cell in a wide range of B concentrations in the media and conferred tolerance to high B concentration in the media. BOR1 homologs present in plants and yeast function as efflux B-transporters and regulate cellular B concentrations.

T07-006

The regulatory role of the acetohydroxyacid synthase small subunit in Arabidopsis

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Acetohydroxyacid synthase (AHAS) catalyzes the first committed step in branched-chain amino acid biosynthesis and is notably the site of action for multiple classes of commercially important herbicides. The AHAS holoenzyme is likely a heterotetramer comprised of two subunits: a catalytic large subunit and a regulatory small subunit. The small subunit has been shown to restore feedback sensitivity as well as boosting activity when added to purified AHAS large subunit protein in vitro. This is consistent with its role in prokaryotes. While there is just a single AHAS large subunit gene (AHASL) in Arabidopsis thaliana, it possesses two AHAS small subunit genes termed AHASS1 and AHASS2, each encoding a distinct variant. This is consistent with surveys of gene sequences from other plants in that each species appears to possess two distinct small subunits. Here we examine the role of each of these differentially expressed genes in arabidopsis using loss of function mutants. Each gene appears to have distinct functions based on their respective expression patterns and their biochemical phenotypes. We also demonstrate that in tobacco co-expression of AtAHASL S653N(csr1-2) and AtAHASS1 have improved imidazolinone tolerance and greater resistant AHAS activity than tobacco expressing AtAHASL S653N(csr1-2) alone. The improved activity of the AHAS enzyme may provide greater tolerance to herbicides in crops.

T07-007

Functional analysis of cell wall components by expressing a xyloglucanase in Arabidopsis thaliana

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Xyloglucan is a component of the plant cell wall and is believed to cross-link cellulose microfibrils and thereby contribute to the strength of the wall. Furthermore, xyloglucan metabolism seems to be involved in cell growth. The aim of the project is to describe the effect of altering the xyloglucan composition in plants on growth and development and on the physicochemical properties of the cell wall.
A xyloglucanase (xyloglucan specific endo-1,4-ß-glucanase) from Aspergillus aculeatus was transformed into A. thaliana using two different vector-systems (pBinAR, pSRN). The pBinAR vector contains a CaMV 35S promoter, which normally leads to high constitutive expression of the gene, whereas pSRN has an ethanol inducible expression system. No A. thaliana transformants were obtained when the xyloglucanase was expressed behind the CaMV 35S promoter, suggesting that high expression of the xyloglucanase is lethal to the plant. The xyloglucanase was successfully transformed into A. thaliana using the pSRN vector. After treatment of the transformed plants with low concentrations of ethanol, the xyloglucanase could be detected by Western blot analysis. The expression level of the xyloglucanase had a direct correlation with the amount of ethanol used for induction. Increased xyloglucanase expression lead to inhibition of growth and formation of necrotic spots. Further phenotypic parameters of the xyloglucanase expressing plants will be presented and discussed.

T07-008

A second starch phosphorylating enzyme is required for normal starch degradation in Arabidopsis leaves: the phosphoglucan, water dikinase (PWD)

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Recent findings showed that degradability of transitory starch is strongly influenced by its phosphorylation state. Until now only one starch phosphorylating enzyme has been characterised, the a-glucan, water dikinase (GWD, EC 2.7.9.4; formerly designated as R1) (1). In GWD-deficient sex1 mutants of Arabidopsis starch breakdown is strongly impaired resulting in a starch excess phenotype (2). However, the link between phosphorylation and degradation of starch is not understood at present.
In order to identify enzymes whose activity depend on phosphate esters in starch we searched for proteins with high affinity to phosphorylated starch. In an in vitro binding assay we could identify a previously unknown protein, preliminarily called OK1 (At5g26570; GenBank-Acc. AJ635427), which exhibits a much higher interaction with phosphorylated starch compared with non-phosphorylated starch. Interestingly, the OK1 sequence displays homology to the GWD sequence. Similar to GWD, the recombinant OK1 protein posseses starch phosphorylating activity. However, its activity strictly depends on preceding phosphorylation of the starch substrate by GWD. Further characterisation of the OK1 activity revealed that OK1 incorporates the b-phosphate of ATP into phosphoglucans, whereas the g-phosphate of ATP is transferred to water. An autocatalytically phosphorylated OK1 protein occurs as an intermediate of this reaction. Unlike GWD, which phosphorylates glucose units of starch both at C-6 and C-3 positions at a ratio of about 2:1, OK1 links the phosphate predominantly to C-3. As revealed by cell fractionation and subsequent immunoblotting using a polyclonal anti-OK1 antibody OK1 is localised in the chloroplast. Transgenic Arabidopsis plants with reduced OK1 expression exhibit a starch excess phenotype.
From these data we conclude that OK1 is a chloroplastidic phosphoglucan, water dikinase (PWD) which is required for normal degradation of leaf starch in Arabidopsis.

(1) Ritte et al. (2002) PNAS 99, 7166-7171.
(2) Yu et al. (2001) Plant Cell 13, 1907-1918.

T07-009

Arabidopsis thaliana P450 transcript profiling and functional genomics

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In plants, P450s function in the biosynthesis of lignins, pigments, defense compounds, fatty acids, hormones and growth regulators as well as in the metabolism of herbicides, insecticides and pollutants. Towards defining the function of the 272 P450 genes existing in Arabidopsis, we have analyzed microarrays containing gene-specific elements for 265 P450s, 40 biochemical pathway markers and 322 physiological function markers for constitutively expressed and chemically inducible P450 transcripts. This analysis has highlighted a number of P450 transcripts expressed in most, if not all tissues, under normal growth conditions and detailed the array of P450 transcripts expressed in response to plant signaling molecules (JA, SA), fungal defense activators (BTH) and environmental stresses (mannitol, cold). Together, these analyses detail the unique and overlapping responses of P450 and biochemical pathway loci to internal and external chemical cues. Heterologous expression of P450s and P450 reductases in baculovirus-insect cell systems have allowed us to co-incorporate these endoplasmic reticulum localized enzymes into a soluble nanodisc system that is suitable for substrate binding and turnover analysis as well as sorting and characterization of other membrane-bound proteins. Molecular models have been developed for a number of P450s whose substrate binding capacities have been defined by these approaches.

T07-010

P, A and L boxes do it together: A statistical approach for the investigation on stress inducible cis-elements in Arabidopsis

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In the model plant A. thaliana, phenylpropanoid metabolism feeds the biosynthesis from simple hydroxycinnamic acids, as soluble and wall-bound phenolics, to lignin-precursors and complex flavonoids, isoflavonoids, and anthocyanins. These products play important roles in plant structure and development, as well as defense responses against biotic and abiotic stresses. The enzymatic steps involved in the biosynthesis of phenylpropanoids are increasingly well understood. Many of the corresponding genes have been cloned, but knowledge about cis-acting elements mediating the response to regulatory factors that orchestrate rapid, coordinated induction of phenylpropanoid is sparse.
Boxes P and L , together with a frequent but variable co-occurring box A may be involved in coordinated expression, were initially identified by in vivo footprinting as UV and elicitor-responsive regions in the PAL1 gene promoter of parsley.
Here we show that the recently identified At4CL4 gene promoter also contains both P and L boxes at TATA proximal positions. Remarkably, these two boxes occur in the same order as in the At4CL1~3 and the initially studied Pc4CL1~2 gene promoters.
Genome wide scrutinizing of available promoter regions with MotifMapper (www.motifmapper.de) helped identifying these boxes in many genes of the phenolpropanoid pathway. Moreover, other promoters containing common elements like the P, A or L-boxes putatively underlay similar regulation by the same upstream transcription factors.

T07-011

Regulation and Localization of the Beta-cyano-L-alanine Converting Enzyme Nitrilase 4 in Arabidopsis thaliana

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There are two groups of nitrilases in Arabidopsis thaliana which differ in their substrate specificities. Nitrilases 1-3 of the first group accept a wide range of nitriles as substrates, and are proposed to be involved in the metabolism of glucosinolates. The only member of the second group, nitrilase 4 (NIT4), specifically hydrolyzes beta-cyano-L-alanine (Ala(CN)), which is the first product of cyanide detoxification in higher plants [1], to the amino acids asparagine and aspartate, respectively [2].
To investigate the role of NIT4 for cyanide detoxification in vivo, we generated a collection of transgenic plants:
NIT4-promoter-GUS-fusions were constructed to study the expression of the NIT4 gene under various conditions. With these plants, we found, that the NIT4-promoter activity is higher in plants treated with L-serine, but not in plants treated with D-serine, O-acteylserine, L-cysteine or Ala(CN). This activation of NIT4-transcription leads to an increase of NIT4 activity in the plant. Another class of substances, which increase the NIT4-promoter activity, are the reducing agents DTT, DTE and gluthathione (GSH). The higher the reducing potential in the medium, the more the promoter-activity increases.
Physiological NIT4 knockout plants using the RNA inhibition (RNAi) strategy and NIT4 overexpressing plants were generated to investigate the metabolism of cyanide and Ala(CN) in these plants. The NIT4-RNAi and NIT4-His-overexpression plants do not show different phenotypes at normal growth conditions. With Ala(CN) added to the medium, we found a higher survival rate of plants with higher NIT4 content.
To determine the intracellular localization of the NIT4-protein, we created a NIT4-GFP-fusion for transient transformation of Arabidopsis thaliana. The localization of NIT4 is cytoplasmatic.

[1] Blumenthal-Goldschmidt et al. (1963), Nature 197: 718
[2] Piotrowski et al. (2001), JBC 276: 2616

T07-012

Analysis of the involvement of AtNIT1 homologous enzymes in the metabolism of glucosinolate-derived nitriles

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The covalent bond connecting a carbon to a nitrogen is of basal importance in nature. Therefore a huge variety of enzymes capable of forming and cleaving such bonds exists. One class of such enzymes are the nitrilases, which catalyse the hydrolysis of nitriles to the corresponding carboxylic acids. In the Arabidopsis thaliana genome genes coding for four different nitrilases called NIT1-NIT4 are present. The enzymes encoded by those genes can be divided into two distinct groups. One group is formed by NIT1/NIT2/NIT3, whereas the second group is formed by NIT4. All four nitrilases where initially identified during the attempt to elucidate the biosynthesis of the auxin indole-3-acetic acid. However in recent years it has been shown that heterologously expressed nitrilases show only a very weak activity with the proposed indole-3-acetic acid precursor indole-3-acetonitrile [1]. A general role for nitrilases in the biosynthesis of indole-3-acetic acid therefore seems to be unlikely. For NIT4, which could be shown to be a beta-cyano-L-alanine hydrolase/nitrilase, a role in detoxifying cyanide arising during ethylene biosynthesis is proposed [2]. NIT4 homologs have been found in many phylogenetically distant plant species, but homologs of the Arabidopsis NIT1/NIT2/NIT3 have so far only been found in plants belonging to the order of Brassicales (Capparales). Heterologously expressed NIT1/NIT2/NIT3 exhibit a very broad substrate range with the highest enzymatic activities achieved with nitriles that can be deduced from glucosinolates, a class of secondary metabolites also almost exclusively found in plants belonging to the Brassicales. Therefore a role for NIT1/NIT2/NIT3 in the metabolism of glucosinolate-derived nitriles seems feasible. To further substantiate this assumption it appears necessary to test the activity of further glucosinolate-derived nitriles especially those derived from glucosinolates, which represent the major glucosinolates of Arabidopsis thaliana. To this end we have established a procedure to produce the glucosinolate-derived nitriles through a combination of precursor isolation from the plant and enzymatic conversion. To gain a broader view over the nitrilases present in the Brassicales we are also looking for nitrilase activity in plants apart from Arabidopsis thaliana and will try to isolate cDNAs of present nitrilases.

[1] Vorwerk, S., et al. (2001) Planta 212: 508-516 [2] Piotrowski, M., et al. (2001) J. Biol. Chem. 276: 2616-2621

T07-013

Expression profiling and functional definition of Arabidopsis CYP86A and CYP94B proteins as fatty acid hydroxylases

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Plants contain a significantly larger number of genes in their cytochrome P450 monooxygenase (CYP) superfamily than other eukaryotic systems (272 in Arabidopsis thaliana, 456 in rice, 56 in human, and 83 in Drosophila). But, the physiological and chemical functions have been defined for relatively few of the encoded proteins. In the Arabidopsis CYP86 and CYP94 cytochrome P450 families, which share some sequence homology with the animal and fungal CYP4 and CYP52 families that have been characterized as fatty acid ω-hydroxylases, only CYP86A1 and CYP86A8 have been functionally defined as fatty acid ω-hydroxylases. With these activities, these and other fatty acid hydroxylases have potential roles in the synthesis of cutin, production of signaling molecules and preventing accumulation of toxic levels of free fatty acids. To define function for the other members of the Arabidopsis CYP86A and CYP94B subfamilies, we have heterologously expressed each of them in baculovirus-infected Sf9 cells, shown that they ω-hydroxylate lauric acid (a prototype short chain fatty acids) and defined a range of long chain fatty acids capable of binding in the catalytic site of each of these enzymes. RT-PCR and microarray analyses have demonstrated very distinct regulation patterns for each of these fatty acid hydroxylases under normal growth conditions and in response to environmental stresses and chemical treatments. We will discuss the potential physiological functions of these fatty acid hydroxylases through a comparison of the regulation patterns with their predicted cis-acting regulatory elements and substrate specificities. We will also describe a Nanodisc system that enables the solubilization and incorporation of membrane-bound P450s and P450 reductases into nanobilayers suitable for sensitive substrate binding and turnover.

T07-014

Proanthocyanidin metabolism in Arabidopsis: dissecting biosynthetic and regulatory functions in developing seed coat

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Proanthocyanidins (also called condensed tannins) are polymeric end-products of the flavonoid biosynthetic pathway. These secondary metabolites influence seed quality (e.g. by increasing seed dormancy and longevity or by depreciating the nutritive value of oleoproteaginous seed cakes) and have positive effects on human health when present in food (e.g. by protecting against neurodegenerative and cardiovascular diseases). Therefore, an interesting purpose for crop improvement is the possibility to modulate proanthocyanidin (PA) biosynthesis in plants (amount and tissue/organ specificity).
In Arabidopsis, PAs are produced specifically in the seed coat, to which they confer a brown color after oxidation during seed maturation and desiccation. Recently, Arabidopsis has become a model of choice to dissect PA metabolism, particularly thanks to the isolation and characterization of many seed coat pigmentation mutants (transparent testa [tt], tt glabra [ttg], banyuls [ban], tannin-deficient seed [tds]). Here we present a spatio-temporal analysis of PA deposition in Arabidopsis developing seeds and correlate it with the activity of the BANYULS (BAN) gene promoter, in wild-type and regulatory mutant backgrounds. BAN encodes an anthocyanidin reductase, i.e. a core enzyme in PA biosynthesis. In parallel, preliminary results on the characterization of the tt15 mutant are also presented.
PA-accumulating cells were localized histochemically in the inner integument (seed body and micropyle) and pigment strand (chalaza). BAN promoter activity (assessed with GUS and GFP reporters) was detected specifically in these cells in wild-type background, demonstrating that BAN expression is regulated mainly at the transcriptional level. Mutations in regulatory genes of PA biosynthesis abolished BAN promoter activity (tt2, tt8, ttg1), modified its spatial pattern (tt1, tt16), or had no influence (ttg2), thus revealing complex regulatory interactions at several developmental levels. Gain-of-function experiments showed that a 86-bp promoter fragment was functioning as an enhancer specific for PA-accumulating cells.
We isolated several new alleles of the tt15 mutant, among which four happened to be T-DNA-tagged. The cloning of the corresponding gene is underway. The analysis of flavonoid metabolism in the tt15 mutant has been undertaken by histochemistry and LC-ESI-MS-MS approaches. Data on gene and flavonoid characterization will be discussed.

T07-015

Chemical Analysis of Arabidopsis Mutants in the Phenylpropanoid Pathway

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The identification of rate limiting steps in metabolic pathways is a prerequisite for designing strategies to manipulate plant metabolism through genetic engineering. To identify target genes for engineering the phenylpropanoid pathway, we carried out chemical analysis on a collection of Arabidopsis mutant lines with knockouts in genes involved in the biosynthesis pathway of sinapoylcholine (sinapine). These mutants were obtained either by direct acquisition from TAIR, i.e. SALK lines, or by screening Agriculture and Agri-Food Canada's Arabidopsis knockout population.

Chemical analysis carried out on seeds of wild type and homozygous mutant lines revealed that the main storage form of phenolics in the seed is sinapine, which represents over 50% of total extracted phenolics. Knockouts in phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H) and coumarate 3-hydroxylase (C3H) genes had no detectable effect on the level of sinapine, but caused the accumulation of some other intermediate phenolic compounds. On the other hand, knockouts in the caffeic acid O-methyltransferase (COMT), ferulic acid hydroxylase (FAH) and sinapoylglucose: choline sinapoyltransferase (SCT) genes resulted in significant reduction in sinapine content. Reduction in the sinapine content of these three knockout mutants also resulted in the accumulation of some intermediate phenolic compounds, such as ferulic acid and sinapoylglucose. Unlike the COMT and SCT mutants, low sinapine accumulation in the FAH mutant (<20% of wild type) was accompanied by a similar reduction in total phenolics content. Our analysis suggests that genes affecting later rather than earlier steps in the biosynthesis pathway have more impact on qualitative and quantitative profiles of phenylpropanoids in Arabaidopsis seeds.

T07-016

Genetic dissection of the regulation of cell wall matrix polymer biosynthesis by UDP-D-glucose 4-epimerase isoforms in Arabidopsis thaliana

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The biosynthesis of complex cell wall polymers requires the concerted action of glycosyltransferases, nucleotide sugar transporters and nucleotide sugar interconversion enzymes [1]. Most cell wall carbohydrates contain D-galactose, the de novo biosynthesis of which crucially depends on UDP-glucose 4-epimerase (UGE). All plant genomes investigated so far contain multiple UGE genes and mutations in the Arabidopsis thaliana UGE4 gene that is allelic to the root hair defective 1 (rhd1) mutant locus have revealed a non-redundant role of an individual UGE isoform in cell wall matrix polymer biosynthesis. Using genetic analysis it was shown that the flux route of UDP-D-galactose into cell wall polymers is regulated by ethylene in a auxin polar transport-dependent manner [2]. Further genetic dissection of the uge4/rhd1 mutant phenotype identified the SHORT ROOT (SHR) gene as another potential regulator of nucleotide sugar flux. The observation that the uge4/rhd1 mutant phenotype is modulated by mutants defective in root epidermal patterning and the expression pattern of UGE4, both indicate that nucleotide sugar metabolism is tightly regulated at a cell type dependent manner. The reverse genetic analysis of the Arabidopsis thaliana UGE gene family, consisting of five genes, indicates an essential partially overlapping role for UGE4 and UGE2 in cell wall matrix biosynthesis throughout plant development that is independent of the ubiquitously expressed UGE1 gene.

1. Seifert 2004 Curr Opin Plant Biol 7:277
2. Seifert ea. 2002 Curr Biol 12:1840
3. Seifert ea. 2004 Plant Cell 16:723

T07-017

A biotechnological approach to reduce photorespiratory losses. Effects of the expression of Glycolate oxidase in plastids of Arabidopsis thaliana.

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The oxygenase reaction catalyzed by RubisCO is the first reaction of the C2-photosynthetic carbon cycle. The prime function of the C2-pathway is to salvage glycolate-2-P by conversion to glycerate-3-P, which re-enters the C3-reductive cycle. In C3-plants, both CO2 and NH3 are released within the mitochondria, resulting in a loss of at least 25% of the CO2 fixed in ambient air. In this way, photorespiration is considered to be an energy wasting process. But, it is also involved in the prevention of photooxidation and the regeneration of metabolites. We attempt to introduce two alternative glycolate catabolic cycles into chloroplasts of A. thaliana in order to create an autoregulatory cycle, which results in an attenuation of the photorespiratory pathway. In both cycles, the first step is catalyzed by glycolate oxidase (GOX). Arabidopsis thaliana plants were transformed with a construct harboring an endogenous GOX-cDNA cloned downstream of a plastidic transit peptide and the CaMV35S-promoter. Selected homozygous lines were analyzed by Southern blot for the number of transgene insertions. GOX plants are characterized by retarded growth and yellowish leaves during the first weeks. RT-PCR analyses showed no co-suppression of the endogenous GOX expression. By week 5, GOX activity and the levels of glyoxylate and H2O2 were increased in the transformants, accompanied by decreasing photosynthetic efficiencies. By week 7, the transformants resemble the WT, showing the same H2O2 level as the WT, and also the electron transport rate tends to approach the WT level. The GOX phenotype can be reverted by growing the plants under high CO2 or by co-expressing catalase in the plastids. Co-expression of tartronate-semialdehyde reductase in the plastids did not revert the GOX phenotype. Here, we present the strategies used to characterize the transgenic plants overexpressing GOX in plastids and conclude that GOX plants are suitable for further engineering of the C2-photosynthetic carbon cycle.

Tolbert, N. (1997) The C2 oxidative photosynthetic carbon cycle. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 1-25.

T07-018

A gene family in Arabidopsis with cystine-lyase and tyrosine aminotransferase-activities

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In the genome of Arabidopsis thaliana seven genes are annotated to be tyrosine aminotransferase(TAT)-like proteins with sequence similarities between 43 and 78 %. While the function of some of these genes has not been elucidated so far, for At4g23600 (CORI3), which is coronatine-inducible (1), the main activity was shown to be a cystine lyase-activity (2). For At2g20610, a participation as a C-S lyase in the glucosinolate metabolism has been demonstrated (3) and for At5g52970, tyrosine aminotransferase-activity could be measured. Both enzymes use pyridoxal phosphate as a cofactor and participate in amino acid metabolism. Characterization of heterologously expressed CORI3 shows that the cystine lyase has a strong preference for the substrate cystine while djenkolic acid, S-methyl- and S-ethyl-cysteine are used to a lesser extent. The enzyme has a broad pH- and temperature-optimum. Expression studies applying semi-quantitative RT-PCR indicate that, while some members of the family are not or hardly transcribed, inducibility with coronatine, octadecanoids and herbicides for the others varies drastically. Total specific tyrosine aminotransferase-activity in Arabidopsis rises twofold upon activation with coronatine, while total specific cystine lyase-activity increases by a factor of 15 ¯18. Expression of the genes in different organs of the plant show divergent patterns. Transgenic plants have been made using a plasmid with the 35-S promotor, BASTA-resistance and the complete cDNA sequence of CORI3. Characterization of these plants showing the expression of CORI3 on the RNA level using semiquantitive RT-PCR and on the enzymatic level will be presented.

1:Lopukhina et al,Plant Physiol 126,1678(2001); 2: Jones et al,JBC278,10291(2002); 3:Mikkelsen et al,Plant J 37,70(2004)

T07-019

Functional Analysis of Plant Nucleotide Metabolism: The Purine and Pyrimidine Phosphoribosyltransferase Gene Family in A. thaliana

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Purine and pyrimidine nucleotides have important functions in a multitude of biochemical and developmental processes during the life cycle of a plant. Central enzymes of the nucleotide metabolism are the phosphoribosyltransferases (PRT's), including the small gene families of PRPP-amidotransferases (ATases), the adenine-and uracil phosphoribosyltransferases (APT's and UPRT's) as well as the hypoxanthine guanine phosphoribosyntransferase (HGPRT). These enzymes contain a conserved motive that is known as the phosphoribosytransferase domain that forms the PRPP-binding site. The phosphoribosyltransferases are involved in the process of the purine biosynthesis and in the purine and pyrimidine salvage pathways, two principle routes to synthesize nucleotides in plants.
The ATase catalyzes the initial step of the purine biosynthesis. Adenine and uracil phosphoribosyltransferases are involved in the nucleotide salvage and catalyzing the Mg2+-dependent transfer of the phosphoribosyl group of PRPP to the nucleo base in order to form the respective nucleoside monophosphate.
The ATases are encoded by three genes. All ATase genes encoding proteins which are predicted to be targeted into the chloroplast. The APT's including the HGPR cover a subfamily of six members with predicted localizations in the chloroplast and the cytosol. The UPRT's consist of 6 members with predicted localizations in the cytosol and chloroplast.
The organization as small multigene families raises the question of what metabolic function the different members of the subfamilies have, especially if the different isoforms are predicted to be localized in different cell compartments. In order to address this question we are currently investigating and characterizing several mutants with defects in the PRT function.

T07-020

Oxylipin-signaling system of chloroplasts from Arabidopsis thaliana: Searching for the initial lipase(s)

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Oxylipins, such as 12-oxophytodienoic acid (OPDA) and jasmonic acid (JA), are very important components in the regulation of plant development and in the interaction of plants with their environment (e.g., Creelman and Mullet, 1997; Weber, 2002). OPDA is an intermediate of the JA biosynthetic pathway and it is derived from chloroplastic membrane lipids for example sn1-O-(12-oxophytodienoyl)-sn2-O-(hexadecatrienoyl)-monogalactosyl diglyceride (MGDG-O) by a lipolytic reaction (Stelmach et al., 2001). All genes from Arabidopsis encoding enzymes of JA biosynthesis have been cloned and some of them (lipoxygenase, allene oxide synthase and allene oxide cyclase) were shown to be localized in chloroplasts (e.g., Laudert et al., 1996; Maucher et al., 2000). Based on the identification of the phospholipase DAD1 (dad1 = defective in anther dehiscence1) in flower buds from Arabidopsis (Ishiguro et al., 2001) as an enzyme catalyzing the initial step of the above mentioned biosynthesis we decided to quest for a lipase releasing OPDA from MGDG-O in chloroplasts from Arabidopsis leaves. Using database search and informations from chloroplast proteomics (Sacha Baginsky, personal communication), eight genes encoding proteins homologous to DAD1 according to their sequences were chosen to analyse. We persecuted different approaches to answer our question. Northern blot analyses gave no satisfactory conclusions, probably resulting from a low mRNA content. To focus on the lipase(s) of interest, a chloroplastic localized enzyme, every lipase gene was fused to the gene encoding green fluorescent protein (GFP) and the constructs were transiently transformed in Arabidopsis thaliana and Nicotiana tabacum leaves. Two lipases could be excluded because of their cytosolic localization.
Further studies with mutant plants should clearly indicate which enzymes are possible canditates for releasing OPDA from its lipid precursor. However, T-DNA mutant lines for only two of the lipases are available at the moment. In parallel, we try to answer our question by an enzymatic characterization of all six remaining heterologously expressed proteins in E.coli BL21(DE3) codon+. Substrate specificities could give important clues to the in vivo functions of the lipase(s).
It seems quite possible that these lipases not only act in the JA biosynthesis, but moreover fulfil other tasks in the lipid-based signaling system.

T07-021

Equilibrative nucleoside transporters (ENT) influence physiology and development in Arabidopsis

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Members of the protein family of equilibrative nucleoside transporters (ENTs) have been identified in diverse organisms like mammals, protists, and fungi. Nucleoside transporters are reportedly involved in the salvage pathways of nucleotide synthesis. We confirmed that nucleoside uptake and salvage occurs in Arabidopsis leaves and Ricinus cotyledons. The Arabidopsis ENT family comprises of eight members in total. The heterologous expression in yeast cells allowed us to characterize the transport properties of AtENT1, 3, 4, 6 and 7 for purine and pyrimidine nucleosides and related compounds. The subcellular localisation has been revealed by GFP-fusion constructs and transient expression in tobacco protoplasts. We studied the tissue specificity of all eight AtENTs applying quantitative RT-PCR and furthermore analysed the cell type specific expression of some AtENTs by promoter-GUS-fusion. Moreover, we identified growth conditions that affect the regulation of AtENT gene expression like pathogen-attack, wounding, senescence and nutrient-limitation. In addition, knockout plants for several AtENTs display phenotypes under distinct growth conditions pointing to an involvement of these proteins in physiology and development in Arabidopsis. Results of the previously listed experiments will be presented on our poster.

T07-022

Identification of new glucosinolate-biosynthesis genes induced by coronatine

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The phytotoxin coronatine is a structural analog of the octadecanoid signal molecules jasmonic acid and 12-oxophytodienoic acid, which are important for activation of plant defense genes. In order to isolate novel coronatine-regulated genes from Arabidopsis thaliana, differential mRNA display was performed (Lopukhina et al., 2001). One of the genes identified by this method, CORI-7, is annotated as a member of the sulfotransferase family. The cDNA for CORI-7 was obtained and expressed in Escherichia coli. The purified protein showed sulfotransferase activity towards several desulfo-glucosinolates transferring the sulfate group of 3'-phosphoadenosine-5'-phosphosulfate to the desulfo-glucosinolates and thereby catalyzes the last step in the biosynthesis of the glucosinolate core structure. Glucosinolates are secondary compounds found in many species of the Brassicales (Capparales) and are involved in herbivore- and pathogen-defense reactions in these plants. It is known for more than ten years that plants treated with jasmonic acid contain increased amounts of glucosinolates (e.g. Bodnaryk, 1993), and we therefore suppose that CORI-7 is involved in the biosynthesis of glucosinolates in vivo. The regulation of the CORI-7 gene by several herbivore- or pathogen-defense signaling compounds and the impact of coronatine on glucosinolate biosynthesis in comparison to jasmonic acid was studied.
Interestingly, other genes found to be up-regulated by coronatine in this screen, like a C-S lyase and a methyltransferase, do, in principle, also catalyze reactions taking place during the biosynthesis of glucosinolates and may therefore also be involved in this pathway.

Bodnarik (1993) Phytochemistry 35:301-305
Lopukhina et al. (2001) Plant Phys. 126:1678-1687

T07-023

Translational/post-translational regulation of a boron transporter AtBOR1

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Boron is an essential element for plant growth with a very narrow window between deficiency and toxicity. Arabidopsis BOR1 is a boron exporter for xylem loading essential for protecting shoots from boron deficiency (Takano et al., 2002). Here we report on the regulation of BOR1 by boron availability. Uptake studies using stable isotopes of boron indicated that xylem loading of boron by BOR1 was upregulated upon boron starvation. However, quantitative RT-PCR showed that mRNA levels of BOR1 were not significantly affected by boron conditions, suggesting translational and/or post-translational regulation of BOR1.
We then studied protein regulation using transgenic plants expressing BOR1-GFP fusion protein under the control of cauliflower mosaic virus 35S RNA promoter. Since expression of BOR1 in yeast conferred tolerance to high concentrations of boron presumably by lowering boron accumulation in cells, we expected that the overexpression of BOR1 in plants might confer tolerance to high concentrations of boron by extruding boron from root surface. However the 35S::BOR1-GFP transgenic plants did not show such a phenotype because of the translational/post-translational regulation. BOR1-GFP mRNA accumulated in several independent transgenic lines and mRNA levels were not affected by boron conditions. However, immunoblot analysis using an anti-GFP antibody showed that BOR1-GFP protein levels were upregulated upon boron starvation and the response was reversible upon re-supply of boron. Furthermore, confocal imaging of BOR1-GFP in root tip cells of transgenic plants showed a change in the sub-cellular localization in response to B supply. Under boron starvation, BOR1-GFP was observed at the periphery of the cells, indicating plasma membrane localization. However, after addition of a high concentration of boron, BOR1-GFP moved into dot-like structures in the cytoplasm and then disappeared. The results suggest endocytosis and subsequent degradation of BOR1 protein in response to B supply.

Takano et al., (2002) Arabidopsis boron transporter for xylem loading, Nature 420, 337-340

T07-024

Respiratory supercomplexes of plant mitochondria

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Supercomplexes are defined associations of protein complexes which are assumed to be important for several cellular functions. This “quintenary” organization level of protein structure recently was also described for the respiratory chain of plant mitochondria (Eubel et al. 2003, 2004). Except succinate dehydrogenase (complex II), all complexes of the oxidative phosphorylation (OXPHOS) system were found to form part of supercomplexes (complexes I, III, IV and V). Compositions of these supramolecular structures were systematically investigated using digitonin solubilizations of mitochondrial fractions and two-dimensional blue-native polyacrylamide gel electrophoresis. The most abundant supercomplex of plant mitochondria includes complexes I and III at a 1:2 ratio (I1+III2 supercomplex). Furthermore, some supercomplexes of lower abundance could be described which have I2+III4, V2, III2+IV1, III4+IV2, and I1+III2+IV1-4 compositions. Supercomplexes consisting of complexes I plus III plus IV were proposed to be called “respirasome”, because they autonomously can carry out respiration in the presence of ubiquinone and cytochrome c. Plant specific alternative oxidoreductases of the respiratory chain did not associate with supercomplexes under all experimental conditions tested. However, formation of the I1+III2 supercomplex possibly regulates alternative respiration, because it might limit access of alternative oxidase to its substrate (ubiquinone). Functional implications will be discussed.

Eubel et al. 2003, Plant Physiol. 133, 274-286
Eubel et al. 2004, Plant Physiol. 134, 1450-1459

T07-025

gamma-aminobutyric acid (GABA) metabolism in plants

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gamma-aminobutyric acid (GABA) is a ubiquitous, non-protein amino acid that has been found to accumulate in plants under various stress conditions. A physiological function, however, has not been assigned to GABA with the exception that it guides pollen tubes to micropyles (Palanivelu et al., 2003). To get more insights into plant GABA metabolism and function, we isolated knock out mutants for two single copy genes of Arabidopsis GABA catabolism, GABA-transaminase (gaba-t) and succinic semialdehyde-dehydrogenase (ssadh). Both mutants are phenotypically impaired, which has also been described by Palanivelu et al., 2003 and Bouche et al., 2003, respectively. The severity of the ssadh mutant phenotype indicates that succinic semialdehyde (SSA) is toxic to the plant. Growth of wild-type seedlings on agar plates supplemented with SSA provides further evidence for this. Growth characteristics of wild-type and gaba-t seedlings on agar plates containing different concentrations of GABA and nitrogen suggest that Arabidopsis senses GABA. A moderate concentration stimulates, whereas higher concentrations inhibit seedling growth. Furthermore, the contents of GABA and other metabolites in wild-type and mutant plants were determined using GC/MS.

Palanivelu et al., 2003 - Cell 114: 47-59
Bouche et al., 2003 - PNAS 100: 6843-8

T07-026

ISI1, a phloem located regulator of carbohydrate allocation in Arabidopsis

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All organisms coordinate their growth and metabolism with the availability of resources such as carbohydrates. In higher plants photosynthate produced in leaves is transported systemically via the phloem to support growth and storage in other organs. We have identified a novel plant-specific gene named IMPAIRED SUCROSE INDUCTION1 (ISI1) in a mutant which shows restricted carbohydrate allocation to plant growth and seed set, elevated chlorophyll levels, and reduced sugar induction of starch biosynthesis. These physiological phenotypes are consistent with altered sugar responsive expression of genes involved in starch, anthocyanin and cell wall biosynthesis, as well as chloroplast function. The isi1 mutant shows responses that are typically associated with reduced carbohydrate availability, despite having increased sugar levels. The genetic pathway identified by isi1 is independent of ABA or hexokinase mediated sugar response pathways and also does not affect the sugar repression of genes involved in nitrogen metabolism or the glyoxylate cycle. The ISI1 gene is expressed in the phloem of leaves following their developmental transition from sink to source status. ISI1 identifies a novel regulatory pathway that monitors whole-plant carbohydrate availability and co-ordinates photoassimilate production in leaves with its utilisation by carbohydrate-dependent processes.

T07-027

Expression Analysis of Nucleotide Metabolism Genes in Arabidopsis

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Nucleotides are central metabolites in all species. As components of nucleic acids, phytohormones, cofactors, and energy rich precursors they are involved in vital cellular processes. Whereas the purine nucleotide ATP is the major energy donor in most organisms, in plants the pyrimidines UTP, UDP and UDP-sugars serve as co-substrates for sucrose metabolism and the synthesis of cellulose and other glycosides. Enzymes in the de novo synthesis of nucleotides are often encoded by single genes whereas steps involved in salvaging and inter-conversion processes are always encoded by multi-gene families.
The temporal and tissue specific expression analysis gives valuable information on the regulation of each gene involved in nucleotide metabolism. Using semi-quantitative Real-Time PCR we examined a subset of 54 genes involved in the nucleotide metabolism of Arabidopsis. These data will help us to interpret the physiological function of nucleotides in general and the contribution of each individual enzyme to the metabolism of plants.

T07-028

Expression patterns of five Arabidopsis thaliana UDP-D-glucose-4-epimerase genes in leaves .

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Conversion of UDP-D-glucose to UDP-D-galactose is an essential step during the biosynthesis of galactosylated cell wall matrix components like hemicelluloses, pectin and AGP. This reaction is catalyzed by UDP-D-glucose 4-epimerase (UGE). Arabidopsis contains five isoforms of UGE genes. Differentiation of leaf cells during leaf development results in remarkable variations in cell shape and in cell wall composition, which may be in part related to differential expression of the UGE genes. Moreover Arabidopsis transports traces of raffinose in the phloem and the synthesis of one of its precursors, galactinol, requires UDP-D-galactose as a substrate. Thus, in leaves of Arabidopsis, UGE is required for galactinol biosynthesis. We analyzed the tissue specificity of expression of the GUS gene under control of the promoters of five UGE genes in Arabidopsis leaves. In fully expanded source leaves, four of five UGE genes were expressed in all tissues of the leaf but the expression levels showed a tissue specific pattern. Expression of one isoform was confined to guard cells. Expression patterns of UGE genes were also analyzed during leaf developmental stages, and by mining of public global expression profile data. Possible functions of different UGE isoforms during leaf development are discussed.

T07-029

Genetically encoded sensors for metabolites

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Abstract

During the last decade many of the genes encoding important transporters and metabolic enzymes have been identified. Using heterologous expression systems it has been possible to study the biochemical properties of the corresponding proteins in great detail. It is expected that within the next ten years biochemical functions will have been assigned to many of the products of the approximately 30,000 Arabidopsis genes. Analysis of knock-out mutants will provide insight into the biological function of the proteins. Complementing metabolomic approaches provide information on changes in cellular ion and metabolite profiles in the mutants, thus providing information essential for the construction of integrated cellular and whole plant models.

However, one important set of information especially relevant to multicellular organisms with specialized organ function is lacking: the knowledge of the spatial and temporal profiles of metabolite levels at cellular and subcellular levels. To address this issue, we have developed protein-based nanosensors providing a set of tools for real time measurements of metabolite levels with subcellular resolution. The prototypes of these sensors were shown to function in yeast and in mammalian cell cultures. One future goal is to expand the set of sensors to a wider spectrum of targets by using the natural spectrum of periplasmic binding proteins from bacteria and by computational design of proteins with altered binding pockets. Application of nanosensor technology to plant cells and tissues will help to elucidate the special and temporal distribution of ions and metabolites.

T07-030

The Arabidopsis plastidic Glucose 6-Phosphate/ Phosphate translocator GPT1 is essential for pollen maturation and female gametogenesis

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Plastids of non-green tissues can import carbon in the form of glucose 6-phosphate by the glucose 6-phosphate/phosphate translocator (GPT). The genome of Arabidopsis contains two homologous GPT genes, AtGPT1 and AtGPT2. Expression of these genes in yeast cells revealed that both proteins represent functional glucose 6-phosphate translocators. When ectopically expressed in a plastidic phosphoglucoisomerase-deficient mutant line (pgi), both Arabidopsis GPTs complemented the low-starch leaf phenotype of this mutant, indicating that both GPTs are functional also in planta. By reverse genetics two mutants were isolated, gpt1-1 and gpt1-2, harboring T-DNA insertions in the GPT1 gene. In the homozygous state both mutations resulted in lethality. In both mutant lines a distorted segregation ratio together with a reduced male and female transmission efficiency indicated profound defects on gametogenesis. The mutant female gametophyte development was arrested at a stage before the polar nuclei fuse. The mutant pollen development was associated with less vesical and lipid body formation and a disintegration of the membrane system. The pleiotropic effect on gametophyte development could be fully reversed by introducing the AtGPT1 gene under transcriptional control of its authentic promoter in the gpt1-2 mutant. On the other hand, disruption of GPT2 had no obvious effect on growth and development under green house conditions. Taken together, our results implicate that import of glucose 6-phosphate by GPT1 into non-green plastids is crucial for pollen maturation and female gametophyte development. It is most likely that glucose 6-phosphate serves as substrate for the oxidative pentose phosphate cycle to create reducing power, which is required for fatty acid biosynthesis.

T07-031

Analysis of a putative plastidic transporter affecting photosynthesis in Arabidopsis thaliana

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Membranes are the major barriers between the cytoplasm and cell organelles. Therefore, a variety of transporters are needed to mediate the exchange of metabolites, ions and proteins between these compartments.
We are analysing a family of five proteins, with putative transport function. A comparison between serveral prediction programmes (http://aramemnon.botanik.uni-koeln.de/) revealed that these proteins contain more than five transmembrane spanning regions and different subcellular targeting signals. For the two plastidic predicted members of this family, targeting of the proteins was confirmed experimentally by GFP fusion constructs. For each member homozygous T-DNA insertion mutants were isolated. Mutants defective for the plastidic proteins (dap1, dap2) exhibit drastic phenotypes. They are characterised by dwarfish growth, yellowish leaves and a delay in flowering time. In addition, dap1 has a significantly lowered electron transport rate, chlorophyll- and starch content. This gene is transcribed only in green tissue, which may implicate an association with photosynthetic processes. Therefore, these mutants are named dwarf affected in photosynthetic electron transport rate (DAP). Experiments to determine the substrate specificities of the respective transport proteins and the resulting impact on electron transport are in progress.

T07-032

Functional analysis of the nucleotide sugar conversion pathway in Arabidopsis

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Significant parts of the plant cell wall consist of polysaccharides. It has been generally accepted that these polysaccharides are synthesized from a large number of different NDP-sugars which act as activated precursors. These NDP-sugars are interconnected to each other via extensive interconversion networks.
During the last years an explosion of knowledge concerning nucleotide-sugar conversion pathways took place. However, for example the synthesis of UDP-L-rhamnose, the proposed precursor of cell wall derived L-rhamnose, remains unclear. Mutants in one of the proposed three genes encoding UDP-L-rhamnose synthases did not display any morphological or biochemical phenotype in the plant cell wall (Usadel et al. 2004). However, a reduction of the whole UDP-L-rhamnose synthesizing gene family facilitating an RNAi approach resulted in a decrease in cell wall bound rhamnose. Moreover, we show that these plants display drastic visible phenotypes such as dwarfism and increased production of anthocyans. Details concerning these mutants such as in-depth cell wall analysis, and possible implications for cell wall synthesis and development will be discussed.
In addition, a co-response network analysis of most nucleotide sugar converting enzymes from Arabidopsis was performed in an attempt to establish the metabolic network leading to the synthesis of the wall and the identification of the key enzymes involved in this process

T07-033

The role of the oxPPP during the development of Arabidopsis thaliana.

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The oxidative pentose phosphate pathway (oxPPP) plays a crucial role in plant cells. In plastids, the oxidative part of the oxPPP produces reducing power (i.e. NADPH), which is required for fatty-acid synthesis, the assimilation of inorganic nitrogen and protection against oxidative stress. The non oxidative part of the oxPPP provides metabolic intermediates for several biosynthetic processes like the synthesis of nucleotides and the Shikimate pathway. Altough the basic features of the oxPPP are well-characterized, it is unknown how the oxPPP influences processes like plant development and stress response. In this work we focus on the impact of genes coding for plastidic enzymes of the oxPPP on the development of Arabidopsis thaliana using a reverse genetic approach. Because each of the enzymes of the oxPPP is encoded by multiple genes, we first concentrated on proteins providing Glc-6-P for the oxPPP in the plastids. For this purpose we investigated T-DNA insertion lines in genes coding for the glucose6-Phosphate/phosphate transporters (GPT1 and GPT2) and plasidic phospho-gluco isomerase (PGI1). Analysis of mutant lines of AtGPT revealed that a knockout of GPT2 does not affect plant development, whereas disruption of the GPT1 gene results in lethality (see T07-30). Yu et al. (2000) isolated an Arabidopsis pgi1-1 EMS mutant with decreased PGI activity and starch content. However, the functional analysis of a complete knockout of AtPGI1 was not described so far. We characterized a T-DNA insertion mutant of AtPGI1. The inability to isolate homozygous lines implicates a crucial role of PGI1 for plant development. In addition we analyse mutant lines in genes for 6-phosphogluconate dehydrogenase, which is a key enzyme of the oxPPP. 6-PGDH catalyzes the oxidative decarboxylation of 6-phosphogluconate, the second of the initial steps of the oxPPP. The Arabidopsis genome encodes three 6-PGDH isozymes, however none of them posess an apparent transit peptide sequence. The comparison of the Arabidopsis 6PGDH isozymes with related sequences from spinach suggests that two of the Arabidopsis genes encode plastidic forms of the enzyme. Expression analysis showed ubiquitous expression with a similar pattern of all three genes in Arabidopsis. Until now we have sucessfully isolated a homozygous mutant line for the predicted cytosolic isozyme. The isolation and characterization of mutant lines for the other 6-PGDH isozymes is in progress.

T07-034

A novel MYB factor involved in the regulation of phenylpropanoid biosynthesis as pathogen response?

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Phenylpropanoids make up a large family of plant secondary metabolites and are believed to reduce the risk of cancer and cardiovascular diseases due to their antioxidant properties. In order to find novel regulators of the phenylpropanoid pathway, a high-throughput screen was used to identify Arabidopsis activation-tagged lines with altered phenylpropanoid contents.
One of the screened lines showed an accumulation of phenylpropanoids in rosette leaves as revealed by HPLC analysis. The phenotype resulted from the overexpression of a gene that encodes a putative MYB transcription factor. The gene-to-trait relationship was confirmed by overexpression of the gene under control of a constitutive promoter and comparing the HPLC profile with those of wild type and the original activation-tagged line.
Using gfp-fusion constructs, we showed that the fusion protein is targeted to the nucleus when expressed in tobacco protoplasts, hence supporting the hypothesis of a transcription factor involved in the regulation of the phenylpropanoid pathway. In addition, microarray data (Chen et al., 2002; Cheong et al., 2002) showed an upregulation of the MYB factor upon pathogen challenge indicating a linking role between pathogen stress and the accumulation of phenylpropanoids.

W.Chen et al., Plant Cell. 14, 559 (2002).
Y.H. Cheong et al., Plant Physiology. 129, 661 (2002).

T07-035

Threonine aldolase, a previously uninvestigated component of plant amino acid metabolism

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Amino acids are not only the building blocks of proteins, but also serve as precursors for other important plant metabolites and constitute an essential part of human and animal diets. Nevertheless, many aspects of plant amino acid metabolism remain unknown. A mutant with a 20-fold increase in seed threonine was discovered in a screen for Arabidopsis with altered seed amino acid levels. Genetic mapping and DNA sequencing identified a missense mutation in a putative threonine aldolase (AT1G08630) as the underlying genetic basis of this phenotype. Threonine aldolase, which catalyzes the reversible interconversion of threonine and glycine + acetaldehyde, has not been studied previously in Arabidopsis or any other plant. Wild type, but not mutant, cDNA clones rescued the glycine auxotrophy of a yeast gly1, shm1, shm2 mutant. In vitro assays with extracts from the transformed yeast strain showed threonine aldolase activity, with an apparent Km for threonine of 7.0 mM for the Arabidopsis enzyme.

Analysis of the Arabidopsis genome identified a second putative threonine aldolase (AT3G04520), which also rescued the yeast glycine auxotrophy. Semi-quantitative RT-PCR experiments demonstrated that the two threonine aldolases have overlapping, but not identical expression patterns, indicating different roles in plant metabolism. Analysis of a T-DNA insertion in the AT3G04520 threonine aldolase showed that this gene may be essential for the life of the plant. It was not possible to isolate a homozygous T-DNA insertion mutant, and 25% of the seedlings from seeds of heterozygous plants abort early in development. Ongoing and future work with Arabidopsis threonine aldolases will reveal the role that these previously uninvestigated enzymes play in plant amino acid metabolism.

T07-036

Spatial and temporal expression of sucrose synthase gene family members

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Sucrose synthase (SuSy) is a key enzyme involved in sucrose metabolism catalysing the reversible conversion of sucrose and UDP to UDP-glucose and fructose. Therefore, its activity, localization and function has been broadly studied in various plant species. It has been shown that SuSy can play a role in supplying energy in companion cells for phloem loading (Fu and Park, 1995); provide substrates for starch synthesis (Zrenner et al., 1995) as well as supply UDP-glucose for cell wall synthesis (Haigler et al., 2001).
Analysis of the Arabidopsis genome identifies 6 different SuSy isoforms (Baud et al., 2004). In order to further understand the functions of these enzymes we have investigated their spatial and temporal expression patterns. We have done this using promoter reporter gene fusions and correlate these data with quantitative real-time RT-PCR.
Promoter sequences for each Susy isoform were cloned into the GUS reporter vector: pGPTV-KAN and transformed into Arabidopsis. Tissue and cell specific localizations were observed including vascular bundles, roots, leaves, stomata and seeds. Analysis of transcript levels using real-time RT-PCR detected expression of all isoforms in several plant organs and tissues. For some isoforms the expression was highly tissue-specific (e.g. in seeds, roots and developing siliques) whilst for some of them it was generally lower and constant throughout the whole plant. These data support some of the previously reported roles for Susy and suggest the occurence of isoforms with specific functions.

T07-037

a mutation in the sucrose transporter gene SUC2 leads to changes in metabolic gene expression

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In Arabidopsis the sucrose-proton symporter, SUC2 is responsible for the movement of sucrose into the phloem for transport from leaves to other parts of the plant. Molecular analysis of the Arabidopsis pho3 mutant revealed a single nucleotide change in the gene encoding this sucrose transporter. The mutation led to the accumulation of sucrose and other carbohydrates to high levels, providing a tool to investigate the genomic response to sucrose accumulation using microarray analysis. Wild type and mutant plants were grown in soil to the mature rosette stage for analysis of gene expression using the Affymetrix ATH1 chip. Small but significant decreases were observed in the expression of many genes encoding enzymes and regulatory proteins involved in primary carbon assimilation, suggesting that in mature leaves of Arabidopsis there is limited feedback regulation on gene expression by sugars. Levels of Rubisco small subunit mRNA and protein were reduced by 30-50% but chlorophyll fluorescence imaging of seedlings indicated that photosynthesis was not affected. In contrast, notable changes to the expression of some genes involved in plastid carbon metabolism were found, including increases in transcripts for the large subunits of ADP-glucose pyrophosphorylase. The study also revealed a striking increase in expression of the plastid glucose 6-phosphate/phosphate translocator, characteristically expressed only in heterotrotrophic tissues. This indicated a change in the nature of metabolite exchange between the plastid and cytosol in the pho3 mutant. Very large increases were observed in the expression of transcription factors and enzymes involved in anthocyanin biosynthesis.

Lloyd JC and Zakhleniuk OV (2004) Responses of metabolism to sugar accumulation. J Exp Bot May adv access

T07-038

Contribution of the myo-inositol oxygenase (miox) gene family of Arabidopsis thaliana to ascorbate biosynthesis

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There is evidence of the operation of three biosynthetic pathways for vitamin C (ascorbate, AsA) in plants: the mannose/L-galactose pathway, a D-galacturonic acid pathway and a route that resembles the “animal pathway”. Our group recently published molecular and biochemical evidence for an additional route using myo-inositol (MI) as the initial substrate. A MI oxygenase (MIOX) gene was identified in chromosome 4 (miox4) of Arabidopsis. AsA levels increased 2- to 3-fold in homozygous Arabidopsis lines over-expressing the miox4 open reading frame thus suggesting the role of MI in AsA biosynthesis and the potential for using this gene for the agronomic and nutritional enhancement of crops. According to sequence similarity analysis, miox4 (ORF At4g26260) belongs to a family of four members in the Arabidopsis genome. Its homologs are located in chromosomes 1, 2 and 5 (named miox1, miox2 and miox5, ORFs At1g14520, At2g19800, At5g56640, respectively). Analysis of the protein structure showed that Miox4/Miox5 and Miox1/Miox2 are closely related. We have cloned all ORFs and confirmed the enzymatic activity of a truncated Miox2, containing the catalytic domain that is common to all members, in bacterially expressed recombinant protein. RT-PCR experiments performed with RNA extracted from 5-week old plants showed that miox1 is predominantly expressed in cauline leaves and siliques, and miox5 in flowers, while miox2 is highly expressed in all tissues. Assays performed with homozygous T-DNA knockout lines of miox1, 2, 4 and 5 under different light conditions revealed that miox1 is the member of the family that contributes the most to the AsA content of leaf tissue in Arabidopsis. Silencing of all miox genes caused an arrest of growth in the cotyledonary stage of the T1 generation plants transformed with an RNAi construct indicating the importance of the miox family not only for AsA biosynthesis, but also to normal growth and development.

T07-039

Identification and characterization of a putative glucuronic acid reductase in Arabidopsis thaliana

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There is evidence of the operation of at least three biosynthetic pathways for vitamin C (ascorbate, AsA) in plants: the mannose/L-galactose pathway, a D-galacturonic acid pathway and a route that resembles the “animal pathway”. Recently our group published molecular and biochemical evidence for an additional route using myo-inositol (MI) as the initial substrate. A MI oxygenase (MIOX) gene was identified in chromosome 4 (miox4) of Arabidopsis ecotype Columbia, and its enzymatic activity was confirmed in bacterially expressed recombinant protein. MIOX is an enzyme containing non-heme iron that catalyses a four electron oxidation with the transfer of only one atom of oxygen into the product D-glucuronic acid (GlcUA). There are only two additional enzymatic steps necessary for the conversion of GlcUA to AsA. The first of those reactions is the oxidation of GlcUA to L-gulonic acid. This conversion is catalyzed by a GlcUA reductase (EC1.1.1.19), also called gulonate dehydrogenase or L-hexonate dehydrogenase. GlcUA reductase belongs to the family of aldo/keto reductases and has been extensively studied in mammalian models, however, there are no reports of its enzymatic activity in plants. Our database search revealed no plant enzymes annotated as GlcUA reductase (or any of its synonyms). We expanded our analysis and found that there are close to 40 members of the aldo/keto reductase family in the Arabidopsis genome. Screening of T-DNA knockout lines using HPLC and spectrophotometric-based assays of some of the members of the aldo/keto reductase family allowed us to identify the SALK line 119576, which had a substantial reduction (~50%) of AsA leaf content compared to wild type plants. This line has a T-DNA inserted in ORF At2g37770. We have cloned this ORF from leaf cDNA. Experiments are in progress to test the enzymatic activity of the recombinant protein in bacteria and to examine AsA biosynthesis in transgenic Arabidopsis plants over-expressing this gene.

T07-040

Reduction of cytokinin biosynthesis genes

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Although the hormone cytokinin has long been studied for its ability to stimulate plant cell division in culture, fundamental processes that absolutely require cytokinin for normal growth and development have not yet been identified. Previous work in the field has succeeded in up-regulating levels of the hormone in-vivo by either exogenous cytokinin application or transgenic expression of bacterial isopentenyl transferase genes. Heterologous expression of a gene that inactivates and stores cytokinins, zeatin O-glucosyltransferase (Martin et al., 2001 In Vitro Cell Dev Biol - Plant 37:354-360) reduces cytokinin levels, while constitutive overexpression of cytokinin oxidase genes (which degrade a subset of cytokinin compounds) has also been shown to reduce both cytokinin and auxin levels (Werner et al., 2001 PNAS 98:10487-10492) Although cytokinins were reduced in these studies, they were not completely eliminated. The first plant cytokinin biosynthesis genes, the Arabidopsis isopentenyl transferase genes (AtIPTs), have recently been identified (Takei et al., 2001 JBC 276:26405-26410; Kakimoto, 2001 Plant Cell Physiol 42:677-685). We are currently working with the AtIPTs to develop plants that lack the ability to produce cytokinins. AtIPTs consist of a seven member family, and their expression patterns overlap throughout the plant (Miyawaki et al., 2003 Plant J 37:128-138). Expression analysis of the AtIPT gene family with our GUS lines and RT-PCR data will be presented, as well as the characterization of T-DNA insertion lines and RNAi lines.

T07-041

A Systems Approach to Nitrogen Networks

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Our long-term goal is to identify networks in the Arabidopsis genome that are transcriptionally controlled by C and N metabolic sensing and signaling pathways. Visualizing and modeling such metabolic regulatory networks should enable us to devise predictive models that may permit intervention for agricultural traits. As a proof of principle, we have begun to use genomic, bioinformatic and systems biology approaches to model and visualize metabolic regulatory networks controlled in response to carbon and nitrogen metabolites. We have used several math tools to design systematic spaces that enable us to cover a large experimental space of treatment conditions with a small number of experiments. One approach we have used to do this is called Combinatorial Design. We have used Affymetrix whole genome chips to identify genome wide responses to this matrix of C:N treatment conditions. Hierarchical cluster analysis of the treatments reveals three types of genome-wide responses to C and/or N treatments. We have identified gene clusters that are transcriptionally regulated by N sensing or CN sensing. We have used several approaches to analyze these clusters to define biologically relevant processes controlled by N and CN networks. In one network analysis we queried the clusters for biological themes using GO or MIPS functional annotations, performed statistical tests for significance of the response and display the results in a color-coded network graph. In another type of network analysis, we used Cytoscape to build a metabolic and regulatory network model of Arabidopsis using the information from KEGG, AraCyc and Transfac databases. These network models enabled us to define several biologically relevant subnetworks within the N-regulated gene networks. This analysis has revealed that a number of cell biological, metabolic and regulatory processes are part of a N-regulatory network in Arabidopsis. This work has also generated biological hypothesis for mechanisms of CN sensing/signaling which we are testing using Arabidopsis mutants.

T07-042

Elevated plastid-derived isoprenoid synthesis in the prl1 mutant of Arabidopsis thaliana

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Isoprenoids comprise a wide spectrum of primary and secondary plant metabolites. Primary metabolites are present in all plants and essential for their survival, e.g. as part of the photosynthetic machinery (phytol moiety of chlorophylls and carotenoids), whereas the dispensable secondary metabolites provide the plant with benefits for the interaction with its environment. Both groups of metabolites can be synthesized either in the cytosol through the MVA (mevalonate) pathway or in the plastid via the MEP (methylerythritol phosphate) pathway. Although enzymes in both pathways have been identified in bacteria and plants, little is known about the interaction between the two pathways and their spatial and temporal regulation. We performed a resistance-screen in Arabidopsis thaliana activation-tagged lines using fosmidomycin, an inhibitor of the DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase) of the MEP pathway. In the presence of fosmidomycin, wild-type Arabidopsis seedlings cannot develop green cotyledons due to a block in the synthesis of pigments through the MEP pathway in plastids. We reasoned that the MEP pathway and/or the cross-talk between the MEP and the MVA pathways (Kasahara et al., 2002) might be modulated in fosmidomycin-resistant (fre) mutants.

Here, we report the isolation and characterisation of a fre mutant with a T-DNA insertion in the PRL1 (pleiotropic regulatory locus 1) gene. PRL1 is a regulatory protein with conserved WD motifs, and is proposed to function as a pleiotropic regulator of glucose and hormone responses in Arabidopsis (Nemeth et al., 1998). The mutants are not only resistant to fosmidomycin, but also to ketoclomazone inhibiting the DXS (1-deoxy-D-xylulose 5-phosphate synthase) of the MEP pathway and to fluridone, inhibiting the phytoene desaturase in carotenoid biosynthesis. We show that the resistance of the prl1 mutants to various herbicides blocking the plastid-derived isoprenoid synthesis is, at least in part, due to an upregulation of protein levels of the MEP-pathway enzymes and provide biochemical evidence for an elevated flux through the MEP pathway. A possible regulatory role of PRL1 in isoprenoid synthesis is discussed.

Kasahara et al. (2002) J. Biol. Chem. 277, 45188-45194
Nemeth et al. (1998) Genes Dev. 12, 3059-3073

T07-043

The three desulfo-glucosinolate sulfotransferase proteins in Arabidopsis have different substrate specificities

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All members of the sulfotransferase (SOT, EC 2.8.2.-) protein family use 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as sulfuryl donor and transfer the sulfonate group to an appropriate hydroxyl group of several classes of substrates. In Arabidopsis thaliana (L.) Heynh. 18 genes encoding sulfotransferase proteins have been identified. For most of the sulfotransferases the respective substrate specificities and therefore their functions in the organism are not known yet. Three out of 18 sulfotransferase proteins are good candidates for desulfo-glucosinolate sulfotransferases (SOT16-SOT18). None of these three SOT genes contains introns. Therefore the encoding sequences were amplified from genomic DNA isolated from Arabidopsis, ecotype C24, and the respective proteins were expressed in E. coli as fusion proteins with the 6xHis-tag. The affinity-purified proteins were used in enzyme activity assays testing different desulfoglucosinolates. The reaction products were analysed by HPLC. First results on substrate specificities for SOT16, SOT17, and SOT18 will be presented.

T07-044

High Sugar Response Mutant 5 encodes a F-box protein: A link between regulation of carbohydrate resource allocation and SCF ubiquitin ligase mediated protein degradation?

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Plants co-ordinate the processes of carbohydrate production, utilisation for growth and storage in response to the availability of carbohydrates. The mechanisms by which plants determine how much carbohydrates are available and how their allocation to the different processes is regulated remain largely unknown. In order to identify mutants with altered carbohydrate resource allocation a genetic screen was performed and several high sugar response mutants (hsr) were isolated (1). Here we report the characterisation and cloning of HSR5. The hsr5 mutant shows enhanced seedling growth in response to glucose and sucrose at low concentrations and is hypersensitive to arrest of seedling development caused by high sugar concentrations. When grown on 3 % sucrose the hsr5 seedlings accumulate 50% more starch and show reduced chlorophyll and elevated anthocyanin levels. These phenotypes are consistent with changes in expression levels of sugar responsive genes involved in starch, storage protein and anthocyanin biosynthesis, sugar transport and photosynthesis. When grown on soil the hsr5 mutant has a reduced growth rate, flowers later and produces bigger but fewer seeds. The hsr5 mutant shows unaltered responses to the plant hormones ABA, ethylene and cytokinin. These phenotypes suggest the hsr5 mutation effects the co-ordination of carbohydrate resource allocation directly or the plant fails to monitor its carbohydrate status correctly and therefore is unable to adjust the carbohydrate allocation in accordance with the available carbohydrates. We have cloned hsr5 by mapbased cloning and it encodes a protein with a F-box motive. In a yeast two hybrid assay the HSR5 protein interacts via its F-box with ASK1/SKP1 subunit of ubiquitin ligase. Recently it has been reported that PRL1 competes with ASK1/SKP1 for the binding to plant Snf1-related protein kinases (SnRK) (2,3,4,) Since the prl1 mutant shows a hypersensitivity to glucose and sucrose which is remarkably similar to the hsr5 phenotype and Snf1 related kinases play a central role in the regulation of glucose signalling in yeast and animals we are tempted to speculate that HSR5 is part of a SCF complex that is regulated by SnRKs and PRL1.

1. Plant Physiol. 134: 81-91
2. Genes & Development 12: 3059-3073
3. PNAS 96: 5322-5327
4. EMBO 20: 2742-2756

T07-045

Overexpression of a specific Sucrose-Phosphat-Synthase (SPS) isoform from Arabidopsis thaliana sensitive to phosphorylation

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SPS is a key enzyme in carbohydrate metabolism of plants. It regulates sucrose synthesis and therefore connects the metabolism of the chloroplasts with that of non?photosynthetic tissue. As derived from the genome sequence, there are 4 putative SPS isoforms in Arabidopsis thaliana.
To investigate characteristic features and regulation of this enzyme, we over-expressed one isoform, the SPS1(At5g11110), in Escherichia coli. This isoform carries the well-studied RISS-phosphorylation motif. The his-tagged protein had been isolated to homogenity via IMAC (immobilised metal affinity chromatography) and FPLC (fast performance liquid chromatography). Molecular and kinetic properties of the purified enzyme are determined.
Based on this model protein we developed a “mass western” for the detection of single low abundant protein species in complex samples via mass spectrometry.
A further aim is to get inside in the mechanisms of SPS-regulation via Ser-phosphorylation.

T07-046

Phosphorylation studies on sucrose-phosphate synthase based on mass

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Sucrose-phosphate synthase (SPS) is a highly regulated enzyme that catalyzes
the penultimate step in sucrose synthesis in plants. The enzyme plays a key role in carbohydrate metabolism, and its regulation is therefore of special interest. SPS activity is known to be strongly regulated posttranslationally by phosphorylation at three different serine residues in response to dark/light modulation and various stresses such as water stress and cold stress.

Using synthetic peptides that contain the putative regulatory phosphorylation sites of the four SPS isoenzymes from A. thaliana as potential targets for kinases, in vitro phosphorylation studies were performed in a multiparallel assay [1]. Identification and quantification of phosphopeptides was achieved on a triple-quadrupole mass spectrometer. The
studies revealed different substrate specificities among the putative phosphorylation sites and increased kinase activities in the starchless mutant plant (PGM) that lacks plastidic phosphoglucomutase, as compared to the wild-type (WT) plant.

The multiparallel kinase assay facilitated furthermore the assessment of various protein kinases for their specific substrate affinity towards each SPS peptide. This enabled us to screen for upstream kinases potentially involved in SPS regulation in vivo. So far, several calcium-dependent protein kinases (CDPKs) from A.thaliana and N. tabacum were investigated.

To gain more insight into the mechanisms of regulation of the SPS enzyme we
studied the molecular properties of the protein. We therefore overexpressed a His6-tagged isoform of SPS from A. thaliana, SPS1 (At5g11110), which contained the conserved phosphorylation motif RISS, in E. coli and purified the enzyme to near homogeneity by metal affinity chromatography and two subsequent hydrophobic interaction chromatography steps on butyl sepharose.

[1] Glinski et al. (2003) Rapid Communications in Mass Spectrometry 17(14):1579-84

T07-047

Characterization of two splicing variants of AtUPS 5

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UPS (Ureide Permeases) constitute a novel family of polytopic membrane proteins with 5 members in Arabidopsis initially identified as allantoin transporters (Desimone et al., 2002). Recently, two members of the Arabidopsis family, AtUPS1 and AtUPS2, as well as a homologue in Phaseolus vulgaris, PvUPS1, have been characterized in detail (Pelissier et al., 2004; Schmidt et al., 2004). AtUPS1 and AtUPS2 seem to be main transporters for uracil, while in nodulated legumes, UPS may serve for long distance transport of allantoin. The present work provides information about possible functions of AtUPS5. Two different AtUPS5 mRNAs encoding for 413 and 378 amino acid proteins have been identified. The predicted structure for the larger protein (AtUPS5) is similar to the other AtUPS members, while the shorter protein (AtUPS5s) is lacking two transmembrane domains. Yeast cells expressing AtUPS5 were able to transport allantoin and other substrates with different affinities. In constrast, the functionality as a transporter could not be shown for AtUPS5s. Co-expression of both proteins severely reduces the capacity of yeast cells for allantoin uptake, suggesting that AtUPS5s plays a regulatory role in AtUPS5 mediated transport. To study potential protein-protein interaction between AtUPS5 and AtUPS5s via co-immunoprecipitation, epitope tagged proteins were generated and their functionality was investigated. The presence of both splicing variants was studied in plants under different nutritional conditions by using RT-PCR. To elucidate the physiological role of AtUPS5 in planta, a T-DNA insertion line is being currently analyzed.

Desimone et al.(2002) Plant Cell 14:847
Pelissier et al.(2004) Plant Physiol. 134:664
Schmidt et al.(2004) JBC submitted

T07-048

Oxygen sensing and adaptive metabolic responses to low internal oxygen in plants

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Plants lack an efficient distribution system for oxygen throughout their tissues, even though oxygen is a vital substrate for energy (ATP) metabolism by mitochondrial respiration. Because of the poor permeability, oxygen concentrations within plant tissues can become very low. Recent studies with many different plant species, including Arabidopsis document that plants decrease their oxygen consumption in response to these low concentrations, thus avoiding internal anoxia (1). The adaptive response consists of a reduction of respiration as well as the inhibition of a wide range of biosynthetic energy consuming processes. Where possible, energy conserving metabolic pathways are preferred when oxygen becomes limiting. Besides, high energy demanding storage metabolism of e.g. oil or protein is more strongly reduced by decreasing oxygen than starch metabolism is. Also phloem transport is affected by the low internal oxygen concentrations. Not only is the oxygen tension within the vascular bundles very low, also oxygen restriction within sink tissues influences sink strength and thereby phloem transport (2).
All these physiological changes are induced at oxygen concentrations much higher than those that are limiting for ATP synthesis by mitochondrial respiration, indicating that a sensing and signaling mechanism must exist to detect low internal oxygen and to trigger coordinated regulation of adaptive responses. Over-expression of plant hemoglobins increased the oxygen concentration and thereby storage synthesis, but it is not known yet what function hemoglobins actually have in the oxygen sensing and signal transduction cascade. We are currently using microarray gene expression analysis to detect genes that are sensitive to changing oxygen in Arabidopsis. Further on, the natural variation between Arabidopsis ecotypes as well as genetic modification of cardinal genes will be used to reveal the oxygen sensing pathway in plants.

(1) Geigenberger (2003) Curr Opin Plant Biol 6, 247-256
(2) Van Dongen et al. (2004) Plant Physiol (in press)

T07-049

Expression profile and functional characterization of the Nucleobase-Ascorbate Transporter multigene family in Arabidopsis thaliana

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Nucleobases and ascorbate, although structurally unrelated, are transported by the nucleobase-ascorbate transporter (NAT) family, also known as the nucleobase/cation symporter2 family. The NAT family is highly conserved in fungi, prokaryotes, plants and mammals. Members of this family transport purines, pyrimidines and, in the case of mammals, ascorbate. In plants, the Leaf Permease1 (Lpe1) from maize is the only NAT characterized so far. Lpe1 was shown to transport xanthine and uric acid and it can bind but not transport ascorbate. The plant NAT proteins can be classified into five subfamilies. All the members are highly hydrophobic, possess 13 membrane spanning domains and share a conserved exon-intron structure. A total of 12 genes encoding putative NATs are present in the genome of A. thaliana. A complete understanding of plant physiology require also the knowledge of protein localization patterns at the supra- and subcellular levels. Here, we describe the expression pattern of Arabidopsis NATs by using promoter-reporter gene (GUS and GFP) fusions. Each of the NAT genes studied exhibited a unique pattern of organ- and tissue specific expression, although some are preferentially expressed in the vascular tissues. Cross-sections through leaf blades and roots allow us to identify the tissue-specific localization in the vasculature. We also studied the effect of the first intron of some AtNAT genes on the activities of the promoter-reporter gene constructs in transgenic plants. It was possible to show that AtNAT9 is a pseudogene and that the NAT genes exhibit distinct but partially overlapping expression patterns. This high level of redundancy could explain the lack of obvious phenotypes observed in all T-DNA insertion lines evaluated. We will present data on the characterization of double mutants as well as results of approaches used to gain insight into the transport specificities.

T07-050

ANALYSIS OF PROTEINS HOMOLOGOUS TO PLASTIDIC PHOSPHATE TRANSLOCATORS IN ARABIDOPSIS THALIANA

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Several proteins (PTh) that show homology to nucleotide-sugar transporters (NSTs) and to plastidic phosphate translocators (pPTs) were recently identified in Arabidopsis and other organisms (Knappe et al., 2003). The PTh proteins that belong to the drug/metabolite transporter superfamily split into three new families named the KD, KT and KV/A/G families according to conserved sequence motifs. The PTh, pPT and NST proteins might share two conserved substrate binding sites facing to different sites of the membrane. Two of these transporters belonging to the KV/A/G family possess a N-terminal presequence that direct the proteins to plastids. In order to analyze the function of these two proteins, the cellular and tissue specific expression of the corresponding genes were determined by RT-PCR and promoter-reporter gene fusion studies, with GUS and GFP as reporter genes.
The characterization of mutant lines with insertions in these two genes revealed no distinct phenotype, suggesting an overlaping function of both proteins. The generation of double mutants, lacking both genes, might lead to a mutant phenotype which could provide new insights in the physiological role of these newly identified transporters.

Knappe, S., Flügge, U.I. and Fischer, K. (2003) Plant Physiol. 131, 1178-1190

T07-051

Nutrient-dependent and hormonal regulation of sulfate transporters in Arabidopsis

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High-affinity sulfate transporters SULTR1;1 and SULTR1;2 are responsible for the initial acquisition of sulfate in Arabidopsis roots. The promoter-GFP plants for SULTR1;1 and SULTR1;2 showed specific localization of GFP in the root hairs, epidermis and cortex, and typical sulfur responses as well, that correlate with the changes in their mRNA contents; accumulation of GFP was induced by sulfur limitation, but was repressed in the presence of Cys and GSH. SULTR1;1 and SULTR1;2 were primarily regulated by sulfur, however the supply of nitrogen and carbon amplified their basal expression levels. It is suggested that metabolic connections between reductive sulfur assimilation and O-acetylserine synthesis may cause general regulation of high-affinity sulfate uptake systems in roots, coordinated with the flux of nitrogen and carbon metabolisms.
Among the plant hormones, cytokinin significantly down-regulated the expression of SULTR1;1 and SULTR1;2, which was correlated with decrease in the uptake of sulfate. The cytokinin-dependent regulation of SULTR1;1 and SULTR1;2 was substantially moderated in the cre1-1 mutant, suggesting involvement of CRE1/WOL/AHK4 cytokinin receptor and its downstream signals in the negative regulation of the high-affinity sulfate uptake system. Although cytokinin effectively attenuates the expression of SULTR1;1 and SULTR1;2, their responsiveness to sulfur limitation was still present independent of the cytokinin-derived signal. Sulfur-dependent response was not affected by cytokinin or by cre1-1 mutation. These results indicate that two different modes of regulation, representing the sulfur- and cytokinin-dependent pathways, independently control the expression of sulfate transporters in Arabidopsis roots.

Maruyama-Nakashita A, Nakamura Y, Yamaya T, Takahashi H: Plant Journal 38, 779-789 (2004)

T07-052

Systematic in-depth analysis of nitrogen signalling in Arabidopsis thaliana (L.)

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Nitrate specifically, and nitrogen-status in general, regulates plant metabolism, growth, and development. Despite the importance of nitrogen regulation in plants, little is known about the signalling pathways and regulatory genes involved. We are taking a reverse-genetics approach to identify key genes involved in nitrogen regulation in Arabidopsis.

To identify systematically transcription factors (TFs) that are regulated by changes in N-nutrition and other external cues, we established a quantitative, real-time RT-PCR platform (see abstract: "Real-Time RT-PCR profiling of over 1,400 Arabidopsis transcription factors: Unprecedented sensitivity reveals novel root- and shoot-specific genes") consisting of 1467 annotated transcription factors. Additionally, we used 22K Affymetrix Arabidopsis Full Genome Arrays (ATH1) for transcriptome analysis. Arabidopsis plants were grown in liquid culture with ammonium nitrate, then deprived of nitrogen, and finally exposed to nitrate as sole N-source, prior to transcriptome analysis. Approximately 120 N-regulated genes were identified using the Affymetrix arrays, 96 of which encoded TFs belonging to 25 different families. Additionally, 12 receptor-like kinases, 6 MAP3Ks, 4 protein phosphatases, and 3 CBL-interacting protein kinases were identified. 44 N-regulated TF genes were identified by Q-RT-PCR. To identify genes that are regulated specifically and directly by nitrate, the following procedure was used: i) RNA levels were compared at different times after nitrate re-addition (from 12 min to 3 h); ii) nitrate-regulation of suspect genes in wild-type (Col-0) plants was compared to that of a nitrate reductase mutant impaired in both NIA1 and NIA2; iii) the list of nitrate-regulated genes was compared to lists of genes that were found to be regulated by other macro-nutrients or abiotic stresses. As a result, we selected 24 putative regulatory genes for further functional characterisation. Suspect genes were over-expressed in Arabidopsis under the control of the constitutive CaMV 35S-promotor, and an ethanol inducible promoter. T-DNA knockout lines were also included in the analysis. Loss- and gain-of-function lines are now the subject of physiological and molecular analyses, which include Q-RT-PCR analysis of a set of nutrient-stress marker genes, and GC-MS and HPLC analysis of metabolites. Results of these analyses will be presented.

T07-053

Soluble Cytosolic Heteroglycans Acts as Substrate for the Cytosolic (Pho 2) Phosphorylase

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The subcellular distribution of starch-related enzymes and the phenotype of several Arabidopsis mutants impaired in starch mobilization suggest that the plastidial starch degradation is linked to a complex cytosolic glycan metabolism. In this communication, a soluble heteroglycan (SHG) preparation from leaves of Arabidopsis thaliana L. has been studied. The SHG, whose major constituents are galactose, arabinose, and glucose, comprises several glycans. Using membrane filtration, the SHG can be separated into a <10 kDa (SHGS) and a >10 kDa (SHGL) fraction. As revealed by field-flow-fractionation (FFF), the latter can be resolved into two subfractions, designated as I and II. Subcellular location of the various glycans was determined by non-aqueous fractionation of leaf material. The various non-aqueous fractions obtained were analysed by HPAEC-PAD and by FFF-DRI. All soluble glycans are located outside the chloroplasts. The low molecular weight glycans (SHGS) possess the same distribution as cytosolic marker proteins, such as Pho 2 and DPE 2. The two glycans of SHGL exhibited an unequal distribution: Subfraction I cofractionated with the cytosolic markers whereas subfraction II did not. Thus, both SHGS and subfraction I of SHGL are cytosolic glycans whereas subfraction II resides outside the cytosol. In in vitro assays subfraction I acted as glucosyl acceptor for the cytosolic (Pho 2) phosphorylase whereas subfraction II did not. Both subfractions possess a similar monomer pattern that, due to the high proportion of arabinose and galactose, somehow resembles arabinogalactans. However, the soluble glycans, especially SHGS and subfraction I, possess a more complex structure, as they possess a variety of minor compounds, such as glucosyl, mannosyl, xylosyl, rhamnosyl, and fucosyl residues. In Arabidopsis mutants that are defective in distinct starch-related enzymes both SHGS and subfraction I are affected whereas subfraction II remains unchanged. As an example: In the Arabidopsis mutant defective in the plastidial phosphoglucomutase (pPGM) the cytosolic glycans differ from these of the wild type in the monomer composition and the molar mass distribution. Interestingly, the pPGM mutant possesses a twofold higher Pho 2 activity.

T07-054

Two interacting high-affinity sulfate transporters regulate the uptake of sulfate in response to sulfur conditions.

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SULTR1;1 and SULTR1;2 are the two high-affinity sulfate transporters responsible for the initial sulfate acquisition in Arabidopsis roots. Studies on transgenic plants expressing the fusion gene constructs of their promoter region and green fluorescent protein demonstrated that SULTR1;1 and SULTR1;2 co-localize at epidermal and cortical cells of roots. The inducibility of their expression was different in response to the sulfur status; SULTR1;1 mRNA was strongly up-regulated by sulfur limitation in parallel with the increase in the sulfate uptake capacity of roots, whereas the abundant isoform, SULTR1;2, was less responsive to the changes in sulfur conditions. Contribution of SULTR1;1 and SULTR1;2 to the total sulfate uptake was investigated by the comparative analysis of sultr1;1, sultr1;2 and sultr1;1 sultr1;2 double knockout. The uptake of sulfate decreased both in sultr1;1 and sultr1;2 single mutants under low-sulfate conditions. The sultr1;1 sultr1;2 double knockout failed to absorb sulfate from μM concentration, and showed severe growth defects. The actual sulfate uptake capacity measured in the wild-type plants was significantly higher than the total contribution of SULTR1;1 and SULTR1;2, which was estimated from the sum of sulfate influx in sultr1;1 and sultr1;2 single mutants, suggesting that SULTR1;1 and SULTR1;2 may synergistically function in maximizing the sulfate uptake capacity under sulfur limited conditions. Co-expression of SULTR1;1 and SULTR1;2 in yeast system also resulted in increasing the sulfate influx rate under sulfur-deficient conditions; however, this multiplying effect was abolished by the addition of organic sulfur. These results strongly suggest the interplay of SULTR1;1 and SULTR1;2 transporters as an essential regulatory mechanism that controls sulfate uptake capacity in response to sulfur conditions fluctuating at the root surface.

T07-055

The PMEI-RP family: Inhibitors of one single protein family interact with apparently unrelated classes of target enzymes

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The post-translational regulation of vacuolar and cell wall enzymes via inhibitory proteins has emerged as a specific mechanism for rapid silencing of enzyme activity. Shortly after cloning of the first invertase inhibitor cDNAs from tobacco (for review see [1]) and the in vitro and in vivo proof of function of a cell wall and a vacuolar isoform (NtCIF, NtVIF), the direct sequencing of a Kiwi fruit pectin methylesterase inhibitor (AcPMEI; [2]) revealed a significant sequence similarity to the invertase inhibitors.
We will use the sequenced A. thaliana genome as the platform to unravel the function of 39 genes with significant homology to NtCIF, NtVIF or AcPMEI. This protein family is referred to as PMEI-RPs (pectin methylesterase inhibitor related proteins), as most of the members seem to be inhibitors of pectin methylesterase. In a functional genomics approach, we will characterize the targets for all 39 members of this diverse protein family and elucidate their roles during critical stages of plant development and in response to biotic and abiotic stress. Furthermore, by studying the structure and function of selected PMEI and C/VIF isoforms [3], we will gain knowledge of so far poorly understood but important mechanisms for post-translational control of enzymes involved in plant metabolism and growth regulation.
In particular, we would like to address the following questions:
1) Are all or only some pectin methylesterases and invertases under control of inhibitory proteins?
2) During which developmental processes (or stress responses) are these regulatory mechanisms required?
3) What are the structural features relevant for their specificity towards PME, CWI or VI?
First functional genomics data for selected members of the PMEI-RP family will be presented.

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T07-056

Functional Characterisation of the ERD6 Sugar Transporter Family

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In higher plants, the uptake of monosaccharides is an essential part in the supply of heterotrophic sink-tissues with nutrients. The analysis of the Arabidopsis genome revealed the existence of a superfamily of potential sugar transporters, including 53 ORFs with significant homology to known monosaccharide transporters. These ORFs can be grouped into seven distinct families.
The AtSTP family was almost completely characterised by our group.
We have demonstrated that the AtSTPs are active monosaccharide/H+ symporters of the plasma membrane with a broad range of monosaccharide substrates and sink-specific expression profiles.
Within the Arabidopsis Functional Genomics Network (AFGN) we started to investigate another group of putative monosaccharide transporters, the ERD6 family. Comprising 19 members, this family was named after the putative sugar transporter ERD6 (early-responsive to dehydration). Another member of this family, SFP1, was isolated as a senescence-induced gene.
In tissue-specific expression analyses by RT-PCR most of the ERD6 genes show distinct expression patterns, whereas “leaf-flower-root” seems to be the predominant site of expression. We firstly concentrated on ERD6.5 and ERD6.7. In the RT-PCR analysis both genes exhibit relatively strong expression in most tissues. First examination of ERD6.5 and EDR6.7 promoter:GUS plants showed that both are active in seedlings and mature leaves and that ERD6.7 expression is strongly wound-induced.
Due to the homology of the ERD6 genes to the known AtSTPs, a similar function was assumed. Since ERD6.5 and ERD6.7 do not complement a hexose transporter deficient yeast mutant, we investigated the subcellular localisation of the ERD6 proteins using cDNA/GFP fusions. In yeast, these fusion proteins were located in the vacuolar membrane. In Arabidopsis protoplasts, transient expression indicates an identical localisation. Preliminary Western analyses using an ERD6.5 specific antibody and isolated vacuoles confirm these results.
Our current data suggests a possible function of the ERD6 genes in storage or remobilisation of monosaccharides during certain developmental stages or stress situations like senescence, wounding/pathogen attacks, C/N-starvation as well as diurnal changes during transient storage of sugars in the vacuole.

T07-057

A FUNCTIONAL GENOMICS APPROACH TO PLANT SOLUBLE PYROPHOSPHATASES

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Being the by-product of many anabolic reactions in plants (like starch formation, protein synthesis, DNA and RNA synthesis), pyrophosphate is hydrolyzed to orthophosphate by the action of soluble or vacuolar pyrophosphatases to make these reactions thermodynamically irreversible. Since the cytosolic pyrophosphate concentration was found to be fairly high (~ 0.3 mM), it has been hypothesized that soluble pyrophosphatase activity in plants is restricted mainly to plastids, whereas cytosolic isoforms of soluble pyrophosphatase are active only in rapidly dividing cells. By using Arabidopsis thaliana as a model system, we are trying to understand the regulation of soluble pyrophosphatase activity during growth and differentiation, and under different environmental conditions, using promoter::GUS and ::EGFP fusion constructs of six pyrophosphatase isoforms (one plastidic and five cytosolic). In an RNAi approach, by knocking out the most strongly expressed two isoforms of Arabidopsis thaliana, we will investigate the importance of cytosolic soluble pyrophosphatases during plant development and particular stress responses.

T07-058

Functional analysis of the CYP76 family of P450 genes in A. thaliana

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Systematic genome sequencing revealed that cytochrome P450 form one of the largest superfamilies of proteins in higher plants, including 272 members in Arabidopsis thaliana. They are a group of haem-containing proteins which catalyse various oxidative reactions. In higher plants, they play important roles in the biosynthesis of cell wall constituents, signal molecules, secondary metabolites, and in the conversion of diverse xenobiotics into non toxic (pesticide detoxification) or toxic compounds (proherbicide activation) (Werck-Reichhart et al, 2002). Despite recent progress in the understanding of plant P450 functions, the vast majority of these functions remain to be established. CYP76s form a family of nine genes, eight of them belong to the CYP76C subfamily (including 1 pseudogene) and a single one to the CYP76G subfamily. CYP76C1 to C4 form a small cluster on chromosome 2, and appear to have arisen from a gene duplication. The expression of two genes of the cluster has been compared in two independent studies. CYP76C1 was shown to be highly expressed in flower, and at lower levels in stem, silliques and leaves (Mizutani et al., 1998). Its expression is decreased by wounding. CYP76C2 expression was associated with stress response, being very low in young tissues, but induced during hypersensitive response to pathogens, senescence, after wounding or heat-shock (Godiard et al, 1998). We have chosen this family of P450s to study the evolution of duplicated genes and acquisition of different expression patterns and functions in the plant physiology. By the use of post genomic tools such as microarrays, promoter-reporter gene fusions, overexpression, RNAi silencing or reverse genetics, and screening for potential substrate using heterologous expression, we aim at determining a physiological role for each member of this family. It is probable that such a systematic approach will reveal new functions in plant defense against pathogens or chemicals and plant development.

Werck-Reichhart et al 2002 Arabidopsis Book 1
Godiard et al 1998 FEBS-438 245
Mizutani et al 1998 Plant Mol Biol-37 39

T07-059

In Arabidopsis thaliana, the invertase inhibitor isoforms AtC/VIF1 and 2 show distinct target enzyme specificities and developmental expression profiles

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In plants, cell wall (CWI) and vacuolar invertases (VI) play important roles in carbohydrate metabolism, stress responses and sugar signalling. Addressing the regulation of invertase activities by inhibitor proteins (C/VIF, cell wall/vacuolar inhibitor of fructosidase), we have identified two C/VIFs from Arabidopsis thaliana. AtC/VIF1 was specific for VI, whereas AtC/VIF2 inhibited CWI and VI alike. High expression of AtC/VIF1 was restricted to specific organs, whereas AtC/VIF2 was weakly expressed throughout plant development. Promoter::GUS transformants confirmed pronounced differences of tissue/cell type-specific expression between both isoforms. Growth of an AtC/VIF1 T-DNA KO mutant was unaffected, but VI activity and hexose content were slightly increased

T07-060

The role of CP12 in the co-ordination of chloroplast metabolism

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The chloroplast protein, CP12, has been shown to regulate the activity of both NADP-GAPDH and PRKase in vitro, by light-driven reversible dissociation of a complex involving PRK/CP12 and GAPDH. Unexpectedly, CP12 antisense plants have a severe growth phenotype with abnormal leaf and floral morphology, reduced apical dominance and, in some lines, the flowers were sterile. Photosynthetic carbon assimilation rates were reduced by up to 25% in these plants but this could not be the cause of this dramatic change in growth. Further biochemical analysis revealed that the enzyme glucose-6-phosphate dehydraogenase was active in the light, suggesting that a futile cycle involving the OPP pathway was occurring. This would have the effect of reducing the availability of photosynthetic carbon for export for the synthesis of end products such as sucrose, starch and also the synthesis of compounds via the isoprenoid and shikimic acid pathways. These data provide in vivo evidence that CP12 has an important role in regulating the allocation of carbon from the Calvin cycle. In Arabidopsis there is a small CP12 multigene family and sequence comparisons have revealed that two of these proteins have 86% similarity at the amino acid level. The third CP12 protein shares less than 60% similarity with the other CP12 proteins. To date there is no information on the role of the third CP12 gene. We will report on our studies on the role of the Arabidopsis CP12 gene family in the co-ordination of metabolism in the chloroplast.

T07-061

Structure-Function Relationship within the Invertase/ Pectinmethylesterase Inhibitor Family of Arabidopsis thaliana

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Upon secretion into the extracellular cell-wall compartment, a variety of plant enzymes do escape cellular control mechanisms. This lack of direct regulation appears to be compensated through production of inhibitory proteins. In particular, these inhibitors seem to be important during processes denoted by fast metabolic or developmental switches. We are focusing on the analysis of such an inhibitory protein family acting towards plant acid invertase and pectin methylesterase (PME). By sequence homology, currently 39 open reading frames have been assigned to this family, referred to as PMEI-RP (pectin methylesterase inhibitor related proteins), in Arabidopsis thaliana. Recently, the biotechnological potential of the PMEI-RPs has been pointed out [1,2].

We have used a structural biology approach to investigate in three dimensions, how members of this inhibitory family achieve their puzzling specificity towards apparently unrelated enzymes. Based on several crystal structures of an invertase inhibitor from tobacco [3] and a pectin methylesterase inhibitor from Arabidopsis in concert with detailed biochemical analysis we suggest a novel module within in these proteins to be critical for the inactivation of the target enzymes. Based on our findings, we will present a model for protein-protein interaction based enzyme regulation in the plant cell wall.

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T07-062

QTL analysis of carbohydrates and growth-related traits in a new recombinant inbred population derived from the Ler x Kond cross

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To study the relation between carbohydrate metabolism, plant growth and flowering related traits and to unravel the genetic background of these characteristics, Arabidopsis natural variation was used. Screening of 22 accessions and 3 mutants (pgm, sex and adg) for their diurnal patterns of carbohydrate accumulation in leaves, revealed 2 classes: the first one accumulates sugars (glucose, fructose and sucrose) and starch during the day, while the second class exhibits a nearly constant level of carbohydrates over the day. Large variations between these accessions were recorded for dry weight, seed weight and relative growth rate but not for water content (El-Lithy et al., 2004). For quantitative trait locus (QTL) analysis, 127 recombinant inbred lines (RILs) (F9 generation) derived from the cross between Landsberg erecta (Ler) and Kondara (Kond), were used. These RILs were genotyped using the SNPWave™ technique (van Eijk et al., 2004) with the addition of some PCR markers (microsatelites and INDELs).
RILs were grown on hydroponics solution as well as in the greenhouse. QTLs for carbohydrates, relative growth rate (RGR), chlorophyll fluorescence, dry weight, fresh weight, root length, flowering time and other traits related to flowering could be detected. Co-location of QTLs for different traits was observed at various locations. These co-locations suggest that these traits might be controlled by the same gene(s) and thus are pleiotropic, or they indicate the presence of a number of closely linked genes.

El-Lithy et al. (2004) Plant Physiology, 135, 444-458

van Eijk et al. (2004) Nucleic Acids Research 32 (4):e47

T07-063

Photorespiration and glycolate cycle: Old subject, new insights

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Photorespiration was one of the first subjects to be investigated in the plant model system Arabidopsis thaliana over 20 years ago. Since these fundamental works the structure of the glycolate cycle is an integral part of botanical textbooks and interest into this field of plant metabolism weakened. The availability of the Arabidopsis genome sequence and of mutant lines for most of its genes offered us the oppurtunity to investigate the glycolate cycle in more detail. We identified at least 23 genes in the Arabidopsis genome to be involved in this metabolism. We isolated and analysed a variety of mutants for several steps of the photorespiratory cycle like 2-phosphoglycolate phosphatase, glycine decarboxylase (GDC), serine hydroxymethyl transferase and hydroxypyruvate reductase. Molecular and biochemical analysis of some mutants confirmed parts of the textbook knowledge about this pathway. But there are also cases with surprising phenotypes and aspects: The mutated locus in glyD, the first known GDC mutant, revealed to be different from all known GDC components. Loss of hydroxypyruvate activity does not lead to a lethal phenotype like in other photorespiratory mutants. Further results of our analysis and conclusions about the role and function of the glycolate cycle will be presented.

T07-064

ENZYME REGULATION THROUGH PROTEIN-PROTEIN INTERACTIONS: GAPDH-CP12-PHOSPHORIBULOKINASE SUPRAMOLECULAR COMPLEX IN Arabidopsis thaliana

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Fine regulation of photosynthetic carbon metabolism is a complex feature of photosynthetic organisms involving redox control by thioredoxins, enzyme sensitivity to both pH and Mg2+ oscillations, effects of metabolites and coenzymes and reversible formation of supramolecular complexes. The activity of chloroplast glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) are virtually affected by all these factors in a tightly concerted manner, but the molecular basis of regulation are not yet fully understood.

Arabidopsis contains a small protein, namely CP12, with limited but significant homology with the C-terminal extension (CTE) of GapB subunits of GAPDH. CP12 is classified as an intrinsically unstructured protein (IUP) which promotes the formation of a supramolecular complex between GAPDH and PRK. Internal disulfide bridges are required both for the interaction of CP12 with partner enzymes (1,2) and the autonomous regulation of GapB-containing GAPDH (AB-GAPDH) (3). Except for the CTE, GapA and GapB subunits are almost identical. Due to the absence of CTE, GapA is not autonomous in regulation, but it is strongly regulated by the concerted action of CP12 and PRK.

We have produced recombinant GapA, GapB, CP12 and PRK in a heterologous system and purified recombinant proteins have been used to build GAPDH-CP12-PRK supramolecular complexes in vitro. GapA, but not PRK, binds CP12 with high affinity provided that NAD, not NADP, occupies GapA active sites. CP12 binding to GapA has limited affects on activity, but in the presence of PRK the formation of the GapA-CP12-PRK complex results in strongly inhibited GAPDH activity. AB-GAPDH can also interact with CP12 provided that CTE is reduced and coenzyme sites are occupied by NAD. Autonomous (CTE-dependent) and CP12-dependent regulation of GAPDH clearly share similar molecular basis and are currently under thorough investigation. The reversible formation of GAPDH-CP12-PRK complex seems to play a crucial role in fine tuning of carbon metabolism in chloroplasts.

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2. Graciet et al (2003) Biochem 42: 8163
3. Sparla et al. (2002) JBC 277: 44946

T07-065

Loss of the hydroxypyruvate reductase, AtHPR1, does not lead to lethality under ambient CO2 conditions

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The majority of carbon molecule losses by oxygenation of ribulose-1, 5-bisphosphate are rescued by the glycolate cycle. During the conversion of 2-phosphoglycolate to 3-phosphoglycerate the reduction of hydroxypyruvate to glycerate is one of the last steps within this cyclic pathway. This reaction is catalysed by hydroxypyruvate reductase (HPR) and in Arabidopsis thaliana this enzyme is encoded by the single copy gene AtHPR1. We isolated a mutant for this gene, hpr1-1, and it revealed to be viable under ambient air conditions, unlike other mutants with defects in photorespiratory cycle. The mutant shows an altered phenotype in normal air. In contrast these phenotypic alterations disappear under elevated CO2. The loss of AtHPR1 leads to an increase of photorespiratorial serine. Interestingly, we could only detect low amounts of residuing HPR activity in the mutants, indicating that AtHPR1 confers the majority of HPR activity. Therefore, we conclude that the reduction of hydroxypyruvate in the glycolate cycle is circumvented by another, yet unknown, reaction.

T07-066

Investigating the active sites of Arabidopsis thaliana cytochrome P450 monooxygenases hydroxylating aromatic rings

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Cytochrome P450 monooxygenases (P450s) are heme thiolate proteins that catalyze difficult and extremely diverse chemistries. Despite the fact that they share less than 13% sequence identity, they display common structural folds that allow bacterial and mammalian P450 crystal structures to be used for modeling of plant P450 sequences. To better understand the role of specific amino acid residues in defining the ability of plant P450s to hydroxylate aromatic substrates, four Arabidopsis P450s (CYP73A5 CYP84A1, CYP75B1, CYP98A3) capable of hydroxylating aromatic rings with substituents at the 4-, 5- and 3-positions, respectively, have been homology modeled using the MOE program. Analysis of the models by Ramachandran plot, PROSA II and Profiles 3D as well as MD simulations have indicated that the P450 models are correctly folded and thermodynamically stable. Substrate docking using SYBYL site ID, MOE dock and Insight II Affinity has identified residues potentially contacting each substrate. Among these programs, MOE dock and Insight II Affinity consistently positioned the aromatic ring of the substrate in a similar orientation in all four proteins. Each aromatic ring was predicted to be contacted by SRS6, the N-terminal of SRS5 and the C-terminus of SRS4 and each substrate tail was predicted to be contacted by SRS1, SRS2, the N-terminal of SRS4 and the C-terminal of SRS5. Fifteen residues spanning all SRS regions were selected for replacement mutagenesis in the active site of CYP98A3 (p-coumaroylshikimic acid hydroxylase). Yeast expression of mutants containing one or two replacements within the proposed SRS contact regions followed by CO difference analysis indicated that most mutants produced stable and correctly configured P450s with only two single mutants (in SRS4) being completely unstable and two double mutants (in SRS1) being partially unstable. Three geometric isomers of p-coumaroylshikimic acid were chemically synthesized, purified by preparative HPLC and used for characterization of these mutant proteins. Comparisons of hydroxylation rates have indicated that mutations in SRS1, SRS2 and the N-terminus of SRS4 discriminate between the isomers demonstrating that these regions are important in recognizing the shikimic acid moiety of this substrate. Also, mutations in SRS5 and SRS6 significantly impact catalytic activity without destabilizing the catalytic site as is consistent with the substrate binding mode predicted for this Arabidopsis P450.

T07-067

Small molecular weight Phospholipase A2 proteins in Arabidopsis.

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In plants a number of gene families are known to encode enzymes with acyl-hydrolase activity that can release fatty acids from the sn-2 position of phospholipids. The products of a PLA2 catalyzed reaction, free fatty acids and lyso-phospholipids, are thought to stimulate the plasma membrane H+-ATPase activity or several protein kinases. Recently, a protein with PLA2 activity has been isolated from developing Elm seeds (Stahl et al., 1998) and expressed sequence tag (EST) clones from Rice encoding similar proteins were subsequently identified (Stahl et al., 1999). The amino acid sequences of the plant PLA2s showed that these enzymes are related to the class II animal PLA2s. These animal secretory PLA2 (sPLA2) are small <20 kDa enzymes that require milimolar concentration of Ca2+ for catalysis, have a high disulfide bond content and are best known from their presence in the venom of various snakes.
In Arabidopsis thaliana, five genes (AtsPLA2 (a-e) are present which encode proteins that are similar to these plant and animal sPLA2s. The similarity with the animal sPLA2s, however, is confined to the region near the active site of the protein and the region involved in Ca2+ binding. Based on the amino acid sequences we have grouped plant sPLA2 proteins into three major families. AtsPla2a and g are members of separate proteins families whereas AtsPLA2b, d and e are part of a third class of plant sPLA2 proteins. To elucidate the function of these plant PLA2 proteins, we have analyzed the expression and localization of these PLA2 proteins in Arabidopsis.
Confocal microscopic analysis of transgenic plants expressing AtsPLA2-GFP fusion proteins indicates that these proteins are localized in different cellular compartments. For example AtsPLA2b which has been implicated in auxin mediated cell elongation (Lee et al., 2003) is localized in the endoplasmic reticulum and does not appear to be secreted as has previously been suggested.
Northern blot analysis showed that AtsPLA2-a__b and g are expressed in all examined tissues. Furthermore, elevated levels of AtsPLA2-g mRNA were detected in senescing leaves suggesting that this protein might be involved in remobilization of membrane components.

T07-068

Sugar signals interact with thioredoxin-mediated light activation of ADPglucose pyrophosphorylase in Arabidopsis leaves

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The reaction catalysed by ADP-glucose pyrophosphorylase is the first committed step in starch biosynthesis. This enzyme is subject to allosteric regulation by 3PGA/Pi ratio and to transcriptional regulation. It has recently been shown in potato tubers that this enzyme is also subject to a novel redox-dependent post-translational regulation, leading to a stimulation of starch synthesis in response to sucrose supply [1]. Redox-activation involves the reduction of an intermolecular disulfide-bridge which is formed between the two AGPB subunits of the heterotetrameric holoenzyme. An analogous mechanism is also operating in photosynthesising leaves of potato, pea and Arabidopsis, where AGPase redox-activation is independently modulated by light and sugars [2]. Currently, the genetic resources of Arabidopsis are being used to investigate the components of the signalling pathways leading to AGPase redox-activation in response to these inputs. On the basis of these results it will be discussed how the sugar signal is transferred to the chloroplast and by which mechanism it interferes with the ferredoxin/thioredoxin system that is involved in the light-dependent redox-activation of the enzyme. Evidence will be presented that this novel regulatory network is also linked to other biosynthetic processes such as lipid biosynthesis.

References:
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T07-069

Structure-Based Design of 4-Coumarate:CoA Ligase Variants with New Catalytic Properties

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4-Coumarate:CoA ligase (4CL) is the branch point enzyme of general phenylpropanoid metabolism from which a large variety of plant secondary products is derived. Most 4CLs catalyze the conversion of the cinnamic acid derivatives coumarate, caffeate and ferulate to their corresponding CoA esters, but enzymes with different substrate utilization profiles have also been described. Arabidopsis 4CL2 (At4CL2) is unusual because its preferred substrate is caffeate, whereas ferulate and sinapate are not converted. To reveal the structural principles determining substrate specificity of 4CL, the crystal structure of the phenylalanine activation domain (PheA) of gramicidin S synthetase was used as a template for homology modeling. According to this model, twelve amino acid residues form the 4CL substrate binding pocket (SBP) and function as a signature motif determining substrate specificity. We utilized this specificity code to create At4CL2 gain-of-function mutants. Substitution of selected bulky amino acids by smaller residues resulted in enzymes that use ferulate instead of caffeate as preferred substrate. Deletion of selected amino acids generated sinapate activating At4CL2 variants. Substitution of charged or polar by hydrophobic amino acids strongly enhanced conversion of cinnamate. These results demonstrate that a size exclusion mechanism controls the accessibility of the At4CL2 SBP for mono- and dimethoxylated substrates, whereas activation of cinnamate is regulated by the hydrophobicity of the SBP. The new enzyme variants are suited tools to investigate and influence metabolic channeling mediated by 4CL. Knowledge of the 4CL substrate specificity code will facilitate the prediction of substrate preference of numerous uncharacterized Arabidopsis 4CL-like proteins.

Schneider et al. (2003) PNAS 100 (14), 8601-8606

T07-070

Biochemical links between growth, nitrogen, and carbon utilisation in Arabidopsis thaliana ecotypes

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Metabolic pathways do not function as isolated units. Rather, they form a network of reactions and regulations. Consequently, a good understanding of a specific metabolic response requires the experimental detection of several parameters in or outside the investigated pathway. Such a global approach has so far been hampered by the cost and difficulties of routinely determining that many variables. To counteract this problem, we are developing a platform of enzymatic assays applied to the rapid detection of metabolites and enzyme activities in the major metabolic pathways of Arabidopsis thaliana. The assays are fast and sensitive. In addition, they do not require any previous knowledge of the genome sequence. As such, they are adaptable to another organism.

A set of assays was tested to investigate the biochemical links between growth, carbon and nitrogen use. The following parameters were measured in 24 ecotypes grown in short days: biomass, nitrate, aspartate, glutamate, amino acids, proteins, starch, chlorophyll, sugars, aminotransferases, glutamate dehydrogenase, and phosphoenolpyruvate carboxylase. The study made use of natural variations of these parameters in Arabidopsis ecotypes. Indeed, though each parameter taken individually yields little information, conclusions can be drawn based on correlations between different variables. The ecotypes exhibit variations in use of nitrogen and carbon. These variations conditioned significant differences in biomass. These results illustrate the potency of our approach. Indeed, the conclusions were drawn based on correlations between parameters from different metabolic pathways. Hence, they required a rapid detection of many parameters.

T07-071

Mutants in medium long and long chain acyl-CoA oxidase activity demonstrate that long chain acyl-CoA activity is important in seed viability and essential for seedling establishment.

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The Arabidopsis acyl-CoA oxidase (ACX) family comprises isozymes with distinct fatty acid chain-length specificities that together catalyse the first step of peroxisomal fatty acid ß-oxidation. We have isolated and characterised T-DNA insertion mutants in the medium long-chain (ACX1) and long chain acyl-CoA oxidases (ACX2). In acx1 seedlings, medium-long (C10:0-C20:0)-chain acyl-CoA oxidase activity was greatly reduced by greater than 95 percent for C16:0. In acx2 mutant seedlings, specifically long-chain activity was reduced, by greater than 90 percent for C18:0. Whilst lipid catabolism during germination and early post-germinative growth was unaltered in the acx1 mutant and only slightly delayed in the acx2 mutant, both mutants accumulated acyl-CoAs. Three-day-old acx1 seedlings accumulated mainly medium/long chain acyl-CoAs, whilst acx2, accumulated only long chain acyl-CoAs. In acx1 and acx2, seedling growth and establishment in the absence of an exogenous supply of sucrose, was unaffected. Furthermore, no alterations in vegetative or reproductive phenotype were obvious throughout the remaining life cycle of the acx1 or acx2 plants.

Seedlings of the double mutant acx1acx2 exhibited a 98% reduction in C16:0 activity, were unable to catabolise seed storage lipid and accumulated long chain acyl-CoAs. In the absence of an exogenous sucrose supply, acx1acx2 seedlings were unable to establish photosynthetic competency. However when rescued on sucrose at the seedling stage, no alterations in vegetative or reproductive phenotype were seen throughout the remaining lifecycle. Germination frequency was less than 30 percent of wild type seeds, and was unaffected by the addition of exogenous sucrose. This phenotype resembles the comatose (cts2) mutant.

We propose that the acx1 and acx2 mutants are partially complemented by ACX2 and ACX1 respectively, and that acx1acx2 seedlings require sucrose for seedling establishment because they have a more severe restriction in long-chain acyl-CoA oxidase activity. Furthermore, long chain acyl-CoA oxidase activity plays an important role in seed viability.

T07-072

Reserve Mobilisation in the Arabidopsis Endosperm Fuels Hypocotyl Elongation in the Dark, is Independent of Abscisic Acid and Requires the PHOSPHOENOLPYRUVATE CARBOXYKINASE1 Gene

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Arabidopsis is used as a model system to study triacylglycerol (TAG) accumulation and seed germination in oilseeds. Here we consider the partitioning of these lipid reserves between embryo and endosperm tissues in the mature seed. The Arabidopsis endosperm accumulates significant quantities of storage lipid, and this is effectively catabolised upon germination. This lipid differs in composition from that in the embryo and has a specific function during germination. Removing the endosperm from wild-type seeds resulted in a reduction in hypocotyl elongation in the dark, demonstrating a role for endospermic TAG reserves in fuelling skotomorphogenesis. Seedlings of two allelic gluconeogenically compromised PHOSPHOENOLPYRUVATE CARBOXYKINASE (PCK1) mutants show a reduction in hypocotyl length in the dark compared to wild-type, but this is not further reduced by removing the endosperm. The short hypocotyl phenotypes were completely reversed by the provision of an exogenous supply of sucrose. The PCK1 gene is expressed in both embryo and endosperm, and the induction of PCK1:GUS at radical emergence occurs in a robust wave-like manner around the embryo suggestive of the action of a diffusing signal. Strikingly, the induction of PCK1 promoter reporter constructs and measurements of lipid breakdown demonstrate that while lipid mobilisation in the embryo is inhibited by abscisic acid (ABA), no effect is seen in the endosperm. This insensitivity of endosperm tissues is not specific to lipid breakdown as hydrolysis of the seed coat cell walls also proceeded in the presence of concentrations of ABA that effectively inhibit radical emergence. Both processes still required gibberellin however. These results suggest a model whereby the breakdown of seed carbon reserves is regulated in a tissue specific manner and shed new light on phytohormonal regulation of the germination process.

T07-073

Role of chloroplast lipids in photosynthesis and oxidative stress

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Thylakoids harbor the photosynthetic complexes and constitute the predominant membrane system in leaf mesophyll cells. The lipid composition of chloroplasts is unique, because galactolipids (mono- and digalactosyldiacylglycerol, MGDG, DGDG) and prenyl lipids (e.g. carotenoids, xanthophylls, plastoquinone, tocopherol) are abundant in thylakoid membranes. Different mutants were isolated from Arabidopsis affected in the synthesis of the galactolipid DGDG and of the antioxidant tocopherol (vitamin E) by forward and reverse genetics. Analysis of the DGDG deficient mutants dgd1, dgd2 and the double mutant dgd1dgd2 revealed that DGDG is essential for photosynthesis. Biosynthesis of DGDG is stimulated during phosphate deprivation in order to replace phospholipids and to save phosphate for other cellular processes. Surprisingly, mutants affected in tocopherol synthesis (vte1, vte4, hpt1) revealed no strong alteration in growth as compared to wild type. To study the role of tocopherol in oxidative stress, double mutants of vte1 (deficient in tocopherol cyclase) with vtc1 (ascorbate deficient) and cad2 (glutathione deficient) were generated.

Porfirova et al. (2002) Proc Natl Acad Sci USA 99, 12495-12500
Kelly et al. (2003) Plant Cell 15, 2694-2706

T07-074

The Arabidopsis seed mucilage mutant mum5 encodes a putative pectin methylesterase

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Pectins represent the most chemically diverse family of plant cell wall polysaccharides. Pectins are a structural component of all primary cell walls, and have been suggested to also have a role as signaling molecules. Despite their prevalence and importance in plants, very little is known about their biosynthesis and modification. Upon seed imbibition in Arabidopsis, pectin is extruded from a single layer of cells on the outside of the seed coat. This pectin, or mucilage, is non-essential for viability, and compared to cell walls it is structurally simple. Therefore, Arabidopsis seed mucilage makes an excellent genetic and biochemical model system. Two previously identified mutants, mucilage modified 3-1 and mum5-1 (1), have seed mucilage with aberrant physical properties. Specifically, the mum seed mucilage does not form a coherent gel-like halo as wildtype seed mucilage does, but rather diffuses away quickly.

Map-based cloning coupled with a candidate gene approach using the mum5-1 mutant revealed a single basepair change in a putative pectin methylesterase (PME). The pectin methylesterase contains a signal sequence, an extension domain, a PME inhibitor domain, and a PME domain. PME inhibitors are proteins which bind PME and inhibit their activity. To date, the regulation of any protein containing both a PME inhibitor and PME domain has not been described. Studies with MUM5 expressed in Pichia pastoris and epitope-tagged versions expressed in planta are being carried out in an effort to understand the regulation of the protein.

Arabidopsis contains 66 putative PMEs, 42 of which also contain PME inhibitor domains. Other than mum5 no mutants in any plant PME exist. Expression and phenotypic analyses of mum5 will aid in determining the biological role of PMEs in plants.

(1) Western et al. 2001. Plant Physiol. 127: 998-1011

T07-075

Genome wide analysis of Arabidopsis gene expression under sulfur starvation reveals the involvement of key transcription factors controlling sulfur assimilation metabolism

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Sulfur, a macronutrient that is essential to plant growth, is contained in a variety of cellular components and plays critical biochemical roles in a number of cellular processes, such as redox cycles, detoxification of heavy metals and xenobiotics, and metabolism of secondary products (Hell, 1997). In a cascade of enzymatic steps inorganic sulfur is converted to the nutritionnaly important sulfur-containing amino acids cysteine and methionine (Ravanel et al., 1998, Droux et al., 2000).

Currently, no knowledge is available concerning the regulatory elements, such as transcription factors, controlling sulfur assimilation and metabolism in plants. Therefore, we see a necessity to screen for transcription factors responding to nutrient alterations, especially sulfate.

Recently, the application of genomic tools as transcriptomics has provided further insight using seedlings or hydroponically grown plants under sulfur starvation (Nikiforova et al., 2003, Maruyama-Natkashita et al., 2003, Hirai et al., 2003). However, because of the astonishingly high number of genes responding to sulfur starvation, the main question is the specificity of the response. This is probably because the stress was too strong or present for a too long period, leading to many secondary answers.

For this reason we applied a moderate sulfate starvation on a short period to Arabidopsis hydroponics, roots and leaves being harvested separately. We also resupplemented the starved plants and applied in parallel to other plants signalling metabolites. This allowed us to eliminate most of the unspecific or secondary reactions. Differential expression was scored using full genome Affymetrix Genechips. Indeed, it indicates a low number of genes responding to sulfate depletion, and marker genes as metabolites measured evidence for a sulfur starvation of the plants.

We selected a set of candidate genes encoding transcription factors that may be involved in the control of sulfur metabolism in plants. The corresponding Arabidopsis mutants analysis are in progress.

Droux M et al., 2000. In Sulfur Nutrition and Sulfur Assimilation in Higher Plants (Brunold C, ed). Bern: Paul Haupt, pp. 73-92.
Hell R, 1997. Planta 202, 138-148.
Hirai MY et al., 2003. Plant J. 33, 651-663.
Maruyama-Natkashita A et al., 2003. Plant Physiol. 132, 597-605.
Nikiforova V et al., 2003. Plant J. 33, 633-650.
Ravanel S et al., 1998. Proc. Natl. Acad. Sci. USA 95, 7805-7812.

T07-076

Multiple regulations on the antagonistic cross-talks between jasmonate- and salicylate-signaling pathways

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It was reported that MPK4 kinase activity is required to repress salicylic acid (SA)-induced systemic acquired resistance (SAR) and to activate jasmonic acid (JA)-responsive gene expression. We have generated a transgenic Arabidopsis transformed with AtMPK4, and observed that the plant constitutively expresses jasmonate-responsive genes such as JR2, PDF1.2 and ERF1. In parallel with the experiment, we have isolated the AtHMGA gene encoding an AT-hook transcription factor binding to the promoter of JMT, a JA-inducible gene. A transgenic Arabidopsis over-producing the gene contained elevated level of SA and constitutively expressed SA-responsive genes, PR1 and PR2. By contrast, JA-response genes JR2 and AOS were highly expressed in AtHMGA-suppressing mutants. Thus, AtHMGA transcription factor may act as an activator for the SA-responsive genes, and as a repressor for the JA-responsive genes. In another set of experiments, we found that PR1 was not induced by SA treatment in a transgenic Arabidopsis integrating a rice SA methyltransferase gene OsSAMT. It was recently reported that the SAMT gene AtBSMT1 was activated by JA treatment. Taken together, JA-induction of SA methylation might be responsible for the antagonistic effect of jasmonates on SA-mediated gene activation. Overall, our data suggest that the two signaling pathways cross-talk at multiple regulatory points; signal transduction, transcriptional regulation, and SA inactivation.

T07-077

Obtusifoliol 14alpha-demethylase mutants reveal that sterols regulate plant growth and development via brassinosteroids-dependent and independent pathways

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Plant CYP51 encodes obtusifoliol 14α-demethylase involved in early step of sterol biosynthetic pathway. Arabidopsis genome contains two putative CYP51A genes, CYP51A1 and CYP51A2 with 72% of amino acid sequence identity and a differential gene expression pattern. As the first step to study the functional roles of both CYP51A genes during plant growth and development, we have isolated 1 mutant allele (cyp51A1-1) for CYP51A1 and 3 mutant alleles (cyp51A2-1 to 3) for CYP51A2, respectively, from T-DNA insertion lines using systematic reverse genetics. Loss-of-function mutants for CYP51A2 gene showed multiple defects in plant growth and development such as stunted hypocotyl and root, reduced cell elongation, and seedling lethality. cyp51A2 mutant has no defect in early embryogenesis, in contrast to other sterol mutants such as smt1, fackel, and hydra1. The phytochemical analysis of cyp51A2 mutant revealed that it accumulates obtusifoliol, the substrate of CYP51, and a high proportion of 14α-methyl-∆8-sterols. In contrast, the phytosterol content (campesterol and sitosterol mainly) was greatly reduced in the mutant. The defect in hypocotyl elongation of cyp51A2 mutants was not rescued by the exogenous application of brassinolide, although BR signalling was normal in the mutant. No defects in cyp51A2 were rescued by the ectopic expression of CYP51A1, indicating that CYP51A1 may encode an enzyme nonfunctional in the 14α-demethylation reaction due to a rare mutation in the heme-binding region. Our results suggest that phytosterols play their own specific roles to control plant growth and development via brassinosteroid-dependent and -independent pathways.

T07-078

Analysis of Putative Signal Termination Mutants from Arabidopsis Thaliana

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Inositol 5-Phosphatases(At5PTases) catalyze the hydrolysis of a 5' phosphate from second messenger inositol phosphates and phosphatidylinositol phosphate(PIP) substrates. These enzymes are an integral part of the IP3 signaling pathway and are thought to be involved in the termination of signal transduction events in animals,yeast and plants.The Arabidopsis genome encodes 15 At5PTase genes. The substrate specificity of At5PTase1 and At5PTase11 has been determined(Plant physiol.,2001.126:801-810 & Plant Physiol.,2004 in press). The function of At5PTase1 as a signal terminating enzyme has been investigated in plants overexpressing the At5PTase1 gene(Plant Physiol.,2003,132:1011-1019). To determine whether mutations of the At5PTase1 gene affects the IP3 signaling function in Arabidopsis plants,we studied the function of At5PTase1 in knockout mutants.T-DNA insertion mutants for At5PTase1 were selected from the Syngenta and Salk collections. Homozygous mutants from three independent lines were identified using PCR screening and the position of the T-DNA insertion within the At5PTase1 sequence was verified by DNA sequencing. Expression analysis indicated that the At5PTase1 gene was not expressed in the knock-outs. The wild type,At5PTase1 overexpressors and mutant plants grow normally and show no differences in phenotype. We are currently testing the response of the At5PTase1 knock-out mutants to biotic and abiotic stimuli, which should provide information on pathways that require inositol containing second messengers. Analysis of At5PTase1 mutant seeds germinated on media containing ABA or paclobutrazol,a gibberellin biosynthesis inhibitor,has revealed that the mutants are hypersensitive to these compounds. The At5PTase1 mutant seedlings also show altered growth as measured by the hypocotyl length when grown in the dark. Preliminary studies also indicate differences in the response of these mutant plants to drought stress. To determine if the substrates for At5PTase1 are altered in the knock-out mutants,levels of IP3 and IP2 were measured with mass assays.The results indicate that a loss of function of At5PTase1 results in increases in both IP3 and PIP2 as expected. The above data suggest that At5PTase1 is required for proper second messenger hydrolysis and function of several physiological pathways in plants.

T07-079

Biochemical and Molecular Analysis of Constitutive and Inducible Terpene Volatile Emission from Arabidopsis thaliana

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Terpene secondary metabolites have been described to exhibit important functions in direct plant defenses against herbivores and pathogens and as volatile signaling compounds in indirect defense or the attraction of pollinators. Recent results have led to exciting new insights into the potential role of volatile terpenes in thermotolerance and the protection of plants against ozone and oxidative stress.
We are using Arabidopsis as model system to gain deeper insights into the biochemistry, regulation and biological functions of terpene volatile biosynthesis. The Arabidopsis genome contains a large family of over thirty terpene synthase (AtTPS) genes. Terpene synthases catalyze the formation of monoterpenes (C10), sesquiterpenes (C15) or diterpenes (C20) from geranyl diphosphate (GPP), farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP), which are central intermediates of the isoprenoid pathway. Applying a functional genomics approach, we have identified several terpene synthases which are constitutively expressed in Arabidopsis roots and flowers and are responsible for the emission of a complex blend of mono- and sesquiterpenes volatiles (Chen, Tholl et al., 2003). Promoter-GUS analyses revealed that floral AtTPS genes are expressed in several different organs including sepals, anther filaments, ovules, stigma and nectaries. A detailed investigation of several Arabidopsis ecotypes showed variation and differential regulation of floral volatile emission. We are currently investigating particular ecotypes and floral TPS gene knock out and overexpression lines to explore the potential role of floral sesquiterpenes in protection of the reproductive tissues against oxidative stress conditions or microbial attack. Similar molecular approaches are applied to analyze antioxidant, signaling or defense roles of stress inducible terpene volatiles released from Arabidopsis rosette leaves. Moreover, we are undertaking comparative biochemical and molecular analyses of terpene biosynthesis and emission from A. thaliana and its close relatives including A. lyrata and Boechera drummondii. Overall, the results should lead to new insights and a more precise understanding of the ecophysiological significance, regulation and evolution of plant terpene volatile biosynthesis in response to abiotic and biotic stress factors.

Chen, F.*, Tholl, D.*, D'Auria, J., Farooq, A., Pichersky, E., and Gershenzon, J., 2003, Plant Cell, 15, 481-494

T07-080

Uniform stable isotope labeling of Arabidopsis thaliana opens hetero-nuclear multi-dimensional NMR-based metabolomics

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As any plant scientist does not doubt importance of plant metabolomics in post-genomics-proteomics era, recent methodological advances of FT-MS analysis lead in this field. However, the MS technology has potentially disadvantages to differentiate identical molecular mass isomers and low reproducibility due to ion-suppression effect. Therefore, NMR will become a key technology in plant metabolomics with the use of stable isotope labeling and advanced hetero-nuclear NMR methodologies. Since the NMR-based approach has an advantage in comparison with different samples, spectral subtraction between different mutants or stimuli enable quantification of increased or decreased metabolites among those samples. To demonstrate the power of this approach, we performed multi-dimensional hetero-nuclear NMR analysis of metabolic movement of carbon and nitrogen nuclei in Arabidopsis thaliana [1]. First, distinct ethanol-stress response was clearly investigated by the uniformly 13C-Glc-labeled plants in the wild type and an ethanol-hypersensitive mutant [2]. Furthermore, inexpensive, and non-harmful uniform 13C labeling was achieved by photosynthetic incorporation of 13CO2. We monitored time-dependence and tissues dependent changes of 13C incorporation by use of multi-dimensional NMR. Over two weeks growth in 13CO2 atmosphere, 1H-detected multi-dimensional NMR measurements such as 2D-1H-13C HSQC, 3D-1H-13C-HCCH-COSY exhibit to detect reasonable number of cross-peaks to perform non-targeting metabolomics analysis. In addition to above extracted, in vitro sampling, the NMR-based methods can be used non-invasively and can yield (albeit limited) spatial information about gradients of solute concentrations. Therefore, we followed nitrogen fluxes in 15N-labeled seeds during the initiation of germination in vivo. Although above advantages in NMR-based methodologies, their low sensitivity offers to restrict measurements of relatively high concentration compounds. This disadvantage is being overcome due to progress in NMR technology, like as the world highest magnetic-field (920 MHz) NMR spectrometer [3] and the achievement of the coolest (4.5 K) high-sensitive cryogenically cooled probe [4], both developed by our groups.

[1]Kikuchi et al(2004)P.C.P. [2]Hirayama et al(2004)P.C.P. [3]Kiyoshi et al(2004)I.T.A.S. [4]Yokota et al(2004)A.C.E.

T07-081

Identification and functional analysis of cis-prenyltransferases in Arabidopsis thaliana

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In higher plants, a variety of Z,E-mixed polyisoprenoid alcohols, including dolichol and polyprenol, have been detected. They show two different distribution patterns in the chain length, which are C50-60 and C70-120. However, only one Arabidopsis cis-prenyltransferase (CPT), which catalyzes the formation of C75-90 polyprenyl products, has been identified in higher plants. In aspect of biological function, dolichol is known to play an important role as a glycosyl carrier lipid in the biosynthesis of glycoproteins, while physiological role of polyprenol is hardly elucidated. In order to identify CPTs corresponding to Z,E-mixed polyprenyl products found in higher plants and to understand the physiological function of CPTs and their products, we cloned and characterized genes encoding CPTs in Arabidopsis thaliana comprehensively.

In Arabidopsis genomic sequences, 9 genes that have high homology to CPTs identified from other organisms were found and termed AtCPT1 to AtCPT9. Among them, 6 AtCPT cDNAs were cloned according to the expression pattern of these genes in various tissues. Expression of these cDNAs in yeast SNH23-7D mutant strain, which is deficient in CPT activity, suppressed temperature-sensitive phenotype of the yeast mutant. Moreover, all of membrane fraction proteins prepared from yeast strains harboring each AtCPT cDNA showed distinct CPT activity, catalyzing the formation of C80-C100 polyprenyl products. Furthermore, soluble crude proteins extracted from AtCPT4-expressed cells showed prenyltransferase activity, catalyzing the formation of C50-C65 polyprenyl products. These results indicate that all of AtCPTs isolated in this study function as CPT in Arabidopsis, and that AtCPT4 contributes to the formation of medium-chain Z,E-mixed polyprenyl products and could be regulated by some factors to change the chain-length of its products. Expression analysis of AtCPTs in the seedlings treated with various abiotic stresses revealed that some of AtCPTs were up-regulated by dehydration and cold stress, suggesting that products of AtCPTs, Z,E-mixed polyisoprenoid alcohols, could function in response to abiotic stress in higher plants.

T07-082

Using microarray data to examine co-regulation in the amino acid metabolic pathways of A. thaliana

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It is generally anticipated that genes in a metabolic pathway will show a high degree of co-regulation in plants, as has been shown for yeast (1). However, this hypothesis has yet to be tested for a large number of genes in metabolic pathways in plants. Using a whole genome microarray data set designed to test the response of genes in A. thaliana roots to carbon and nitrogen treatments, an informatic tool called PathExplore (http://www.cs.nyu.edu/~pathexp) was used to test this hypothesis by analyzing the co-regulation of genes in amino acid biosynthetic pathways. When building pathways, PathExplore includes nutrient and metabolite transporters and identifies subcellular transporters that are potentially involved so that they can be included in the analysis.

Of the N-metabolic and amino acid pathways analyzed, only the N-uptake/assimilation (nitrate to glutamate, glutamine, or asparagine), arginine, and cysteine biosynthetic pathways have a statistically significant number of co-regulated genes as compared to randomly selected sets of genes. Thus, it appears that these CN treatment conditions coordinate the response of a specific subset of N-assimilatory and amino acid metabolic pathways. This analysis also gives insight into the function of different genes involved in N-assimilation. For example, OMT1 (chloroplastic 2-oxoglutarate/malate transporter) and DCT1 (mitochondrial dicarboxylate transporter) both transport C-skeletons required for N-assimilation, but only OMT1 is co-regulated with NIR (nitrite reductase), which is required for the reduction of nitrate and is co-regulated with many other genes in the N-uptake/assimilation pathway, suggesting that OMT1 has a role in nitrate assimilation, but DCT1 does not.

1. Ihmels, J., Ronen, L., Barkai, N. (2004) Nature Biotechnology 22, 86-92.

T07-083

Functional Analysis of Plant Nucleotide Metabolism: The Nucleoside Mono- and Diphosphate Kinase Gene Families in Arabidopsis thaliana

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Nucleotides have essential functions in a multitude of biochemical and developmental processes during the life cycle of a plant. As components of nucleic acids, phytohormones, and energy rich precursors for carbohydrate metabolism (e.g. UDP-glucose) nucleotides are involved in whole plant physiology. Predominantly in form of the purine ATP but especially important for plant sucrose and cell wall metabolism in form of the pyrimidines UTP and UDP nucleotides are co-substrates for most energy consuming or conserving reactions.
Whereas the enzymes in the de novo synthesis of nucleotides are often encoded by single genes the steps involved in salvaging and interconversion processes are always encoded by multigene families. The aim of this project is to elucidate the physiological function of genes necessary in supplying nucleoside mono- and diphosphates for metabolism and growth in Arabidopsis. We present a functional analysis of all nucleoside mono- and diphosphate kinase genes with mRNA expression analysis using semiquantitative RT-PCR, subcellular distribution studies using expression of GFP reportergene fusion proteins, and by enzyme activity characterisation after heterologous expression and purification of the respective proteins. In order to finally characterise the in vivo function of all nucleotide kinases we are currently investigating several mutants with defects in their nucleotide kinase gene expression.

T07-084

Biosynthesis and distribution of glutathione in developing Arabidopsis embryos

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Embryo development is a crucial part of the life cycle of plants, during which the body plan of the daughter plant is established, storage products required for germination are accumulated and desiccation tolerance develops, which enables both seed and embryo to overcome prolonged periods with unfavourable conditions. The tripeptide glutathione (GSH, gamma-glutamylcysteinyl glycine) is an ubiquitous low molecular weight thiol in plant cells with cytosolic concentrations between 0.1 and 3 mmol/L. Representing the major thiol redox buffer in the cell GSH plays various important roles in plant metabolism and is believed to be required during responses to environmental stress and also for normal plant development. A single nucleotide substitution in the gene AtGSH1 encoding of the first enzyme of GSH biosynthesis renders the Arabidopsis thaliana mutant root meristemless1 (rml1) almost GSH-deficient (Vernoux et al, 2000). The mutant lacks post-embryonic cell division, but despite lack of GSH-biosynthesis capacity the embryo still develops to maturity without any obvious phenotype. To investigate biosynthesis and distribution of GSH in wild-type and mutant embryos, non-destructive GSH-imaging techniques based on the GSH-specific fluorescent probe monochlorobimane together with confocal laser scanning microscopy were used. Quantitative image analysis showed that homozygous rml1 embryos from torpedo stage onwards contain only very small amounts of GSH and thus are not supplied with GSH from maternal tissues at this developmental stage. Additional investigation of a T-DNA insertional mutation in AtGSH1 showed that this knockout results in an embryonic lethal phenotype when homozygous. Thus, the developing embryo requires at least a minimal amount of GSH in order to complete embryogenesis to maturity and this GSH has to be synthesised autonomously by the embryo itself.

Vernoux T et al. (2000) Plant Cell 12: 97-110

T07-85

The IPMS/MAM gene family of Arabidopsis and its role in plant primary and secondary metabolism

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Glucosinolates are an important group of plant secondary compounds that occur throughout the Brassicaceae. At least a part of the cancer chemoprotective activity of cruciferous vegetables (e.g. broccoli) has been ascribed to the presence of glucosinolates whose general structure consists of a -thioglucose and sulfonated oxime, plus a variable side chain derived from an amino acid. Arabidopsis thaliana is known to synthesize more than 35 different glucosinolates. This variability is mostly due to the fact that the side chain of the most dominant class of glucosinolates present in Arabidopsis comes from chain-elongated derivatives of methionine. The addition of one to six methylene groups to the methionine precursor and further modifications later in the biosynthetic pathway result in a glucosinolate profile which can chemotype each individual Arabidopsis ecotype and mutant. The additional methylene groups arise from acetyl-CoA, which becomes condensed to a methionine derived 2-oxo acid resulting in a malate derivative. After an isomerisation and decarboxylation step, the resulting chain elongated 2-oxo acid either can be fed back into the so called chain elongation cycle to undergo a further condensation step with acetyl-CoA or can be converted to its final glucosinolate structure. The condensation of n-methylthio-2-oxo acid and acetyl-CoA to a methylthiolalkylmalate (MAM) is very similar to the condensation reaction catalysed by isopropylmalate synthases (IPMS) in leucine biosynthesis, i.e. the condensation of 2-oxo-isovalerate and acetyl-CoA to form isopropylmalate. From the genome of Arabidopsis four candidate genes have been identified that are similar to genes encoding IPMS in other organisms. Two of these candidate genes are located on the GSL-ELONG locus that controls glucosinolate side chain length and they were named MAM-1 and MAM-L [1]. The remaining two candidate genes are named IPMS1 and IPMS2. Both the MAM-genes and the IPMS-genes have been expressed in E. coli and their recombinant proteins biochemically characterised [2]. Whereas the MAM-enzymes proved to be most active with substrates from glucosinolate biosynthesis (secondary metabolism) and the IPMS-enzymes to be most active with substrate of leucine biosynthesis (primary metabolism), the two classes of enzymes do overlap in their substrate specificities. Biochemical data of the MAM/IPMS protein family has been confirmed by the careful analysis of several Arabidopsis mutants.

[1] Kroyman J. et al (2001) Plant Phys 127: 1077-108
[2] Textor S. et al (2004) Planta, 218(6): 1026-1035

T07-086

A Novel Nuclear Calmodulin-binding Protein Modulates Glucosinolate Accumulation in Arabidopsis

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Glucosinolates, a diverse class of sulfur-containing secondary metabolites, are mainly synthesized by species of the order Brassicales from select protein amino acids and decompose during tissue damage to a range of biologically active products with roles in plant defense and human health. To identify regulatory loci of glucosinolate production, we developed a simple bioassay (Wang et al., 2002)to screen Arabidopsis T-DNA activation tagged mutants for plants with a high-glucosinolate chemotype. Here, we report the cloning and functional analysis of AtGCC7. Expression of a series of gain-and loss-of-function gcc7 alleles in different accessions of A. thaliana correlates with higher and lower levels of glucosinolate accumulation, respectively. GCC7 is predicted to encode a novel protein of 50.5 kD, which contains putative nuclear localization signals and several motifs known to mediate calcium-dependent and calcium-independent binding of calmodulin. We show that a GCC7-GFP fusion protein is targeted to the cell nucleus and that recombinant GCC7 binds to calmodulin in a calcium-dependent fashion. Histochemical analysis of tissue-specific GCC7::GUS expression in a GCC7 gene trap line reveals GCC7 promoter activity in vascular tissues of roots, hypocotyls, leaves, stems, young and mature siliques. Interestingly, the observed GCC7 expression domains are strikingly similar with the patterns reported for several genes encoding enzymes of the glucosinolate pathway. Analysis of steady-state mRNA levels of glucosinolate pathway genes in gain-and loss-of-function gcc7 mutants indicates that GCC7 affects expression of multiple genes with roles in glucosinolate metabolism. Since GCC7 gene expression is regulated by jasmonic acid, the prospect arises that GCC7 integrates a subset of jasmonate-mediated responses in plant defense and development, such as glucosinolate production, via calcium/calmodulin signaling. This work is supported by the US Department of Agriculture.

Wang et al. (2002) Direct analysis of single leaf disks for chemopreventive glucosinolates. Phytochem. Anal.13:152-157.

T07-087

Flavonol glycosyltransferases in Arabidopsis thaliana

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Flavonol glycosides constitute a prominent class of secondary metabolites in A. thaliana as well as several crop plants. In Arabidopsis, mainly kaempferol and quercetin occur in glycosylated forms with carbohydrate residues attached at different positions. However, the conjugating enzymes have been elusive so far. Arabidopsis thaliana harbors more than 100 UDP-sugar dependent glycosyltransferase genes (UGT). Based on sequence homologies to known flavonoid glycosyltransferases from other plant species, the UGT78D and UGT73B/C subgroups were targeted as candidate flavonoid-UGTs from Arabidopsis. Indeed, metabolic profiles of T-DNA knockout lines showed the reduction of quercetin-3-O-rhamnoside-7-O-glucoside (ugt73C6 and ugt78D1) as well as flavonol-3-O-rhamnoside-7-O-rhamnosides (ugt78D1) and two flavonol-3-O-glucoside derivatives (ugt78D2). The latter two mutants indicated that UGT78D1 and UGT78D2 were responsible for the initial 3-O-glycosylation of flavonols but discriminated between rhamnosylation and glucosylation. Interestingly, UGT78D2 was recently also found to be upregulated in an anthocyanin-overaccumulating mutant and shown to be responsible for anthocyanin-3-O-glucosylation as well (Nisiyama et al, 2004). In accordance with these mutant data, recombinantly expressed UGT78D1 and UGT78D2 catalyzed the rhamnosylation of the 3-OH group of quercetin and kaempferol, whilst UGT73C6 catalysed the glucosylation of the 7-OH group of kaempferol- and quercetin-3-O-rhamnosides. Thus, these analyses identified the first A. thaliana flavonol glycosyltransferases. The respective mutants and double mutants created will be used for analyzing the function of differential and complex glycosylation of flavonols in Arabidopsis.

Nisiyama Y et al. (2004) Plant Cell Physiol., Supplement 45: s157.

T07-088

MOLECULAR AND GENOMIC ANALYSIS OF NITROGEN REGULATION OF AMINO ACID PERMEASE I (AAP1) IN ARABIDOPSIS

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In higher plants, amino acids are the currency of nitrogen exchange between source and sink tissues at every stage of plant growth and development. We are investigating AAP1 as a prototypical example of a plant proton-amino acid symporter that plays an important role in amino acid partitioning. We've previously shown that AAP1 expression is regulated by changes in nitrate, ammonia, and amino acid abundance, as well as changes in carbon metabolism.

Semi-quantitative RT-PCR showed nitrate and glutamate are metabolite signals that induce AAP1 expression after nitrogen starvation. Microarray analysis showed that application of exogenous nitrate, glutamate and glutamine differentially regulate the expression of AAP1 and several other genes involved in key aspects of plant metabolism and assimilate partitioning. Significantly, there was little congruence between the genes regulated by each of these metabolic signals. This observation was supported in a T-DNA insertion knockout of AAP1 in which a short fragment of the AAP1 promoter was sufficiently long to retain nitrate regulatory elements, but the same region was too short to preserve sensitivity to glutamate regulation.

AAP1:GUS plants demonstrated that AAP1 expression is developmentally regulated in both source and sink tissues. In situ hybridization identified the cell-specific AAP1 expression in the phloem cells of minor veins, consistent with a role in phloem loading of amino acids. Overexpression of AAP1 results in shorter roots and altered leaf shape in seedlings, while RNAi knockouts flowered later than wild type plants. These data support the hypothesis that AAP1 plays an important role in nitrogen partitioning.

Taken together, the evidence presented here shows that there are multiple nitrogen-metabolite mediated regulatory pathways that differentially modulate gene expression in response to dynamic changes in nitrogen status.

T07-089

Accelerated senescence and changes in primary and secondary metabolism due to the overexpression of a novel transcription factor

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Considerable cross-talk can be observed between signalling pathways of different stress responses. On the other side, response specificity can be achieved by selective activation of specific transcription factors. Among those, transcription factors containing the AP2/ERF domain play a central role in plant stress responses. Here we describe that the over-expression of a member of the AP2/ERF transcription factor family leads to plant death. Cell death apart of being the last step of a developmental process can be induced prematurely by environmental conditions such as pathogen infection, nutrient or water stress or oxidative stress induced by ozone or UV-B light. Significant metabolic changes were observed in the transgenic plants that show a "switch" towards secondary metabolism as well as the induction of jasmonic acid related genes

Cheong YH, Chang H, Gupta R, Wang X, Zhu T, Luan S.
Kunkel BN, Brooks DM.
Beers EP, McDowell JM.

T07-090

Potential role of a member of the PHO1 gene family in Pi

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PHO1 has been identified as a protein involved in the loading of inorganic phosphate into the xylem of roots in Arabidopsis. The genome of Arabidopsis contains 11 members of the PHO1 gene family. The cDNAs of all PHO1 homologs have been cloned and sequenced. All proteins have the same topology and harbour a SPX tripartite domain in the N-terminal hydrophilic portion and an EXS domain in the C-terminal hydrophobic portion. The SPX and EXS domains have been identified in yeast (Saccharomyces cerevisiae) proteins involved in either phosphate transport or sensing, or in sorting proteins to endomembranes. Phylogenetic analysis indicated that the PHO1 family is subdivided into at least three clusters. PHO1;H1 is the closest homolog to PHO1 having 46% of amino acid identity and 61% of amino acid similarity. PHO1;H1 is able to complement the pho1 mutant when expressed under the control of the PHO1 promoter. Reverse transcription-PCR revealed an expression of PHO1;H1 in leaves, roots and stems. Analysis of the activity of the promoter of PHO1;H1 homolog using promoter-glucuronidase fusions showed its predominant expression in the vascular tissues of roots, leaves and stems. Upregulation of PHO1;H1 transcription upon Pi stress, as well as its pattern of expression in the phosphate response mutants backgrounds (phr1 and pdr1), suggests that the expression of PHO1;H1 is closely linked to the Pi status of the plant. RT-PCR and Northern analysis revealed increased expression of PHO1;H1 in older and senescensing leaves compared to the young leaves. Taking into account the complementation of pho1 mutant by PHO1;H1, as well as the expression profile of the gene in leaves and its regulation by Pi, we hypothesise the role of PHO1;H1 in displacing Pi from source to sink tissues under Pi stress and senescence.

T07-091

TRANSCRIPTOME AND METABOLIC PROFILE ANALYSIS TO CHARACTERIZE PLANT RESPONSE TO METHANOL TREATMENTS

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Several reports have demonstrated the effect of methanol on growth and development of different plant species. However, most of these studies show morphological observation or measurement of basic parameters providing limited information which does not allow an understanding of the mechanism involved in the plant response to methanol treatment. Despite these significant insights into methanol effect, much remains to be elucidated about the molecular mechanism by which methanol affects plant metabolism. Preliminary results obtained in our group clearly show that methanol applied to the foliar tissue promotes the growth in tobacco, tomato and Arabidopsis thereby increasing fresh and dry weight. In order to gain a broader insight on the events involved in methanol responses in plants, transcript profiling from Arabidopsis plants treated and nontreated with methanol were analysed by using affymetrix microarrays. Preliminary results of such data will be presented.

The project is supported by FONDECYT 102424

T07-092

Identification of Arabidopsis Seed Color Mutants with Altered Oil Content

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Yellow seeded varieties of Brassica napus have been shown to contain higher oil content than black seeded varieties. To identify the gene(s) responsible for the high oil phenotype we measured seed oil composition in yellow and tan seeded mutants of Arabidopsis thaliana. Using a map based cloning approach we identified a mutant allele of tt18 (leucoanthocyanidin dioxygenase (LDOX)) in a mutant producing seed with altered coat color and higher oil content. Accumulation of LDOX transcript requires the transcription factor TTG1. We measured seed oil levels in three alleles of ttg1 (ttg1-1 as well as two newly identified alleles) and found significant increases in oil content in all three mutant lines. By complementation, we confirmed that mutations in the LDOX and TTG1 genes are responsible for both the seed color and high oil content phenotypes.

T07-093

Regulation of the Anthocyanin Pathway by bHLH, Myb, and TTG1 proteins in Arabidopsis

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The bHLH proteins GL3, EGL3, and TT8 are regulators of anthocyanin production in Arabidopsis. egl3 and tt8 single mutant seedlings show a reduction in anthocyanins while multiple mutant combinations such as gl3/egl3 and gl3/egl3/tt8 seedlings show no anthocyanin accumulation at all. The WD40 protein TTG1 is also an anthocyanin synthesis regulator with ttg1 mutants showing a complete lack of pigment. The PAP1 and PAP2 genes of Arabidopsis that both encode R2R3 Myb transcription factors are candidate anthocyanin regulators because overexpression of either one results in overproduction of anthocyanins. We have previously shown that, like ttg1 mutant, bHLH mutants show decreased expression levels of the structural anthocyanin gene TT3 (DFR) but not TT4 (CHS). In this study we used a Q-PCR approach to analyze the expression levels of structural anthocyanin genes TT18 (LDOX), TT19 (a GST), and Ban (LAR) in bHLH mutants, ttg1 mutant, and wt plants. Also, bHLH, PAP Myb, and TTG1 gene expression levels were analyzed in various mutants and in light vs. dark grown wt seedlings to test for the possible light-regulation of these anthocyanin regulators.

T07-094

Functional analysis of sterol-C24-methyltransferase in arabidopsis

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S-adenosyl-methionine sterol C-methyltransferases are involved in two distinct and non consecutive methyl group transfers in phytosterol biosynthesis. SMT1 catalyses preferentially the C24 methylation of cycloartenol whereas SMT2 catalyses preferentially the C241 methylation of 24-methylene lophenol. SMT2 acts at a branchpoint of the sterol pathway defining then two biosynthetic segments: the 24-methyl sterol (campesterol) and the 24-ethyl sterol (sitosterol) segments. Polypeptides of the first and the second class of SMTs share around 40% identity. The Arabidopsis genome contains three genes encoding SMTs. The biochemical and physiological functions of AtSMT1, AtSMT2;1 and AtSMT2;2 were examined using a set of mutants or transgenic plants in which the expression of one of each SMTs was modulated. These plant lines were characterized by a high cholesterol, high campesterol or high sitosterol profile. Expression analysis (RT-PCR, GUS fusions, in situ) showed transcription patterns for all three genes. However, a knock-out in SMT2;2 did not show any dramatic phenotypic variations compared with the wild-type. Modulation of the expression of the two other SMTs had an impact on sterol profile and morphology; certain organs or tissues were more responsive to modified ratios of 24-methyl to 24-ethyl sterols than others. Behaviour of smt lines in adverse environmental conditions was studied: high sitosterol plants displayed an increased tolerance to salt stress compared to the wild-type. Regulation of the Arabidopsis sterol metabolism and its physiological implications will be further discussed in the view of transcription profile analysis.

T07-095

Comparative analysis of the regulatory events that modulate the plastidic MEP isoprenoid pathway in Arabidopsis.

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Plants produce an enormous variety of natural products, from which isoprenoids constitute the major proportion. Isoprenoids participate in a variety of central cellular processes in all living organisms as growth regulators, structural components of membranes and pigments, among others. In addition, many isoprenoids are of biotechnological value. In plants the biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the two structural blocks for isoprenoid biosynthesis, takes place by two pathways localized in different cellular compartments. The mevalonic pathway that operates in the cytoplasm and the methyl-D- erythritol 4-phosphate (MEP) pathway that operates in plastids. In the past few years an important progress has been accomplished with the identification of all the genes and enzymes for the MEP pathway. In contrast to the mevalonic pathway, much less it is known about the regulation of the MEP pathway in plants. To get new insights into some of the regulatory mechanisms that control the expression of the transcripts and proteins in this pathway, a comparative analysis of the complete MEP route was performed at the transcript and protein levels under different conditions. At the transcript level we found that all genes of the pathway are regulated in a coordinate in response to several conditions, including development. This expression analysis also has revealed that post-transcriptional events are key players in the regulation of specific proteins of this biosynthetic route. The results obtained in the present study demonstrate that the 1-deoxy-D-xylulose 5-phosphate synthase (DXS) protein, the first enzyme of the pathway, is feedback regulated in response to alterations in the pathway flow. This regulation is observed in different mutants of the pathway, during seedling development and with the use of specific inhibitors of the route. This study represents the first comparative analysis of the complete MEP route in any organism at the transcript and protein level.

T07-096

Genomewide diurnal and circadian changes in transcript levels of A. thaliana revealed by microarray analysis

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So far microarray based analysis of circadian changes in transcript levels was used to find genes dependent on the molecular clock. We are interested in the question which genes of the metabolism and gene families are subjected to diurnal and circadian changes due to the light-dark rhythm that leads to changes in the metabolite contents of a plant. We use oligonucleotide based microarray analysis to semi-quantitatively determine the genomewide transcript levels in A. thaliana. RNA samples from total non-flowering plants were obtained every 4 hours at 6 timepoints over a 12 h/12 h day/night cycle. They were hybridised against the Affymetrix ATH-1 gene chip array. By using the MapMan software that displays transcript and metabolite datasets on user generated maps (Thimm et al., 2004) we intuitively take a genome-wide picture of genes that are regulated by diurnal and circadian changes in plant metabolism. We show which pathways and gene groups are harbouring genes with oscillating transcript levels, which are most active and the amplitudes. Transcripts of several hundreds of genes are oscillating and distributed in nearly all functional categories. The corresponding genes are displayed in pathway and family context. The dataset serves as a basis for additional gauntlets/experiments which induce deviations from the normal time dependent expression status of genes. It is also useful to analyse quantitative transcript-protein relationships.

T07-097

Ionomics: Gene Discovery in Aid of Plant Nutrition, Human Health and Environmental Remediation

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Increasing the ability of plants to take up minerals could have a dramatic impact on both plant and human health. Furthermore, understanding the pathways by which metals accumulate in plants will enable the engineering of plants to exclude toxic metals or to extract toxic metals from the soil. We have employed mineral nutrient and trace element profiling (via ICP-MS or ICP-AES) as a tool to determine the biological significance of connections between a plant's genome and its elemental profile. Our focus is on genes that control uptake and accumulation of solutes, including N, P, Ca, K, Mg (the macronutrients in fertilizer), Cu, Fe, Zn, Mn, Co, Ni, Se, Mo, I (micronutrients of significance to plant and human health), Na, Pb, Al, As, and Cd (metals causing agricultural or environmental problems). The ion profile of 4429 mutants from the commercially available yeast knockout collection has been completed, yielding new insights into the role of the vacuole and the mitochondria in metal metabolism. We have also completed a proof-of-concept screen of over 6000 FN mutagenized Arabidopsis plants (Lahner et al., 2003 Nat. Biotechnol. 21:1215) and we are currently screening the Salk collection of T-DNA insertion alleles. The long-term goal is to quantify the functional contribution to mineral nutrition and ion homeostasis of every gene in Arabidopsis. To maximize the value of this ionomics approach, we have developed a searchable online database containing ionomic information on over 22, 000 plants.

http://hort.agriculture.purdue.edu/Ionomics/database.asp

T07-098

Functional Genomics of Carbon-Nitrogen Interactions

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Carbon-nitrogen interactions provide a good system to explore the use of system-orientated approaches to improve understanding of complex biological systems. The use of carbon and nitrogen are closely intertwined with each other, and has important consequences for a wide range of downstream processes, including metabolism but also in growth-related processes and development. Nevertheless, many basic structural features of the network are known, at least in the area of primary metabolism, which aids experimental planning and the interpretation of the results, I will discuss how a range of phenotyping platforms including commercial expression arrays, multiplexed real time RTR-PCR for > 1200 transcription factors, robot-based enzyme assays and metabolite profiling are being used to characterise the response to changes in the carbon and nitrogen supply. Various bioinformatics approaches are used to analyse the results, including MapMan, a simple software tool that allows genomics data sets to be displayed on diagrams of biological processes. The results will be used to illustrate some general points about the relation between changes of transcripts, proteins/enzyme activities and metabolites, to provide a detailed molecular characterisation of the global response to changes in nitrogen and carbon, and to show how this approach allows novel genes to be identified that can be highlighted for subsequent details functional analysis.

T08 Long Distance Transport
(Signals Including Silencing and Metabolites)

T08-001

Involvement of DIR1, a putative lipid transfer protein, in long distance signaling during Systemic Acquired Resistance

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Systemic acquired resistance (SAR) is elicited in response to local necrotizing infections which induce the production of a long distance signaling molecule that is perceived in distant tissues resulting in resistance to normally virulent infections in distant parts of the plant. Our previous studies using dir1-1(defective in induced resistance), a SAR-defective mutant, indicate that dir1-1 can perceive the SAR signal present in petiole exudates (enriched for phloem sap) from wild type SAR-induced plants, but dir1-1 exudates do not contain this signal (Maldonado et al, 2002). Protein Gel Blot analysis demonstrated that DIR1 protein is present in petiole exudates of SAR-induced wild type, but not dir1-1 plants. These results suggest that DIR1, which encodes a putative lipid transfer protein (LTPs contain a lipid binding pocket), may be involved in the production of the SAR mobile signal or in transporting a lipid signal to distant tissues to establish SAR. DIR1 can be detected in intercellular washing fluids of wild type, but not dir1-1 plants indicating that the predicted cell wall signal sequence does localize DIR1 to the cell wall.
We hope to address how DIR1, a cell wall-associated protein translocates, such that it is found in petiole exudates after SAR induction. Our data suggest that DIR1 may be transporting a lipid signal via the phloem to distant tissues during SAR long distance signaling. Therefore, a number of approaches are being taken, including characterization of transgenic dir1-1 plants containing a signal sequence-less DIR1 gene fused to the GUS reporter gene, under the control of the native DIR1 promoter. We predict that mis-expression of DIR1 in the cytoplasm will provide insight into the movement and role of DIR1 during long distance signaling during SAR. Other transgenic lines have been generated, including a native DIR1 promoter-GUS line to determine the cellular location of DIR1 before and after SAR induction. DIR1's presence in peitole exudates after SAR induction suggests that DIR1 may be expressed in phloem-associated companion cells. The results of preliminary experiments with these transgenic plants will be presented and should provide more information about DIR1 and its role in long distance signaling during SAR.

A. Maldonado, P.Doerner, R.A.Dixon, C.J. Lamb, *R.K. Cameron. Nature, 419: 399-403, 2002

T08-002

How are signalling pathways involving Jasmonate and Calcium linked to the wound response in Arabidopsis.

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Jasmonate (JA) is a key hormone in the regulation of plant defence against herbivory and responses to wounding. Wounding induces the biosynthesis of JA at the site of damage and subsequent activation of JA responsive genes both locally and systemically in non-wounded tissues. It is not known what form the systemic wound signal is transmitted. Here we used mutants defective in biosynthesis of JA (dad1 and opr3) and defective in response to JA (coi1,16) and micro grafting to investigate the form of the systemic wound signal.
The luciferase reporter gene driven by the JA responsive promoter from the vegetative storage protein (VSP) gene was introduced into the mutants. The transgenic mutants were grafted as scions onto wild type rootstocks and vice versa. The rootstock was wounded and the scion was then imaged with a low level light camera to determine if there was a transmissible wound signal. Luciferase activity could be detected in dad1 and opr3 scions grafted onto wild type rootstocks after wounding. This indicates that JA synthesis via DAD1 and OPR3 in the scion is not required for the systemic wound response. By contrast, luciferase activity could not be detected in coi1,16 scions grafted onto wild type rootstocks after wounding. This indicates that the JA responsive gene COI is required for a systemic wound response. Therefore JA's are candidates as the systemic wound signal in Arabidopsis. Results show that the OPR3 gene is required for a JA dependent wound response as no luciferase activity is detected in opr3 mutants after wounding. In contrast the DAD1 gene is not required for a JA dependent wound response. Luciferase activity was higher in dad1 compared to wild type suggesting the DAD1 gene may function by inhibiting the JA signal activating VSP activity in Arabidopsis.
Calcium is a universal secondary messenger in eukaryotes. To test it's function in the JA dependent wound response, wild type and JA mutants containing the calcium reporter aequorin fused to the 35 S Cam promoter shows calcium to be involved in signalling locally but not systemically in wounded tissues.

T08-003

Patterning of plants by auxin

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Plants exhibit highly regular arrangements of leaves and flowers around the stem. The pattern of organ position is referred to as phyllotaxis, the greek word for “leaf arrangement”. The most widespread phyllotaxis is spiral; other phyllotactic patterns involve distichous (alternate) and decussate (opposite) organ position. Under certain natural or experimental conditions, for example at the onset of flowering, phyllotactic patterns can change from one stable pattern into another, indicating that they are based on the same underlying mechanism.

The origin of phyllotaxis is in the shoot apical meristem where the leaves and flowers are formed. There, founder cells are selected at predictable positions, depending on the relative position of preexisting primordia. Auxin plays a pivotal role in the selection of the founder cells, and as the trigger of organ outgrowth. At later stages, auxin is instrumental in the specification and patterning of the vascular system of the organs.

The patterning mechanism involves specific cellular transporters that catalize the influx and efflux of auxin. Their polar subcellular localization results in upward auxin transport into the meristem. Preexisting primordia function as auxin sinks, thus allowing for auxin accumulation only at certain minimal distances from primordia. The cells that are located at the auxin peak are selected to become founder cells, and grow out. The resulting new primordium will soon act as a new sink, thus providing positional information for the next round of founder cell selection. The resulting feedback mechanism is responsible for the regularity, and for the reiterative nature of phyllotaxis.

Reinhardt et al. (2000) Plant Cell 12, 507-518.
Reinhardt et al. (2003) Nature 426, 255-260.

T08-004

Expression Pattern of an Arabidopsis Dehydrin, Homologous to an Iron Transport Protein from Ricinus

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Dehydrins or Group 2 LEA (Late Embryogenesis Abundant) proteins are thought to be important in the response to different kinds of abiotic stresses, which cause a decrease in water potential. Therefore dehydrin genes are commonly found to be induced by drought, cold- and salt (osmotic)- stress. They are also found to be expressed late in embryogenisis (hence the name!). Here we describe the expression pattern of the Arabidopsis dehydrin gene AtITP, which is homologous to an iron binding protein from Ricinus communis phloem sap (Krüger et al., 2002). By Northern blot and promoter reporter gene analysis we didn?t detect any increase in AtITP expression after stress treatment, but we were able to localise the main site of expression in the phloem of leaves, stems and roots. Besides there promoter activity was found close to the xylem in the pith, in pollen and in the endosperm. We also studied the effect the intron from the AtITP gene has on the activity of the promoter reporter gene construct in transgenic plants, by generating an uidA gene harbouring the Intron in a similar distance to the TATA box as in the native AtITP gene. The implications of the constitutive expression of a dehydrin, which probably has the ability to bind iron, in the tissues mentioned above, will be discussed.

Krüger, C., Berkowitz, O., Stephan, U. and Hell, R. 2002. J Biol Chem 277: 25062-25069.

T08-005

Expression profiling of membrane transporters in Arabidopsis

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Membrane transporters were shown to be important for diverse processes like photoassimilate allocation, cell expansion, salt/metal tolerance, defense responses and senescence. Furthermore, the efforts to improve crop yield by means of overexpressing metabolic enzymes could be hampered by a limitation in the maximal transport capacity of the transporter(s) for the required substrates, thereby creating a bottleneck. Thus, it is important to identify and characterise all plant transporters to understand the complex transport pathways and their regulation. To date, only a small number of plant transporters has been fully characterised. In the framework of the GABI genome project we focus on the identification and characterisation of transporters in Arabidopsis. Using the criteria of proteins containing more than three transmembrane regions, a set of 2000 (putative) transporters has been selected from the ARAMEMNON database and gene specific PCR products for those were spotted on macro- and microarrays. Comparison of these dedicated transporter arrays with commercial oligonucleotide arrays shows a good correlation between the data obtained from the different sources. We present here the results from expression profiling experiments using our dedicated arrays under a variety of developmental- and nutrient stress/deficiency conditions.

T08-006

Nutritional regulation of cytokinin biosynthesis: a possible role for long-distance signaling molecule

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Inorganic nitrogen has crucial effects on growth and development of plants, providing cellular components and modulating gene expression. Recent studies suggest that, in addition to nitrate ion itself, cytokinin (CK) plays an important role as a communicating substance of nitrogen status between root and shoot. Spatial and temporal studies on the accumulation and the translocation of CK in response to nitrate replenishment in maize showed subsequent accumulation of various CK species in the roots, xylem sap and leaves [1]. In Arabidopsis thaliana, similar response was observed [2, 3]. These studies suggest that CK metabolism and the translocation could be commonly modulated by the nitrogen availability in higher plants.
To understand the mechanism for nitrogen-dependent accumulation of CK, we analyzed the spatial expression pattern of Arabidopsis adenosine phosphates-isopentenyltransferase genes (AtIPT1, AtIPT3 to AtIPT8) and the effect of inorganic nitrogen sources on their regulation [3]. In a long-term treatment, the accumulation level of AtIPT5 transcript was correlated with the concentrations of nitrate and ammonium ions in the growth medium. However, under a nitrogen-limited condition, AtIPT3 expression was rapidly induced by nitrate in the seedlings accompanying the accumulation of cytokinins, whereas AtIPT5 expression was little affected. The nitrate-dependent accumulation of both the AtIPT3 transcript and the CKs was markedly reduced in a mutant of AtIPT3. These results suggest that nitrogen-availability differentially regulates expression of AtIPT3 and AtIPT5, and that AtIPT3 is a key determinant of CK biosynthesis in response to rapid changes in the availability of nitrate. The AtIPT3 expression was also correlated with the concentration of sulfate and phosphate but not that of K+, Mg2+ or Ca2+. These results also imply that AtIPT3 is involved in regulation CK biosynthesis in response to the inorganic macronutrients. The biased distribution of trans-zeatin riboside and isopentenyladenine riboside in the xylem and phloem, respectively, implied that the CK-mediated nutritional signal is translocated along both routes by different means. Putting together these findings, we will discuss the physiological function of CK as a long-distance nutrient signal in higher plants.

1. Plant Cell Physiol. (2001) 42: 85-93.
2. J. Exp.Bot. (2002) 53: 971-977.
3. Plant Cell Physiol. (2004) 45: in press.

T08-007

Novel proteins in the phloem of Brassicaceae.

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Sieve element/ companion cell-complexes form the functional long distance transport system, called phloem, in higher plants. This tissue is responsible for the translocation of nutrients from the site of their synthesis to sink tissues (i.e. developing leaves, flowers, roots). In addition, the phloem transport stream contains proteins at reasonable concentrations. According to the fact that mature sieve elements are not capable to transcribe and translate, it is generally suggested that this specific set of proteins is synthesized in companion cells and transported into their dependent sieve tubes via specialized plasmodesmata. So far, there exists only limited information about the identity and function of those proteins. Identification was hampered mainly by the limited suitability of genetic model plants, like Arabidopsis, to sample sufficient amounts of phloem sap.
In contrast, Brassica napus, a relative of Arabidopsis, allows the collection of a sufficient amount of phloem sap to perform two-dimensional gel electrophoresis (2D PAGE) and about 1000 soluble proteins can be separated using this technique. To identify these proteins, visible spots are excised, digested with trypsin and partially sequenced by ESI MS/MS.
As a complementary strategy, a cDNA expression library from complete RNA of Brassica napus was created. This library was screened with antibodies directed against total proteins from phloem exudate.
Both approaches led to the identification of a large set of formerly unknown phloem sap proteins that should allow a deeper understanding of the vital processes occurring inside this transport stream.

T08-008

Functional analysis of the transcription factor TF55

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Brassinosteroids (BRs) comprise a group of polyhydroxysteroids and represent a recently identified class of phytohormones that show structural similarities to steroid hormones of mammals and arthropods. They occur ubiquitously in plants and control various developmental and metabolic processes and are integrated into a complex regulatory network, which includes several other plant hormones.
Several BR-biosynthetic mutants have hitherto been isolated including the BR-deficient mutant dwarfs cbb1/dwf1/dim and cbb3/cpd as well as the BR-insensitive cbb2/bri1.
In order to gain insight into the molecular mechanisms underlying BR action, BR-regulated genes have been isolated by means of subtractive suppression cDNA cloning.
Among approximately 200 clones checked about 20 proved to be differentially expressed upon BR-treatment including cDNAs encoding OPR3, CAB, Rubisco, RSZp22/21 protein, Retrovirus-like sequences, chloroplast-proteins and several proteins of unknown function. The BR-inducible gene “TF55“ thus identified encodes a putative Zn- and RING-finger containing transcription factor and has been chosen for detailed analysis. It´s only homologue in Arabidopsis is also expressed ubiquitousely throughout the plant but is not BR-regulated.
Analysis of putative loss of function T-DNA insertion mutants of both genes derived from the SIGnAL collection as well as of transgenic plants with altered gene expression revealed no visible phenotypic alterations.
Affymetrix gene chip analysis conducted with k.o. mutants of “TF55” and its homologue and with 35S::TF55 overexpressors suggest antagonistic functions of both genes in stress and defense pathways.

T08-009

C8 GIPK, a GAI-interacting protein kinase that controls hypocotyl elongation in Arabidopsis

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Gibberellic acid (GA) regulates a variety of plant developmental processes including germination and elongation. In Arabidopsis thaliana, several downstream players of GA signaling have been described, the best known of which are the DELLA proteins. These proteins are highly conserved and thought to act as repressors of GA signaling. GAI and RGA, two members of the DELLA protein family, have been implicated in mediating hypocotyl and stem elongation. Here, we report on the trans-membrane receptor protein kinase C8 GIPK. C8 GIPK is an active protein kinase that interacts in the yeast two-hybrid system with GAI and RGA. Confocal microscopy analyses demonstrate that C8 GIPK is localized to the plasma membrane. C8 GIPK antisense lines germinate faster than the wildtype. Moreover, hypocotyl and stem elongation are increased. Based on our results, we believe that C8 GIPK is a negative regulator of GA-mediated processes.

T08-010

Essential role of riboflavin pathway in jasmonate signaling

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The Arabidopsis coi1 mutation defined a key regulator in the JA signaling pathway. The recessive coi1 mutants fail to respond to jasmonate and coronatine, a phytotoxin structurally similar to jasmonic acid, displaying defects in JA-regulated gene expression, exhibiting male sterility and showing susceptibility to insects attack and pathogens infection. The COI1 gene encodes a 592-amino acid protein containing an F-box motif and 16 leucine rich repeat sequences (LRRs), which interact with Arabidopsis CULLIN1, RBX1 and Skp1 like proteins ASK1 or ASK2 to assemble SCFCOI1 complexes in planta. The SCFCOI1 is assumed to regulate the abundance of the substrate proteins, which may suppress a set of transcription factors and/or affect the expression of appropriate target genes essential for JA responses.

To better understand the molecular mechanism via which COI1 regulates JA responses, we conducted a screen for suppressors of the coi1 mutant. A cos1 (coi1 suppressor 1) recessive mutant was identified and found to regain wild type-like phenotypes of JA-sensitive root elongation, gene expression, senescence and defense response in the coi1 background (Xiao et al 2004). The COS1 gene was cloned using map-based approach and found to encode a lumazine synthase, a key component in riboflavin pathway that is essential for diverse yet critical cellular processes. The riboflavin pathway is speculated to act downstream of COI1 and to be required for suppression of the COI1-mediated root growth, senescence and plant defense.

Xiao, S., Dai, L., Liu, L., Wang,Z., Peng, W., and D Xie. (2004) Plant Cell 16:1132-1142

T08-011

Molecular analysis of plasmodesmata

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In wild-type Arabidopsis plants the AtSUC2 promoter directs the expression of the AtSUC2 sucrose-H+ symporter gene only in companion cells. It was demonstrated, that GFP expressed under the control of the AtSUC2 promoter, can traffic from companion cells through plasmodesmata into sieve elements. With the stream of photoassimilates the GFP is transported to different sink tissues such as petals, funiculi, anthers, young rosette leaves and root tips. Through plasmodesmata GFP can be symplastically unloaded into the sink tissues of the plant, such as mesophyll cells of sink leaves or cells of the root tips and can migrate cell to cell by the way of post-phloem transport. Therefore a strong GFP fluorescence is detectable although the promoter is not active in these sink tissues.
Here we studied the identification of genes that may be involved in structure and development of plasmodesmata or in the regulation of the size exclusion limit of these organelles. Therefore we subjected the AtSUC2::GFP Arabidopsis line described above to T-DNA mutagenesis resulting in 14000 independent mutant lines. Until now about 7000 lines were screened for plants lacking the unloading of GFP especially into the mesophyll cells of the root tips. Among these 7000 lines we have identified seven interesting plants showing no GFP unloading in the cells of the root tip. In addition there is one mutant line showing no GFP unloading even in all other sink tissues mentioned above. By different PCR methods the T-DNA insertions in these mutant plants were located and possible affected genes of interest were identified. Complementation- and RNAi-experiments on the respective lines and analysis of the genes will be discussed.

Imlau, A. Truernit, E. Sauer, N. (1999)
Plant Cell 11: 309-322

T08-012

Expression of AtMHX, a transporter involved in long distance metal transport, is governed at both the transcriptional and translational levels

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AtMHX is an Arabidopsis thaliana tonoplast transporter that can sequester Mg, Zn, and Fe ions into the plant vacuole [1]. Vacuoles play a major role in ion homeostasis in plants. To understand the regulation of AtMHX expression, we fused reporter genes to its promoter, and expressed the chimeric genes in transgenic Arabidopsis plants. We found that AtMHX expression is particularly high in stems, where expression can be seen in the cortex, at the phloem region, and at the xylem parenchyma. The parenchyma of vascular cells (and stem tissue in general) play a buffering role in long distance mineral transport. These tissues absorb minerals when the later are in ample supply, and release them in times of deficiency. Thus, vacuolar sequestration by AtMHX may regulate the extent of metal loading and unloading at both the xylem and the phloem. AtMHX is expressed at other plant tissues as well, and its expression is subjected to developmental and hormonal regulation. A preliminary promoter deletion analysis was also carried out.
Besides transcriptional regulation, AtMHX expression is affected by post-transcriptional mechanisms. In vitro and in vivo studies showed that the 5' untranslated region (5'UTR) of AtMHX can repress the translation of downstream coding sequences. We performed detailed analyses to determine the role of different motives within the 5'UTR in this repression. These observations could account for our recent finding that the levels of MHX proteins are significantly lower in leaves of A. thaliana then in leaves of the closely related Zn hyperaccumulator species Arabidopsis halleri. This difference is regulated at the post-transcriptional level, as transcript levels are higher in leaves of A. thaliana. A working hypothesis to explain the role of MHX transporters in the two plant species will be presented.

1. EMBO J. (1999) 18: 3973-3980.

T08-013

Phosphate signaling in Arabidopsis.

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Arabidopsis responds locally and systemically to phosphate (Pi) starvation by altering growth patterns and inducing starvation responses that aim to reduce Pi demand, while increasing Pi acquisition and mobilization within the plant. Systemic adaptation to non-uniform Pi availability leads to the suppression of starvation responses in roots exposed to low Pi, if other parts of the root system are exposed to high external Pi. Thus, a systemic signal has been posited to coordinate Pi homeostasis in the plant (1).
We examined systemic signaling by two approaches: We analyzed systemic responses to the local perception of Pi in phosphate-starved Arabidopsis, and dissected the requirements for the induction of Pi-starvation responses. To analyze the kinetics of systemic signaling, we focused on responses to perception of phosphate in Pi-starved plants, because starvation-induced gene expression is only a late response to the absence of Pi. Gene expression responses in systemic roots are rapid and down-regulation of starvation-induced gene expression can occur within a few hours, implying the rapid movement of a signal. Surprisingly, Pi is systemically mobilized very rapidly through the root system of starved plants, preceding systemic gene expression responses and raising the possibility that Pi translocation orchestrates systemic responses. Gene expression responses in systemic roots of cytokinin-insensitive plants are not significantly different from the wild-type, suggesting that cytokinin is not the systemic signal. We examined the induction of Pi-starvation responses in different genetic backgrounds and under different environmental conditions. We find that C and N availability, as well as cytokinin perception, strongly affect the magnitude and timing of phosphate starvation responses. This effect is, at least in part, caused by altered partitioning of growth and Pi between plant organs under these conditions. Therefore, previously described cytokinin-dependent suppression of Pi-starvation induced gene expression may the consequence of altered growth control under these conditions.
These studies are beginning to reveal details of a systemic metabolic and signaling network in phosphate homeostasis. Our data suggests that this network is cued by the magnitude of Pi-demand and not only by the generation of a systemic signal as proxy for Pi supply. This suggests that the cognate signaling network includes feed-back regulation.

1. Burleigh, S. H., et al. (1999) Plant Physiology 119, 241-248.

T08-014

Determinants of polar localization of PIN proteins in Arabidopsis

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Auxin mediates multiple aspects of plant growth and development. It is transported in a strictly regulated, polar fashion utilizing influx/efflux carriers-based mechanism. PIN proteins are important regulators of auxin efflux. The Arabidopsis PIN gene family consists of eight members, five of which have been characterized in detail in relation to their expression, localization and role in plant development. PIN proteins are expressed in different tissues and they show specific asymmetric cellular localization, impressively correlating with the known direction of auxin flow. We address question of how the polar localization of PIN proteins is controlled with a particular focus on cell-type-based vs. primary sequence-based determinants. Following ectopic expression, different PIN proteins display different polarities of their localisations in different cell types of the Arabidopsis root tip. This data suggests that the particular localization of the PIN proteins is differently regulated in characteristic cell types, suggesting both sequence- and cell type-specific control of PIN proteins polarity.

T08-015

Non-Genomic Effect Of Auxin On Protein Trafficking

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The plant hormone auxin is one of the major regulators of plant development implicated in variety of processes such as organ initiation, directional growth (tropism), meristem activity and apical dominance. Auxin is distributed throughout the plant by a polar auxin transport system, which requires asymmetrically localized regulators from the PIN family. Using a specific agent for inhibiting recycling of proteins in cells - brefeledine A- we show that many plant plasma membrane proteins exhibit constitutive cycling. In animals signalling molecules such as hormones can regulate protein activity by modulating their constitutive cycling. We present data demonstrating that auxin is able to modulate cycling of PIN proteins and thus regulate their levels at the plasma membrane. This auxin effect is not dependent on protein and mRNA synthesis and does not require known components of auxin signalling pathway. Our data imply a model how auxin, by influencing protein trafficking, can regulate various cellular processes including its own efflux.

T08-016

Novel Feedback Regulations in Efflux-Dependent Auxin Distribution

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More than a century of physiological work as well as recent contributions from molecular genetics identified auxin as a prominent intercellular signal in plants. Auxin distributed over long distances largely contributes to the coordination and integration of growth at the whole plant level. On the other hand, directional, active, cell-to-cell transport over short distances mediates local, differential auxin distributions (gradients), required for various patterning processes, including apical-basal axis formation and organogenesis. Also growth responses to environmental cues such as light or gravity utilize a similar mechanism involving auxin gradients. Differentially expressed auxin transport regulators of the PIN family, each with specific polar, subcellular localization form a network for auxin distribution and formation of these local gradients. The activity of PIN proteins can be regulated at the single cell level by changes in their vesicle trafficking-dependent polar targeting in response to developmental and environmental cues. Thus, this auxin distribution network, whose directional throughput is modulated by both endogenous and exogenous signals, provides, by means of mediating auxin fluxes and creating local gradients, a common mechanism for the plasticity and adaptability of plant development. New insights in the complex regulatory mechanism for controlling expression, polarity and activity of auxin efflux components will be presented.

T08-017

Role Of Protein Phosphorylation In Polar Auxin Transport In Arabidopsis

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The plant hormone auxin plays a crucial role in plant development by regulating basic cellular processes such as cell division, cell elongation and differentiation. Auxin also functions as signal between cells, tissues and organs. Auxin is synthesized in young shoot apexes from where it is polarly transported down towards the root tip. Polar auxin transport (PAT) through plant cells and tissues is mediated by at least two biochemically and structurally distinct classes of specific plasma membrane associated carriers, namely auxin influx carriers and auxin efflux carriers. These are probably encoded by AUX1 and PIN gene families respectively. The direction of auxin transport is supposed to be determined by polar, localization of the efflux carriers within cells. Physiological and genetic evidence indicate that phosphorylation/dephosphorylation processes are involved in the regulation of the activity of the carrier systems. Kinases and phosphatases inhibitors, were shown to inhibit also PAT and influence plant growth and development that depend on it. In Arabidopsis, mutants in protein kinase PINOID (PID) and protein phosphatase RCN1, display developmental defects related to auxin transport. However, the molecular mechanism of these effects has not been identified yet. Novel findings about the regulation of PAT, auxin distribution and pattern formation in various loss and gain-of function phosphorylation mutants will be presented. Based on these results we propose an integrative model, how PID-dependent phosphorylation regulates polar auxin transport and thereby local auxin distribution for plant pattern formation.

T08-018

Genetic dissection of RNA silencing movement in Arabidopsis

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RNA silencing is a conserved eukaryotic pathway mediating sequence-specific inhibition of gene expression through the involvement of 21-24nt RNAs known as short interfering (si)RNAs. These small RNAs are processed from RNA precursors with double-stranded(ds) features, by the action of homologues of the RNase-III Dicer. In plants, (si)RNAs are in two size classes. 24nt-long siRNAs seem to mediate transcriptional silencing of DNA repeats and transposon loci, while 21nt-long siRNAs target cytoplasmic RNA for degradation. The latter process forms the basis of a plant innate antiviral response with the outstanding capacity to move between cells and over long distances. A further sophistication of this defense is that it is amplified: having detected only a few molecules of the viral dsRNA, the plant can mount a large systemic silencing response against the pathogen.
This process can be mimicked in Arabidopsis by phloem-specific expression of transgenes producing long dsRNA. RNA silencing triggered in companion cells moves from cell-to-cell and eventually invades most of the lamina. This extensive movement is dependent upon SDE1 and SDE3, encoding a putative RNA-dependent RNA polymerase and RNA helicase, respectively. Inactivation of both genes results in silencing movement being limited to 10-15 cells.
Our previous analysis suggested that this short distance silencing involved trafficking of 21nt-long, but not 24nt-long, siRNAs. Extensive movement was associated with de novo synthesis, by SDE1 and SDE3, of secondary siRNAs belonging exclusively to the 21nt size class. We proposed that both movements were in fact manifestations of the same process, whereby extensive cell-to-cell spread of RNA silencing resulted from re-iterated short-distance signaling events involving amplification and movement of 21nt, but not 24nt siRNAs.
Here, I will present the results of a genetic screen aimed at identifying Arabidopsis mutants with compromised short distance silencing movement. Some mutants have lost the capacity to produce siRNAs of both size classes. Other mutants still produce siRNAs but are either unable to display the movement phenotype or, on the contrary, exhibit enhanced silencing movement outside the veins. Analysis of those mutants together with our recent identification of a silencing movement-deficient mutant that is specifically unable to produce 24nt siRNAs provides direct support to our model of silencing cell-to-cell spread in Arabidopsis.

T08-019
Long Range Signalling

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Co-ordination of plant physiology, growth and development requires effective communication between cells, tissue and organs. Such long range signalling has been known for more than a century and played an important part in the identification of hormonal signals such as auxin. More recently, the range of signals has expanded, with suggestions that nutrients, proteins and RNAs are all important in long-distance communication within the plant. It seems likely that many new signals will be discovered.

A good example is the control of shoot system architecture. Balancing root and shoot growth, and primary and secondary shoot growth, necessarily requires long-distance signalling between the root and the shoot, and between primary and secondary shoot apical meristems. Classically both shoot-derived auxin and root-derived cytokinin have been implicated in these processes. More recently, additional components of the network of signals that regulate shoot branching have been identified. Mutants at 4 new arabidopsis loci, called MAX1-MAX4, with increased shoot branching appear to define an additional branch-inhibiting pathway, also found in pea and petunia, that has roles both downstream of, and independent of auxin. Grafting studies have demonstrated that three of these loci are involved in the production of a long-range graft transmissible signal that inhibits bud growth, and this is consistent with the molecular identities of these genes. We are working to understand this network of signals that controls shoot branching and our progress will be presented.

T08-020
Functional analysis of the CHoR protein

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CHoR (calcium homeostasis regulator) represents a novel protein identified in a functional expression cloning approach in Xenopus laevis oocytes, designed to screen for proteins involved in ion transport and homeostasis in plant cells. Initially, poly(A+)-RNA isolated from guard cell-enriched young potato leaves was used to generate a cDNA library that was injected into oocytes which were subsequently analyzed by two-electrode voltage clamp measurements. The StCHoR (Solanum tuberosum CHoR) cDNA was then cloned using a cDNA subpooling strategy. The StCHoR protein, when expressed in oocytes, induces the activation of a well-documented Ca2+-dependent chloride channel. Further electrophysiological studies suggested that StCHoR triggers an increase in intracellular Ca2+ concentration either by the release of Ca2+ ions from internal stores or uptake from the external medium. StCHoR has been shown to be tightly associated with cellular membranes by expression in insect cells (Baculovirus system). Homologs of StCHoR only occur in higher plants. We have cloned the CHoR cDNA from Arabidopsis thaliana (AtCHoR). Both CHoR mRNA-levels are decreased in response to drought, osmotic stress and treatment with ABA. The subcellular localization of C-terminal CHoR-GFP fusion proteins was studied in Nicotiana tabacum BY2 and Arabidopsis leaf protoplasts and it could be shown that the CHoR proteins are localized in the plastids. These observations are consistent with the results of tissue northern analysis and promoter-GUS studies on AtCHoR showing that the mRNA respectively promoter activity is mainly detectable in green above-ground organs. At the cellular level AtCHoR promoter activity was confined to photosynthetically active cells. In a yeast-two-hybrid screen AtCHoR showed interaction with chloroplastic proteins associated with photosystem II and the O-2-evolving complex, both of which are known to require calcium for proper structural and functional organization. Preliminary data on transgenic Arabidopsis overexpressing the AtCHoR cDNA show a slight decrease of photosynthetic efficiency in pulse amplitude modulation (PAM) measurements. Our working model that we are currently testing is that the CHoR protein is involved in the regulation of chloroplastic calcium homeostasis. Ongoing studies include the screen for interacting proteins using a biochemical approach, in-vivo measurement of calcium concentration in the chloroplast stroma using the aequorin reporter system, and the analysis of stable and inducible suppression lines.

T09 Genetic Mechanisms
(Transcriptional and Chromatin Regulation)

T09-001

Brca2 is essential to meiosis in Arabidopsis

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Mutations in theBRCA2 gene are major cause of breast cancer susceptibility in human. As observed for RAD51, disruption of the BRCA2 gene causes an accumulation of double-strand breaks and is embryo-lethal in mice. Since the Brca2 and Rad 51 proteins were shown to interact in vivo, they are considered to be implicated in the same pathway of DNA repair in mammals.

Two BRCA2-like sequences are present in the Arabidopsis genome. Both genes are expressed in flower buds and encode nearly identical proteins which contain four BRC motifs. In a yeast two hybrid assay, the Arabidopsis Brca2 proteins interact with Rad51 and Dmc1. These results were confirmed in vitro by co-IP. RNAi constructs aimed at silencing the BRCA2 genes at meiosis triggered a reproducible sterility phenotype which was associated with dramatic meiosis alterations (1). We obtained the same phenotype upon introduction of RNAi constructs aimed at silencing the RAD51 gene at meiosis in dmc1 mutant plants (2). The meiotic figures we observed strongly suggest that homologous recombination is highly disturbed in these meiotic cells, leaving aberrant recombination events to repair the meiotic double strand breaks. The “brca2” meiotic phenotype was eliminated in spo11 mutant plants. Our experiments point to an essential role of Brca2 at meiosis in Arabidopsis. We also propose a role for Rad51 in the dmc1 context. We are now examining other genetic functions that might be required for the “brca2” phenotype.

(1) Siaud N et al (2004) EMBO J inpress
(2) Couteau F et al (1999) Plant Cell 11, 1623-1634

T09-002

Arabidopsis Mutants Enhanced in RNA Silencing

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In spite of extensive effort, previous genetic and molecular studies had identified only five plant genes required for RNA silencing. To identify additional silencing-related genes, we screened M2 populations of Arabidopsis ecotype “Columbia 0” for EMS mutants with enhanced RNA silencing (ESI) of a green fluorescent protein (GFP) reporter gene. Fifteen recessive mutants showing high GFP expression in cotyledons, but no GFP expression in true leaves fell into 5 complementation groups (ESI1-ESI5).
The esi1 and esi2 mutants studied in detail showed some variability in growth rate, but were otherwise normal in appearance. Nuclear run-on transcription experiments and the presence of GFP siRNAs verified that esi1 and esi2 mutants chosen for study exhibit RNA silencing and suggested that both mutants act upstream of siRNA production. We did not detect marked effects of esi1 and esi2 on either susceptibility to virus infection or virus-induced gene silencing (VIGS) as judged from inoculations of mutants, the high-GFP expressing wild-type line, and a silent GFP reporter line with Cucumber mosaic virus (CMV) and with RNA representing the genome of a recombinant Turnip crinkle virus (TCV) with the coat protein ORF replaced by a GFP- phytoene desaturase transcriptional fusion (TCV GFP-PDS DCP). The CMV 2b protein has been shown to suppress RNA silencing and is believed to act in the cell nucleus. Our most interesting finding was that GFP silencing was not suppressed in esi2 mutants infected with CMV. These results, and the fact a GFP reporter gene is constitutively silenced in esi2, lead us to propose that ESI2 is part of host silencing-suppression pathway, which is activated by the CMV 2b protein.
This work was supported in part by the Novartis Research Foundation and by Grant No. BBW 00.0224-2 from the Swiss Office for Education and Science as part of the European Union Gene Silencing in Transgenic Plants Project No. QLK3-2000-00078.

T09-003

Nuclear transcriptional control of chloroplast functions: analysis of 101 nuclear transcriptomes reveals distinct regulons and their relationship to metabolism and chromosomal gene distribut

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Post-endosymbiotic evolution of the protochloroplast was characterized by gene transfer to the nucleus. Hence, most chloroplast proteins are nuclear-encoded and the regulation of chloroplast functions includes nuclear transcriptional control. The expression profile of 3292 nuclear Arabidopsis genes, most of them encoding chloroplast proteins, was determined from 101 different conditions. The 1590 most-regulated genes fell into distinct groups of co-regulated genes (regulons). Genes of some regulons are not evenly distributed among the five Arabidopsis chromosomes. Most regulons are heterogeneous and consist of genes coding for proteins with different subcellular locations or contributing to several biochemical functions, implying that different organelles and/or metabolic pathways are coordinated at the nuclear transcriptional level. The co-expression of nuclear genes coding for subunits of the photosystems or for plastid ribosome polypeptides implies that ribosome abundance involves nuclear control. Co-regulation of genes for photosystem and plastid ribosome proteins escapes a previously described general control of nuclear chloroplast proteins imposed by a transcriptional master switch. This is taken as evidence for the existence of additional mechanisms controlling the transcription of nuclear chloroplast genes. From the evolutionary standpoint, the results provided indicate that domestication of the protochloroplast and its functional integration in the eukaryotic cell was associated with the establishment of different hierarchies of nuclear transcriptional control.

T09-004

Effects of mutations causing reduced DNA methylation on interhomologue chromosome association in Arabidopsis thaliana.

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Each individual chromosome occupies a discrete space (a so-called “territory”) in the interphase nucleus, and radial positioning of a chromosome in the nucleus from interior to periphery may have an intimate correlation with the size and gene density for each chromosome. Chromatin organization is likely maintained by multiple types of interactions between chromatin fibers and their protein modifiers. Currently, the question of whether nucleosome modifications are involved in the global organization of chromosomes within the nucleus is being investigated in different model systems. We have recently reported the possible association between homologous chromosome loci in living guard cells by using the GFP-tagging system (Kato and Lam, 13th Arabidopsis meeting). We report here the influence of DNA methylation on the association between transgene loci by using two mutants (ddm1 and ddm2) with reduced DNA methylation. The GFP-tagging system contains concatenated Lac operator arrays, and these arrays are largely methylated in wild type Arabidopsis plants. DNA methylation on the transgene loci was reduced in either the ddm1 or ddm2 background, and the number of GFP spots detected by expression of nuclear targeted GFP-LacI protein in living guard cells was compared between widl-type and mutant backgrounds. The different results between the ddm1 and ddm2 mutants and the possible role of these gene products in organization of chromosome in a nucleus will be presented and discussed.

T09-005

FUNCTIONAL CHARACTERIZATION OF ARABIDOPSIS AtSWI3 GENES ENCODING HOMOLOGS OF A CORE SUBUNIT OF YEAST SWI/SNF CHROMATIN REMODELING COMPLEX

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ATP-dependent chromatin remodeling complexes in yeast, Drosophila and humans play essential roles in transcriptional regulation and share similar core ATPase subunits that are represented by the SWI2/SNF2, ISWI and Mi-2 ATPase families. In yeast, the SWI2/SNF2 ATPase is found in association with SNF5 and two copies of SWI3 proteins in the minimal functional core of SWI/SNF complex that can remodel chromatin in vitro. Although bioinformatics analyses of sequenced Arabidopsis and rice genomes predict the existence of several potential homologues of core subunits of SWI/SNF complexes, thus far little information is available about the functions of plant chromatin remodeling complexes. Here we report on the characterization of Arabidopsis genes that encode homologues of SWI3, representing potential core components of canonical SWI/SNF complexes. The Arabidopsis SWI3 gene family consists of four members: AtSWI3A, AtSWI3B, AtSWI3C and AtSWI3D. Our data indicate that AtSWI3B is a functional homologue of yeast SWI3 and, together with AtSWI3A, is probably involved in the control of flowering through its interaction with the RNA-binding FCA protein. Members of the Arabidopsis SWI3 family show homo- and heterodimerisation in vitro and in the yeast two-hybrid system. In addition, AtSWI3A and AtSWI3B interact with the BSH protein, an Arabidopsis homologue of yeast SNF5, that represents another conserved subunit of plant SWI/SNF complexes. Characterization of T-DNA knockout mutations demonstrates that both AtSWI3A and AtSWI3B genes are essential for viability and early embryonic development. By contrast, plants carrying T-DNA insertions in the AtSWI3C and AtSWI3D genes are viable, but show dwarfism and characteristic developmental defects in their flowers and leaves. These results suggest that diverse members of the AtSWI3 gene family are implicated in distinct regulatory pathways controlling plant development.

T09-006

Genetic dissection of early meiotic prophase events in maize and Arabidopsis.

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We are combining cytological, genetic, and molecular approaches to identify regulatory networks involved in the initiation and progression of early events in meiosis. Meiosis is initiated by the switch from the somatic to the meiotic cell cycle, which in maize is controlled by the ameiotic1 (am1) gene. am1 regulates the meiotic chromosome structure and condensation, as well as installation of the meiotic recombination machinery on chromosomes. In most mutant alleles of am1, meiocytes undergo mitosis instead of meiosis or arrest in pre-division interphase. The am1 gene encodes a protein with several conserved domains of unknown function and two predicted coiled-coil domains, frequently associated with protein-protein interactions. AM1 has also a potential binding site for PCNA, a DNA “sliding clamp” protein involved in the progression through the S phase and known to interact with cyclins and chromatin remodeling proteins. We are now testing a hypothesis that AM1 interacts directly with PCNA and in this way changes the identity of the S phase from mitotic to meiotic.

We are also investigating another key meiotic protein in maize, encoded by the poor homologous synapsis1 (phs1) gene. phs1 is required for proper pairing of homologous chromosomes during the meiotic prophase. In phs1 complete loss of function mutants, homologous chromosome pairing is nearly completely replaced by synapsis between non-homologous chromosomes. The mutants are also defective in meiotic recombination. Our data suggest that phs1 plays a role in ensuring that pairing occurs exclusively between homologous chromosomes, and it is involved in a multistep process to coordinate meiotic chromosome pairing and recombination. The phs1 gene encodes a novel meiosis-specific protein with several short conserved domains but with a low overall level of evolutionary conservation. We have identified an Arabidopsis homolog of phs1, and are currently testing if it fulfills the same function as the phs1 gene in maize despite the relatively low evolutionary conservation.

T09-007

Two BRCA2-like genes are needed for homologous recombination repair in Arabidopsis

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The BRCA2 gene was identified as a breast and ovarian cancer susceptibility gene and has been implicated in the response to DNA damage. The evidence for a role in DNA repair came from the observation that BRCA2 binds directly with RAD51, the bacterial RecA homolog, which is required for homologous pairing and DNA strand exchange during homologous recombination repair. Furthermore, BRCA2 null mouse embryos that do not survive past day 8 of embryogenesis are hypersensitive to gamma-irradiation.
Two BRCA2 genes, AtBRCA2L1 and AtBRCA2L2, are present in Arabidopsis genome (Siaud et al. 2004). Both AtBRCA2L1 and AtBRCA2L2 have four BRC motifs that interact with Rad51. Yeast two-hybrid assay shows AtBRCA2L1 and AtBRCA2L2 interacts with AtRad51, suggesting that both AtBRCA2L1 and AtBRCA2L2 are involved in homologous recombination repair. The biological roles of AtBRCA2L1 and AtBRCA2L2 proteins in Arabidopsis were ascertained by obtaining homozygous mutant plants containing the AtBRCA2L1 or AtBRCA2L2 genomic sequences interrupted by a Ds insertion. Both mutant plants showed increased sensitivity to gamma-ray radiation, suggesting that AtBRCA2L1 and AtBRCAL2 have a role in double strand break repair.
We have crossed AtBRCA2L1 and AtBRCA2L2 mutants to produce double mutants and observed developmental abnormality in double mutant plants.

T09-008

An inversion of dominance between epialleles in polyploid Arabidopsis

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Polyploidy, the presence of more than 2 complete chromosome sets, is frequent among higher plants, especially in plants cultured for human use. The success of polyploids may lie in increased genetic redundancy supporting subsequent diversification. Although doubling the genome does not generate diversity per se, polyploid formation is associated with rapid genomic rearrangements, changes in DNA modification and altered gene expression patterns 1.
Several independent tetraploid Arabidopsis thaliana lines, derived from the same diploid strain uniformly expressing a transgenic locus over many generations, showed transcriptional silencing of the transgene. These silent states were stably inherited to tetraploid progeny and diploid derivatives. The loss of expression after polyploidization appears to be due to epigenetic modification accompanied by hypermethylation of the silent loci.
Surprisingly, the transcriptionally inactive epialleles reduce the expression of an active allele if combined in the same tetraploid (but not in a diploid) genome 2. The active allele therefore loses its dominance (with different degrees of penetrance) and becomes hypermethylated. Moreover, genetic segregation data indicate that the suppressive effect is lasting even after genetic separation from the silencing allele, thus resembling paramutation. Therefore, epigenetic states can be inherited even if the chromosome carrying this information is not transmitted itself. This leads to functional epigenetic homozygotization of alleles and, thus, to conversion of new recessive alleles into traits expressed in early polyploid generations. Such interactions might contribute to rapid adaptation and evolution of polyploid plants.

1 Osborn et al. (2003) Trends Genet 19:141-147.
2 Mittelsten Scheid et al. (2003) Nat Genet 34: 450-454

T09-009

Exploring early signaling pathways in phytochrome B-regulated seedling de-etiolation

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Phytochrome B (phyB) is a member of the Arabidopsis phytochrome family (phyA-E) of red and far-red light absorbing photoreceptors. While phyA is the primary phytochrome functioning in far-red light, phyB dominates photosensitive responses in red light, particularly the inhibition of hypocotyl cell elongation (Tepperman, et. al., 2004). The biologically active form (red light activated, Pfr conformer) of phyB is localized into the nucleus and is thought to interact with PHYTOCHROME INTERACTING FACTOR (PIF)3 and PIF4. PIF3 and PIF4 belong to the AtbHLH (Arabidopsis thaliana basic helix-loop-helix) superfamily of transcriptional regulators. By sequence similarity studies, followed by biochemical analysis, we have identified two new phytochrome interacting factors, PIF5 and PIF6. Both bind specifically to the biologically active form of phyB. We are further analyzing the protein-protein interaction motifs responsible for the specific interactions of the PIF proteins with the biologically active form of phyB. Using reverse genetics, we have identified pif5 and pif6 mutant lines. The pif5-mutant seedlings display a hypersensitive morphological phenotype in red light, and the PIF5 (OX) transgenics exhibit a hyposensitive phenotype. At present, we are characterizing the pif6 mutants for phenotypes. It is likely that the different PIF proteins modulate phyB-regulated pathways with spatial, temporal and developmental specificities. Functional analysis of the interactions between the PIF proteins and phyB (Pfr) will provide insights into the early signaling mechanisms in phyB-mediated responses.

Reference
Tepperman J.M. Hudson, M.E., Khanna R., Zhu T., Chang S.H., Wang, X. and Quail, P.H. (2004) Expression profiling of phyB mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. Plant J. in press

T09-010

Functional identification of microRNA targets in Arabidopsis

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The molecular mechanism of miRNA action requires complementary base-pairing between miRNA molecules and target mRNAs. In animals, where the primary mode of miRNA action is translational repression, there is only modest complementarity between miRNAs and their targets. In plants, it appears that most miRNAs can trigger cleavage of target mRNAs similar to perfectly complementary siRNAs (short interfering RNAs). For this reason, computational identification of miRNA targets has focused on a high degree of complementarity to the miRNA (0-3 mismatches). Many predicted targets have been verified in vitro, some also in vivo, mostly by showing that the targets are indeed cleaved. However, there are also experimentally verified examples of targets with more than 4 mismatches, which can still be cleaved in a siRNA-related manner.

We have been asking for common rules of functional miRNA target sequences in Arabidopsis thaliana. For that purpose we created several transgenic lines overexpressing miRNAs from different families. Genome-wide expression changes have been analyzed in the overexpression lines. Inferences from these experiments will be presented.

This study is supported by the Max-Planck-Society

T09-011

TT2, TT8, and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in Arabidopsis thaliana.

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Genetic analyses have demonstrated that together with TTG1, a WDR protein, TT2 (MYB), and TT8 (bHLH) are necessary for the correct expression of BANYULS (BAN). This gene codes for the core enzyme of proanthocyanidin biosynthesis in Arabidopsis thaliana seed coat. The interplays of TT2, TT8, and their closest MYB/bHLH relatives, with TTG1 and the BAN promoter have been investigated using a combination of genetic and molecular approaches, both in yeast and in planta. Results obtained using Glucocorticoid Receptor (GR) fusion proteins in planta strongly suggested that TT2, TT8, and TTG1 can directly activate BAN expression. Experiments using yeast two- and three-hybrid clearly demonstrated that TT2, TT8, and TTG1 can form a stable ternary complex. Furthermore, although TT2 and TT8 were able to bind to the BAN promoter when simultaneously expressed in yeast, the activity of the complex was correlated to the level of TTG1 expression in A. thaliana protoplasts. In addition, transient expression experiments revealed that TTG1 acts mainly through the bHLH partner (i.e. TT8 or related proteins) and that TT2 can not be replaced by any other related A. thaliana MYB proteins to activate BAN. Last and consistently with these results, ectopic expression of TT2 was sufficient to trigger BAN activation in vegetative parts, but only where TTG1 is expressed. Taken together, these results indicated that TT2, TT8, and TTG1 can form a ternary complex directly regulating BAN expression in planta.

T09-012

Identification and characterisation of Aurora-like kinases in Arabidopsis thaliana.

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Aurora-like kinases have been shown to play a key roles in regulating chromosome segregation, condensation and cytokinesis, yet, similar kinases has not been isolated in plants. Here we identified three A. thaliana protein kinases, designated At-Aurora1, At-Aurora1 and At-Aurora3 which share high amino acid identities with the serine/threonine kinase domain of yeast Ip11, fly and mammalian aurora. A strong expression of all Aurora-like kinases was detected in mitotically and meiotically active tissues of Arabidopsis. At-Aurora1 displays the dynamic properties of a 'chromosomal passenger' protein. At metaphase, a notable feature is that the At-Aurora1-signals are restricted to the centromeres of chromosomes where the cell-cycle dependent phosphorylation of histone H3 occurs. In cytokinesis, immunostaining revealed a specific At-Aurora1-labelling along the developing cell plate. A preferential phosphorylation of histone H3 was demonstrated by an in vitro kinase assay using baculovirus-recombinantly produced At-Aurora1. Separate inactivation of At-Aurora1 (T-DNA „knock out") and At-Aurora2 (RNAi-approach) does not effect H3 phosphorylation and the mitotic segregation behaviour of chromosomes. The missing phenotype of At-Aurora1/2 "knock out" plants suggests a mutual compensation of the At-Aurora kinases.

T09-013

Developmental silencing and gene knockout analysis of the Ser/Arg-rich splicing factor SR45

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SR45 is a plant-specific splicing factor protein originally identified by yeast two-hybrid analysis to binding to U1-70K, a U1 snRNP protein, and SR33, a plant specific Ser/Arg-rich protein related to human SC35 (Golovkin and Reddy, 1999). This interaction of SR45 with U1-70K, which is situated at the 5' splice site of introns during the early stages of intron splicing, suggested that this protein functions in the process of plant intron recognition. The structure of SR45, which is unique in that it contains two SR domains separated by a single RBD where the SR domains are used in protein-protein interactions and the RBD domains bind RNA. Overexpression analysis of the SR45 protein attempted by crossing transgenic parental mGFP-SR45 lines to transgenic wtGFP lines resulted in developmental silencing of the mGFP-SR45 transgene beginning at 4 weeks and progressing as plants mature to 5-9 weeks. Genomic DNA methylation analysis and GFP fluorescence analysis are consistent with silencing being mediated at a non-heritable post-transcriptional level. Analysis of the effects that this silencing has on other alternatively and constitutively spliced transcripts indicates that mGFP-SR45 transcript depletion generates different types of downstream effects. Analysis of a T-DNA knockout line (SALK_063528) carrying an insertion immediately upstream from the transcription start site of the SR45 gene depletes endogenous SR45 transcripts and generates the same classes of downstream effects as seen in SR45 silenced plants. We are currently analyzing the range of transcripts affected by this depletion.

Golovkin, M. and Reddy, A.S.N. (1999) J. Biol. Chem. 274, 36428-36438.

T09-014

Palindromic ACGT-core motifs, the designated bZIP transcription factor binding sites, gather near putative transcriptional initiation sites in front of the ATG

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Basic region/leucine zipper (bZIP) transcription factors have a basic region that binds DNA and a leucine zipper dimerization domain. In Arabidopsis about 75 putative genes containing a bZIP signature are known, which could be clustered into ten groups by structural and functional information.
Their function appears to be diverse and their involvement in pathways such as stress, pathogen, light, hormone, senescence and development is understood best for some of the members.
Despite their differences on structure/functional level, they all were found to bind to variants of the conserved palindromic ACGT-core motif. For proper binding it is assumed that homo- or heterodimerisation of bZIP proteins is essential.
Most of the characterized binding motifs represent short hexanucleotide sequences known as A (TacgtA)-, T (AacgtT)-, C (GacgtC)- or G (CacgtG)-boxes. The ability of bZIP proteins to bind to one or the other element is varying and it is not well understood how they can trigger between different signalling pathways.
Here we examined the occurrence of these boxes in 25538 promoters of Arabidopsis with the help of the MotifMapper Package (www.motifmapper.de). We found preferred locations for the presence of certain elements. Furthermore, we provide evidence for the different hexanucleotide boxes functioning in different signalling pathways.

T09-015

Expression and Functional Connections of Arabidopsis Two-Component Signaling Elements

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Two-component systems have emerged as important sensing / response mechanisms in higher plants. In Arabidopsis thaliana these systems are composed of hybrid histidine kinases (AHKs, 8 genes), histidine-containing phosphotransfer domain proteins (AHPs, 6 genes) and response regulators (ARRs, 12 type A and 12 type B genes) which are biochemically linked by His-to-Asp phosphorelay. AHKs perceive signals in the environment and the signal is subsequently transduced through AHPs to ARRs that mediate the cellular responses. In plants two-component systems play a major role in cytokinin perception and signaling and contribute to ethylene signal transduction and osmosensing. Furthermore, developmental processes like megagametogenesis in A. thaliana involve two-component elements. Because of the molecular mode of signaling plant two-component systems also appear to serve as intensive cross talk and signal integration machinery. However, less is known about their detailed molecular composition in plant tissues. We introduced TaqMan probes based quantitative PCR for accurate monitoring of two-component genes at the transcription level to establish one of the cornerstones for elucidation of two-component signaling network.

T09-016

Towards a complete transcript map in Arabiodopsis thaliana mitochondria

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Even in an autotrophic organism such as Arabidopsis thaliana, working mitochondria are a prerequisite for normal growth and development. Mitochondria contain their own genome and an appropriate apparatus for its expression. RNA, the first product in gene expression, is processed in several ways before being fully functional. Besides various other modifications such as splicing and RNA editing, new transcript ends are created by exo- or endonucleolytic cleavage after transcription.

Mapping of primary ends allows the identification of respective cis-elements like promoters or trancription termination sites, whereas analysis of secondary termini permits the description of sequence motives responsible for site-specific nucleolytic cleavage. An assembly of these different cis-elements will provide a database for the future search for trans-factors like transcription factors or processing enzymes.

We initiated a survey of such cis-elements by identifying primary and secondary RNA termini of all mitochondrial transcripts. We perform this analysis mainly by CR-RT-PCR (circularized RNA reverse transcription polymerase chain reaction). This methods allows the simultaneous mapping of the 5' and 3' ends of a given transcript to the nucleotide scale on the complete genomic DNA sequence. The first step in this procedure is circularization of the RNA molecules via ligation by T4 RNA polymerase. Then a cDNA first strand is synthesized from a primer in the 5' region of the open reading frame. The primer points outwards towards the former transcript end, as does its partner primer for the subsequent PCR reaction. This second primer anneals in the 3' part of the reading frame. Usually a second PCR with nested primers is necessary to remove unspecific PCR products. After PCR the products are cloned into an A/T-vector and the exact RNA 5' and 3' termini are determined by sequencing.

To date, we have investigated the major transcript ends of most of the genes of the inner mitochondrial strand. In general, we observe unique 3' ends, whereas we often detect multiple 5' ends in the genes analyzed. The majority of these 5' and 3' termini is most likely derived from processing events.

T09-017

Interaction between pRb and FIE polycomb protein, point at a possible mechanism regulating endosperm development

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Inactivation of the Arabidopsis FERTILIZATION INDEPENDEDENT ENDOSPERM (FIE) protein induces division of the central cell of the embryo sac, leading to endosperm development in absence of fertilization. The mechanism whereby FIE regulates this process is largely unknown. Since FIE protein encode for an LxCxE motif at the its C-terminal, we postulated that activation of central cell division in fie plants may result from improper interaction with the cell cycle regulatory element, the retinoblastoma protein (pRb) which can bind to different proteins, among other, via the LxCxE motif. Pull-down and surface plasmon resonance (Biacore) assays demonstrated that FIE interacts in-vitro with the Arabidopsis (AtRb), maize (ZmRb) and human Rb (HuRb). The interaction of FIE with ZmRB and HuRb in the yeast two-hybrid system, are in agreement with previous results describing interactions between pRb and different members of the polycomb protein family in mammals and plants. Moreover these results support the possibility that FIE-pRb interaction may occur also in planta. Mutational analysis, show that FIE-RB interaction does not occur via the LxCxE motif of the FIE protein nor via the pocket B domain of pRb. These results suggest that FIE may restrain embryo sac central cell division, at least partly, through interaction with pRb, and suppression of pRb-regulated genes.

T09-018

A ROS Repressor-Mediated Binary Regulation System for Control of Gene Expression in Transgenic Plants

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We describe a novel binary system to control transgene expression in plants. The system is based on the prokaryotic repressor, ROS, from Agrobacterium tumefaciens, optimized for plant codon usage and for nuclear targeting (synROS). The ROS protein bound in vitro to double stranded DNA comprising the ROS operator sequence, as well as to single stranded ROS operator DNA sequences, in an orientation-independent manner. A synROS-GUS fusion protein was localized to the nucleus, whereas wtROS-GUS fusion remained in the cytoplasm. The ability of synROS to repress transgene expression was validated in transgenic Arabidopsis thailana and Brassica napus. When expressed constitutively under the actin2 promoter, synROS repressed the expression of the reporter gene GUS linked to a modified CaMV35S promoter containing ROS operator sequences in the vicinity of the TATA box and downstream of the transcription initiation signal. Repression ranged from 32% to 87% in A. thaliana, and from 23% to 76% in B. napus. These results are discussed in relation to the potential application of synROS in controlling the expression of transgenes and endogenous genes in plants and other organisms.

Schäfer et al., (2004) Transgenic Res. 13: 109-118

T09-019

SCL14 ACTIVATES ACTIVATION SEQUENCE-1 (AS-1) THROUGH INTERACTION WITH TGA FACTORS

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Activation sequence-1 (as-1) is a salicylic acid responsive cis element found in the promoter of glutathione S-transferase genes and pathogenesis related gene 1. As-1, which is also activated by several stress conditions like reactive oxygen species and increased levels of auxin, is bound by a family of bZIP transcription factors, called TGA factors. When searching for proteins interacting with TGA2 in a modified yeast two hybrid system, a clone coding for scarecrow-like protein 14 (SCL14) was isolated. The interaction was confirmed in vitro using pull down assays. Transgenic plants ectopically expressing SCL14 show enhanced transcriptional activation from the truncated CaMV 35S promoter that encodes the as-1 element as the only cis regulatory element. In protoplasts, SCL14 is localized both in the cytosol and the nucleus. Addition of nuclear export inhibitor LMB led to the exclusive localization of the protein in the nucleus, indicating that SCL14 can be transported out of the nucleus via the CRM1 pathway. SCL14 overexpressing Arabidopsis lines and knock out lines with reduced SCL14 transcript levels have been identified and will be analysed with regard to stress-responsive gene expression from promoters encoding binding motifs for TGA factors.

Pysh, L.D. et al. 1999. Plant J. 18: 111¯119. Krawczyk, S. et al. 2002. Nucleic Acids Res. 30: 775-781.

T09-020

The Rad17 homologue of Arabidopsis is involved in the regulation of DNA damage repair and homologous recombination

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Rad17 is involved in DNA checkpoint control in yeast and human cells. A homologue of this gene as well as other genes of the pathway (the 9-1-1 complex) are present in Arabidopsis and share conserved sequence domains with their yeast and human counterparts. DNA damaging agents induce AtRAD17 transcriptionally. AtRAD17 mutants show increased sensitivity to the DNA damaging chemicals bleomycin and MMC, which can be reversed by complementation, suggesting that the loss of function of Rad17 disturbs DNA repair in plant cells. Our results are further confirmed by the phenotype of a mutant of the 9-1-1 complex (Rad9), which is also sensitive to the same chemicals. AtRAD9 and AtRAD17 seem to be epistatic as the double mutant is not more sensitive to the chemicals than the single mutants. The mutants show a delay in the general repair of double-strand breaks (DSBs). However, frequencies of intrachromosomal homologous recombination (HR) are enhanced. Nevertheless, the mutants are proficient for a further induction of HR by genotoxic stresses. Our results indicate that a mutant Rad17 pathway is associated with a general deregulation of DNA repair, which seems to be correlated with a deficiency in non-homologous DSB repair.

T09-021

Arabidopsis AtMut11, related to a subunit of trithorax-like complexes, is required for gene silencing and heterochromatin maintenance

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Post-transcriptional histone modifications can affect chromatin states by directly modulating nucleosome structure and/or by creating binding surfaces for chromatin structural/modifying factors. Histone lysine residues, particularly in the N-terminal tails, are frequently acetylated or methylated. In most eukaryotes, dimethylation of histone H3 lysine 9 (H3K9) has been linked to repressive chromatin, whereas di-/trimethylation of histone H3 lysine 4 (H3K4) has been correlated with transcriptionally permissive/active chromatin. Complexes carrying out the latter modification contain trithorax-like methyltransferases and a conserved WD40-repeat protein, whose Arabidopsis homolog is encoded by AtMut11. We have found that disruption of AtMut11 by a T-DNA insertion results in embryo lethality, whereas AtMut11-suppressed RNAi lines show conspicuous developmental abnormalities and deficiency in H3K4 monomethylation. Interestingly, these strains also display gene silencing defects, significant reduction in H3K9 dimethylation and relaxation of heterochromatic chromocenters, without detectable changes in CpG DNA methylation. Our results indicate that AtMut11, unlike its mammalian homolog which participates in transcriptional activation, is required for the maintenance of silent chromatin. These findings also suggest that AtMut11 functions in association with multiple histone methyltransferase activities and concurrent alterations in several epigenetic marks may be required to disrupt heterochromatic structure.

T09-022

Molecular basis of vernalization requirement and response

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The timing of the floral transition has significant consequences for the reproductive success of plants. Plants need to gauge when both environmental and endogenous cues are optimal before undergoing the switch to reproductive development. To achieve this a complex regulatory network has evolved consisting of multiple pathways that quantitatively regulate a set of genes (the floral pathway integrators) whose activity causes the transition of the meristem to reproductive development. The Dean group has focused on the acceleration of flowering by a long period of cold temperature or 'winter', a process known as vernalization. Multiple genetic pathways regulate whether plants require and can respond to vernalization.
Vernalization has been shown to epigenetically regulate RNA levels of a floral repressor, FLC. Prolonged cold results in a reduction in FLC RNA levels, which then remain constant at that lower level when plants are moved back to warm temperatures or when cuttings are made from the mother plant. Identification of vrn mutants, defective in vernalization response, has defined VRN genes required to cause the initial down-regulation of FLC and maintain the cellular memory of vernalization. The Dean lab is focused on understanding how these mediate vernalization.

Vernalization antagonizes the function of FRIGIDA, which up-regulates FLC. These combined activities thus prevent flowering until winter has passed. In contrast, vernalization works in parallel to genes such as FCA and FY to repress FLC expression. FCA encodes an RNA-binding protein and interacts with FY, a polyadenylation factor. We are also addressing how these pathways interact to regulate FLC expression at different stages in the plant life-cycle and how their predominance has altered in natural Arabidopsis variants adapted to very different growth conditions

T09-023

Role of E2F transcription factor in the control of Arabidopsis cell growth and differentiation

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The balance between cell proliferation and differentiation is crucial in multicellular organisms, where it is regulated by complex gene expression networks. This is particularly relevant in plants since organogenesis is a continuous post-embryonic process. Transcription factors E2F/DP are a complex multigenic family, crucial for controlling expression of genes involved in cell cycle transitions, both in animals and plants.
In Arabidopsis, an in silico search of genes potentially regulated by E2F, revealed that this transcription factor may be implicated not only in direct control of cell cycle-related gene expression, but also in the regulation of other functional process, e.g. transcription, stress and defence or signalling. The analysis of Arabidopsis transgenic plants expressing a DP gene containing a truncated DNA binding domain, which likely has a dominant-negative effect on AtE2Fa, b and c, three typical E2F members which require DP for efficient DNA binding, show alteration in the expression levels of some of the identified targets.
In addition, a novel subfamily of E2F transcription factors has been identified, showing an atypical functional domain distribution. We have investigated the function of Arabidopsis E2Ff, an atypical member of the E2F family, which acts independently of a dimerization partner (DP). The study of its promoter activity indicates an interesting tissue-specific expression pattern. Transgenic plants with altered E2Ff expression levels show morphologic alterations in hypocotyl and root development with a deficient cellular expansion and tissue-specific deregulation of genes involved in several processes. Altogether, our results strongly support that the E2F pathway plays a crucial role in regulating both cell cycle-dependent and independent pathways.

T09-024

Sub-nuclear localization of chromatin remodeling factor DDM1

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Decrease in DNA methylation 1 (DDM1) protein plays a crucial role in the maintenance of DNA methylation status in Arabidopsis thaliana. Depletion of DDM1 function also results in the aberrant pattern of histone H3 methylation at the Lysine 9. DDM1 is a member of the broad SWI2/SNF2 protein family. We have recently demonstrated that recombinant DDM1 is an ATP-dependent chromatin remodeling enzyme. Here we present results of the study on sub-nuclear localization of DDM1. This study demonstrates DDM1 enrichment in pericentromeric heterochromatin. We also show the analysis of the effect of various mutations on DDM1 localization.

T09-025

In vivo investigation of the transcription, processing, endonucleolytic activity and functional relevance of the spatial distribution of a plant miRNA

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In eukaryotes, miRNAs are processed from predicted intergenic stem-loop precursors and mediate RNA silencing through mRNA degradation or translational inhibition. In plants, the targets for most identified miRNAs have been described; they belong mainly to transcription factor families with key developmental roles. By contrast, little is known about the transcription, processing, activity and functional distribution of plant miRNAs. We show here that the Arabidopsis miR171 gene has a modular structure and is an independent transcription unit, with the predicted precursor being sufficient for miRNA processing and sequences upstream of the stem-loop containing highly tissue-specific promoter elements. miR171 processing was based on structural rather than sequence information. Its function in vivo was compromised by mutations affecting target pairing at the centre and the 5', but not the 3' region of the miRNA sequence. In planta analysis of miR171 activity by sensor transgene constructs required prior inactivation of SDE1, which encodes an RNA-dependent RNA polymerase, indicating that miRNAs can direct transitive RNA silencing of foreign RNAs. Compared analysis in the sde1 background revealed a near-perfect spatial overlap between the patterns of miR171 transcription and activity, strongly supporting the idea that plant miRNAs enable cell differentiation and tissue identity.

T09-026

Genomic imprinting of the FWA gene in Arabidopsis endosperm

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The FWA gene encodes a homeodomain-containing putative transcription factor, which was initially identified from late flowering epigenetic mutants. The mutations cause ectopic FWA expression associated with heritable hypomethylation of repeats around transcription starting site (1). To understand this epigenetic regulation, we investigated the expression and methylation status of wild type FWA allele (2). Interestingly, wild type FWA is specifically expressed in endosperm, and the expression is associated with demethylation of the 5Åf repeats of the gene. Silencing and hypermethylation of the 5Åf repeats are maintained in embryo and other tissues. Furthermore, the FWA gene displays imprinted (maternal-origin-specific) expression in endosperm. The FWA imprint depends on the maintenance DNA methyltransferase MET1, as is the case in mammals. Unlike mammals, however, the FWA imprint is not established by allele-specific de novo methylation. It is established by female gametophyte-specific gene activation, which depends on a DNA glycosylase gene DEMETER . Since endosperm does not contribute to the next generation, the activated FWA gene need not be silenced again. The mechanism of genomic imprinting of the FWA gene and those in mammals will be discussed.

1. W. J. Soppe, et al. (2000) Mol Cell 6, 791-802.
2. T. Kinoshita, et al. (2004) Science 303, 521-3. Epub 2003 Nov

T09-027

Histone methylation and heterochromatin assembly in Arabidopsis thaliana

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The N-terminal residues of nucleosomal core histones are subjected to a variety of post-translational modifications such as acetylation, phosphorylation, methylation and ubiquitination. The acetylation and in particular the methylation of lysine residues of histones H3 and H4 concerted with the cytosine methylation of DNA seem to play a crucial role in heterochromatin formation in a variety of organisms. Each methylatable histone residue can be either mono-, di- or trimethylated (Paik and Kim 1971). Using specific antibodies against the three different methylation states of histone H3 at lysine K9 and K27 we performed immunostaining experiments on interphase nuclei of Arabidopsis thaliana to analyse the distribution of these isoforms between heterochromatic and euchromatic subdomains. The heterochromatin of A. thaliana, mainly composed of tandem and dispersed repeats, is located around centromeres (and NORs) and identifiable as distinct densely stained chromocenters in interphase nuclei (Fransz et al. 2002). Immunostaining revealed mono- and dimethylated H3-K9 predominantly at chromocenters (Jackson et al. 2004). The occurrence of trimethylated H3-K9 in plants is still a matter of discussion. For H3-K27 methylation all three methylation states were found dispersed over the interphase chromatin, but the mono- and dimethylated H3-K27 were enriched at the chromocenters. Together with previous findings, these data suggest that wildtype heterochromatin in Arabidopsis is characterized by high levels of DNA methylation, mono- and dimethylated H3-K9 and H3-K27 and low levels of histone H3 and H4 acetylation (except for H3K18 and H4K16 which show an increased acetylation at chromocenters during and after replication) and dimethylated H3-K4.
The transcriptionally permissive euchromatin in contrast shows high levels of acetylation of histones H3 and H4, dimethylation of H3-K4 and moderate methylation of H3-K27 (mono-, di- and tri-).
In order to identify genes responsible for the distinct types of histone methylation and to characterize the interdependence of the different chromatin modifications putative chromatin mutants of Arabidopsis are being tested. Similarities and differences as to heterochromatin assembly between Arabidopsis and other organisms are discussed.

Jackson et al. (2004) Chromosoma 112:308-15
Fransz et al. (2002) PNAS 99:14584-9
Paik and Kim (1971) Science 174:114-9

T09-028

Contribution of target transgene position and structure to RNA-directed promoter methylation and TGS

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RNA-directed transcriptional gene silencing (TGS), the specific repression of transcription of a gene in correlation with extensive promoter DNA methylation in the presence of double stranded (ds) RNA with homology to the respective promoter, is an epigenetic mechanism so far only known from plants. For its study, transgene systems employing the nopaline synthase promoter (NOSpro), a moderately strong constitutive promoter widely used in T-DNA constructs, have proven very useful [1]. In tobacco and Arabidopsis thaliana, transcription of dsRNA from a silencer transgene with a NOSpro inverted repeat (IR) can trigger TGS of unlinked homologous target promoters in trans. Similar to other silencing mechanisms involving dsRNA, the NOSpro dsRNA is processed to short interfering RNAs predominantly 21-24 nucleotides in length. Both, TGS and DNA methylation at the target NOSpro are dependent on the NOSpro dsRNA, upon its removal, NOSpro-driven reporter-gene expression is reactivated and the NOSpro DNA methylation is lost. But reactivation and release from DNA methylation are not immediate, indicating that there is some level of maintenance of the silenced state in the absence of the inducing RNA signal. Testing Arabidopsis thaliana mutants known to affect TGS in other systems identified the DNA methyltransferases DRM1/DRM2 and MET1 as well as the putative SWI2/SNF2 chromatin remodelling factor DDM1 as essential for RNA-directed transcriptional gene silencing. New genetic screens yielded additional mutations, of which one was mapped to histone deacetylase HDA6, a candidate enzyme for histone modification. Interestingly, not all target transgenes containing NOSpro-driven reporter genes show the same susceptibility to RNA-directed TGS, indicating that the chromosomal location of target transgenes and / or the particular arrangement of NOSpro copies in the target transgenes might contribute to the silencing process. To approach this problem in a systematic way, collections of well characterized transgenes with the same structure integrated at different chromosomal positions [2] or of transgenes with differing structures integrated at the same chromosomal positions are being challenged by a silencer transgene providing NOSpro dsRNA. Initial results of the analysis of the levels of induced transcriptional repression and NOSpro DNA methylation will be presented. The work is supported by DFG grant ME 2122/1-1.

[1] Matzke et al. (2004) Biochim Biophys Acta 1677:129-141
[2] Forsbach et al. (2003) Plant Mol Biol 52:161-176

T09-029

Control of Arabidopsis development by Polycomb-group dependent histone methylation

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Modifications of histones play important roles in epigenetic gene regulation both in plants and animals. It was recently shown in Drosophila and mammals that Polycomb-group (Pc-G) protein complexes catalyse methylation of lysine 9 and/or 27 of histone H3. These marks are correlated with stable, mitotically heritable repression of homeotic genes and thus have an important function in development.

Initially, we showed that the Arabidopsis Pc-G protein CURLY LEAF (CLF), a homologue of the Drosophila histone methyltransferase ENHANCER OF ZESTE, represses the transition to flowering. Curly leaf mutants are early flowering and display a dwarf phenotype mainly due to ectopic expression of the floral homeotic gene AGAMOUS (AG). We now use Chromatin Immunoprecipitation to investigate if AG repression/expression can be correlated with certain histone modifications at the AG locus. Indeed, repression of AG is correlated with methylation of lysine 9 and 27 of histone H3 and these marks are lost in clf mutants. Vice versa, methylation of lysine 4 of histone H3, a mark for actively transcribed genes, is gained in clf mutants. This is the first evidence that CLF acts on AG chromatin and that floral homeotic genes are regulated by histone modifications. We are currently investigating if AG is a direct target of a CLF complex and if other putative CLF targets are modified in a similar way.

Interestingly, despite local changes at the AG locus, global histone methylation levels are not significantly altered in clf mutants. However, loss of histone methylation of many more loci in clf mutants is obscured by the partial redundancy with the homologous gene SWINGER/EZA1 (SWN). swn clf double mutants show a severe enhancement of the clf phenotype: they are minute and display severe disorganized growth after germination. To assess the role of CLF and SWN during leaf and flower development we introduced a conditional CLF allele in the double mutant background. Interestingly, some of the phenotypes are also found in transgenic plants showing co-suppression of FERTILIZATION INDEPENDENT ENDOSPERM whose gene product forms a complex with CLF and SWN (Katz et al. 2004).

Therefore, these data indicate that many more genes are likely to be directly controlled by a CLF/SWN-complex and histone methylation, respectively, than just AG and emphasize the importance of epigenetic gene regulation in plants.

Katz A, Oliva M, Mosquna A, Hakim O, Ohad N. 2004. Plant J. 37:707

T09-030

Control of plant development by the miR-JAW and miR-159 microRNAs

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MicroRNAs are ubiquitous in plants, as they are in other eukaryotes. Animal microRNAs, like the canonical let-7 and lin-4 from C. elegans, bind to imperfect matching sequences in the 3'UTR of target mRNAs rendering in translation attenuation. However, evidence has accumulated suggesting that some plant microRNAs can guide cleavage of their targets, in a manner similar or identical to RNAi.

We identified the JAW locus using an activation tagging screen in Arabidopsis thaliana (random insertion of viral enhancers). A microarray survey identified 5 TCP transcription factors downregulated in the mutant. These TCPs share an almost invariant 20 bases motif in their RNA. We showed that JAW encodes a microRNA (miR-JAW/miR-319a) that targets the TCPs through this conserved box. In the jaw-D mutant the expression of miR-JAW is increased around 50 times, being also broader its expression domain. In vitro and in vivo evidence demonstrate that miR-JAW is able to direct the cleavage of the target mRNAs and fragmentation products can be detected in plants, being mapped the cleavage site to the middle of the miRNA matching sequence. Mutated versions of the TCPs were prepared in which the miRNA target sequence was modified, but not the encoded amino acids. MicroRNA-guided cleavage is necessary to prevent aberrant activity of the TCP4 gene expressed from its native promoter, which unchecked leads to embryo-patterning defects.

In addition, overexpression of wild-type and microRNA-resistant TCP variants demonstrate that mRNA cleavage is largely sufficient to restrict TCP function to its normal domain of activity. JAW and its target sequences are found in a wide range of species, indicating that microRNA-mediated control of leaf morphogenesis is conserved in plants with very different leaf forms.

miR-JAW mature sequence has similarity to five other miRNAs from Arabidopsis: miR-319b, miR-319c, miR-159a, miR-159b and miR159c, that are predicted to target members of the TCP and Myb families of transcription factors. Overexpression of miR-159a or miR-159b caused stamen defects and sterility. An analysis of the system will be presented.

Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. Nature 425, 257-263.

T09-031

Alternating partnerships of FIE with SET domain PcG members, mediate different developmental programs in Arabidopsis

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Specification of cell differentiation is determined by establishing and maintaining patterns of regulatory gene expression. Polycomb group (PcG) proteins constitute a cellular memory system to maintain the repressed state of homeotic gene expression. In animals, PcG proteins repress their target genes by modifying histone tails through deacetylation and methylation, possibly generating a PcG-specific histone code. The PcG histone code further recruits other chromatin remodeling proteins to establish a stable and heritable mechanism of epigenetic expression control.
In Arabidopsis, structurally conserved PcG proteins that we and others have identified were found to control multiple aspects of plant development (Katz et al. 2004; Kinoshita et al. 2001). Morphological, molecular and biochemical characteristics of mutated fie (FERTILIZATION INDEPENDEDENT ENDOSPERM), mea (MEDEA) and clf (CEURLY LEAF) plants contributed to the hypothesis that FIE function is mediated by association with different polycomb proteins, including members of the SET domain family as in the case of MEA and CLF, resulting in differential regulation of gene expression throughout the plant life cycle.
Remarkably, as in animals, the Arabidopsis PcG proteins also functions in maintenance of homeotic gene repression including members of the homeobox and MADS-box gene families (Katz et al. 2004; Kinoshita et al. 2001). Interestingly, de-repression of MEA was observed in both clf and FIE-silenced plants (Katz et al. 2004). This result may indicate that in wild type plants the regulatory PcG complex, which contains both FIE and CLF, down-regulate MEA expression in the sporophyte to facilitate the formation of FIE-CLF complex. Supporting evidence for this hypothesis will be presented.

Katz A, et .al (2004). Plant J 37: 707-719
Kinoshita T, et .al (2001). Proc Natl Acad Sci U S A 98: 14156-14161

T09-032

Arabidopsis thaliana AtPOLK encodes a DinB-like DNA polymerase that extends mispaired primer termini and is highly expressed in a variety of tissues

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Cell survival after DNA damage depends on specialized DNA polymerases able to perform DNA synthesis on imperfect templates. Most of these enzymes belong to the recently discovered Y-family of DNA polymerases, none of which has been previously described in plants. We report here the isolation, functional characterization and expression analysis of a plant representative of the Y-family. This polymerase, which we have termed AtPolk(kappa), is a homolog of E. coli pol IV and human pol kappa, and thus belongs to the DinB subfamily. We purified AtPolk and found a template-directed DNA polymerase, endowed with limited processivity, that is able to extend primer-terminal mispairs. The activity and processivity of AtPolk are enhanced markedly upon deletion of 193 amino acids from its carboxy-terminal domain. Loss of this region also affects the nucleotide selectivity of the enzyme, leading to the incorporation of both dCTP and dTTP opposite A in the template. We detected three cDNA forms which result from the alternative splicing of AtPOLK mRNA and have distinct patterns of expression in different plant organs. Histochemical localization of beta-glucuronidase (GUS) activity in transgenic plants revealed that the AtPOLK promoter is active in endoreduplicating cells, suggesting a possible role during consecutive DNA replication cycles in the absence of mitosis

T09-033

Arabidopsis Cellular Responses to DNA Damage

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Genetic fidelity is challenged continuously by DNA damage from endogenous and environmental causes. Cellular repair pathways are activated upon DNA damage. Repair mechanisms involve recognition of damaged DNA, removal of damaged DNA, DNA synthesis and DNA ligation. Plants are unique in their fixed life mode in response to environmental damages. We are trying to understand DNA repair mechanisms in Arabidopsis thaliana from two themes. Since protein phosphorylation is important in DNA repair, we analyzed protein phosphorylation status stimulated by DNA damage. We have had interesting candidates and are in the process of detailed characterization. Secondly, we are using proteomics approaches to analyze whole cellular protein responses to DNA damage. Our preliminary data showed that we have identified proteins involved in protecting DNA from damage. In summary, we are approaching DNA repair from the biochemical and molecular levels using Arabidopsis model system. Plants are different from Mammals in many ways. Hopefully we can contribute our knowledge in plants to human health research.

T09-034

The PCF-like subfamily of TCP proteins in Arabidopsis thaliana : molecular and genetic studies.

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TCP proteins, named for TEOSINTE BRANCHED 1 (TB1) in maize, CYCLOIDEA (CYC) in Anthirrinum majus and PCF in rice (Cubas 1999), constitute a widespread family of transcription factors described in many angiosperms species and involved in development control.These factors have a DNA binding domain with a predicted secondary structure similar to bHLH transcription factors but their primary amino acid sequence is unrelated to that of all other bHLH proteins (Cubas 1999). The TCP proteins can be classified into two sub-families according to the primary structure of the DNA binding domain. The C-subfamily (TCP-C) consists of proteins sharing conserved regions with CYC and TB1, whereas proteins more similar to PCF belong to the P-subfamily (TCP-P). Although several experiments have revealed the role of TCP-C proteins in plant development, function of the TCP-P proteins is much less documented. They may be involved in control of cell division (Kosugi and Ohashi 1997, Tremousaygue et al, 2003). Genetic and molecular analysis on the Arabidopsis TCP-P proteins have been initiated to get more insight into the role of these proteins . Results concerning two members of this family, AtTCP9 and AtTCP20, will be presented.

Kosugi 1997 Plant Cell 13, 1437-1452. Cubas et al 1999 Plant J. 18, 215-222. Tremousaygue et al 2003 Plant J 33, 957-966

T09-035

AtERF2 is a Positive Regulator of Defence Gene Expression in Arabidopsis

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Ethylene Response Factors (ERFs) are a large family of plant transcription factors that are known to interact with the conserved GCC-box sequence (AGCCGCC) found in the promoters of several defence-related genes, including the antifungal peptide PDF1.2. The role of one of the members of this gene family, ERF1, is relatively well characterised while little is known about the roles of the other members of this gene family. To identify new members of this gene family with potential roles in regulating plant defence responses, expression patterns of 10 selected Arabidopsis ERFs were examined by Real-Time Quantitative RT-PCR in tissue samples collected from Alternaria brassicicola-inoculated or methyl jasmonate (MJ) treated plants. Of these, AtERF2 showed induction over time in response to both Alternaria and MJ treatments. In an effort to better understand the function of AtERF2, transgenic Arabidopsis plants overexpressing AtERF2 were generated. Transgenic lines with increased expression of AtERF2 showed a proportionate increase at the transcript levels of PDF1.2 as well as other GCC-box containing genes such as basic chitinase (Chi b). . The Arabidopsis lines over-expressing AtERF2 are currently being further studied for increased resistance to a range of necrotrophic fungi.

T09-036

FLC the epicentre of an expression domain

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Plant developmental processes such as the transition to flowering and seed development are under both genetic and epigenetic regulation. Epigenetic changes in gene expression are mediated by two distinct, but not completely independent processes: methylation of DNA and chromatin remodelling. Vernalization, the promotion of flowering by prolonged exposure to low temperatures, has the hallmarks of an epigenetically regulated phenomenon. The vernalization signal is inherited through mitotic divisions, but is reset during meiosis or seed development. FLC, a gene encoding a repressor of flowering that plays a key role in the vernalization response, is down-regulated in response to vernalization or by decreased levels of DNA methylation. The down-regulation of FLC by low temperatures is maintained throughout vegetative development but is reset at each generation. Acetylation of histone H3 at the FLC gene is also decreased by vernalization or decreased methylation. During our study of vernalization we have found that a small gene cluster, including FLC and its two flanking genes, is co-ordinately regulated in response to genetic modifiers, to the environmental stimulus of vernalization, and in plants with low levels of DNA methylation. Genes encoded on foreign DNA inserted into the cluster also acquire the low temperature response. When inserted at other chromosomal locations, FLC maintains its response to vernalization and confers a similar response to a flanking gene. This suggests that FLC contains sequences that direct changes in gene expression extending beyond FLC itself, perhaps through chromatin modification.

T09-037

Efficient gene targeting in plants by transient inhibition of non-homologous recombination

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Agrobacterium tumefaciens T-DNA normally integrates into random sites in the plant genome and frequencies of gene targeting in plants are very low. It would be an advantage for analysis of gene function and for exploitation of genetically modified organisms if the frequency of targeted integration could be increased.

Experiments with yeast mutants have shown that gene targeting can be enhanced by mutations in the DNA repair pathway of non-homologous end joining (NHEJ) (van Attikum H and Hooykaas PJJ (2003) Nucl Acid Res 31, 826-832). We are currently testing if components of the NHEJ DNA repair play a role in T-DNA integration in plants and if inactivation of NHEJ genes or proteins facilitates targeted integration of the T-DNA.

We have isolated and characterized T-DNA insertion mutants in genes involved in NHEJ (Bundock P et al (2002) Nucleic Acids Res 30, 3395-3400; Bundock P and Hooykaas P (2002) Plants Cell 14, 2451-2462). These mutants as well as wild type Arabidopsis are now being tested in gene-targeting experiments using the PPO system (Hanin et al (2001) Plant Journal 28, 671-677). Since constitutive inhibition of the NHEJ DNA repair pathway results in telomere lengthening and maybe other DNA rearrangements, we are applying RNAi technology combined with an inducible promoter system for the temporary knock-down of NHEJ genes.

T09-038

DNA-binding function and physiological function of the Dof transcription factors conserved only in higher plants

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Dof proteins are members of a major family of plant specific transcription factors. These proteins have a highly conserved DNA-binding domain, named Dof domain. The Dof domain, which is composed of 52 amino acid residues, is similar to Cys2/Cys2 zinc finger DNA-binding domains of GATA1 and steroid hormone receptors, but has a longer putative loop than these zinc finger domains. However, the DNA-binding properties of Dof proteins are yet unclear. AOBP is one of the Dof proteins and binds to 5'-upstream region of ascorbate oxidase gene. Although AOBP has been suggested to function as a suppressor of ascorbate oxidase gene, little is known about the function of AOBP in transcriptional regulation. In this study, we investigated the DNA-binding function of a Dof domain of AOBP and the homologues in pumpkin, Arabidopsis and tobacco by gel retardation analysis and the inductively coupled argon plasma mass spectrometer (ICP-MS). The results showed that the characteristic longer loop of the Dof domain was essential for DNA-binding activity. The Dof domain could not function as a Cys3/His or a Cys2/His2 type, but the Dof domain truly contained zinc ion. Thus, the Dof domain was proved to function as a Cys2/Cys2 zinc finger domain. Furthermore, in order to elucidate the physiological function of a Dof protein AOBP, we analyzed the transgenic tobacco plants expressing the antisense RNA of AOBP. These transgenic plants showed an abnormal phenotype in the leaves which became spindly and rigid. The cells in these abnormal leaves were more elongated when compared with wild type leaves. It has been shown that ascorbate oxidase is induced by auxin and highly expressed during elongation of plant cells. Thus, we suggest that AOBP participates in transcriptional regulation of genes involved in cell growth of plants.

Umemura Y. et al. (2004) The Plant Journal, 37, 741-749

T09-039

Plant specific GAGA-binding proteins regulate MADS-box gene expression through DNA remodelling

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In Drosophila the Trithorax-like locus encodes several GAGA-binding proteins (GBPs) that bind to GAGA-DNA repeats and regulate the expression of several genes, including themselves and the homeotic genes Ultrabithorax and Engrailed (reviewed in 1). The GBPs have been reported to co-localize with polycomb response elements and trithorax response elements and have been reported to be important in both gene repression and activation pathways. This regulation is mediated by the interaction of GBP with several protein complexes like NURF, SIN3 and SAP18 or directly with PC or TRX.
In Soy bean, Barley and Arabidopsis proteins that bind to GA-rich DNA-elements have been reported recently (2,3,4). Though they seem to be unrelated to the Drosophila GBPs, they share a surprising number of functions.
We show that the Arabidopsis GBP Basic Penta Cystein 1 (BPC1) binds to a DNA motif RGARAGRRA, which is several times present in the regulatory sequence of the homeotic MADS-box gene STK as well as in the regulatory sequence of BPC1 and BPC2. The expression of these genes is altered in the bpc1 and bpc2 mutants, showing that in Arabidopsis the homeotic gene STK and BPC1/2 are regulated by BPC1/2. With TPM-experiments we show that BPC1 is able to form stable DNA-loops by binding to several GAGA-elements present in the STK regulatory sequence. Like in Drosophila multiple binding sites are required for the formation and stabilization of these loops. This loop is possibly mediated by the formation of dimers or oligomers, since we show that the BPC1 and BPC2 proteins are able to form homo or heterodimers, like the Drosophila GBPs.

1 Trends in Genet. 20, 15-22
2 Plant Physiol. 129, 1788-1794
3 Plant J. 37, 426-438
4 Plant J. 34, 813-826

T09-040

The influence of the light period on redox regulation and stress responses

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Under natural conditions, plants are subjected to an environment in which several parameters such as light or temperature can change dramatically for periods which can last for seconds or even for days. Plants possess a large set of mechanisms, such as the malate valve, to prevent damages in the short-term. However, when environmental alterations persist, a response on the genetic level is induced, possibly mediated by altered redox states in the chloroplast. The type of acclimation strictly depends on the developmental stage of the plants and and on the duration of the light period. To investigate the different responses, Arabidopsis plants were grown in low light (150 µE) either in short days (7.5 h light) or long days (16 h light period), and then transferrred into high light (350-800 µE) at 12°C. The plants grown in short days responded with a quick increase in NADP-malate dehydrogenase activation state, but persisting overreduction revealed a new level of regulation of the malate valve. Activity measurements and Northern-blot analyses indicate that upregulation of the NADP- malate dehydrogenase transcript amount starts a few hours after the onset of the stress. Using macroarrays, additional changes in gene expression were identified. The transcripts of several enzymes of glutathione metabolism and of some photosynthetic genes increased. The cellular glutathione content increased, but its redox state remained unchanged. A completely different situation was obtained in plants grown in long-day conditions. Here, neither NADP-malate dehydrogenase nor other photosynthetic enzymes changed, but the expression of several antioxidative enzymes increased strongly. We conclude that the endogenous systems that measure the daylength interact with redox regulation, and override the interpretation of the signals, i. e. they redirect redox-mediated acclimation signals from a more efficient light usage and from redox poising in short days towards the prevention of oxidative damages in long days.

This work is financially supported by a grant from the Deutsche Forschungsgemeinschaft (FOR 387, TP1 and TP3).

T09-041

Effector of Transcription (ET): A novel plant protein family repressing gibberellin mediated processes

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Current models of gibberellin (GA) action and signal transduction are based on a derepressible system and a number of candidate regulators have been identified. We describe a new family of negative regulators of GA perception designated as Effectors of Transcription (ET). Structurally they are characterised by a C-terminal zinc and DNA binding domain with a highly conserved cysteine pattern. The gene family is restricted to the plant kingdom and members have been found both in monocot (Hordeum vulgare, Oryza sativa) and dicot (Brassica napus, Vicia faba) species as well as in mosses (Physcomitrella).
Ectopic expression of a Brassica napus ET factor (BnET) both in Arabidopsis and tobacco leads to phenotypes which include dwarfism due to shorter internodes as well as phenotypes like reduced germination rate, increased anthocyanin content and finally reduced xylem lignification. Data from transient expression assays support the notion that this most likely is due to a direct transcriptional repression of GA controlled genes.
The Arabidopsis genome contains three ET-genes with ecotype specific structural differences. Microarray experiments based on a knock out mutant in the AtET2 gene demonstrate that AtET2 might act as a repressor of several other transcription factor genes. Further analysis of this mutant reveals gene functions during seed maturation and germination. A current working hypothesis suggests that ET factors contribute to the maintenance of the ABA/GA balance involved in the decision between maturation and germination.

T09-042

The chromatin-remodelling complex FACT associates with actively transcribed regions of the Arabidopsis genome

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The packaging of the genomic DNA into chromatin in the cell nucleus requires machineries that facilitate DNA-dependent processes such as transcription in the presence of repressive chromatin structures. The two major classes of activities that make the DNA template accessible for transcription are the ATP-dependent chromatin remodelling complexes and enzymes that covalently modify histones. Using co-immunoprecipitation we have identified in Arabidopsis cells the FACT (facilitates chromatin transcription) complex, consisting of the 120-kDa Spt16 and the 71-kDa SSRP1 proteins. Indirect immunofluorecence analyses revealed that both FACT subunits co-localise to nuclei of the majority of cell types in embryos and roots, while FACT is not present in terminally differentiated cells such as mature trichoblasts or cells of the root cap. In the nucleus, Spt16 and SSRP1 are found in the cytologically defined euchromatin of interphase cells, but the proteins are not associated with heterochromatic regions and condensed mitotic chromosomes. FACT can be detected by chromatin immunoprecipitation over the entire transcribed region (5´-UTR, coding sequence, 3´-UTR) of actively transcribed genes, whereas it does not occur at non-transcribed heterochromatic or intergenic regions. FACT localises to inducible genes only after induction of transcription, and the association of the complex with the gene correlates with the level of transcription. Collectively, these results indicate that FACT assists transcription elongation through plant chromatin.

Belotserkovskaya, R. and Reinberg, D. (2004) Facts about FACT .... Curr. Opin. Genet. Dev. 14, 139-14

T09-043

Signal pathway of the endoplasmic stress response

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In eukaryotic cells, accumulation of malfolded proteins in the endoplasmic reticulum (ER) induces expression of various genes such as those for the ER chaperones. This phenomenon is called as the ER stress response. Mechanism of signal transduction for the ER stress response has been extensively characterized in yeast and mammals, while little of that has been known in plants. The aim of our study is clarification of signaling mechanism of the ER stress response in plants using Arabidopsis thaliana. Since bZIP transcription factors are involved in signal transduction of the ER stress response in yeast and mammals, we hypothesized that some bZIP proteins also play roles in the ER stress response in plants. According to this idea, we surveyed bZIP genes in Arabidopsis genome and found AtbZIPER that was specifically induced by tunicamycin, an inhibitor of protein glycosylation. AtbZIPER has a transmembrane domain suggesting its localization in the membrane. When transmembrane domain was removed and truncated protein was fused with GFP, the fusion protein localized to the nucleus suggesting possible translocation of native protein to the nucleus after proteolysis. Expression of AtbZIPER was also induced by other drugs such as DTT that caused the UPR. Analysis using GUS reporter gene indicated that AtbZIPER strongly expressed in immature seeds without artificial stress. A knock out mutant of AtbZIPER grew without clear visible phenotype. However, when profile of gene expression was compared with wild type, induction of genes for one of three BiPs, Sar1 (small G protein involved in the COPII pathway) and other putative transcription factors associated with the ER stress response dismissed in mutants. (The presence of multiple BiP genes seems to be specific for plants in contrast to single BiP gene in yeast and mammals.) This observation strongly suggests that AtbZIPER regulates those genes directly or indirectly. Being consistent with this idea, genes repressed in the mutants contain sequence similar with ERSE (ER stress responsible element) identified in mammals. In summary, we identified a novel transcription factor involved in the ER stress response in Arabidopsis. We also found that there are multiple signal transduction pathways for the ER stress response in plants as like in mammals. However the mechanism is clearly different from that of mammals.

Plant Cell. 15: 2273 (2003)
Biochem. Biophys. Res. Com. 301: 225 (2003)
Plant Physiology 127: 949 (2001)

T09-044

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

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

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

Strahl, B.D., and Allis, C.D. (2000), Nature 403, 41-45.
Turner, B.M. (2000), Bioessays 22, 836-845.

T09-045

Epigenetic control of seed development

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In flowering plants, seed development begins with the fertilization of the egg cell and the central cell by the male gametes, giving rise to the diploid embryo and the triploid endosperm, respectively. Although the genomes present in the embryo and the endosperm are identical, the fates of the fertilization products are different. In most species, the endosperm initially develops as a syncytium. After a defined set of mitotic events, the endosperm cellularizes and differentiates into specific subdomains. The embryo, endosperm and surrounding maternal tissue interact and coordinate their development. Mutants of the fertilization independent seed (fis) class have defects in the coordinated development of embryo and endosperm. In fis mutants, endosperm development initiates in the absence of fertilization. If fertilization does occur, the endosperm proliferates abnormally and the embryo aborts. The FIS genes encode Polycomb group (PcG) proteins, a class of proteins that produce a mitotically stable repression of their target genes. We are interested to elucidate how this repression is established and maintained. To address this question, we started a biochemical characterization of the FIS complex. We could show that the FIS complex has a similar size compared to a homologous complex from animals and identified MSI1, a WD-40 domain protein, as a new subunit of this complex. All of the identified FIS complex subunits have close homologs in animals, indicating a strong evolutionary conservation of PcG function. Using the msi1 mutant we provided functional proof for MSI1 being a subunit of the FIS complex. Loss of MSI1 protein in female gametophytes results in the initiation of endosperm development without fertilization, phenocopying the other fis mutants. To identify genes regulated by this complex we hybridized microarrays with RNA from two fis mutants (medea (mea) and fertilization independent endosperm (fie)). This identified the type I MADS-box gene PHERES1 (PHE1) as a primary target of the FIS complex. PHE1 is expressed in the endosperm and the embryo until the globular stage, then the expression is restricted to the chalacal endosperm. However, in fis mutants PHE1 expression remains high until embryo growth is arrested at heart stage. Reduced expression of PHE1 in mea mutant seeds can suppress mea seed abortion, indicating that PHE1 is a regulator of seed development.

T09-046

Mitochondria and plastids: Complex machineries transcribe simple genomes

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In higher plants, a small nuclear gene family encodes mitochondrial as well as chloroplast RNA polymerases homologous to the T7-bacteriophage enzyme. The genome of Arabidopsis thaliana contains three such genes, RpoTm, RpoTp and RpoTmp. According to GFP import studies, the RpoTm enzyme is targeted to mitochondria and RpoTp is found in plastids, whereas RpoTmp is present in both mitochondria and plastids. Plastids have in addition retained their bacterial-type transcription system and the corresponding RNA polymerase (rpo) genes. Arabidopsis therefore seems to rely on two phage-type RNA polymerases to transcribe mitochondrial genes and may require two enzymes of this type besides the plastid-encoded RNA polymerase for plastid gene transcription. Accordingly, the plastid genome harbours sigma70-type promoters comprising -10 and -35 regions, as well as promoters that deviate from this architecture and are recognised by a nucleus-encoded RNA polymerase (NEP). To address the question of individual roles of RpoTp and RpoTmp in plastid RNA syntheses, we have analysed the utilisation of different NEP promoters in transgenic plants with introduced copies of the RpoTp cDNA preceded by the CaMV promoter. RpoTp overexpression in these plants was found to correlate with an enhanced transcription of the plastidial atpB gene from NEP promoters.

RpoT-dependent transcription of organellar genes likely requires a complementation of the RpoT enzymes with specificity factors. Candidate cofactors will be tested in in vitro transcription experiments. In order to have promoter sequences available for these experiments, and to learn about promoter specificities of the organellar transcription machineries in Arabidopsis, we have employed an RT-PCR-based method to map transcription initiation sites of selected mitochondrial and plastid genes of this plant. In addition to a previously predicted mitochondrial promoter motif (CATAAGAGA), sequences were found at transcription start sites in mitochondria that differ from this motif. Most genes showed simultaneous activity of more than one promoter, while no differences in promoter utilisation were observed between leaf and flower tissues. This may indicate minor regulation of mitochondrial genes on the transcriptional level.

While higher plants require rather complex machineries for the transcription of their organellar genomes, we have found no more than one phage-type RNA polymerase gene in the green alga Chlamydomonas reinhardtii.

T09-047

Expression of nuclear genes for organellar RNA polymerases in Arabidopsis

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The Arabidopsis thaliana nuclear genome encodes three organellar phage-type RNA polymerases (RpoTm, RpoTmp, RpoTp). While we could show that RpoTm is targeted to mitochondria and the amino terminus of RpoTp targets the proteins to plastids, RpoTmp is dually targeted to both mitochondria and plastids.

To gain first insights into the division of labor among the different organellar RNA polymerases, we have investigated RpoT transcript abundance in Arabidopsis tissues by quantitative real-time RT-PCR. Relative determination of transcript levels revealed a higher abundance of RpoTp mRNA in leaf tissue, whereas RpoTm transcripts levels were higher in root, stem and flower tissue. All RpoT transcripts were found to accumulate to higher levels in young tissues.
Histochemical GUS assays of transgenic Arabidopsis plants showed differences in RpoT promoter activity, especially in seedlings and flowers. Whereas promoter activities of RpoTm and RpoTmp were detectable in some meristematic tissues like root tips and young primary leaves, in leaf veins, stigmatic papillae and carpels, RpoTp promoter GUS activity was only visible in leaf tissue and sepals. In cross sections of stems, RpoTp promoter activity showed high GUS staining intensity predominantly in the primary cortex, while detection of RpoTm and RpoTmp GUS signals were restricted to the stele. Analyses of transcript levels of the RpoT genes using in situ hybridization corroborate the findings obtained by GUS staining.
We generated transgenic Arabidopsis plants expressing RpoT antisense transcripts. The lower expression of the RpoT genes affects the transcription of the organellar genes in these plants. RpoTm- and RpoTmp-antisense plants were green and showed retarded growth, whereas RpoTp-antisene plants displayed bleached leave tissue.

T09-048

Analysis of a suppressor mutant of the immunophilin-like twisted dwarf1 (twd1) gene mutation

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Null mutations of the recessive Arabidopsis FKBP-like immunophilin gene TWISTED DWARF1 (TWD1) cause a pleiotropic phenotype characterised by reduction of cell elongation and disorientated growth caused by impairment of polar auxin transport. This results in plants with a severe dwarf phenotype and twisted organs above and below ground.

Screens for suppressor mutants of the twd1 mutation resulted in the isolation of a plant that shows an intermediate phenotype between wild-type (wt) and the dwarf phenotype of the null mutation. The size of most organs is only slightly reduced whereas the stems and siliques still show twisted growth behavior. This plant was called twd-sup.

The suppressor screens has been performed with a null mutant induced by T-DNA insertion in the fourth intron. This insertion leads to a complete knock-out of the TWD1 gene and results in the twd1 phenotype. Complementation analysis between twd1 mutants and twd-sup plants revealed genetic dominance of twd-sup over twd1 mutation. Analysis of more than 30 000 offspring of a cross between wt and twd-sup revealed tight genetic linkage between the twd1 mutation and the twd-sup mutation. Intragenic suppressor mutation cannot be ruled out. Surprisingly, no sequence alteration has been found in the TWD1 gene sequences of twd1 and twd-sup plants. No structural rearrangements of the inserted T-DNA could be found in both lines.

A striking difference between twd1 and twd-sup, however, can be found in the methylation pattern of the NPTII gene of the T-DNA which serves as a selection marker of T-DNA transformed plants. In twd-sup plants we found silencing of the NPTII gene which leads to sensitivity to kanamycin. Twd1 plants that have the identical genetic makeup concerning the T-DNA insertion are completely kanamycin resistant. Silencing of the NPTII gene has never been observed for this line. The silencing of the NPTII gene can be shown by RT-PCR. Treating twd-sup plants with 5-aza-cytidine reactivated the expression of the silenced NPTII gene and resulted in kanamycin resistant twd-sup plants.

Kamphausen et al., (2002) Plant J. 32, 263
Geisler et al., (2003) MBC 14, 4238
Matzke et al., (1989) EMBO J. 8, 643

T09-049

Arabidopsis HAF2 encoding transcription coactivator TAFII250 is required for leaf greening and genetically interacts with photomorphogenic regulators

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Light signals received by a set of specific photoreceptors are integrated by transcription factors to activate plant gene expression and growth. A number of light-responsive transcription factors has been functionally identified. However, the mechanism by which the transcription factors activate plant light-inducible gene expression remains unknown. In this work, we report the characterisation of a mutation within the Arabidopsis HAF2 gene encoding transcription cofactor TAFII250. This mutation (haf2) affected young leaf greening processes including chlorophyll accumulation, light-responsive gene expression and chloroplast development, while the hypocotyl length of the mutant appeared normal. Transcriptome analysis revealed that the mutation altered the expression of about 8,7% of the Arabidopsis genes in rosette leaves. Analysis of double mutants of haf2 with long hypocotyl mutants hy that affect phytochrome (hy1-1) and cryptochrome 1 (hy4-1) synthesis showed that haf2 was epistatic to the hy1 and hy4 mutations in red/far-red and blue light, respectively. In the white light, haf2 appeared to interact genetically with hy4-1 with respect to hypocotyl elongation. These data suggest that HAF2 is involved in integration of both red/far-red and blue light signals. Accordingly, haf2 showed a synergistic effect on hypocotyl elongation with hy5-1, a mutation of a light signalling positive transcription factor gene, in all tested light wavelengths including white, red, far-red and blue lights. In addition, light inducible gene expression was lower in haf2/hy5-1 than in either single mutant. These results suggest that HAF2 functions in a separated transcription pathway that interacts with HY5 to regulate light-dependent gene expression. Therefore, this work identifies HAF2 (TAFII250) as a light-responsive transcriptional cofactor.

T09-050

Specific heterodimerization of group C and group S Arabidopsis thaliana bZIP transcription factors

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In tobacco, the bZIP transcription factor BZI-1, which is involved in pathogen defence, forms specific heterodimers with a group of bZIP transcription factors that are regulated by diverse stresses like light, sugars, cold, senescence or IAA (BZI-2,-3,-4). It was proposed that the heterodimerization might lead to a signal integration of these different stresses. To further address this question heterodimerization was analysed on a genome wide basis in Arabidopsis.
Specific heterodimerization between members of group C (homologues to BZI-1, 4 members) and group S (homologues to BZI-2,-3,-4, 17 members) of Arabidopsis bZIP transcription factors was analysed using yeast two-hybrid assays. The results show that all group C members form specific heterodimers only with a subgroup of group S.
Tissue specific expression of a part of these TFs was analysed using promoter-GUS fusions, because colocalisation is required for heterodimerization.
A common feature of the subgroup of group S that heterodimerizes with group C is that they all have a conserved uORF that mediates sucrose-induced repression of translation, indicating that carbohydrates might play an important role in the function of the heterodimers.

T09-051

The INCURVATA2 gene is involved in chromatin-mediated cellular memory

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The incurvata2 (icu2) mutant of Arabidopsis thaliana is early flowering and displays homeotic transformations similar to those caused by hypomorphic apetala2 mutations, leaf incurvature and ectopic derepression of the AGAMOUS gene in vegetative leaves. Microarray analysis and ulterior quantitative RT-PCR validation demonstrated that other genes are also derepressed in the vegetative leaves of the icu2 mutant, including APETALA1, APETALA3, PISTILLATA, SEPALLATA3, CAULIFLOWER, FRUITFUL and FLOWERING LOCUS T, among others. In addition, we found that the curly leaf, terminal flower2 and embryonic flower2 mutations interact with icu2 in double mutants. The ICU2 gene was positionally cloned and found to encode a DNA polymerase subunit. Taken together, our results strongly suggest that the product of the ICU2 gene is involved in chromatin-mediated cellular memory.

T09-052

A molecular and structural analysis of introns that reside in non-coding sequence.

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We have been investigating the introns that reside within non-coding sequence of Arabidopsis genes through the alignment of expressed sequence data to genome sequence. Given the relatively short length of the 5'UTR regions (average unprocessed length =193 bases) we were surprised to find over 18% of these expressed genes contain introns. In contrast the 3'UTR is slightly longer (average unprocessed length =243 bases) but less than 5% appear to contain introns.

Introns in the 5'region of genes have been implicated in a post-transcriptional regulation, a phenomenon known as Intron Mediated Enhancement (IME). We were interested in analyzing these non-coding sequence introns to discover if there was sequence information that could define the molecular parameters associated with the process.

Three features were of immediate interest: the position of the introns within the 5'UTR, sequence conservation of around the intron-exon junction and the size distribution of introns that reside within the 5'UTR regions. The analysis of these features will be discussed.

We have also developed a transient assay system to test the role of the 5' UTR introns and will present data that describes these features and their role in genome organization and posttranscriptional regulation.

T09-053

Specific Methylation-Mediated Silencing of 4CL::GUS Transgene - Expression

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Lignin is an important biopolymer that is deposited in secondary cell walls of specialized plant cells (e.g. xylem elements and fiber cells). Biosynthesis of lignin monomers occurs via the phenylpropanoid pathway, in which the enzyme 4-coumarate:CoA ligase (4CL) plays a key role by catalyzing the formation of hydroxycinnamoyl-CoA esters. These are subsequently reduced to corresponding monolignols (hydroxycinnamoyl alcohols). 4CL is encoded by a family of 4 genes in Arabidopsis thaliana (4CL1-4CL4), all of which are developmentally regulated, together with other genes involved in the phenylpropanoid pathway. In order to identify regulatory genes involved in the developmental regulation of 4CL and other phenylpropanoid and lignin specific genes, we generated EMS mutagenized populations of 4CL1::GUS and 4CL2::GUS Arabidopsis transgenic lines, and screened several thousand progeny for reduced or altered GUS _expression. Several lines with reproducible and striking patterns of reduced GUS _expression were identified. However, the GUS _expression phenotypes segregated in a non-Mendelian manner in all lines identified, suggesting that epigenetically silenced paramutants had been isolated. 4CL::GUS transgene silencing was also observed at low frequency in non-mutated 4CL::GUS transgenic plants. 5-azacytidine treatment restored wild-type 4CL::GUS _expression in the paramutant lines, and additional molecular data further suggest the involvement of methylation in 4CL::GUS transgene silencing. The paramutant plants were otherwise phenotypically normal, and endogenous 4CL gene _expression was not always affected in silenced lines. Thus, introduction of ectopic copies of the 4CL promoter appears to consistently trigger methylation-induced silencing of the 4CL-GUS transgene in transgenic Arabidopsis.

T09-054

Helper component proteinase (HC-Pro) as a tool to dissect the mechanisms of RNA silencing and microRNA (miRNA) mediated RNA degradation

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HC-Pro is a multifunctional, viral protein that suppresses RNA silencing, a defense mechanism against viruses, transposons and aberrant genes. HC-Pro also appears to disrupt the miRNA directed degradation of some target mRNAs involved in development and other processes. A yeast two-hybrid interaction screen identified several tobacco proteins that interact with HC-Pro. One of these proteins is closely related to members of the Arabidopsis RAV family of transcription factors and is termed ntRAV. To determine if ntRAV plays a role in RNA silencing, wild type plants and plants overexpressing ntRAV were crossed to a tobacco transgenic line containing a silenced GUS sense-transgene, and offspring were analyzed for expression of the ntRAV and GUS genes. The endogenous ntRAV gene is expressed at high levels in seedlings and then decreases precipitously at about 3 weeks, the same time that silencing of the GUS sense-transgene initiates. The expression of high levels of ntRAV is extended by about two weeks in the ntRAV overexpressing line, and the onset on RNA silencing is delayed correspondingly. A high level of ntRAV is therefore correlated with inability of plants to initiate GUS sense-transgene silencing. These results raise the possibility that members of the RAV gene family of transcription factors directly or indirectly control expression of components of the silencing machinery. We are also using HC-Pro in transient Agrobacterium-infiltration assays to examine the mechanism by which two model plant miRNAs negatively regulate expression of their target mRNAs. Coinfiltration of genes encoding miR171 and one of it targets, SCL6-IV, reduced accumulation of the derived SCL6-IV mRNA as compared to infiltration of the SCL6-IV gene alone. This reduction was accompanied by the accumulation of SCL6-IV siRNAs and both the miR171 induced reduction in SCL6-IV mRNA and the accumulation of siRNAs was blocked by HC-Pro. miR172, which blocks translation of AP2 mRNA in Arabidopsis, also induces degradation of AP2 mRNA in the Agro system. However, unlike the case with miR171 and its target, HC-Pro did not inhibit miR172 induced degradation of AP2 mRNA, but rather enhanced it. This finding suggests that there are at least two pathways for miRNA directed mRNA degradation in plants, those sensitive and those insensitive to HC-Pro. The role of deadenylation and decapping in miRNA mediated degradation is currently under investigation.

T09-055

Histone H1 is required for maintaining the pattern of DNA methylation in Arabidopsis

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Histone H1 is an abundant component of eukaryotic chromatin that is thought to stabilize higher-order chromatin structures. In contrast to extensive knowledge about the functional significance of core histones, very little is known about the biological function of histone H1. Despite its evolutionary conservation and binding at a critical position on the nucleosome surface, histone H1 has been shown to be non-essential in both protista and fungi. In higher eukaryotes, the presence of many isoforms of this protein has made assessment of the global function of H1 more difficult.
We have used double-stranded RNA silencing to suppress all the H1 genes of Arabidopsis thaliana. Plants with a >90% reduction in H1 expression exhibited a spectrum of phenotypic defects, resembling the pleiotropic phenotype observed in various DNA methylation-deficient mutants. These phenotypic defects increased in subsequent generations and segregated independently of the H1 silencing dsRNA construct. We have shown that in plants lacking histone H1 the level of DNA methylation is changed. We observed both increases and decreases of DNA methylation occurring in a locus-specific manner. These findings reveal an important and previously unrecognized biological function of linker histones in maintaining specific patterns of DNA methylation.

T09-056

Chromatin assembly and gene silencing during development involve MSI1-like proteins

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MSI1- and RbAp46/48-like proteins are components of several complexes involved in nucleosome assembly, histone acetylation or deacetylation and other chromatin modifying processes. Previously, mutants in MSI1-like genes were described only in budding yeast and C. elegans. In the model plant Arabidopsis, there are five genes encoding MSI1-like proteins. Recently, one member of this family, MSI1, has been shown to form a complex with chromatin assembly complex (CAC) subunits CAC1 and CAC2 of Arabidopsis, and this complex has nucleosome assembly activity in vitro (Kaya et al., 2001). To elucidate the role of MSI1-like proteins in vivo, we studied transgenic plants with altered MSI1 protein levels and T-DNA insertion mutants. While plants with strongly elevated MSI1 protein levels appear mostly normal, development of plants with reduced MSI1 levels is strongly affected. The phenotype becomes progressively more severe through the plants life cycle, indicating a role of MSI1 in maintaining correct patterns of gene expression through successive rounds of cell divisions. Interestingly, the observed phenotypes only partially overlap with the phenotypes of the CAC1 and CAC2 mutants fas1 and fas2, strongly suggesting major roles of MSI1 in addition to chromatin assembly (Hennig et al., 2003). During seed development, MSI1 is part of the FERTILISATION INDEPENDENT SEED complex that represses seed development in the absence of fertilization (Köhler et al., 2003). Here, we will present results of a comparative analysis of mutants in FAS1, FAS2 and in MSI1-like genes. Importantly, loss of different MSI1-like proteins leads to clearly distinct phenotypes indicating strong functional diversification in this protein family and making Arabidopsis an ideal model system to study the function of MSI1-like proteins.

Hennig et al. (2003) Dev. 130, 2555-65
Kaya et al. (2001) Cell. 104, 131-42
Köhler et al. (2003) EMBO J. 22, 4804-14

T09-057

MicroRNA regulation of lateral organ separation in Arabidopsis

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MicroRNAs (miRNAs) are ~21-nucleotide (nt) RNAs that negatively regulate gene expression in plants and animals. Most known plant miRNAs target transcription factors that influence cell fate determination. Here we identify a developmental role for miR164-directed regulation of Arabidopsis NAC-domain genes, which encode a family of transcription factors that includes CUP-SHAPED COTYLEDON1 (CUC1) and CUC2. We found that constitutive overexpression of miR164 recapitulates cuc1 cuc2 double-mutant phenotypes, including cotyledon and floral organ fusions. miR164 overexpression also leads to phenotypes not previously observed in cuc1 cuc2 mutants, including leaf and stem fusions. These likely reflect the misregulation of other NAC-domain mRNAs, including NAC1, At5g07680, and At5g61430, for which we detect miR164-directed cleavage products. Expression of a miR164-resistant version of CUC1 mRNA from the CUC1 promoter causes alterations in Arabidopsis embryonic, vegetative and floral development, including cotyledon orientation defects, loss of rosette leaf petioles, dramatically misshapen rosette leaves, one to four extra petals, and one or two missing sepals. These results demonstrate that miR164-directed regulation of CUC1 is necessary for normal embryonic, vegetative, and floral development. They also show that proper miR164 dosage or localization is required for separation of adjacent embryonic, vegetative, and floral organs, thus implicating miR164 as a common regulatory component of the molecular circuitry that controls the separation of different developing organs and thereby expose a posttranscriptional layer of NAC-domain gene regulation during plant development.

T09-058

Developmental defects triggered by ectopic expression of microRNAs in Arabidopsis

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miRNAs regulate the activity of target genes through either translational suppression or cleavage of target mRNAs. Many miRNA targets encode transcription factors that control development. In Arabidopsis, recent studies demonstrate that miRNAs such as miR319 (JAW) and miR172 direct normal leaf and flower development by controlling transcription factor abundance. To identify other developmental pathways regulated by miRNAs, members from each known family of Arabidopsis miRNAs were overexpressed in plants by placing the sequences corresponding to precursor hairpins under the control of a constitutive promoter. Several miRNAs (JAW, 156, 157, 165, 167, 170, 171 and 172) elicited pronounced phenotypes in transgenic plants. In general, plants that overexpress miRNAs displayed defects associated with disruption of either normal meristem patterning or organ morphology. Arabidopsis plants that overexpress miR167 (targeting ARF6 and ARF8) had defects in flower development and leaf morphology, and displayed growth defects similar to those observed in auxin signalling mutants. Plants that overexpress miR171 (targeting SCARECROW-LIKE factors) displayed loss of axillary meristem maintenance and defects similar to plants with altered gibberellic acid metabolism. Results showing how these miRNAs integrate with hormone signalling pathways will be presented.

T09-059

Inheritance of methylation patterns of transgenes displaying post-transcriptional gene silencing in Arabidopsis thaliana

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The role of transgenic plants in research and agriculture increased over the last decades and will be of major interest in future. Unfortunately, independent transgenic lines generated with the same construct often vary considerably with respect to transgene expression levels and gene silencing is frequently observed. In this work cytosin methylation of transgene sequences as a hallmark of post-transcriptional gene silencing (PTGS) was analysed in Arabidopsis thaliana T-DNA lines. Applying digestion with methylation sensitive restriction enzymes followed by Southern blot analysis or PCR detection, we examined CNN, CNG and CG methylation of transgene sequences in transgenic lines harbouring different copy numbers of the ß-glucuronidase (GUS), streptomycin-phosphotransferase (SPT) or green fluorescent protein (GFP) genes under the control of the CaMV 35S promoter. PTGS of the different transgenes was found to be highly correlated with methylation of sequences located in the transcribed transgene regions. In contrast, methylation of promoter sequences was not detected regardless of whether the plants expressed a particular transgene highly or whether they displayed transgene silencing. We exploited the finding that the progeny of silenced plants showed fully restored transgene expression early in plant development to analyse the heritability of CNN, CNG, and CG methylation. Our results indicate that methylation patterns are partially maintained in the progeny of silenced lines despite the fact that the plants express the transgenes highly. Thus, methylation of sequences located in the transcribed transgene regions per se does not interfere with transgene expression.

T09-060

Transcriptional and chromatin regulation: a dynamic affair

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The genomes of eukaryotic cells are packaged into a complex structure called chromatin. The basic unit of chromatin is the nucleosome, which comprises ~ 146 bp of DNA wrapped twice around a core histone octamer containing two copies each of histones H2A, H2B, H3 and H4. A linker histone, such as H1, interacts with the nucleosome core and with adjacent linker DNA. Nucleosome arrays fold into higher-order structures, reaching their most compact state in metaphase chromosomes during mitosis. Chromatin structure influences all DNA-related nuclear processes including transcription, replication, recombination and repair. Variations in chromatin structure control access of underlying DNA to regulatory factors and hence modulate gene expression. In general, highly condensed chromatin is associated with transcriptionally silent genes whereas decondensed chromatin contains actively expressed genes. Changes in chromatin configuration are mediated by a variety of enzymes that covalently modify DNA or histones, or disrupt histone-DNA contacts and displace nucleosomes. Recent years have witnessed major advances in understanding how these enzymes complexes act in concerted ways to regulate chromatin structure and gene transcription. I will summarize some of these findings and discuss emerging evidence that RNA plays a key role in targeting DNA and histone modifications to specific regions of the genome.

T09-061

A gene-specific RNA sensing mechanism, not position effects, triggers silencing in T-DNA transformants

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Pronounced variability of transgene expression and transgene silencing are commonly observed among independent plant lines transformed with the same construct. Single-copy T-DNA lines harbouring reporter genes of various kind and number under the control of a strong promoter were established in Arabidopsis for a comprehensive analysis of transgene expression. Characterisation of 132 independent transgenic lines revealed no case of silencing due to site of T-DNA integration. Below a certain number of identical transgenes in the genome, gene copy number and expression were positively correlated. Expression was high, stable over all generations analysed, and of a comparable level among independent lines harbouring the same copy number of a particular transgene. Conversely, RNA silencing was triggered if the transcript level of a transgene surpassed a gene-specific threshold. Transcript level mediated silencing effectively accounts for the pronounced transgene expression variability seen among transformants. It is proposed that the RNA sensing mechanism described is a genome surveillance system that eliminates RNA corresponding to excessively transcribed genes, including transgenes, and so has an important role in genome defence.

T09-062

Functional characterization of the Polycomb group protein MEDEA from Arabidopsis thaliana

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The medea (mea) mutant was identified in a genetic screen for gametophytic mutants showing a maternal effect. MEA encodes a SET domain protein similar to the Enhancer of zeste (E(Z)) protein from Drosophila and regulates growth of embryo and endosperm in Arabidopsis. MEA is localized in a 600 kDa protein complex together with the WD40 domain proteins FIE and MSI1. FIE and MSI1 are homologs of the E(Z) interacting proteins ESC and p55 from Drosophila, respectively. Polycomb group proteins form multimeric protein complexes that maintain transcriptional expression states of target genes by altering the chromatin structure. Interestingly, their structure and function is conserved between plants and animals. In both, they affect homeotic gene expression and cell proliferation. It has been demonstrated that the E(Z)/ESC complex in Drosophila has histone methyltransferase activity. The structural similarities of the E(Z)/ESC complex with the MEA/FIE complex suggests that the MEA/FIE complex might have histone methyltranferase activity as well. Based on the structural conservation of the SET domain between different organisms, we follow genetic and biochemical approaches to characterize the composition and function of the MEDEA protein complex.

T10 Novel Tools, Techniques and Resources

T10-001

A global view of cellular identity in the Arabidopsis root

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Organs are composed of mosaics of highly specialized cells, of which higher plants have at least 40 distinct types. A critical task in development is regulating gene expression to create the differentiated cells types that make organs function. We have developed a cell-type specific profiling method that breaks apart the plant root into constituent cells by protoplasting. GFP marked cell types or tissues are isolated using a fluorescence activated cell sorter and their transcriptional contents are analyzed with microarrays. We have analyzed the global expression profile of five cell types and tissues of the Arabidopis root, which contains about 10 major cell types, and three different stages of root development. This has resulted in a root map of gene expression, which shows that the majority of genes are differentially regulated within the root. We will present analysis of gene duplicates in the root, which shows two distinct trends. 1) There is a high potential for genetic redundancy; for example, 70 percent of the 300 or so transcription factors expressed in the root have a duplicate with at least partially overlapping expression. 2) On the other hand, many genes have another family member that is expressed in a distinct domain within the root. In some cases, entire pathways are duplicated in different domains of the root. Thus, gene duplication appears to play a role in tissue and cell type specialization.

T10-002

TILLING - high throughput functional genomics

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One possible definition of "functional genomics" is: Development and application of genome-wide experimental approaches to assess gene function by making use of the information and reagents provided by structural genomics (Hieter & Boguski, 1997).
An effective approach to do genome-wide functional genomics can be provided by the reverse genetics method TILLING. Targeting induced local lesions in genomes was first performed by the lab of Steven Henikoff, Seattle. It is a general method to identify chemically induced point mutations which can be applied to almost any organism.
The basic methodology for TILLING is: Samples from the organism of choice undergo random mutagenesis. These samples are then used to generate a founder population which finally is crossed. Gene segments from these mutagenized samples are amplified using asymmetrically labelled fluorescent primers, and products are denatured and reannealed to form heteroduplexes between the mutated sequence and its wild-type counterpart. These heteroduplexes are nicked at mismatched sites by the endonuclease CEL I. Following cleavage, products are analyzed on denaturing polyacrylamide gels using the LI-COR® DNA analyzer system.
With this technique not only knock-out but also partial loss-of-function genes can be created/detected. No transgenic steps are required and it is perfect for breeding and long term storage.
A recent publication (Comai et al., 2004) also proves this technique to be ideal for the detection of natural polymorphisms: "Ecotilling" allows the rapid and cost effective detection of genetic variation in many individuals. Not only single nucleotide polymorphisms (SNPs) are identified but also insertions and deletions (1-30 bases).
The instrument which is uniquely suited for TILLING and Ecotilling is the DNA analysis system from LI-COR® Biosciences (Colbert et al., 2001; Till et al., 2003; Wienholds et al., 2003). This is due to the use of two-colour infrared fluorescence detection which provides high sensitivity, a wide dynamic range and two true gel images during electrophoresis. This system has demonstrated an outstanding throughput performance of up to 2000 samples and up to 2 million bases screened per day in the Arabidopsis Genome.

T10-003

Quantitative Immunodetection using Infrared Technology

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A new technology for sensitive and quantitative 2-colour immunodetection - the Odyssey® Infrared Imaging System - has recently been developed by LI-COR® Biosciences.
The system combines unparalleled sensitivity in fluorescence detection with precise quantification and provides new perspectives for quantitative analysis of immuno-stained samples. The Odyssey® is unique in that it combines two independent Infrared (IR) laser/detection systems for simultaneous detection of two targets: e.g. in signal transduction experiments both total protein and phosphorylated protein can be simultaneously detected and quantified.
In-Cell Western ¨ a brand new application for the Odyssey® - provides a new tool for drug discovery and signal transduction research. This application enables antibody mediated protein detection and quantification in the whole cell environment. Again two targets can be simultaneously detected in a microplate format and normalised for cell numbers in each well. Consequently this technique is well suited for high throughput quantitative analysis of protein modifications in fixed cells. Compared to traditional Western Blots, experiments are carried out in a 96 or 384 well format, saving time while gaining accuracy: Time consuming and error-prone steps such as lysate preparation, gel loading, electrophoresis and membrane transfer are eliminated with In-Cell Westerns.
This flexible and powerful imaging system can also be used for analyzing Northern Blots, Southern Blots and EMSAs, tissue section and small animal imaging, as well as for documentation of 1D/2D-Coomassie Gels.

T10-004

K+ channel interactions detected by a system optimized for systematic studies of membrane protein interactions

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Organization of proteins into complexes is crucial for many cellular functions. Yet most proteomic approaches primarily detect protein interactions for soluble proteins, which are less suitable for membrane-associated complexes. We constructed a mating-based split ubiquitin system (mbSUS) for systematic identification of interactions between membrane proteins. mbSUS allows in vivo cloning of PCR products into a vector set, detection of interactions via mating, regulated expression of baits, and improved selection of interacting proteins. Cloning is simplified by introduction of l attachment sites for GATEWAY. Homo- and heteromeric interactions between Arabidopsis K+ channels KAT1, AKT1 and AKT2 were identified. Tests with deletion mutants demonstrate that the C-terminus of KAT1 and AKT1 is necessary for physical assembly of complexes. Screening of a sorted library of 84 plant proteins with potassium channels as bait revealed different oligomerization properties between KAT1, AKT1 and AtKC1, and allowed detection of new putative interacting partners of KAT1 and AtKC1. Together with the detection of AMT1 ammonium transporter oligomers (Ludewig et al., 2003), this shows that mbSUS is suited for systematic analysis of membrane protein interactions.

Ludewig, U., Wilken, S., Wu, B., Jost, W., Obrdlik, P., et al. (2003) Journal of Biological Chemistry 278, 45603-45610

T10-005

Functional Genomics using RIKEN Arabidopsis Full-length cDNAs

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Full-length cDNAs are essential for the correct annotation of genomic sequences and for the functional analysis of genes and their products. Using the biotinylated CAP trapper method, we have constructed full-length cDNA libraries from Arabidopsis plants and isolated 224,641 RIKEN Arabidopsis full-length (RAFL) cDNA clones. They were clustered into 18,127 nonredundant cDNA groups, about 70% of predicted genes1). We have determined full-length cDNA sequences of 13,427 RAFL cDNA clones2). Full-length sequencing of the RAFL cDNA clones are in progress. We have also used the RAFL cDNAs for the microarray analysis3) of expression profiles in more than 200 different experimental conditions.
Furthermore, we have applied the RAFL cDNAs to transgenic analysis, such as overexpression, studies on protein-protein and protein-DNA interactions using wheat germ cell-free protein synthesis system, and structural analysis of Arabidopsis proteins. We have determined the domain structure of 31 proteins containing plant specific-type transcription factors.
In this meeting, overview of our RAFL cDNA project will be presented.

1) Seki et al. (2002) Science 296, 2) Yamada et al. (2003) Science 302, 3) Seki et al. (2004) J. Exp. Bot. 55.

T10-006

Professor

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Systems Biology Maps: tools and approaches for the integration and analysis of genome-scale data

Post-genomic era research is focusing on studies to attribute functions to genes, their encoded proteins, and to describe the regulatory networks controlling biochemical, protein synthesis and signal transduction pathways. To facilitate the analysis of experiments using post-genomic technologies, new concepts for linking the vast amount of raw data to a biological context have to be developed. Visual representations of pathways help biologists to understand the complex relationships between components of metabolic networks and provide an invaluable resource for the knowledge-based integration of transcriptomics, proteomics and metabolomics data sets.

A visual interface, termed Systems Biology Maps, for an annotation database covering biochemical and cell biological pathways in the model plant Arabidopsis thaliana is presented. The Systems Biology Maps tool allows the knowledge-based integration and analysis of genome-scale datasets (oligonucleotide microarrays, proteomics, metabolomics, flux control measurements, phenotyping), which is demonstrated using various experiments with Arabidopsis.

T10-007

A Comparison of Global gene Expression and MPSS profiling in Arabidopsis thaliana.

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MPSS stands for Massively Parallel Signature Sequencing, a technique invented and commercialized by Lynx Therapeutics, Inc. of Hayward, California. Brenner et al. have described MPSS and related technologies in publications (Nature Biotechnol. [2000] 18:630-634, and PNAS [2000] 97:1665-1670). Like SAGE (Serial Analysis of Gene Expression), MPSS produces short sequence tags produced from a defined position within an mRNA, and the relative abundance of these tags in a given library represents a quantitative estimate of expression of that gene. The MPSS tags are 17 bp in length, and can uniquely identify >85% of all genes in Arabidopsis.
To date, eleven Arabidopsis thaliana MPSS libraries are available for analysis at the following URL (http://www.dbi.udel.edu/mpss). The in silico transcriptional data obtained from these libraries (callus, inflorescence, shoot, root, silique etc) has been compared to global gene expression data obtained by hybridization of cRNA derived from the same RNA populations. The DNA microarrays used were the new Agilent Arabidopsis 3.0 microarray (42k format); this array contains 60-mers synthesized in situ array based on the 26,207 annotations for protein encoding transcripts in the TIGR/TAIR ATH1 annotation (version 5.0). Another 11,000 features were included in the array which include both unannotated 10,000 transcripts identified by whole genome tiling arrays and by MPSS data, and about 1000 mi RNAs.We also included some replicated “housekeeping genes” as internal controls on the array. Total features in this array are 37,478 leaving an additional c4500 features available for further upgrades of this array. We examined the detection limits of the Agilent microarray and detection system to measure changes in relative abundance of low (single) copy transcripts, by reference to the Arabidopsis MPSS database; the MPSS data identifies the specific genes from which the signatures are derived, and a quantitative abundance level in parts per million (PPM). We present our preliminary results from this comparison.

T10-008

Proteomic analysis of nuclear components from Arabidopsis suspension cells and Arabidopsis plants

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The chromatin structure plays a crucial role in the processes of development and differenciation, and it undergoes complex epigentic regulation. The chromatin proteome can be viewed as a gene-regulatory system in metastable (fluid) equilibrium. The regulatory switches of the system are stochastic outcomes of the fluctuations in local concentration of the hetero- vs. euchromatin proteins (competing for the available binding sites) and the environmental conditions (eg. temperature). The proteomic analysis of the Arabidopsis nuclei has been performed, with a goal of comparison of two chromatin proteomes ¯ isolated from suspension-grown A. thaliana cells and axenically-grown A. thaliana plants of the laboratory strain Columbia. The nuclear protein components have been isolated, resolved on the 2D gels, and analysed by mass spectrometry. By using the multidimensional protein identification technique (MudPIT) the identification of several protein isoforms is in progress, and it focuses on the comparison of different histone variants and their modifications (known as a “histone-code”) found in Arabidopsis plants. More than 160 proteins have been reliably identified in both systems and they were subjected to the Internet-based tools of bioinformatic analysis, in order to predict their subcellular localization and biochemical properties. One example of such identified protein is the HD2-type histone deacetylase, which we plan to follow as a marker of histone modifications. These studies are also followed up by a similar analysis with the Arabidopsis nuclear and chromatin proteomes isolated from four climatic ecotypes differing in response to transient cold (vernalization phenomenon), and from mutant lines missing specific nuclear proteins (eg. variants of histone H1, chromatin-mediated histone methylase, SWI3 type chromatin rearrangement factors).

T10-009

Bimolecular fluorescence complementation - a novel tool for in planta protein interaction studies

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Selective interaction between proteins is a crucial step in the regulation of their function. However, a universal reliable method has still to be found for demonstration of protein-protein interaction in living plant cells.
Recently, bimolecular fluorescence complementation (BiFC) assay was successfully used to demonstrate cross-family interactions between bZIP and Rel proteins in mammalian cells (Hu et al., 2002). This approach is based on reconstitution of fluorescent YFP by its two non-fluorescent fragments, which is mediated by protein-protein interaction. We adapted this assay to verify both inter- and intra-family interactions of plant proteins in plant cells. Comparable results were obtained from several independent transient expression approaches, namely biolistic transformation of onion cells, transformation of Arabidopsis protoplasts and Agrobacterium infiltration of tobacco leaves. We were able to distinguish between abilities of particular proteins to form homo- and/or heterodimers as well as to identify the subcellular localization of specific dimers. By BiFC analyses we also confirmed results obtained from yeast two-hybrid screen, which depicted LSD1 as potential cytosolic retention factor for bZIP10 (LSD1, lesions simulating disease 1, is a negative regulator of plant cell death). Our results clearly demonstrate the main advantages of BiFC: on one hand, it is comparatively simple, on the other, it allows not only verifying protein-protein interactions but also to visualize their specific localization and dynamics.
Note added: The usefulness of BiFC in plant protein-protein interaction research is also demonstrated on a poster presented by K. Shichrur, K. Bracha-Drori, M. Oliva, A. Katz, R. Angelovici, N. Ohad and S. Yalovsky "Plant Bimolecular Fluorescence Complementation (PBFC) ¯ a system for detecting protein-protein interactions in plants".

Hu C.-D., Y. Chinenov, T.K. Kerppola (2002) Molecular Cell, 9: 789-798.

T10-010

Identification of apoplastic plant proteins by Transposon Assisted Signal Trapping (TAST)

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Plant cell walls are composed of independent but interacting networks of carbohydrates, proteins, and aromatics. Since protoplasts have no cell walls, but are able to regenerate a new wall within a couple of days they are very useful for examining cell wall biosynthesis, in particular the assembly of polymers in the apoplast.
Our work focuses on the identification of potential proteins that are involved in the wall assembly process specifically in the apoplast. To identify those candidate genes a newly developed approach, named Transposon Assisted Signal Trapping (TAST), was used.
TAST involves the generation of a cDNA library derived from regenerated protoplasts of an Arabidopsis cell suspension culture. The cDNA library was tagged with a transposon containing a secretion signal less ß-lactamase as selectable marker and was transformed into E.coli. The E.coli host cells can only grow on selective medium in which an in-frame fusion of ß-lactamase and a gene containing an N-terminal secretion signal has taken place.
Using the TAST system on regenerating protoplasts, 600 tagged colonies were selected and the upstream or downstream gene sequences of the transposon insertions sites were determined. The corresponding sequences assembled into 154 different Arabidopsis contigs. Sixty percent of the isolated genes were predicted by SignalP to contain a signal peptide targeting the protein to the apoplast.
The results demonstrate that TAST is a useful tool to identify gene sequences that encode apoplastic proteins.

T10-011

Plant resources database at the MPI-MP

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The MPI for Molecular Plant Physiology has a large and scientifically as well as financially precious collection of transgenic plants, mutants, RIL- and NIL lines. Further material is produced in high-throughput projects. In addition, plant material for RNA-expression and metabolite profiling is cultivated in tightly controlled and monitored environments. To make optimal use of these resources for genomics projects, we installed a laboratory information management system that provides information on genetic features and environmental history of each plant grown at the MPI. The technical realisation is based on the commercially available, Oracle-based LIMS system Nautilus™. Data import and export is realised via XML protocols. In the system, all processes altering the genetic status of the plant (e.g. transformation, crossing) are modelled as workflows that link parent to offspring. Location tracking of the plants in the greenhouse is based on barcode labels and portable scanners, thus reducing work costs for data entry. Scientist interface the system via html-pages or SQL queries. Automatically generated sample numbers provide a link to the analytical platforms (e.g. transcript and metabolic profiling). Thus, the systems provides background information for data mining in genomics and helps to make optimum use of the genetic and analytic resources of the MPI-MP.

T10-012

Development of a quality-controlled cDNA micro-array method for expression profiling

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RNA-Expression profiling on micro-arrays is used to identify candidate genes or map gene expression networks. The major advantage is the multiparallel measurement for a high number (>104) of genes. However, the high cost restricts the number of replicates n. Theoretical power analysis based on GLM models indicates that 2fold induction will be overlooked in more than 50 % of the genes unless between-sample variation is reduced to CV < 70 % if n < 6. To reduce technical variation, we developed a quality-controlled protocol for RNA expression profiling based on commercially available cDNA micro-array slides. mRNA is isolated with magnetic beads and first-strand cDNA synthesis is performed on bound RNA. Subsequent melting and 2nd strand synthesis leaves RNA intact for further analysis. The quality of the mRNA and the subsequent cDNA synthesis is controlled by RT-PCR. cDNA is labelled by covalently binding fluorescent dyes to the nucleotides. After hybridisation of the slides, laser-scanner fluorescence images are automatically analysed with a high reproducibility (CV £ 6 %) for more than 90 % of the genes. Finally, data are quantile-normalised. Analysis of 6 biological replicates processed by this method indicated a total CV of £ 45 % for 90 % of the genes. In these genes, n = 6 allows the detection of 2fold changes with a reasonable type II error rate (10 %). (BMBF: 0312632C).

T10-013

Report of Resource Project in RIKEN BRC

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RIKEN BRC has joined the National Bioresource Project of Japan, and preserves and distributes various biological materials. Plant resources including Arabidopsis seeds and cDNA clones are distributed from the Experimental Plant Division. Since 2002, we have distributed approx. 7,000 plant materials to the world. Early in this year, we succeeded to the wild type and mutant collections of SASSC. We will continue our efforts to serve for plant science community in the world.

T10-014

A molecular atlas of transcription factor expression patterns in Arabidopsis

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We are undertaking a systematic survey of spatial and temporal patterns of Transcription Factors in Arabidopsis using high-throughput mRNA in situ hybridisation (ISH). By generating an extensive map of expression patterns in roots, shoots and siliques, we have provided another criterion with which to identify candidate genes controlling plant development. Having identified candidate genes we can use reverse genetics to determine their function in the plant. Using this approach we have already identified candidate genes involved in root epidermal development and cell cycle regulated chromatin organisation processes. By standardising the ISH technique we can provide spatial information about the expression patterns of large numbers of genes which complements the information gained by other means such as microarrays. The approach also opens up the potential for large scale comparison of gene expression patterns between species.

T10-015

A method to isolate chloroplasts from specific cell types of Arabidopsis thaliana

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In multicellular organisms, cells and their organelles differentiate to perform specific functions. In plants this is exemplified within a leaf where different cell types contain chloroplasts of different sizes and structures, enabling photosynthesis to be efficient (1). Photosynthetic properties also vary between cell types, for example, recently it has been shown that cells located around the vascular bundles in petioles and stems of C3 plants show characteristics of C4 photosynthesis (2). However, to date one of the limitations to studying photosynthetic properties of individual cell-types is our inability to isolate chloroplasts from specific cell types.

In order to address this problem we are developing a method to tag chloroplasts from specific cell-types and subsequently isolate them. As a tag the gene coding for the yellow fluorescent protein (YFP) was fused to a gene that codes for an outer envelope protein from pea. The fusion protein inserts into the outer chloroplast envelope membrane with YFP located on the cytosolic side of the membrane. We are using tissue specific promoters and enhancer-trap lines to express the fusion construct in chloroplasts of different cell-types of Arabidopsis thaliana leaves and stems. Magnetic beads coated with anti-GFP antibody are used to isolate the YFP-tagged chloroplasts from a mixed population. Our initial results show that we are able to specifically isolate YFP-labelled chloroplasts.

(1) Terashima et al., (1986) Plant Cell Physiol., 27: 1023-1031
(2) Hibberd & Quick, (2001) Nature, 415: 451-454

T10-016

Plant Bimolecular Fluorescence Complementation (PBFC) ¯ a system for detecting protein-protein interactions in plants

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Protein function is often mediated via formation of stable or transient complexes. Currently, however, there are no simple and reliable methodologies for detecting protein-protein interactions in vivo in plants. To detect protein-protein interactions in vivo, we have adapted the recently published bimolecular fluorescence complementation (BiFC) assay (Hu et al., 2002) to plants. The Yellow Fluorescent Protein (YFP) was separated into two non-overlapping N- and C-terminal domains designated YN and YC, respectively. To reconstitute YFP fluorescence, YN and YC were fused with putative interacting protein pairs and co-expressed in plants. The feasibility of the PBFC system was demonstrated with two known interacting proteins pairs: Arabidopsis protein farnesyltransferase (PFT) A and B subunits and the polycomb proteins FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) and MEDEA (MEA). Reconstitution of YFP fluorescence occurred following transient expression of either protein pair in leaf epidermal cells of Nicotiana benthamiana or Arabidopsis. The fluorescence emission spectra of native and reconstituted YFPs were identical, confirming their specificity. Furthermore, no YFP fluorescence was detected following co-expression of non-fused YN and YC or non-interacting protein pairs. Monoclonal antibody tags fused to YN and YC enabled cross verification of protein expression and interactions by immunoblots and co-immunoprecipitations. Reconstitution of YFP fluorescence was detected in the respective subcellular compartment (cytoplasm and nuclei) for each protein pair. The simplicity PBFC and the ability to detect protein-protein interactions at different subcellular compartments make it an attractive method for analysis of protein interactions and networks.

Note added: The usefulness of BiFC in plant protein-protein interaction research is also demonstrated on a poster presented by Christina Chaban , Michael Walter, Katia Schütze, Oliver Batistic, Claudia Oecking , Wolfgang Werr, Jörg Kudla and Klaus Harter, “Bimolecular fluorescence complementation - a novel tool for in planta protein interaction studies”.

Hu C.-D., Chinenov Y., Kerppola T.K. (2002) Molecular Cell, 9: 789-798.

T10-017

The essentials of tissue-specific protein and metabolite profiling - Laser Microdissection, LC/MS/MS and GCMS

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The vascular bundle is a tissue system of vital importance for higher plants. It appears to have much more functions than only the transport of water and metabolites, since also proteins, nucleic acids and signal substances are transported.
A powerful method for contamination-free collection of specific cell types from tissue sections is Laser Microdissection (LM) coupled to Laser Pressure Catapulting (LPC). This technique has been routinely used in mammals and is just starting to spread in plant sciences.
To demonstrate the applicability of LMPC for protein and metabolite analysis, we collected samples from different stem tissues from Arabidopsis thaliana. One strategy we used for the analysis of proteins was separation of intact proteins by classical two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) coupled to mass spectrometry. After tryptic digest, proteins were identified by matrix assisted laser desorption/ ionization (MALDI TOF MS) or hybrid mass spectrometry (QTOF MSMS). A drawback of 2D-PAGE for small sample amounts is the relatively high amount of material needed. Regarding the limited sample amount obtainable by microdissection, the combination of protease digest of the complex protein mixtures with HPLC separation and subsequent tandem mass spectrometry (LC/MS/MS) provides an alternative, promising tool for tissue specific proteome analysis.
Additionally, we optimise our sample preparation procedures to allow not only proteome analyses but also investigations of tissue-specific metabolite concentrations and even enzyme activities in microdissected samples.

T10-018

AtGenExpress ¯ Expression atlas of Arabidopsis Development

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The activity of genes and their encoded products can be regulated in several ways, but transcription is the primary level, since all other modes of regulation (RNA splicing, RNA and protein stability, etc.) are dependent on a gene being transcribed in the first place. The importance of transcriptional regulation has been underscored by the recent flood of global expression analyses, which have confirmed that transcriptional co-regulation of genes that act together is the norm, not the exception. Moreover, many studies suggest that evolutionary change is driven in large part by modifications of transcriptional programs.

An essential first step toward deciphering the transcriptional code is to determine the expression pattern of all genes. With this goal in mind, an international effort to develop a gene expression atlas of Arabidopsis has been underway since fall 2003. This project, dubbed AtGenExpress, is funded by the DFG and the MPG, and will provide the Arabidopsis community with free access to a comprehensive set of Affymetrix microarray data. As part of this collaboration, we have generated expression data from 79 samples in triplicate focusing on development of wild-type Columbia (Col-0) and various mutants. Samples consist of a wide range of Arabidopsis tissues, including microscopically dissected organs, at various developmental stages. A meta-analysis of the data set will be presented.

T10-019

Novel Gene Discovery in Arabidopsis thaliana

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The latest annotation of the Arabidopsis genome estimates that there are 26,207 protein coding genes. Approximately 62% of these are represented in GenBank by a full length cDNA and another 11% are represented only by ESTs. The remainder has yet to be experimentally defined and only exist based on genome analyses using modeling algorithms that predict gene structure. At TIGR, our efforts have been focused on generating experimental evidence for these hypothetical genes by RACE PCR, various cDNA library construction strategies, and comparative genomics. From these studies, we have detected many instances of alternative splicing and variations in polyadenylation sites, as well as antisense gene expression and dicistronic transcripts. Additionally, through comparison of Arabidopsis and Brassica genomes, approximately 15,000 highly conserved intergenic regions have been identified, thus suggesting the presence of non-annotated novel genes. A pilot study with 192 of these regions confirmed expression of 52 genes suggesting that there may be thousands of genes not yet discovered. From these analyses, Gateway pENTR ORF clones are being produced and will be available through the Arabidopsis Biological Resource Center. These efforts will facilitate novel gene discovery, lead to improvements in genome annotations, generate a set of Arabidopsis ORF clones, and ultimately contribute to the genetic toolkit that is necessary for fulfilling goals of the Arabidopsis 2010 project.

Supported by the NSF 2010 Program

T10-020

RNAi for Plant Functional Genomics

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A major challenge in the post-genome era of plant biology is to determine the functions of all the genes in the plant genome. A straightforward approach to this problem is to reduce or knockout expression of a gene with the hope of seeing a phenotype that is suggestive of its function. Insertional mutagenesis is a useful tool for this type of study but is limited by gene redundancy, lethal knockouts, non-tagged mutants and the inability to target the inserted element to a specific gene. The efficacy of RNAi in plants using inverted repeat transgene constructs that encode a hairpin RNA (hpRNA) has been demonstrated and can overcome many of the deficiencies of insertional mutagenesis listed above. We have determined design rules for using hpRNA constructs and developed a series of vectors for dicots and monocots along with high throughput vectors using an in vitro recombinase system. Further developments of our RNAi vector system and its application will be described.

T10-021

NARC - Norwegian Arabidopsis Research Centre - University of Oslo

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NARC is a national technology platform, sponsored by the Norwegian Functional Genomics initiative (FUGE), that will offer Norwegian plant scientists resources and technology in the Arabidopsis thaliana model system. FUGE is the result of an initiative taken by the Norwegian research establishment; the underlying process has been supported by the Research Council of Norway. FUGE represents a cooperative effort between Norway?s universities and research institutions and the industrial sector.
NARC will establish a solid basis for basic plant molecular biology, applied plant research and plant breeding in Norway. The centre partners provide the basic technologies to utilize the powerful tools of comparative genomics and thereby to promote the quality of plant science in Norway. The establishment of NARC will result in more efficient use of resources and equipment available in the Norwegian plant science community. By taking advantage of the tools already available, gene function and interaction of gene products can be studied in great detail.
NARC is built on existing competence in Arabidopsis research at the Norwegian University of Science and Technology (NTNU), University of Oslo and the Agricultural University of Norway (NLH), and has a close collaboration with other FUGE resource centres, e.g. The Norwegian Micro Array Consortium, imaging and proteomics facilities and bioinformatics expertise centres.
At NARC - University of Oslo we focus on In situ hybridisation for cellular localisation and expression analysis of specific transcripts, and Yeast two-hybrid analysis for protein-protein interactions and identification of binding domains and interacting/assoaciated protein partners. Also researchers outside Norway may get access to our services at a reasonable price (contact e-mail: b.e.sather@bio.uio.no). See also our web-site at http://www.imbv.uio.no/mol/groups/narc/.

T10-022

Imposing rigorously identical water deficits to different Arabidopsis thaliana accessions. An automated system for high throughput analyses of plant responses to soil water deficit.

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In many studies of Arabidopsis thaliana responses to water deficit, drought is imposed either by dehydrating a detached plant on a bench or by withholding water supply at a given date for all studied genotypes. Plant responses identified with the first method are involved in survival to extreme water deficits, rarely encountered in agricultural conditions. The second method is closer to agricultural scenarios but is not reproducible between genotypes, which each deplete soil water at different rates depending on their leaf area and stomatal conductance.
A method was developed to impose identical soil water stati to different Arabidopsis thaliana accessions, thereby analysing rigorously the genetic variability of the responses of leaf expansion and plant transpiration to soil water deficit. This method allows decreasing soil water content rapidly and homogeneously, and subjecting plants to a constant soil water status set at a desired level for the whole period of analysis (days to months). An automaton based on this method was designed for high throughput analyses. It is composed of a steel frame supporting 500 pots and a mechanical arm able to move according to instructions provided by a software developed on Api-Graf IP. Displacement sensors, a balance, a tube for irrigation and a camera are loaded on this arm, allowing to weight, water and acquire a numerical image of each individual pot in the experiment. The whole system was set up in a controlled growth chamber and was used to impose identical water deficit scenario to 9 accessions of Arabidopsis thaliana. During their whole development (2 to 3 months), these accessions have been grown at identical soil water contents. This required different water supplies to each individual plant depending on its leaf area and transpiration rate. The responses of leaf expansion and transpiration at different soil water content could therefore be analysed in a reproducible way.

T10-023

Real-Time RT-PCR profiling of over 1,400 Arabidopsis transcription factors: Unprecedented sensitivity reveals novel root- and shoot-specific genes

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To overcome the detection limits inherent to DNA array-based methods of transcriptome analysis, we developed a real-time RT-PCR-based resource for quantitative measurement of transcripts for more than 1,400 Arabidopsis transcription factors (TF). Using closely spaced, gene-specific primer pairs and SYBR® Green to monitor amplification of double-stranded DNA, transcript levels of 83% of all target genes could be measured in roots or shoots of young Arabidopsis wild-type plants. Only 4% of reactions produced non-specific PCR products, and 13% of TF transcripts were undetectable in these organs. Measurements of transcript abundance were quantitative over six orders of magnitude, with a detection limit equivalent to one transcript molecule in 1000 cells. Transcript levels for different TF genes ranged between 0.001-100 copies per cell. Whole-genome Arabidopsis Affymetrix-chips detected less than 63% of TF transcripts in the same samples, the range of transcript levels was compressed by a factor of more than 100, and the data was less accurate especially in the lower part of the response range. Real-time RT-PCR revealed 26 root-specific and 39 shoot-specific TF genes, most of which have not been identified as organ-specific previously. Finally, many of the TF transcripts detected by RT-PCR are not represented in Arabidopsis EST or MPSS databases. These genes can now be annotated as expressed.

Czechowski et al. ; Plant J. 2004 Apr;38(2):366-79.

T10-024

Search for Tissue-specific Promoters in Arabidopsis

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In order to search for useful new tissue-specific promoters, we used Arabidopsis whole genome information from TIGR Arabidopsis thaliana database. The genes expressed exclusively in leaf, root, or seed were selected. To isolate the promoter regions that are 2 Kb upstream of the selected genes by PCR, a total of 880 primer sets were designed by Primer 3 program. We cloned each PCR product into a final binary vector pBGWFS7 using Gateway cloning system (>270 clones obtained as of May 2004). The promoter clones were transformed into Agrobacterium then subsequently into Arabidopsis by floral spray. The T1 transformants were obtained after Basta treatment and the T2 seedlings were subject for GFP/GUS expression analysis. Up to date more than 40 promoter candidates were analyzed and their expression by either GFP or GUS varied from none to weak tissue-specific to whole seedling regardless of origins of the genes. It was unexpected to find that expression patterns of GFP and GUS by each candidate did not match. Promoter activity through the whole life cycle was investigated only with a few candidates. We keep cloning and transforming the promoter regions to secure more candidates and further analysis of the selected promoter regions is in progress.

T10-025

Fox Hunting: A novel gain-of-function gene-hunting technique.

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Gene tagging is a powerful tool to assign the function of genes. However, even in the model plant of Arabidopsis, in which saturation mutagenesis was achieved from different plant resources, function of many genes belonging to different gene families remains unknown mainly due to the functional redundancy. Gain-of-function technique is an alternative way to address this problem. And the activation tagging is often used as one of them. One drawback of this system derives from the fact that the genes responsible are not always located near the integration site of the T-DNA or transposon in the genome. Therefore, genome walking of some kilo bp is required. In a model plant where the genome sequence is revealed such genome walking takes no time. However, in many non-model plants this genome walking hinders the use of the activation tagging technology for the comprehensive analysis of gene function due to the lack of genome sequence information.
We describe here the establishment of a novel gain of function system, in which genome walking is not required at all. The system was designed to apply it for functional genomics of non-model plants and named as Fox hunting system (Full-length cDNA over-expresser gene-hunting system) for random over-expression of a normalized Arabidopsis full-length cDNA library. Full-length cDNAs from Arabidopsis were sequenced and about 10,000 non-redundant clones were randomly expressed in Arabidopsis plants. The average size of the inserted cDNAs in 106 randomly chosen transformants was 1.4 kb with the range between 0.3 kb and 4.2 kb, and 43 sequenced clones were non-redundant. We observed 93 morphological mutant candidates while growing 1167 T1 transformants. One of these lines, F03024, showed a pale green phenotype with retarded growth that was semi-dominant in the T2 generation. The phenotype was recaptured when a full-length cDNA was rescued from the F03024 plant and re-introduced into wild type plants. In both F03024 and the re-transformed plants the corresponding gene was over-expressed in the pale green plants.
We will establish the similar resources using 13,000 non-redundant rice full-length DNA to introduce them into Arabidopsis plants. Applications and possibilities of this unique system will be discussed.

T10-026

Laser microdissection: A tool to perform tissue-specific transcript profiling using microarray analysis

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The measurement of RNA abundance of a given gene, or even a set of different genes, is one of the major goals of many experiments in the era of functional genomics. In multi-cellular organisms these type of experiments are complicated by the fact that tissues are composed of a range of specialized cell-types with different complex transcription profiles. Most standard experiments are based on the analysis of samples containing a mixture of heterogeneous tissues, thus averaging gene expression of a vast number of different tissue- and cell-types. This often prevents a meaningful interpretation of results, especially because abundant cell types and transcripts can mask rare ones. Attempts to overcome this averaging effect require refining the spatial resolution of analyses to a tissue- or even cell-specific level. Fundamental for the successful analysis of tissue-specific transcripts is the isolation of sufficient cell-specific material. We are using Laser Microdissection coupled to Laser Pressure Catapulting (LMPC), a new technique for touch-free single cell sampling routinely used in medical research.This method allows the collection of specific cell-types from tissue sections avoiding contaminations. We have optimized the protocols for fixation and embedding of Arabidopsis tissues and the isolation of minute amounts of RNA from the collected samples. After linear amplification, we can obtain sufficient amounts of labeled RNA to perform tissue-specific microarray hybridizations using Affymetrix Arabidopsis ATH1 Genome Arrays. Our protocols will enable comprehensive insights into the function of specific cell-types within heterogeneous plant organs.

T10-027

Proteome analyses of different plastid types: A first step towards a “systems” analysis of plastid development and differentiation

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Most chloroplast-localized proteins are encoded in the nuclear genome and imported into the chloroplast after translation in the cytoplasm. Although many chloroplast proteins have already been identified, they represent only a small fraction of the expected number of plastid proteins. We have developed a large-scale and high-throughput analysis of the proteomes from different plastid types including Arabidopsis chloroplasts and rice etioplasts. Altogether 690 proteins were identified with high confidence from Arabidopsis chloroplasts. Most proteins could be assigned to known protein complexes and metabolic pathways, but more than 30% of the proteins have unknown functions, and many are not predicted to localize to the chloroplast. Novel structure and function prediction methods provided more informative annotations for proteins of unknown functions. While near complete protein coverage was accomplished for key chloroplast pathways such as carbon fixation and photosynthesis, fewer proteins were identified from pathways that are down-regulated in the light. Parallel RNA profiling revealed a pathway-dependent correlation between transcript and relative protein abundance, suggesting gene regulation at different levels. The rice etioplast proteome contains proteins that have no homologue in the Arabidopsis chloroplast proteome and the majority of the 231 identified proteins function in plastid gene expression. Interestingly, the most abundant etioplast phosphoproteins are nucleus-encoded plastid RNA-binding proteins (RNPs) that play a role in plastid mRNA metabolism. We have analyzed the response of the etioplast proteome to 2 and 4 hours illumination and detected alterations in the phosphorylation status of these plastid RNPs. Our data support the model that the regulation of plastid mRNA stability by the interplay of endo- and exonucleases with RNA-binding proteins is the key mechanism that determines the development of photosynthetically active chloroplasts.

Baginsky, S. and Gruisssem, W. (2004). J. Exp. Bot., Epub.

Kleffmann, T. et al (2004). Current Biology 14, 354-62.

T10-028

Stable high-level transgene expression in Arabidopsis thaliana using gene silencing mutants and matrix attachment regions

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Basic and applied research involving transgenic plants often require consistent high-level expression of transgenes. Here we show that stable, high-level transgene expression is obtained using Arabidopsis thaliana post-transcriptional gene silencing (PTGS) sgs2 and sgs3 mutants (1, 2). In populations of first generation (T1) A. thaliana plants transformed with a b-glucuronidase gene (uidA) driven by a strong constitutive promoter, the incidence of highly expressing transformants shifted from 20% in wild type background to 100% in sgs2 and sgs3 backgrounds. Likewise, when sgs2 mutants were transformed with a visible marker gene under control of strong promoter, all transformants showed a clear phenotype, whereas such phenotype was only observed in about 1 out of 5 wild type transformants. Transgene expression remained high and steady in T2 sgs2 and sgs3 transformants, in marked contrast to the variable expression patterns observed in wild type T2 populations. For at least two different genes, we further show that flanking the gene expression unit by matrix attachment regions of the chicken lysozyme gene (chiMARs; 3) cause a boost in transgene expression in sgs2 plants, reaching up to 10% of the total soluble proteins. MAR-based plant transformation vectors used in a PTGS mutant background might be of high value for efficient high-throughput screening of transgene-based phenotypes as well as for obtaining extremely high transgene expression in plants (2).

(1) Mourrain 2000 Cell 101:533-542
(2) Butaye 2004 Plant J. in press
(3) De Bolle 2003 Plant Science 165:169-179

T10-029

Cellular and subcellular protein profiling: Comprehensive Shotgun LC/MS/MS analysis in Arabidopsis thaliana

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Determining a complete set of a plant proteome is a challenging task, which is complicated by proteome dynamics and complexity. Several proteome studies of plant cell organells have been reported, including chloroplasts and mitochondria. Information of specific organelle proteome analysis will provide new insights into pathway compartmentalisation and the localisation of proteins. Combining divers cellular and subcellular proteome analysis such as chloroplasts and trichomes (1) it is possible to obtain an overview of a large leave proteome dataset. Differential protein extraction procedures such as subcellular and FPLC fractionation and chloroform/methanol or Urea extraction prior to 2D LC/MS/MS analysis lead to the identification of the whole dynamic range of proteins (2,3). This new dataset allows us to pick out interesting biochemical pathways for further in depth studies.

1 Wienkoop et al. 2004 Phytochem. in press
2 Weckwerth et al. 2004 Proteomics 4:78
3 Wienkoop et al. 2004 RCM 18:643

T10-030

Development of IRES-mediated gene expression system in plants

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In order to produce transgenic plants expressing multiple foreign genes, it is necessary that the DNA fragment is efficiently introduced into the genome and stably maintained in later generations. Standard methods used to introduce multiple genes require transformation with T-DNA plasmid vectors that contain multiple promoter-cDNA-terminator cassettes. Although these strategies are widely used, it is known that the presence of repeated sequences often causes instability of introduced DNA sequences. To simplify the strategy for multigene transformation and for the efficient expression of introduced genes, we investigated the use of IRESes (Internal Ribosome Entry Sites) from animal picornavirus (EMCV) and tobamovirus (crTMV). The IRES element was inserted between ORFs of two reporter genes and tested for its translation enhancement effect by measuring reporter activities. Using transient expression assay by microprojectile bombardment and the analysis of transgenic plants we have shown that the insertion of IRES elements significantly increased translation of the second ORF. We have also investigated the efficiency of the IRES-mediated translation of several IRES-containing expression vectors in plant cells. Although crTMV-IRESes are active in both tobacco and Arabidopsis, our results indicate that the EMCV-IRES is able to direct efficient cap-independent translation in tobacco but not in Arabidopsis. Further examination of transgenic plants indicated a possible involvement of tissue specific regulation in the IRES-mediated translation efficiency.

In an attempt to identify IRES-mediated translation-associated factors, we conducted a series of genetic screening for Arabidopsis mutants with enhanced IRES activity. Seeds from transgenic Arabidopsis harboring the EMCV-IRES inserted between Renilla and firefly luciferase reporter genes were mutagenized by EMS treatment. As the result of screening of 30000 M2 plants by in vivo bioluminescence assay, we obtained 193 lines with higher luciferase activity as candidates for mutants with enhanced EMCV-IRES mediated translation efficiency.

Matsuo, N., Gilmartin, P.M., and Hiratsuka, K. (2004). Plant Biotechnology 21, 119-126.

T10-031

A novel approach to dissect the abscission process in Arabidopsis.

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Isolation and characterization of genes from a particular tissue sample has been used widely as a method to understand and study any process in the development of a plant. However, the origin of the transcripts isolated may have come from a mixture of specialised cells and not only from those involved in a unique process. Recently the use of laser capture microdissection technology and the production of GFP protoplasts, which are sorted by fluorescence, have been reported to resolve this difficulty. The use of the latest technology overcomes the problem of mixture of transcripts, however, manipulation of tissue either to embed the samples for microdissection or the generation of protoplasts may alter the transcript profile in the samples. In previous studies we demonstrated that a polygalacturonase (PGAZAT) from Arabidopsis (Gonzalez, et al., 2002) was expressed specifically in abscission zone cells by fusing the promoter of this gene to the reporter GFP. Using these transgenic lines we have now developed a strategy to generate cDNA and yeast libraries specifically from abscission zone cells of floral organs of Arabidopsis using the GFP tag. This approach has overcome some of the problems previously faced when studying development and it is enabling us to dissect the abscission process in a unique way.

Gonzalez-Carranza, et al. 2002. Plant Physiol. 128 (2): 534-543.

T10-032

Enrichment of phosphorylated proteins from A. thaliana

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Phosphorylation of proteins is one of the most important regulatory mechanism in living organisms. However, mainly due to the low abundance of phosphorylated proteins (about 10 % at any given timepoint) the analysis of protein phosphorylation is difficult.
The enrichment of these low abundance proteins is therefore highly desirable when trying to get a glance at the phosphorylation status.
We compared different methods for the enrichment of phosphoproteins out of sub-stoichiometric mixtures. The methods were evaluated with mixtures of model proteins and one of the methods was used to enrich phosphoproteins out of a complex leaf extract from A. thaliana. The successful enrichment was confirmed by staining an sds-gel with pro-q diamond stain, which selectively stains phosphorylated proteins.
From a digested gel band we identified the small subunit of rubisco as a phosphorylated protein in A. thaliana.
Furthermore, the method was used to enrich the phosphorylated peptides out of a tryptic digest from alpha-casein. The eluate contained only phosphorylated peptides as confirmed by beta-elimination of the phosphate-group. Thus, the proposed method demonstrates applicability even for the enrichment of phosphopeptides.

T10-033

Analysis of gene expression within single cells from Arabidopsis thaliana

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Transcript analysis at single cell level represents a major approach in understanding of plant complexity. The objective of the present project is focused on temporal and spatial expression of genes within single cells.
We have optimised the single cell sampling protocol for Arabidopsis thaliana and are able to collect routinely sap from trichome, basal and epidermal cells. This single cell sampling technique, enabled us to analyse in a minimal-invasive approache genes and transcripts. Samples are analysed not only using hybridisation of nylon filter membranes, also the expression of genes were studied by real time RT PCR experiments. Here we present exemplary results of single cell expression study using real time RT PCR from MYB 23 (At5g40330), an transcription factor high expressed in trichomes, from Arabidopsis thaliana.

T10-034

Gene Identification by Transcript Based Cloning

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1-john innes centre

The identification of genes required for a biological process, usually through mutation and positional cloning, is a critical first step in identifying the molecular players in that process. However, positional cloning can be laborious in organisms with sequenced genomes and impractical in organisms, such as crop plants, with large and complex genomes. We have developed a rapid gene cloning method based on transcript abundance and present here the identification of the Barley RAR1 gene in 1 month, as a demonstration of this technique. Other work cloning the Medicago DMI3 gene over a 3 month period is described elsewhere (Mitra et al PNAS 2004 101:4701-4705). We show that Transcript Based Cloning is a viable approach for gene cloning in crop species with large genomes.
The barley RAR1 gene is required for resistance against a range of pathogenic powdery mildew fungi, triggered by different race-specific resistance (R) genes. Cytological analyses have shown that two key phenotypes of race-specific resistance, hydrogen peroxide accumulation and hypersensitive cell death (HR) are compromised in rar1 mutants. The RAR1 gene has previously been cloned using a map-based cloning approach that took about 5 years to complete.

Raka M. et al A Ca2calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning. PNAS 2004 101: 4701-4705

T10-035

Integrative metabolic networks in plants: What do we get from it?

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For the construction of dynamic metabolomic networks at a systems level a high amount of statistical significant metabolic data is essential. Gas chromatography coupled to time of flight mass spectrometry (GC-TOF) makes these data available [1]. With this approach more than 600 metabolites from Arabidopsis thaliana leaf extracts can be detected in best time including known, classified and unknown chemical structures. Restricted to a set of approximately 80 identified metabolites we distinguished technical and biological variability. Accuracy of identification and quantification of these metabolites is improved by using internal standards and retention index- (RI-) marker. Together with mathematical and statistical analyses we constructed and compared metabolite networks from A. thaliana wild type and starchless mutant (PGM) plants with focus on specific participation of metabolites in different reaction networks and distinct correlations depending on the genomic background.
The throughput of metabolomics is still not achievable with protein profiling or other biochemical profiling strategies. However, the metabolic networks have to be complemented with “orthogonal” data to reveal causal connectivities [2]. In an integrative approach combining protein data represented by increased kinase activities in PGM plants that lack the plastidic phosphoglucomutase and metabolite fingerprints the co-regulation in biochemical networks was studied.

[1] Weckwerth et al. 2004 PNAS 101(20): 7809˜7814
[2] Wagner 1999 Biol. Phil. 14:83-101

T10-036

Protein profiling at the single cell level in Arabidopsis thaliana

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Recently genome profiling based on DNA chip arrays has become a powerful tool for large scale investigation of transcript levels and changes of gene activities in many aspects.
Special sampling techniques and highly sensitive molecular methods enable transcript analysis even at the single cell level.
Thus it is possible to identify genes that control most biological processes in living organisms.
But it is known that characterisation of protein profiling is equally important for understanding those processes as the proteome analysis is a fundamental step in systematic functional genomics.
Therefore a huge number of proteomic tools have been developed. However compared to the genome profiling techniques these methods are less sensitive and so far an application on the single cell level is a more difficult approach. Based on this fact we applied different methods for protein profiling at the single cell level. Novel methods like SELDI (Surface Enhanced Laser Desorption and Ionisation) and shotgun peptide sequencing (nano LC/MS/MS) are used to generate protein profiles from limited amounts of biological material.
We have tested the applicability of these techniques for the creation of protein profiles from specific cell types of Arabidopsis thaliana leaf epidermis. Protein extracts from pooled epidermal pavement and trichome cells were selected to gain insight of the proteome of specific single cells.
The outcome of the applied methods is shown and discussed in comparison to given results from experiments on the transcription level.

T10-037

Genome-wide Analysis of Recombination Frequency in Arabidopsis thaliana

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We use an AFLP map with more than 1250 physically located markers[1] to measure recombination frequencies. Because each AFLP gel contains several markers distributed over the genome, the Arabidopsis genome can be covered with a large number of AFLP markers by performing only a limited number of AFLP reactions. One application of such markers is in the first step of map-based cloning projects and we designed an AFLP-based genome-wide mapping strategy[2,3]. Another application is to measure the effect of certain mutations on genome-wide recombination frequency. We already performed an analysis of recombination events in two reference backcross populations between the Columbia and Landsberg erecta ecotypes. Regions exhibiting recombination frequencies that deviate significantly from the average could be identified. The results obtained provide us with a standard recombination map. As a next step we have started to study the effect of a number of mutations, known to affect mitotic recombination and/or biotic stress response pathways, on meiotic recombination. In addition we plan to use the AFLP markers to investigate the effect of meiotic-recombination mutants on genome-wide recombination frequencies. These mutants were selected based upon an inventory of genes resulting from a cDNA-AFLP transcript profiling experiment on male meiosis in Petunia hybrida. A cluster analysis has been performed on the obtained cDNA-AFLP profiles, grouping transcript fragments based on their expression profiles. We selected a group of as yet unknown genes that cluster with genes that have already been shown to play a role in meiotic recombination. To analyze the phenotype of mutants in such unknown genes, Arabidopsis orthologues have been identified and the corresponding T-DNA lines available from public libraries were obtained for further characterization. Within the framework of the recombination frequency project, the characterization of some of these mutants will focus on the effect of the mutated gene on genome-wide recombination frequencies.

[1] Peters JL et al (2001) Plant Physiol. 127, 1579-1589
[2] Peters JL et al (2003) Trends in Plant Sciences 8, 484-491
[3] Peters JL et al (2004) Theoretical and Applied Genetics 108: 321-327

T10-038

Exploiting Laser Capture Microdissection to elucidate spatial gene expression during plant embryogenesis.

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Embryogenesis in the model plant species Arabidopsis thaliana is a highly co-ordinated developmental process with distinct spatial and temporal patterns of cellular organisation, protein accumulation and gene expression.

Despite a histologically well characterised, and highly predictable level of cellular patterning and cell fate, we still have only a limited understanding of the underlying transcriptional changes required during embryogenesis.

Using a combination of the recent technologies of microarray analysis and laser capture microdissection the analysis of transcriptome changes between different zones of the developing embryo has been facilitated.

Bioinformatic analysis has been undertaken to identify transcription factors of putative importance in apical-basal pattern formation.

T10-039

The Genome Laboratory- Genomic Services for Arabidopsis Research

James Hadfield(1), Leah Clissold(1)

The Genome Laboratory, based at the John Innes Centre, is a genomic service provider for Arabidopsis and other plant and microbial research. It is a state-of-the-art laboratory. with high throughput robotics equipment to help with all your genome based projects.
Services include:
Sequencing
Affymetrix GeneChip processing
Genotyping
Library Screening and Handling
TILLING
PCR and Robotic handling

Gotot: www.jicgenomelab.co.uk for more information

T10-040

An alternative tandem affinity purification strategy applied to Arabidopsis protein complex isolation

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Tandem Affinity Purification (TAP) strategies constitute an efficient approach for protein complex purification from many different organisms. However, the application of such strategies in purifying endogenous Arabidopsis multi-protein complexes has not been reported yet. Here, we describe an alternative TAP system (TAPas) that successfully allows protein complex purification in Arabidopsis. In our newly generated TAP tag we have replaced the tobacco etch virus (TEV) protease cleavage site by the more specific and lower-temperature active rhinovirus 3C protease. In addition, the second purification step can be now performed through two different and alternative epitopes: a 6 His repeat or a 9 MYC repeat. We have generated Arabidopsis transgenic lines expressing different TAP fusions, both N-terminal and C-terminal, of light signaling pathway regulators. The protein expression level and, in specific cases, the complementation of the corresponding mutant phenotypes have been analyzed. We could validate our purification procedure by pulling down the entire COP9 signalosome when we used Arabidopsis plants expressing a TAP fusion to one of its components, CSN3, as starting material.

T10-041

The AGRIKOLA project: systematic RNAi in Arabidopsis

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6-UMR Génomique Végétale, Evry, France

The completion of the Arabidopsis genome sequence has provided an extremely important resource for scientists interested in comparative genomics. However, experimental proof of the function of less than 10% of the 27000 genes predicted by the Arabidopsis Genome Initiative has been obtained so far. Reverse genetics approaches give valuable information on gene function, but in plants, until very recently, there was no feasible way of systematically making targeted gene-knockouts on a large scale. The situation changed with the development of recombinational cloning vectors for expressing hairpin RNA, a potent inducer of RNAi in plants. The EU-funded AGRIKOLA project is aiming to generate a genome-wide resource for reverse genetics in Arabidopsis by constructing 50 000 plasmids each carrying gene-specific tags designed to induce gene-silencing by RNAi of a single target gene, either inducibly or constitutively. So far, more than 5000 hairpin plasmids have been made and more than 1000 of these have been introduced into Arabidopsis. Preliminary analysis of the transformants indicates that (i) phenocopies of previously described knockout mutants can be obtained; (ii) viable 'knockdown' mutants of genes known to be essential can be obtained; (iii) the project will reveal many informative phenotypes by inhibition of genes of currently unknown function.
The project will provide unparalleled resources for Arabidopsis reverse genetics, allowing researchers to quickly and easily produce mutants at will in almost any Arabidopsis gene. This approach will be a valuable complement to existing collections of T-DNA and transposon insertion lines. The resources generated in the project will be distributed via NASC.

Hilson et al. Versatile gene-specific sequence tags for Arabidopsis functional genomics. Genome Res.

www.agrikola.org

T10-042

Functional Analysis of EMB Genes Using Epitope-Tagged Proteins

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An important goal of genomics research is to acquire a comprehensive understanding of protein interactions inside the living cell. Advances in protein purification techniques and mass spectrometry have significantly accelerated the detection and analysis of multiprotein complexes, particularly in model eukaryotes. In our SeedGenes Project (www.seedgenes.org), we have confirmed the identities of more than 30 EMB genes predicted to encode proteins with unknown or uncertain functions. These represent a valuable subset of Arabidopsis genes for tag-based affinity purification methods because the identification of molecular partners may help to reveal their underlying functions. We describe here the molecular complementation of mutant embryos with histidine-tagged (6xHis) and tandem affinity purification-tagged (TAP) proteins. We demonstrate that 6xHis- and TAP-tagged EMB1629 protein can restore normal function in the absence of competing native protein, enabling the recovery of viable homozygous knockouts. The TAP-tagged protein has been detected by western blot analysis and work is underway to isolate potential interacting partners. We also present the results of molecular complementation experiments involving other EMB genes predicted to encode proteins with unknown functions. The epitope tag in some cases appears to interfere with normal protein function because the mutant phenotype is only partially rescued. We conclude that complementation of knockout mutants with TAP-tagged proteins, when followed by recovery and identification of interacting protein partners, represents an effective strategy for understanding the biological functions of many essential genes in Arabidopsis.

Funded by the NSF 2010 Program and the S.R. Noble Foundation.

T10-043

Toward the high throughput identification of the binding sites of TGA transcription factors using a whole-genome promoter array

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TGA transcription factors govern the expression of genes that confer protection against xenobiotic stress and microbial pathogens. In Arabidopsis, TGA2 and TGA3 regulate the transcription of Pathogenesis-Related 1 (PR1) and presumably other host-defense genes, which together contribute to the establishment of systemic acquired resistance (SAR). However, differences in DNA-binding properties within the 10-member Arabidopsis TGA transcription factor family imply that they are likely to have both distinct and shared sets of target genes. This hypothesis is supported by the diversity of expression patterns that we observe in plants expressing TGA promoter-GUS or -GFP fusions. Consequently, one of our goals is to identify the genome-wide binding sites for each Arabidopsis TGA transcription factor. To this end, labeled probes derived from chromatin, immunoprecipitated with antibodies specific to each TGA factor are hybridized to arrays representing Arabidopsis promoter regions (ChIP-chip). The quality of the ChIP samples obtained with anti-TGA3 and TGA2 antibodies was assessed using an array representing approximately 200 Arabidopsis promoters, and was confirmed by real-time PCR. Moreover, in collaboration with NimbleGen we have developed an Arabidopsis chip representing the promoter regions of the 27,100 genes in the TIGR annotation. By hybridizing mock ChIP samples to this array, we were able to exclusively identify promoters that had been artificially enriched. Hybridizations of bona fide ChIP samples from salicylic acid (SA)-treated plants to this whole-genome promoter array are in progress and results will be presented. In parallel, we are using Affymetrix ATH1 arrays to analyze gene expression in response to SA in wild-type and mutant plants. Collectively, the clustered expression profiles, ChIP analysis and spatial patterns of expression will form the foundation for developing a model for the transcriptional network of TGA factors in Arabidopsis.

This work is supported by the National Science Foundation's 2010 Project.

T10-044

Transcriptome and proteome analysis of the light induced greening of an Arabidopsis cell culture

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Light regulates many different aspects of plant development and function such as photosynthesis, photo morphogenesis, phototropism, chloroplast development, and movement. Regulation mechanisms of light dependent gene expression are still largely unknown. The light-induced greening of an Arabidopsis cell culture was used to characterize light dependent changes at mRNA and protein levels. The green cultured cells turned yellow under dark condition and greening can be induced again upon illumination.

We used Affymetrix Genechip® technology for transcriptional profiling of the greening process. In addition, proteome analysis was carried out using an LC/MS/MS technology approach. While genes related to photosynthesis were induced at the mRNA levels, we could not detect significant changes at the protein level. The comparison of transcriptome and proteome data will be discussed.

T10-045

CSB.DB - A Comprehensive Systems-Biology Database

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The availability of genome information facilitated the development and spurred employment of multi-parallel techniques to monitor the cellular inventory. Modern functional genomics encompasses technologies designed for the systematic investigation of gene function at all levels of a living cell. Combined and multi-parallel analyses allow investigation of complex biological processes at full systems level. A future task will be the discovery of functional interaction within and among the levels of the cellular inventory and to extend knowledge from organism specificity towards universality.
The open access comprehensive systems-biology database (CSB.DB) was developed to integrate bio-statistical analyses on multi-parallel measurements of different organisms, such as Escherichia coli, Saccharomyces cerevisiae and Arabidopsis thaliana, to extend knowledge from well understood model organisms to plants. The major goal of CSB.DB is to present a central publicly accessible resource for novel results of large-scale computational analyses on transcript and other profiling data, which mirror the large functional network of the cellular inventory. The data is presented both in tabular format as well as in an overview graph showing gene classes responding to query genes. For our analyses we implicitly make the assumption that common transcriptional control of genes is reflected in co-responding, synchronous changes in transcript levels. CSB.DB gives easy access to the results of large-scale co-response analyses, which are based on the publicly available compendia of transcript profiles. By scanning for the best co-responses among changing transcript levels CSB.DB allows to infer hypotheses on the functional interaction of genes. These hypotheses are novel and not accessible through analyses of sequence homology. The science community will be able to search for pairs and larger sets of genes, which are under common transcriptional control. Statistical and graphical tools are offered to validate and display co-responses of gene pairs, which are discovered by user-directed queries on genes of interest and with focus to a pre-selected organism or specific dataset.
Here we present some examples how to infer hypotheses about the respective functional involvement of genes. CSB.DB is accessible at the URL http://csbdb.mpimp-golm.mpg.de/.

T10-046

Subcellular-Targeting for Efficient Expression of Foreign Gene in Transgenic Plant

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For a high-yield production of foreign gene product in transgenic plants through efficient localization, we have targeted several different GFP constructs to various subcellular compartments such as cytoplasm, apoplast, endoplasmic reticulum (ER), and chloroplast of Arabidopsis. GFP was functionally expressed either in the cytosol or in the apoplast using a rice signal peptide of alpha-amylase. A construct containing the C-terminal KDEL sequence was tested for retention in the ER. In this case, fluorescent spindle-shaped (fusiform) bodies were brightly shown on the ER network. These structures are mostly abundant in stems, but are also found in leaves and roots. The level of GFP expression in the ER is also higher than in the other cell compartments, yielding about 1% of the total soluble protein in mature tissue.

T10-047

Insertional mutagenesis by Ac/Ds transposon system and a phenome analysis of transposon-tagged lines in Arabidopsis

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2-RIKEN Plant Science Center

Arabidopsis is a model plant in which it is possible to carry out saturation mutagenesis for every coding gene. We constructed about 12,000 Ds transposon-mutated lines to collect a series of gene-knockout mutants. To determine the precise locations of insertions, we developed a high throughput analysis of the flanking sequences with a semi-automated method. We analyzed the flanking regions of Ds insertion for all independent lines we have constructed. A searchable database is open now, filing the sequence results, the insertion site in Arabidopsis genome, the closest genes and the results of homology search. Seeds are being deposited to RIKEN BioResource Center as available resource.
In this resource, we can investigate about 4,000 genes which would be disrupted by Ds insertion in gene-coding regions. Recently, we have started a "Phenome Analysis", in which we observe various phenotypes systematically for each Ds insertional line. For 4,000 insertional lines, we are counting the germination and growth rate, and are collecting phenotypic data by morphological observation on each growth stage. We obtained mutants so far showing visible phenotypes including dwarf, albino or pale-green, late flowering, leaf abnormality, waxy stem, flower organ alteration, silique or seed abnormality, and so on. We are classifying the records into one hundred kinds of category of phenotypes. We propose an international effort to develop a “Phenome Analysis” using gene knock-out mutants for every functional gene in Arabidopsis.

Ito T. et al. (2002) Plant Physiol. 129, 1695-1699.
Kuromori T. et al. (2004) Plant J. 37, 897-905.

T10-048

AGRIKOLA: a systematic approach for hpRNA induced gene silencing

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The AGRIKOLA consortium consists of six European laboratories (see related posters) and is aiming for genome-wide determination of gene function by using a reverse genetic approach based on post transcriptional gene silencing. The collection of gene-specific tags created by the CATMA consortium is used to generate 25 000 hair-pin RNA plant expression vectors, each designed to induce silencing of a single gene. As a proof of concept a subset of these constructs is transformed into Arabidopsis thaliana and the transgenic plants are subjected to visual phenotypic and molecular analyses. As our contribution to the AGRIKOLA program we transform 2500 of the intended 5000 constructs into Arabidopsis and select 10 T1-lines per construct, document their phenotypes and recover their seeds to create an initial collection of T2 RNAi lines.
Here we report on our phenotypic studies and give information about the frequency of phenotypes hitherto obtained for 200 constructs. We show examples of lines carrying RNAi constructs for genes with known function mimic the phenotype of the corresponding knock-out mutants. Furthermore, we give biochemical evidence for the down regulation of the target gene and show the efficient transmission of these phenotypes into the T2 generation. Our data indicate high efficacy of gene silencing by the CATMA-GST-based RNAi constructs and demonstrate the usefulness of this approach for large-scale gene function analysis in plants

T10-049

The use of STAIRS for mapping genes controlling reporter gene transfer and integration into Arabidopsis thaliana using Agrobacterium-mediated transformation.

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A protocol to measure the efficiency of Agrobacterium-mediated transformation of A. thaliana has been established and genotypes screened at specific time points after inoculation. Highly significant differences in gus reporter gene expression were identified, initially between the parental genotypes, Columbia-0 (Col-0) and Landsberg erecta (Ler) and also with a set of Chromosome Substitution Strains (CSSs). CSS 5 (which has a Col-0 background and a Ler chromosome 5) was found to perform the most significantly different to Col-0, out of all the available CSS lines. These differences were identified by scoring the presence or absence of the gus expression in root segments, at different time points after inoculation. To further investigate chromosome 5 and identify specific regions along the chromosome, the development of Stepped Aligned Inbred Recombinant Strains (STAIRS) [Koumproglou, R., et al, Plant Journal, 2002. 31(3): p. 355-364.], was carried out. The STAIRS are a set of single recombinant lines (SRLs), in a Col-0 background; when stacked sequentially each successive line has a little more of the Ler chromosome present. STAIRS differ initially by 10-15cM, which will be either Col-0 or Ler-derived, and regions of interest can be further resolved to approximately 1 cM. An initial set of STAIRS has been screened and their responses to Agrobacterium-mediated transformation measured. Target chromosomal intervals have been identified and these regions investigated further by both genotypic and phenotypic analyses. This will allow identification of candidate genes controlling this trait.

Koumproglou, R., et al, Plant Journal, 2002. 31(3): p. 355-364

T10-050

T-DNA insertion mutagenesis: identification of tagged Arabidopsis genes by insert mapping and promoter trapping.

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Induction of knockout mutations by T-DNA insertion mutagenesis is widely used to study plant gene functions. Using different T-DNA tagging vectors, we created a large collection of insertion mutants. To assess the efficiency of this genetic approach, we have sequenced PCR amplified junctions of 1000 T-DNA insertions and analyzed their positions in the Arabidopsis genome. The majority of T-DNA insertions landed in chromosomal domains of high gene density. Around 5% of insertions were found in interspersed, centromeric, telomeric and rDNA repeats. 35% of T-DNAs were found in intervals flanked by ATG and stop codons of predicted genes. The frequency of T-DNA insertions in 3' and 5' regulatory regions of predicted genes, was 2-3 times higher than in any similar intervals elsewhere in the genome. Our estimate for the mutation rate suggests, that nearly 50% of all T-DNA tags could induce knockout mutations in Arabidopsis (1). To monitor the expression of T-DNA tagged plant genes in vivo, a collection of insertion mutants were were generated with the promoter trap vector pTluc, which carries a promoterless firefly luciferase (luc) reporter gene linked to the right T-DNA border. Monitoring bioluminescence in three-weeks old seedlings, nearly 4% of plants showed luciferase expression. To illustrate the use of luc gene fusion system in the identification of specific stress-regulated genes, we have isolated several luc gene fusions, which show specific induction by sugar, salt or ABA stimuli. Sequencing the T-DNA insert junctions isolated from 25 luciferase expressing lines identified T-DNA tags in 5' and 3' transcribed regions, as well as in exons and introns of Arabidopsis genes. Tissue specific expression of 8 wild type Arabidopsis genes was confirmed to be similar to the luminescence patterns observed in the corresponding luciferase-tagged lines. The use of luc gene fusions can therefore provide a facile means for identification of specifically regulated genes in conjunction with identification of corresponding insertion mutations (2). The collection of Arabidopsis lines with mapped insertions and the luciferase trap lines are available for research purposes (see: http://www.szbk.u-szeged.hu/~arabidop/).

This research was supported by EU 5th Framework Program (GVE, no. QLK5-CT-2001-01871), OTKA Grant no. T038375, and OM Biotechnologia2001 grant no. BIO-00118/2001.

1) Szabados L et al., 2002, Plant J. 32:233-242,
2) Alvarado M et al., 2004, Plant Physiol. 134:18-27

T10-051

Development of a novel reporter to monitor homoeologous recombination events in Arabidopsis.

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Homoeologous recombination involves DNA sequences that are very similar but not identical. It has been shown in model eukaryotes such as yeast that sequence divergence can significantly decrease the frequency of recombination between homoeologous sequences (Datta et al., 1997). In plants, it has been proposed that the low recombination rates often encountered when attempting to introgress traits of interest from wild relatives of crop species are attributable to this sequence divergence. To facilitate the identification and characterization of genes involved in limiting recombination between homoeologous sequences, we have developed a novel reporter system based on the GUS gene. The construct consists of a disrupted GUS gene containing two copies of a large intron (626bp). Upon recombination between the two copies of the intron, a functional gene containing a single copy of the intron is produced. Recombination events are visualized as blue sectors on a white background following histochemical staining. As introns are non-coding, mutations can be made in one copy of the intron to study their impact on the recombination frequency between the two introns. The introduction of as few as 3 mutations (0.5% divergence) decreased the recombination frequency more than four-fold relative to a homologous construct with two identical copies of the intron. A maximum of 53 mutations (9% divergence) were engineered and resulted in a 20-fold decrease in the frequency of recombination. This reporter system was used to investigate the role of a key component of the DNA mismatch repair system, MSH2, a gene known to restrict homoeologous recombination in other model eukaryotes (Hunter et al., 1996). An Arabidopsis insertional mutant (SALK line 2708; Atmsh2::T-DNA) was crossed to two reporter lines: one with 0% and the other with 2% sequence divergence. F3 families homozygous for the reporter and for either the MSH2 or msh2 allele were stained. Loss of MSH2 activity had no significant impact on recombination between homologous substrates, whereas it led to a 10-fold increase between homoeologous substrates.

Datta et al., 1997. PNAS 94:9757-62.
Hunter et al., 1996. EMBO J. 15(7):1726-33.

T10-052

Mapping LUX ARRHYTHMO, a novel myb transcription factor essential for circadian rhythms, and other circadian clock mutants by oligonucleotide array genotyping

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Many components of the circadian clock remain undiscovered. In an effort to increase throughput for mutant screens, we performed a novel screen that relies on hypocotyl growth alterations followed by circadian phenotyping as a tool to isolate circadian mutants. Mutations were then mapped using high-density oligonucleotide arrays as a tool to assay several hundred thousand loci in a single assay. The Affymetrix ATH1 GeneChip can detect approximately 8,000 hybridization differences between the Columbia and Landsberg erecta accessions at the DNA level. Using bulk segregant analysis in the F2 generation and treating these polymorphisms as genetic markers, we routinely map mutations down to regions of 6cM or less. This approach assisted in the identification of a mutation in LUX ARRHYTHMO (LUX), a gene that encodes a myb family transcription factor essential for circadian rhythms. Expression of LUX and the core clock component TOC1 are tightly correlated in constant light, short and long day conditions as well as in multiple mutant backgrounds. In constant light, lux mutants lack normal rhythmic expression of TOC1, PRR3, 5, 7, and 9, CCA1, LHY, and ten other clock regulated genes. However, in constant darkness, lux mutants exhibit normal rhythmic CCR2::LUC expression suggesting it is involved in mediating light signaling into the clock. Using this approach, we have also identified several new alleles of known clock genes such as ELF3 and ELF4. Another method to identify a mutant locus is by mapping large deletions caused by fast neutrons bombardment. Without having to make a mapping cross, array genotyping can identify a deleted locus that is either homozygous or heterozygous. We have successfully demonstrated this technique with two well-studied deletions: the 77 kb fkf1 deletion and the 7kb cry2-1 deletion. A new circadian reporter line (TOC1::LUC) bombarded with fast neutrons has yielded several mutants showing multiple types of circadian aberrations. Both duplications and deletions have been detected in these lines. Candidate genes in those regions are currently under investigation.

T10-053

Identification of genetic regions controlling Agrobacterium-mediated transformation of Arabidopsis thaliana

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One of the biggest problems with the transformation of many plant species is the variability in the successful production of transgenic plants. The molecular basis of Agrobacterium-mediated transformation has been an important area of research, however much of this research has been concentrated on the bacterial genes involved in the process. Understanding the role that plant genes play in this process is very important, however as this will facilitate further manipulation of the plant to achieve improved transformation efficiency. Over the last 3 years at the University of Birmingham, a significant amount of work has been carried out to develop and optimise an assay for T-DNA integration using a GUS (beta ¯ glucuronidase) construct. The assay is a simple way of observing transformation by following expression of a reporter gene. By using this assay and the available Chromosome Substitution Strains (CSS) and Stepped Aligned Inbred Recombinant Strains (STAIRS), we are able to fine-map quantitative traits to individual chromosomes and regions of chromosomes respectively. The available CSS (CSS 2 ¯ 5) have now been screened using the assay with the CSS for chromosomes 2 and 5 giving the most interesting results to date, indicating that there may be genes controlling Agrobacterium-mediated transformation found on these chromosomes. A screen of the STAIRS for chromosome 5 has just commenced and chromosome 2 will be screened over the coming months. This should allow identification of specific regions of the chromosomes that may contain candidate genes.

Koumproglou R et al,(2002) STAIRS:a new genetic resource for functional genomic studies of Arabidopsis.The Plant Journal

T10-054

Functional Annotation of the Arabidopsis Genome Using Controlled Vocabularies

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The use of controlled vocabularies in describing genes and gene products allows standardization of annotation and facilitates identification of similar genes within an organism or among different organisms. One of The Arabidopsis Information Resource's (TAIR's) goals is to associate all Arabidopsis thaliana genes with terms developed by the Gene Ontology (GO) Consortium that describe the molecular function, biological process, and subcellular location of a gene product. We have also developed terms describing Arabidopsis anatomy and developmental stages and use these to annotate published gene expression data. We have used computational and manual annotation methods to make ~85,000 annotations to 26,599 unique loci. We focus on associating genes to controlled vocabulary terms based on experimental data from the literature. Each annotation is tagged with a combination of evidence codes, evidence descriptions and references that provide a robust means of assessing data quality. Genes annotated with GO terms and expression patterns can be searched using these vocabularies. Users can also navigate through the ontology structures, explore term relationships and view definitions and annotated data objects using our new Keyword Browser. Controlled vocabulary annotations are displayed on our gene and locus detail pages. The GO annotation bulk download page at http://www.arabidopsis.org/tools/bulk/go/index.html allows researchers to (1) obtain GO annotations for any gene or set of genes using locus names and (2) group large sets of genes such as those coming from microarray experiments into broad categories based on high level GO terms called GOslim terms. The Arabidopsis functional annotation data can facilitate annotation of newly sequenced plant genomes by using sequence similarity to transfer annotations to homologous genes. In addition, complete and up-to-date annotations will make 'unknown' genes easy to identify and target for experimentation.

T10-055

GENOME-WIDE DISCOVERY OF TRANSCRIPTION UNITS AND FUNCTIONAL ELEMENTS IN ARABIDOPSIS

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Complete genome sequences are now available for a wide variety of organisms and many more are on the way! In order to carryout functional analysis in these organisms, accurate determination of gene structures and complete gene inventories will be essential. Computational gene prediction methods are improving but alone are inadequate for new gene discovery and accurate annotation of genomes, in particular for certain gene classes such as non-coding RNA genes. New approaches are required to identify the entire complement of transcription units (protein coding and non-coding), and their associated regulatory elements (e.g. TF/chromatin binding locations and sites of DNA methylation). We are pursuing empirical approaches to decode this information using the genome sequence of Arabidopsis, enabling more rapid assessment of the biological functions of the ~30,000 predicted plant genes. Unbiased mapping of the transcription units is being carried out using third-generation Affymetrix whole genome tiling array (WGA) technology. The high-resolution transcription unit location information is being used to guide the construction of a complete, expression-ready, gene inventory- "the plant ORFeome". We have also begun to utilized WGAs as a universal data-gathering platform for capturing a variety of types of genome-scale information, including the chromosomal locations of DNA methylation sites (and identification of the methylases that target these sites), chromatin/transcription factor binding sites and for discovery of genome-wide allelic variations among geographically isolated Arabidopsis accessions. When coupled with transcriptome mapping data, these unbiased sets of genome-wide regulatory information will begin to allow the construction of an integrated set of cellular/molecular connectivity maps for Arabidopsis.

T11 Modeling the Virtual Plant/Bioinformatics

T11-001

Formation of flower primordia at the shoot apical meristem of Arabidopsis ¯ a quantitative approach to the meristem surface growth

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Formation of flower primordia is a major function of the reproductive shoot apical meristem (SAM). Genes controlling this process are well characterised for Arabidopsis. This knowledge should be complemented by quantification of growth leading to the flower primordium formation. Changes in geometry during the process imply that the growth is unsteady. Moreover, it is anisotropic, i.e. growth rates are different in different directions. Growth quantification accounting for its unsteadiness and anisotropy requires sequential observations of individual meristems and estimation of principal directions of growth. These are the directions in which growth rates attain their minimal or maximal values. In this investigation sequential replica method is used. Dental polymer moulds are taken from the individual SAM surface at time intervals. Epoxy resin casts from the moulds are observed in scanning electron microscopy. Displacements of vertices (cell wall edges) due to growth are used to compute growth variables ([1]). At the earliest stages of primordium formation, growth of the SAM periphery is anisotropic with maximal expansion in the meridional direction (along the meristem radius). The primordium appears as a lateral outgrowth, which cannot yet be delineated from the SAM. Further growth of the primordium is again anisotropic, but principal growth directions change dramatically. Growth rate is negative (contraction) in a meridional direction, while in the latitudinal direction the extension takes place. Areal growth rates are smaller than previously. The primordium becomes a wide shallow saddle. Cells located at the bottom of this saddle continue to grow slowly and anisotropically, which results in formation of a distinct crease (a future axil). The crease delineates the primordium from the SAM. The delineated primordium grows fast and less anisotropically, forming a rounded bulge. Further growth leads to the formation of the first pair of sepals. Now the surface of the primordium becomes partitioned: two creases, delineating the sepals from the remaining primordium surface, are formed. Growth of the creases closely resembles growth of the primordium axil. The presented results allow one to differentiate early stages of flower primordium formation into phases differing significantly in growth, especially in growth anisotropy. This poses additional questions concerning the regulation of early flower development.

[1] Dumais J., Kwiatkowska D. 2002. Plant J. 31: 229-241

T11-002

Bicistronic and fused monocistronic transcripts are derived from adjacent Arabidopsis loci

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Compilations of all available Arabidopsis thaliana cytochrome P450 monooxygenase EST and cDNA sequences have allowed us to reanalyze all of the P450 models. Initially, this identified long monocistronic transcripts derived from tandem loci that fuse two ORFs to create dimeric P450s containing two heme binding domains as well as a P450 fused to a PPR protein. Later, comparisons of all available full-length cDNAs with Arabidopsis genomic DNA sequences indicated that 60 sets of adjacent loci are transcribed into extremely long RNAs spanning two annotated genes. Once expressed, some of these transcripts are post-transcriptionally spliced within their coding and intergenic sequences to generate biscistronic transcripts containing two complete open reading frames (ORFs). Others are spliced to generate monocistronic transcripts coding for novel fusion proteins with sequences derived from both loci. RT-PCR analysis of a number of transcripts has verified the existence of these unusual transcripts and demonstrated that some adjacent loci have the potential to code for three different classes of transcripts corresponding to short transcripts for each of the individual loci and long transcripts containing sequences from both loci. The existence of these unusual transcripts highlight variations in the processes of transcription and splicing that could not possibly have been predicted in the algorithms used for genome annotation and that must be taken into account in describing these and other Arabidopsis loci.

T11-003

MotifMapper: A modular based collection of Visual Basic routines for the analysis of correlative sequence data

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Motif Mapper is a VBA-WsH open-source package containing an association of independent word-counting scripts to assist the analysis of distribution and frequency of DNA elements in genomic and sequence set contexts. All output and input files are ASCII-text files whose data can be manipulated and viewed using array based spreadsheet applications. The package has been extended from an earlier version whose output was included in poster 1-75 at the XIIIth Arabidopsis Conference, and now comes with a GenBank parser. Motif Mapper (www. motifmapper.de) provides a portable means for extending phylogenetic footprinting, by allowing the screening of many queries against large data sets.
Here, we introduce the package format emphasizing the most important algorithms and demonstrate an application based on the analysis of hexanucleotide frequencies in promoters.
As similar studies have already been undertaken and shown of investigative value, we focus on the frequency and distribution of known cis-elements as well as motifs conserved in promoters of different plant species.
In particular, our data reveal motifs that are known to be functional as proximal or distal elements. Moreover, some motifs exhibit distinct behavior near known and putative translation start codons.

T11-004

UniProt and the Swiss-Prot Plant Proteome Annotation Project (PPAP)

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In December 2003, a single, centralized, authoritative resource for protein sequences and functional information, UniProt, was created by joining the information contained in Swiss-Prot, TrEMBL, and the PIR-International Protein Sequence.
UniProt consists of 3 different sections, optimized for different uses:
- The first section is the UniProt Knowledgebase itself, composed of Swiss-Prot and TrEMBL, which have integrated all the PIR-PSD data.
- The second section is an archive (UniParc), containing non curated protein sequences loaded from public databases, including Swiss-Prot, TrEMBL, RefSeq, FlyBase, Patent databases, etc.
- The third section, UniRef, contains three sub-layers, UniRef100, UniRef90 and UniRef50. UniRef100 is a non-redundant version of all the sequences in the knowledgebase. UniRef90 and UniRef50 collapse all the sequences that are respectively at least 90% or 50% identical into a single record.

Completion of the genome sequence of model plant organisms has lead the Swiss-Prot team to initiate the Plant Proteome Annotation Project (PPAP) devoted to plant-specific protein families, with a particular emphasis on Arabidopsis thaliana.
Currently, more than 11'000 plant proteins have been curated and integrated into Swiss-Prot, while 148'000 entries still await manual annotation in TrEMBL, the database regrouping the translations of all the coding sequences (CDS) proposed by authors in their nucleotide sequence submissions to the EMBL database. With 2'800 entries Arabidopsis data represents 25% of Swiss-Prot plant entries, with the rest concerning about 1'200 others species.
For Arabidopsis, the 67 proteins encoded in the chloroplast and the 114 proteins encoded in the mitochondrion are present in Swiss-Prot, with an indication of the amino acid replacements due to RNA editing. Alternatively spliced gene models, including those published by TIGR, that concern a protein present in the Swiss-Prot database have been annotated accordingly and the various isoforms described.
In future, the release of additional plant genomes and full-length cDNAs will offer us the opportunity to extend our scope to the annotation of orthologous proteins.

T11-005

AthaMap, an online resource for in silico transcription factor binding sites in the Arabidopsis thaliana genome

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Gene expression is mainly controlled by the binding of transcription factors (TFs) to regulatory sequences. To generate a genomic map for regulatory sequences, the Arabidopsis thaliana genome was screened for putative transcription factor binding sites. Using publicly available data from publications, alignment matrices for 23 transcription factors of 13 different factor families were used with the pattern search program Patser to determine the genomic positions of more than 2.4 x 10^6 putative binding sites. Due to the dense clustering of genes and the observation that regulatory sequences are not restricted to upstream regions, the prediction of binding sites was performed for the whole genome. The genomic positions and the underlying data were imported into the newly developed AthaMap database. This data can be accessed via an interactive web interface by submitting positional information or by entering The Arabidopsis Genome Initiative identification number. Putative binding sites are displayed in the defined region. Data on the matrices used and on the thresholds applied in these screenings are given in the database.
The upcoming database update will include newly published matrices, describing TF binding sites, as well as potential combinatorial elements important for gene regulation. Additionally, a feature will be introduced which facilitates colocalization analysis of TF binding sites according to user-specified parameters, allowing to analyze new potential combinatorial elements. Considering the high density of sites, this map will be a valuable resource for generating models on gene expression regulation. The data is available at http://www.athamap.de.

T11-006

Metabolite fingerprinting: an ICA approach

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Metabolite fingerprinting is a technology for providing information from spectra of total compositions of metabolites. Here, spectra acquisitions by microchip-based nanoflow-direct infusion QTOF mass spectrometry, a simple and high throughput technique, is tested for its informative power. As a simple test case we are using Arabidopsis thaliana crosses. The question is how metabolite fingerprinting reflects the biological background.
In many applications the classical principal component analysis (PCA) is used for detecting relevant information. Here a modern alternative is introduced - the independent component analysis (ICA). Due to its independence condition, ICA is more suitable for our questions than PCA.
However, ICA has not been developed for a small number of high dimensional samples, therefore a strategy is needed to overcome this limitation. To apply ICA successfully it is essential first to reduce the high dimension of the data set, by using PCA. The number of principal components determines the quality of ICA significantly, therefore we propose a criterion for estimating the optimal dimension automatically. The kurtosis measure is used to order the extracted components to our interest.
We found that ICA could detect three relevant components, two biological and one technical, and clearly outperforms the PCA. The first independent components is usable for separating the Arabidopsis crosses from the background parental lines, the second contains information for discriminating the two parental lines. The third component could be interpreted as a contribution of chemical noise due to increasing contamination of the QTOF skimmer along the analytical sequence.
ICA, together with the proposed criteria, forms an automated analytical procedure that offers a metabolite fingerprinting technique designed for high sample throughput. The described approach is available for public at the MetaGeneAlyse (http://metagenealyse.mpimp-golm.mpg.de), a web-based analysis tool for analyzing biological data from metabolomics, proteomics and transcriptomics.

T11-007

Computational comparison of eukaryotic SNF1 and plant-specific SnRK1 protein kinase phosphorylation motifs on the basis of mutual information (MI).

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The SNF1 (sucrose non-fermenting-1) family of protein kinases consists of the homologous yeast SNF1, animal AMPK and plant SnRK1 counterparts. First identified in Fungi the gene for SNF1 encodes a protein kinase in S. cerevisiae that is activated in response to low cellular glucose levels. The animal homolog of SNF1 is the aforementioned AMP-activated protein kinase (AMPK), while the plant homolog is SNF1-related protein kinase-1 (SnRK1). Despite assuming approximately 1.5 billion years of evolutionary divergence the three members of the SNF1 protein kinase family have a remarkable homology in eukaryotes where they are involved in regulating key aspects of cellular function including cell division, metabolism, and responses to external signals [1]. Today the completed genomic sequences of manifold eukaryotic organisms provide new potentials for comparative structural analysis of diverse SNF1 phosphorylation consensus sequences. On the basis of several suggested common consensus sequences [1, 2] we compared putative phosphorylation sites in the predicted proteomes of organisms belonging to different eukaryotic kingdoms. The systematic analysis was done by adopting a statistical approach using mutual information (MI), a measure of association to reveal species-specific characteristics of conserved sequence motifs [3]. The derived MI-profiles indicate evolutionary differences of known and putative substrate specificities in the SNF1 family of protein kinases. In addition to the kingdom-based approach we also examine resemblances in the SnRK1 motifs of different plants likewise clarifying evolutionary distances by structural and functional distinction of putative phosphorylation motifs.

[1]Halford et al. 2004. JXB 394,35-42
[2]Lunn, MacRae 2003. COPB 6,208-214
[3]Weckwerth, Selbig 2003. BBRC 307,516-521

T11-008

DIAGNOSIS OF PLANT METABOLISM

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Diagnosis of plant metabolism is defined as the process of identifying the physiological state by the analysis of a minimal number of robust markers, e.g. metabolite levels and enzyme activities. Applications range from phenotyping of mutants and natural diversity to evaluation of plant performance. The establishment of a diagnostic platform necessitates the 3 following approaches:

1. Phenotyping through a broad range of growth conditions. Various genotypes are grown under controlled conditions where parameters like temperature, light intensity, nutrient availability or time are varied. Targeted analyses as well as profiling experiments are then performed. In addition we started to collect existing data, from various species.
2. Development of high throughput assays: we have adapted or conceived robust microplate-based assays for metabolites and enzyme activities.
3. Data mining: we build a database to record growth conditions and all the parameters determined for every sample. A module allows the selection of classes of growth conditions and/or genotypes. The classes are then “separated” via a machine learning system (decision trees), providing templates for diagnosis. The ImageAnnotator module will be used to visualise the diagnostic classifications.

To illustrate this, we will present the diagnosis of carbon-starvation in the Arabidopsis starchless mutant pgm (lacking the plastidial phosphoglucomutase).

T11-009

Non-Random Distribution of Transcription Factor Binding Sites in the Arabidopsis thaliana Genome

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Transcription factors (TFs) are essential for regulation of gene expression by controlling transcription through binding to specific regulatory DNA sequences and interaction with other TFs. Specific target sequences of a TF can be determined experimentally and are generally described using a positional weight matrix.
In this study, 31 plant matrices were employed in screenings for putative transcription factor binding sites (TFBSs) with PATSER using the Arabidopsis thaliana genome as target sequence. A software tool was developed to calculate the occurrence of neighbouring TFBSs and to determine the preferred distance between them. Low distances between TFBS were found to be overrepresented indicating a clustering of matches. A distance of up to 50bp was discovered as the most preferred space with a 30 fold higher frequency than expected. Colocalization frequencies remain elevated in distance windows ranging up to 1000 bp. A positional analysis was performed for each matrix in order to consider the genomic context of the TFBSs relative to the neighbouring translational initiation ATG. Several matrices showed a preference for binding to the region located upstream of the next ATG. Others exhibited an indifferent distribution or an accumulation downstream of the next ATG. This study shows that TFBSs are not randomly distributed in the Arabidopsis thaliana genome.

T11-010

New tools for computer visualisation and modeling of cell interactions.

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The genetic control of plant development is mediated by cellular interactions, and an exchange of positional information contributes to the self-organisation and coordination of cells during development. The Arabidopsis root meristem provides an ideal test-bed for probing these interactions. The root meristem grows indeterminately, is genetically amenable, has a simple and transparent architecture, and can be induced to form de novo in adult tissues. We have developed new genetic and optical techniques for following organisation of cells within living meristems, using modified green fluorescent proteins, and have generated a two large libraries of Arabidopsis lines that can be used for targeted gene expression in the root meristem. Confocal fluorescence microscopy techniques allow high resolution imaging, and 3D reconstruction of Arabidopsis root cells during initiation and growth of the meristem.

We have adapted computer segmentation methods for the description of 3D cell arrangements in plant tissue, and in our first steps towards developing a system for dynamic modelling of plant cell interactions, we have generated a 2D description of the physical properties of cells, using a novel double spring model to describe cell wall properties. This physical model provides an engine for the production of cells through enlargement and division. Fields of proliferating cells can then be programmed via a genetic script to produce and respond to different morphogens. We are using this 2D system as a pilot study for modelling cell dynamics and interactions in 3D.

web site: http://www.plantsci.cam.ac.uk/Haseloff

T11-011

Arabidopsis Microarray Resource at TAIR

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The Arabidopsis Information Resource (TAIR) at http://arabidopsis.org has developed a database for Arabidopsis microarray data that supports both spotted microarrays and Affymetrix chips. The database conforms to MIAME standards for publication of microarray data. Current holdings include data produced by large-scale projects, such as the Arabidopsis Functional Genomics Consortium (USA) and AtGenExpress (Germany), and by a few individual labs. In the near future the contents will expand to include all the data produced by NASCArrays (UK), the Compendium of Arabidopsis Gene Expression (Belgium), and Arabidopsis data available in the ArrayExpress and GEO repositories. Researchers are encouraged to submit their data using simple, pre-formatted Excel spreadsheets (arabidopsis.org/info/microarray.submission.jsp). Extensive data curation, annotation, quality control and normalization procedures performed at TAIR make it possible to compare and analyze the results of many microarray experiments stored in the database. Besides raw and normalized data, TAIR provides arithmetic mean, standard error and other related statistical measures of gene expression for each gene from replicated spots per array (if available), replicated hybridizations (if available), and across all arrays. ANOVA calculations are included as a measure of whole array data quality. Data can be searched using several parameters, such as experimental variables, experiment categories, plant tissues, and expression level. Clustered data and complete results files from each slide are available for download from the ftp site at ftp://ftp.arabidopsis.org/home/tair/Microarrays/, together with stand-alone programs for analyzing the data. TAIR also provides updates to the genome mapping of several publicly available array designs (e.g., AFGC, Affymetrix and CATMA), which can be searched from the Array Element Search or downloaded as a set from the ftp site.

T11-012

Quantitative modelling of Arabidopsis thaliana development

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The development and growth of a plant is regulated by the activity of genes which are responding to both the stage of development of the plant and environmental conditions. To understand plant growth, it is necessary to know the structure of the plant at different developmental stages. A descriptive model is a useful tool to summarise information on the timing and growth of all the organs of a plant and to provide a foundation for modelling the spatial and temporal interactions of genes throughout plant development.

In this work we have created a descriptive L-system model of Arabidopsis growth using the L-studio software [1]. The structure of the plant is generated in the model by considering an Arabidospsis shoot to be a series of metamers. During vegetative growth, each metamer consists of three modules: an axillary meristem, subtending leaf (if present) and supporting internode. On flowering, a metamer consists of a flower and its supporting internode. In addition to the structure of the plant, a comprehensive account of growth requires a description of how the size and shape of each module change over time. Changes in size were described by measuring one feature, such as length or width, at a sequence of time-points. Growth functions were fitted to these points to obtain parameters such as the timing, growth rate and maximum size of the modules of each metamer. In addition, the shape of the module was quantified at various stages using several approaches. In the case of leaves, sepals and petals, the shapes were interpolated between time points to obtain a continuous description of the changes in shape over time. This information has been incorporated into a comprehensive three-dimensional model that simulates the growth of an Arabidopsis plant.

The model we present summarises data collected during a time course of Arabidopsis growth and the average properties of a plant at any time point can be obtained from it. Trends in timing of organ formation, growth rate, shape and the maximum size that organs attain have been analysed, providing a means of comparing the development of different genotypes and plants grown in different conditions.

[1] Prusinkiewicz, P. 2004. Acta Horticulturae (ISHS) 630: 15-28

T11-013

DegP/HtrA proteases in plants: A proposal for a new classification and nomenclature

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The family of DegP/HtrA serine endopeptidases is found in most organisms from Archaea to Eukarya. DegP/HtrA proteases are very versatile enzymes: Some homologues seem to act as housekeeping proteases and chaperones in distinct compartments, while other homologues appear to act as more specific proteases in signalling cascades.
We have searched for DegP/HtrA-related amino acid sequences in freely accessible genome databases and found sixteen DegP/HtrA homologues in Arabidopsis thaliana. These sixteen sequences were used for surveying their structural and evolutionary relationships. A comparison of A. thaliana DegP/HtrA proteases with selected members from other taxonomic groups was performed. Based on the evolutionary relationship of the conserved trypsin domain, DegP/HtrA proteases clustered into four groups. These groups differ also in the number and position of the PDZ domains and total sequence length. Thus the DegP/HtrA family appears to be larger and more diverse than previously thought. Furthermore, higher plants seem to be the only taxonomic group containing DegP/HtrA proteases from all four groups. In the progress of studying these enzymes, it became apparent that the currently used nomenclature of these enzymes as DegP-proteases is a source of confusion. A common mistake is to anticipate greater similarity of the plant DegP-like proteases with the well studied DegP/HtrA protease of E.coli than with other DegP/HtrA homologues like DegQ or DegS. To clarify this situation, we propose that the currently used nomenclature for plant HtrA/DegP-like proteases should be modified: A. thaliana proteases of this family should be designated Deg1 to Deg16, in order of discovery, while proteases from other plants should be named according to their most similar A. thaliana homologue.

T11-014

Model of rosette development and expansion in Arabidopsis thaliana subjected to various temperature and incident radiation conditions

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In response to fluctuating environmental conditions, plants have developed various adaptative processes among which the plasticity of leaf area is a major ones because of its direct consequences on the water and energy balances. Recent works have proposed a generic framework to analyse the responses of individual leaf expansion of dicotyledonous to environment including the genetic variability (e.g. Granier and Tardieu, 1999; Dosio et al., 2003). In the proposed approach, the characteristic phases of leaf development and the time-course of the relative leaf expansion rate expressed on a thermal time basis were considered. Based on this formalism, models of leaf expansion have been established in lettuce (Gay, 2002) and in sunflower (Rey, 2003). The possibily of adapting this approach to Arabidopsis thaliana was tested. A model was proposed to simulate the expansion of plant leaf area in this species in response to temperature and incident radiation. The plasticity of the plant development was studied through the changes in leaf initiation rate, relative leaf expansion rate and duration of leaf expansion. These plant variables were estimated from quantitative relationships obtained in Arabidopsis thaliana, ecotype Columbia using temperature, absorbed PAR by the plant and blue light intensity as input environmental variables (see Chenu et al. in the same conference). The leaves were considered as independent organs from each other and the plant leaf area was obtained by adding up the area of individual leaves. The model mimicked well the changes of leaf expansion rates in time as well as the durations of leaf expansion. This resulted in close estimations of the plant leaf area including the variability of individual leaf areas that was observed among experiments. The next step will be to test this approach with fluctuating environmental conditions and to compare the phenotypic plasticity of different genotypes.

Dosio et al. 2003. J. Exp. Bot. 54: 2541-2552
Granier and Tardieu. 1999. P.C.E. 22: 1365-1376

T11-015

Leaf development in response to light in Arabidopsis thaliana: a quantitative approach using 3D virtual plants to compare genotypes

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Plant development is plastic in response to light environment. Both quantitative and qualitative characteristics of light affect plant development resulting in complexe responses. Eight wild ecotypes and mutants of Arabidopsis thaliana with contrasting rosette architecture were grown under a range of light conditions characterised by a level of incident photosynthetically active radiation (PAR) and a blue light intensity proportional to the level of incident PAR.
All the processes involved in rosette development were affected by a reduction of incident radiation. The amplitude of the responses differed among the genotypes. Three main responses to radiation reduction were identified: the leaf initiation was delayed, the relative leaf expansion rate was reduced during the early stage of leaf development and the duration of leaf expansion was increased. The range in genotype response was due either to varying rosette architecture which resulted in differences in the amount of PAR absorbed by the plant or to differences in physiology. To make the distinction between these two types of effects, 3D virtual plants were built and used to estimate the amount of PAR absorbed by the plant for all situations (genotype x light treatment) following a daily time step.
For each genotype, consistent quantitative relationships were found relating (i) the leaf initiation rate and (ii) the initial relative expansion rate to the amount of PAR absorbed by the plant. In contrast, the duration of leaf expansion was not correlated to the amount of absorbed PAR but was negatively related to the blue light intensity under a threshold. Genotypes were compared on the basis of these relationships. The response of relative leaf expansion rate to absorbed PAR was similar in the different genotypes. In contrast, the sensitivity of the leaf initiation rate to the absorbed PAR varied among genotypes. An extreme response was found in a mutant whose leaf initiation rate was totally insensitive to the amount of absorbed PAR.
The use of 3D virtual plants taking into account the plant architecture allowed us to identify physiological differences in the response of the different genotypes. The relationships presented here assist the analysis of the plant development, its phenotypic response to the light environment and the genotype-environment interaction. These relationships were used to establish a model of leaf development in Arabidopsis thaliana (Christophe et al., same conference).

T11-016

PaVESy: Combining profiling data with pathway knowledge

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Since a couple of year's high-throughput profiling methods are entering life sciences. For instance, a gene expression profile contains information about the transcriptional response of a complete genome to different experimental conditions. For scientists this abundance of data is often difficult to interpret. Several statistical methods are developed, and progress in data processing and management makes it more and more feasible to connect the results of a concrete experiment to the confirmed but seemly unmanageable knowledge already known.
The pathway database software PaVESy (http://pavesy.mpimp-golm.mpg.de/PaVESy.htm) offers the possibility to map profiling data (enzymes and metabolites) into established knowledge about biological pathways. PaVESy allows processing a whole data set (profile) in one step. A set of user-defined identifiers occurring in a profile are linked to compounds in the database and stored within a template. According to a given request different templates can be created. In this way a template can be re-used to map different data sets into pathway knowledge or different templates can be applied to one data set. Templates can be used for queries or visualization. Currently implemented queries are reaction and neighborhood queries. Besides output in the form of tables we provide a hierarchical presentation of query results in a navigation tree. The structure of the navigation tree can be adapted according to user specific questions. For visualization in pathways we use a directed graph representation in a graphical user interface where compounds and reactions are shown as vertices and arcs represents their connections. The user interface provides editing functions for the graph elements and an energy layout implementation.
A data set derived from a sulphur starvation experiment was chosen to demonstrate the power of PaVESy. The data set contains information about the changes in concentration levels of 140 metabolites and 5 phytohormones. Out of this amount of data a template with hormone co-regulated metabolites was generated and processed with PaVESy. In this application a database containing data compiled from the KEGG website was used. The pathways possibly affected by hormonal regulation under sulphur deficiency could be pointed out. Results of this data evaluation are discussed in the contribution by C. Birkemeyer et al.

T11-017

GABI-Primary Database: A Comprehensive Database for Plant Genome Data

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The GABI-Primary Database (GabiPD, http://gabi.rzpd.de) was established in 2000 during the first phase of the German Plant Genome Project GABI. In the meantime GabiPD harbours lots of data from different scientific partners and from public resources, e.g. sequence data, mapping and SNP information, results from gene expression experiments, proteome and metabolite analysis and information on availability of biological material.
GabiPD is localized at the RZPD, which provides high quality research material, e.g. clones, DNA, and protein arrays, and services like expression profiling, Affymetrix service, large scale PCR amplification, and cDNA library generation.
GabiPDs benefits are
the high level of data integration, that is clone, sequence, gene expression and mapping data, 2D-gel images and protein information, SNP data and mapping information [1] for example are linked with each other, to provide the user with a comprehensive overview of available information for their particular field of interest,
the linkage of data and biological material as available from different sources, to facilitate access to clones, TDNA insertion lines etc.
the availability of a novel user interface called MapMan, which allows extraction of relevant information from transcript, proteome and metabolite profiling data and graphical mapping of data onto diagrams of metabolic pathways and other biological processes [2],
the integration with different plant genome databases, like MIPS, NASC, SGN, TAIR or TIGR and public genome databases like EMBL or GenBank,
the connection between Arabidopsis genome data and data of other plant species,
a web accessible flexible cooperate database as a backbone, which allows a seamless integration of new data types on the one hand, and which sticks to MGED standards for storing gene expression data on the other hand.
The presentation will give an impression of data currently stored and an introduction about GabiPDs data search options and data visualization tools, including an overview about MapMan.

[1] Rickert et al. (2003), Plant Biotech J 1: 399-410
[2] Thimm et al. (2004), Plant J 37(6): 914-39.

T11-018

MapManXT a generic software tool to functionally assign plant's genome and metabolome enabling integration and display of complementary high-throughput data onto biochemical pathway maps

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The development of high-throughput techniques provides the opportunity to generate complementary 'omic' datasets that are essential to understand developmental processes or stress responses and their underlying regulatory networks. The upcoming flood of information can hardly be interpreted by an individual scientist and biological messages remain often uncovered.
The MapManXT software aids scientists to analyse their data in an intuitive way regardless their field of expertise. MapManXT consists of individual modules that can be used individually or combined into a pipeline that functionally categorises plant's genome (TranscriptScavenger), processes and condenses experimental data (ReadySteady), visualises data onto pathways (ImageAnnatator) and administrates on-line gene assignments and pathway releases (MapManStoreDatabase).
The crucial step for biological-based data display is an accurate functional categorisation. Arabidopsis genes represented by the AffymetrixTM full genome chip were categorised by a semi-automated approach: suitable gene ontologies of public databases (TAIR, GO and Kegg) were imported and translated to functional categories (BINs, Thimm et al. 2004). Wrong annotations were reduced with several manual curation steps. The resulting mapping file is used to generate a 'functional name dictionary' and a 'sequence dictionary'. In a three-step procedure the TranscriptScavenger module uses these dictionaries to transfer the functional categorisation to new genome releases like Tigr5.0: i) blast comparison of dictionary sequences with the target genome sequences ii) identification of dictionary names in the target gene description strings applying a text-mining strategy iii) manual curation of contradictive gene matches.
Using mapping files generated by the TranscriptScavenger the ImageAnnotator displays experimental data BIN-wise on maps of choice at user-defined positions. ReadySteady stores data files in a SQL database and processes data in three ways: i) calculation of ratios from test and control data, ii) generation of overlays of comparisons (Thimm et al. 2004) and iii) revealing BINs that show significantly different data distribution than the rest of the chip (Wilcox and goodness-of-fit tests). Both procedures generate data files that can be directly viewed in the ImageAnnotator module.
MapManXT combines different modules to categorise, administrate, process and display data of interest in a user-friendly and flexible manner.

Thimm, O. et al. (2004). Plant J 37(6): 914-39.

T11-019

The SYSTERS Protein Family Web Server: Shortcut from large-scale sequence information to phylogenetic information

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2-TFH Wildau, Dept. Biosystemtechnik/Bioinformatik
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Motivation:
With this poster, we present the SYSTERS protein family database, an attempt to classify all available protein sequences. In particular, we focus on the capability of the web interface to assist in in-depth analyses of special protein families. We demonstrate this by an analysis of a specific family of transmembraneous metal ion transport proteins characterised by the so called cation efflux domain.
We show three strategies to query SYSTERS: 1. by protein domain name as defined in Pfam, 2. by a set of sequence database accession numbers, and 3. by SYSTERS superfamily ID. All stategies lead to the identification of relevant SYSTERS protein families with description annotations from the source database entries, links to sequences, annotated alignments, family consensus sequences, phylogenetic trees, phylogenetic profiles, functional annotations, and links to other sequence resources. The analysis presented here documents how SYSTERS can help to generate new hypotheses about the function and evolution of protein families.

Results:
The Pfam Cation_efflux domain annotations in SwissProt/TrEMBL proteins serve us to identify 28 CDF transporter families within SYSTERS. Five of these families include proteins from Arabidopsis thaliana.
For selected sequences of the five families including Arabidopsis proteins, we construct an alignment with the alignment tool implemented in the SYSTERS web server. Furthermore, we retrieve a UPGMA tree from the web based on this alignment. The tree is composed of 5 subclades, congruent with the SYSTERS clustering result into 5 protein families. In the alignment, we observe characteristic conserved differences or extentions of the annotated Pfam Cation_efflux domain in the distinct subfamilies. Results from recent publications are discussed.
Other organisms than Arabidopsis contribute sequences to these 5 SYSTERS protein families. Phylogenetic profiles, displayed for three of these families by the PhyloMatrix tool within the SYSTERS web server, show the distribution in the three kingdoms of life.

Availability:
SYSTERS is accessible via the WWW (URL: http://systers.molgen.mpg.de).

T11-020

ARAMEMNON 2: a database and data mining tool for Arabidopsis and rice membrane proteins

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ARAMEMNON is a curated database for Arabidopsis thaliana and rice (Oryza sativa) transmembrane (TM) proteins. The database compiles topology from eleven programs and intracellular targeting predictions from eight programs and displays the results in a directly comparable graphical output format. The reliability of TM predictions is improved by calculating a consensus TM prediction from the eleven individual programs.
The user can search for TM proteins in the Arabidopsis and rice (and soon yeast) genome by free text, BLAST, gene name, transmembrane topology or subcellular localization queries. The output options include protein and DNA sequences plus links to the corresponding NCBI, TIGR TAIR, SwissProt and MIPS accessions, Clustal alignments, individual and consensus TM topology and targeting predictions, hydropathy profile plots, classification of proteins according to sequence similarity, the Transporter Classification (TC) system and Gene Ontologies (GO) terms, a display and list of neighboring genes, paralogs and orthologs in Arabidopsis/rice and three cyanobacteria. The database also contains experimental intracellular localization data collected from the literature, a comprehensive bibliography with links to PubMed and other features for data mining and sequence interpretation of membrane proteins. The database is continuously updated with the latest gene models and will be expanded by adding additional functions and membrane protein sets from more organisms. ARAMEMNON is accessible at the URL

http://aramemnon.botanik.uni-koeln.de.

T11-021

ARABI-COIL - an Arabidopsis Coiled-coil Protein Database

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Long coiled-coil proteins play an important role in the spatial and temporal organization of cellular processes, such as signal transduction, cell division, structural integrity and motility. While several protein classes with long coiled-coil domains have been studied in animals and yeast, our knowledge about plant long coiled-coil proteins is very limited. The repeat nature of the coiled-coil sequence motif often prevents the identification of homologues of animal coiled-coil proteins by sequence similarity searches. Consequently, counterparts of many animal proteins with long coiled-coil domains, like lamins, golgins, or microtubule organization center components, have not yet been identified in plants. On the other hand, the characteristic heptad repeat of the coiled-coil motif allows for the computational prediction of coiled-coil domains.

Using the algorithm MultiCoil on the Arabidopsis thaliana genome, we identified all Arabidopsis proteins predicted to contain coiled-coil domains. Processing software was developed to further select proteins containing long or multiple coiled-coil domains and to build a searchable protein database, ARABI-COIL (http://www.coiled-coil.org/arabidopsis/). This database integrates information on number, size, and position of predicted coiled-coil domains with subcellular localization signals, transmembrane domains, and available functional annotations. ARABI-COIL serves as a data-mining tool to sort and browse Arabidopsis long coiled-coil proteins, therefore facilitating the identification and selection of candidate proteins of potential interest for specific research areas. Using the database, candidate proteins were identified for Arabidopsis membrane-bound, nuclear, and organellar long coiled-coil proteins. The development of a corresponding rice coiled-coil protein database is in progress, which will allow for comparative analysis of long coiled-coil proteins encoded by different plant genomes.

Rose et al. (2004), Plant Physiol. 134: 927-939

T11-022

TAIR (The Arabidopsis Information Resource): New Tools and Data

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The Arabidopsis Information Resource (TAIR) (www.arabidopsis.org) presents researchers with a comprehensive data resource, integrating information on genes, proteins, maps, clones, seed and DNA stocks, biochemical pathways, community members and published literature. In the past year we?ve added several major new features to TAIR, including browsers for keywords and stocks, images of mutants and other stocks available from ABRC, improved microarray data searching and display, a tool for downloading Gene Ontology (GO) keywords associated to a list of genes, a method for researchers to add their publications to their TAIR community record, and improved help documents. TAIR?s ongoing literature-based manual curation effort associates Arabidopsis genes to GO structured vocabulary and TAIR anatomy and development terms. This manual effort has resulted in the association of terms to over 6000 genes, and a complementary computational annotation effort has associated terms to over 18,000 genes. Efforts in the next year will be focused in part on improving gene structures and genome annotation through community data submissions, incorporation of new cDNA and EST data, better incorporation of noncoding RNAs and addition of other genome features such as repeats and cis elements. Other areas of activity in the next year include a website redesign for more intuitive navigation, development of bulk data exchange methods and formats, and curation of phenotype and allele data from the literature.

T11-023

Genomic and systems biology approaches to understand CN signal interactions in Arabidopsis.

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Our long term goal is to have a general understanding of how the assimilation of nitrogen into amino acids in plants is controlled and relates to all other aspects of the plant physiology. Towards this goal, in the past years we have taken biochemical and genetic approaches to understand the regulation of N-assimilation. We have shown that signals such as nitrogen, carbon and light interact to affect nitrogen assimilation. We are now combining mathematical, genomic and system biology approaches to (1) catalog the nature of these interactions, (2) identify the gene networks involved and (3) understand the underlying biological principles.

Here we discuss the results obtained in a systematic exploration of carbon and nitrogen conditions (CN matrix) in Arabidopsis thaliana using Affymetrix whole-genome chips. We found that carbon (C), nitrogen (N) or carbon+nitrogen (CN) treatments elicit global responses in Arabidopsis roots that are readily distinguishable. Interestingly, we identified many genes that respond primarily when plants are treated with CN. Thus CN is an important factor that can induce major gene expression adjustments in plants, comparable in number but distinct in profile to those induced by C alone. We found ample evidence for interactions between C and N that extend beyond metabolic pathways. We used hierarchical clustering to catalogue these patterns and identify the genes with common regulatory strategies in response to C, N or CN. Functional analysis of these groups of genes suggests that CN-responsive genes are involved in protein synthesis and N-responsive genes participate in energy producing metabolic pathways and two-component signal transduction systems. To provide a more holistic view of how the cell molecular network is adjusted in response to these external perturbations, we constructed a model of the plant cell metabolic (KEGG, AraCyc) and regulatory network (Transfac). We integrated gene expression data with this model and identified gene networks that respond to C, N or CN inputs.

Current efforts are directed towards exploring the following input combinations: carbon, nitrogen, light and organs. Because testing all combinations of every input dose is not only impractical but impossible, we are using mathematical methods to generate experimental sets that cover many input/dose combinations in an efficient and economical manner.

T11-024

Detecting Chromosome Features using an Unsupervised Probabilistic Multigram Model: a Case Study of the Arabidopsis thaliana Genome

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DNA feature and structure detection using computational methods becomes increasingly important in navigating through mounting collections of genome sequence data. Well-known methods include sequence alignment, motif-based systems, and stochastic models, among others. These approaches can be classified as supervised or unsupervised, and may or may not crucially employ pre-determined reference data. Alignment-free methods can naturally tolerate reorganizations and insertions common to genome evolution, and when unsupervised permit de novo determination of features and feature association. We develop a notion in an unsupervised, alignment-free context that we call a lexicon, an inductively generated set of nucleotide “words” of varying length devised to represent optimally a given sequence. This unsupervised method applies Expectation Maximization (Dempster, 1977) to a multigram model to estimate the most likely parse of a DNA sequence in the course of acquiring the sequence lexicon (or grammar). The resulting lexicon and parse provide points of departure for sequence analyses utilizing lexicon content, the sequence representation, and sequence information content. This poster illustrates the approach applied to the study of Arabidopsis thaliana chromosomes where familiar feature annotations illustrate and guide assessment of the method’s capabilities. Issues and considerations topical to this poster include sequence segmentation, and lexicon composition and sequence information content applied to chromosome analysis.

Dempster, et al. 1977. Maximum likelihood from incomplete data via the EM algorithm, J. R. Stat. Soc., Series B 39, 1-38

T11-025

The Botany Affymetrix Database: e-Northerns and Expression Angling

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In order to facilitate access to and sharing of gene expression data generated by our Affymetrix facility, we have implemented a web-enabled database based on MIAME and MAGE-ML standards [1,2]. The database currently contains data for approximately 22,000 genes across ~100 samples. Three web-based tools for querying that provide several novel features have been developed: a Project Browser, an Expression Browser, and an Expression Angler. The Project Browser allows users to view all attributes of an experiment, and to download the data associated with it. The Expression Browser allows uses to perform socalled "electronic Northerns", i.e. to input a list of genes, which will be selected across all or user-specified experiments in the database. The Expression Angler is a tool for "fishing" for genes with similar expression profiles, across all samples in the database, from NASCArrays, or in a user-provided set. The results from the latter two tools can be displayed in an extensively annotated HTML format, or plain text. The HTML format contains compact tabular representations of tissue type analyzed, plant age, type of experiment, plus expression levels. For each gene entered functional categories, annotation, and gene aliases, plus links to TAIR are also provided. The Botany Affymetrix Database is accessible for non-commercial use at http://bbc.botany.utoronto.ca:88.

1. Brazma et al. (2001). Nature Genetics 29(4): 365-71

2. http://www.mged.org/Workgroups/MAGE/mage-ml.html

T11-026

Modeling and in vivo live imaging of the Arabidopsis shoot apical meristem

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The shoot apical meristem (SAM) is an amazing dynamical system that provides a basis for the development of the complete aboveground part of a plant. Due to the high complexity of the SAM a mathematical description of the molecular and mechanical dynamics can be a useful tool for illuminating processes during its development. The basic idea is to use current biological knowledge, and to implement a model of the dynamics for simulation on a computer. Models that survive initial validations can be used to test different hypotheses in a much faster and broader way than what is allowed for in experiments. A modeling approach can then be used to guide experiments and laboratory efforts can be directed to those most likely to answer the biological question at hand.

We are applying an approach where in vivo live imaging of proteins and cells is used together with modeling to gain a better understanding of molecular processes within the SAM. The in vivo imaging technique, where proteins are fused to GFP, is used for dynamical tracking of the subcellular locations of important proteins throughout the complete SAM for a time period of days. In parallel we are building a software platform for simulation of developmental systems where the Arabidopsis SAM is used as the main biological target. The software allows for gene regulatory networks, molecular reactions, molecular transport between cells, cell growth and division, and mechanical interactions between cells. The relatively low number of cells in the Arabidopsis SAM allows for an in silico system where all the cells can be accounted for.

In this talk I will concentrate on models for phyllotaxis. Recent studies have indicated that auxin plays an essential role in determining organ position on the shoot apical meristem flanks. In particular, the distribution of auxin, as patterned by the putative efflux carrier PIN1 appears to directly determine the site of primordial emergence. Auxin and PIN1 are the main molecules in our models, and simulations are compared to the dynamics of the in vivo localization of the PIN1 protein. In models of different resolutions we show how regular patterns of auxin concentration peaks which resemble phyllotactic patterns can be obtained. Various simulation results will be presented along with analysis of the models.

http://www.computableplant.org

T11-027

GENEVESTIGATOR: Arabidopsis thaliana microarray database and analysis toolbox.

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High-throughput gene expression analysis has become a frequent and powerful research tool in biology. At present, however, few software applications have been developed for biologists to query large microarray gene expression databases using a web-browser interface. We present GENEVESTIGATOR, a database and web-browser data mining interface for Affymetrix GeneChip® data. Users can query the database to retrieve the expression patterns of individual genes throughout chosen environmental conditions, growth stages, or organs. Reversely, mining tools allow users to identify genes specifically expressed during selected stresses, growth stages, or in particular organs. Using GENEVESTIGATOR, the gene expression profiles of more than 22000 Arabidopsis thaliana genes can be obtained, including those of 10600 currently uncharacterized genes. The objective of this software application is to direct gene functional discovery and design of new experiments by providing plant biologists with contextual information on the expression of genes.
The database and analysis toolbox is available as a community resource at https://www.genevestigator.ethz.ch.

T11-028

Modeling Arabidopsis thaliana from genes to phenotypes

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Computational plant models, or `virtual plants', are increasingly seen as a useful tool for the comprehensive, quantitative understanding of the mechanisms of plant development. The modeling of plants has a relatively long history, but the modeling of the fundamental relationships between genetic regulatory mechanisms, short- and long-distance signaling, and plant form introduces new challenges and requires new methodologies. Their development is a fascinating area of current interdisciplinary research.

In order to harness the complexity of developing plants, computational models decompose them into populations of modules. Depending on the scale, these modules may represent cell components, individual cells, multicellular tissue regions such as primordia, or higher-level architectural units such as internodes, leaves, flowers, and buds. The models integrate the behavior of individual modules into the development of higher-level structures.

My work is focused on L-systems, a modular description of developing organisms first proposed in 1968 by A. Lindenmayer. L-systems are a convenient mathematical and programmatic framework for simulating the development of branching structures. Recent extensions of L-systems make it possible to incorporate genetic regulatory networks into developmental plant models. L-systems also provide a template for constructing models of tissues that are not limited to branching topology.

In collaboration with the groups of Enrico Coen (John Innes Centre), Ottoline Leyser (University of York), and Cris Kuhlemeier (University of Bern), my group is applying L-systems and their extensions to explore the essence of the processes that define the architecture of Arabidopsis shoots. At the focus of this work is genetic and hormonal control of phyllotaxis, transition to flowering, and branching. Sample models capture postulated mechanisms and highlight open questions related to: the interplay between the distribution of PIN1 molecules and the transport of auxins in the apex; the mechanisms of apical dominance; and the determination of the fate of buds in Arabidopsis shoots. The presentation of these models will be illustrated with interactive simulations implemented using L-studio and related plant-modeling software being developed at the University of Calgary.

T11-029

From Genes to Morphogenesis

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Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities with in an organism during its development. However,the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify growth of multi-cellular tissues in 3D space. We have been addressing this problem using a combination of genetic, morphological, computational and imaging ap-proaches in collaboration with the laboratories of Przemyslaw Prusinkiewicz (Calgary) and Andrew Bangham (Norwich). The results provide new insights into how genes interact with patterns of growth to modify plant architecture and organ shape.The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

T11-30

A MapMan-based web-application that extracts gene expression level of your favourite gene from databases like AtGeneExpress and NASC, and visualises it in false-colour code on simple diagrams

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Microarray experiments generate a flood of information that is hard to inter-pret in a comprehensive and systemic manner. Thus the individual scientist cannot benefit from the potential of full genome analyses and biological messages remain uncovered.
Many software tools have been developed during the last years that facilitate the scientist's access to high-throughput datasets, but only few of them are suitable for biologists without any bioinformatic training. The MapMan tool displays large-scale datasets onto familiar pathway maps and aids to analyse profiling experiments intuitively (Thimm et al. 2004). The new web-application provides a complementary data mining approach tracking expression data of a restricted set of favourite genes in public resources (AtGeneExpress, NASC) which are subsequently displayed as virtual in-situ hybridisations/Northern analysis onto treatment-related maps using a web-interface. For a first build diurnal and developmental expression profiles of Blaesing et al. and Weigel et al. were used to reveal gene expression levels during a day/night cycle and in different organs throughout the Arabidopsis lifecycle. Normalised expression data are pre-processed using the in-house ComprehensiveSystemsBiology. DataBase (Steinhauser et al.) and transmitted to a modified MapMan web-application. Gene expression levels are automatically mapped in false-colour code onto diurnal maps and Arabidopsis diagrams of the analysed organs at defined states of development (Lohmann and Schmid). Additionally the user gets a graphical overview if his genes of interest were differentially expressed upon hormone, cold and other treatments.
The presented web tool bundles visually the fast growing quantity of informa-tion stored in public profiling resources and facilitates biologist's everyday life to trace complementary information of their genes of interest and therefore aids scientists to generate biological hypothesis.

Thimm, O. et al. (2004). MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37(6): 914-39.

T12 Non-Arabidopsis
(Limitations of the Arabidopsis Model)

T12-001

Arabidopsis cannot survive long periods of anoxia: analysis of gene expression during mitochondrial biogenesis using rice germination as a model system

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Apart from being an important crop plant, rice is able to withstand long periods of anoxia and can germinate without oxygen. This allows the dissection of genetic and developmental events from environmental influences and separation of mitochondrial biogenesis from respiratory function. We examined transcript abundance of mitochondrial components during germination and in the presence or absence of oxygen using real-time quantitative PCR. Genes encoding mitochondrial proteins were sequentially expressed in embryos during rice seed germination. Genes encoding protein import apparatus subunits were expressed early, followed by the mitochondrial RNA polymerase, mitochondrial components of the tricarboxylic acid cycle and of the non-phosphorylating bypasses of plant respiration. Nuclear-encoded components of the respiratory chain and mitochondrial ribosomal proteins were the final group of genes to achieve maximum transcript levels. When comparing the effect of normoxia and anoxia, a tiered response in gene expression was revealed with some transcripts affected by oxygen availability immediately, others after 4-12 hours, 12-24 hours and others not at all. We also investigated organellar to nuclear genome ratios using real-time PCR and found that anoxia affected mitochondrial to nuclear ratios while mitochondrial morphology, examined using transmission electron microscopy, was unaffected. Coordination of nuclear and mitochondrial gene expression with mitochondrial genome division and communication pathways other than retrograde regulation are thus proposed to be involved in plant mitochondrial biogenesis. Further work will involve determination of signals involved in oxygen sensing and initiation of mitochondrial biogenesis and its regulation.

T12-002

Genome analysis in sugar beet (Beta vulgaris L.)

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Sugar beet (Beta vulgaris L.) belongs to the family of Chenopodiaceae and is an important crop plant for sucrose production in temperate climatic zones. It is characterized by highly specialized anatomical structures such as up to 15 secondary cambium rings in its storage organ, the tap root. Sugar beet physiology allows the accumulation of sucrose up to 20 % of the fresh beet weight in the first year of the biennial life cycle. Sugar beet breeding is directed towards the generation of varieties with improved sugar quality and yield as well as disease resistance. In the last decade molecular genetic tools like molecular markers and genetic maps have been created to facilitate the identification of genetic factors underlying agronomic traits. Using sequence information from Arabidopsis and other species more than 15.000 sugar beet ESTs were annotated and contributed to public databases.
In our group we have developed more than 300 genetic markers mostly based on EST sequences of potential candidate genes for the above mentioned traits. Combining genotypic and phenotypic data from segregating F2 populations we were able to locate Mendelian genes and QTLs for sugar quality and yield as well as for resistance to rhizomania and Cercospora beticola on the nine linkage groups of sugar beet. Additionally, we established cDNA macroarray analysis to identify candidate genes based on their expression pattern during beet development and in beet regions of varying sucrose content.
The presentation will give an overview about our activities with a focus on the identification of candidate genes, SNP mapping and approaches to validate candidate genes in sugar beet. Potentials and pitfalls to use Arabidopsis as a model system for sugar beet will be addressed.

Mol. Breeding 8: 63-74 (2001), TAG 104: 1107-1113 (2002), Plant Biol. 4, 700-710 (2002), Genome 46:70-82 (2003)

T12-003

CYCLOIDEA and Floral Symmetry in Aster family

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Even though flower forms are incredibly diverse, they can be grouped into two classes: radial and bilateral flowers. CYCLOIDEA, a TCP transcription factor, plays an important role in controlling the distinction between these two floral forms in Antirrhinum.

Inflorescences in the aster family (Compositae) have a unique structure, called capitulum (flower head). Although a capitulum superficially resembles a single flower, it comprises a group of flowers (florets). Three floret types [disc floret (radial), ray floret (bilateral) and bilabiate (two-lipped)] and five capitulum types [radiate (consisting of disc florets surrounded by ray florets), bilabiate (consisting of only bilabiate florets), ligulate (consisting of only ray florets), discoid (consisting of only disc florets) and disciform (consisting of two types of disc florets)] are found in nature. In Senecio vulgaris, both radiate and discoid capitula are found and these capitulum forms are controlled by RAY locus. Preliminary data showed that Senecio CYCLOIDEA orthologs (sCYC) are expressed in two different ways. One class is expressed in the adaxial part of every floret, whereas the other class is expressed only in the ray florets. A linkage analysis revealed that sCYCs expressing only in the ray florets were linked to the RAY locus. This suggests that sCYCs expressing only in the ray florets are responsible for determining presence or absence of the ray florets, while, the other sCYCs expressing in the dorsal part of the each floret may be responsible for controlling the flower symmetry.

To confirm that the sCYC is the RAY, further linkage analysis is under way as well as sequence analysis of sCYCs from the different capitula of Senecio vulgaris. Furthermore, we will determine if CYC orthologs are involved in generating different forms of capitula in other aster family species.

T12-004

Assessing the impact of polyploidy by comparative analysis of Brassica genome microstructure

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The cultivated Brassica species are the group of crops most closely related to Arabidopsis. They represent models both for the application in crops of genomic information gained in Arabidopsis and for the study of plant genome evolution. An important aspect of genome structure in Brassica species is redundancy resulting from polyploidy in their ancestry. How will this limit our ability to apply knowledge gained in Arabidopsis? To begin to address this, we have conducted a comparative analysis of genome microstructure. Genomic BAC libraries were constructed for B. rapa (containing the Brassica A genome), B. oleracea (containing the Brassica C genome) and B. napus (a natural tetraploid containing both A and C genomes) and used to undertake a comparative analysis of the microstructure of homoeologous regions of the genomes of Arabidopsis, B. rapa, B. oleracea and B. napus. The genomes of the diploid Brassica species show extensive triplication of an Arabidopsis-like genome structure. These segments show, compared with Arabidopsis, generally well-conserved gene order, but extensive interspersed gene loss (O'Neill et al., Plant Journal 23:233-243, 2000). We found genome microstructure to show extensive, though imperfect, conservation between the A and C genomes as represented in B. rapa and B. oleracea. We also found that the microstructure of the A and C genomes are highly conserved in B. napus, indicating that extensive genome change at the level of microstructure did not occur during the formation of natural B. napus, contradicting interpretations based on the study of resynthesised B. napus (Song et al., Proc. Natl. Acad. Sci. USA 92: 7719-7723, 1995). We interpret our results as indicating that the evolution of plant genome microstructure is an ongoing process, i.e. it is not limited to a burst of activity immediately following polyploid formation. Although the many-to-one relationship of genes in Brassica and Arabidopsis will inevitably complicate the transfer of functional information, our growing appreciation of the structures of the Brassica genomes, and how they evolved, will help overcome the problems.

T12-005

Development of three different cell types is associated with the activity of a specific MYB transcription factor in the ventral petal of Antirrhinum majus flowers

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The petals of insect-pollinated species are much more specialised and elaborate than those of self-pollinated species such as Arabidopsis. The petals of Antirrhinum majus differentiate a number of specialised cell types. Conical petal epidermal cells attract pollinators by focussing light and heat into petals, while trichomes in the corolla tube provide guides for nectar-gathering bees, trap pollen from the surface of pollinators and are also the site of scent production. A cDNA encoding an R2R3 MYB protein (AmMYBML1) was isolated from developing petal tissue and the encoded protein was shown to induce both trichome and conical cell formation in floral tissues. AmMYBML1 is expressed in the ventral petal only, in the trichomes and epidermal cells of the corolla tube, in the epidermal cells of the ventral petal hinge and in the mesophyll on the adaxial side of the ventral petal hinge. The expression pattern of AmMYBML1 suggests that it fulfils three functions in A. majus; trichome production in the corolla tube, conical cell development in the petal hinge epidermis and the re-enforcement of the petal hinge through differential expansion of the adaxial cells of the mesophyll relative to the abaxial cells to provide the landing platform for pollinators. The DIVARICATA (DIV) gene of Antirrhinum is required for the specification of ventral petal identity. In div mutants the ventral petal assumes the identity of lateral petals. div mutants lack specific trichomes in the corolla tube, conical epidermal cells in the region of the ventral petal hinge and the undulations of petal tissue that re-enforce the hinge. Expression of AmMYBML1 is reduced very significantly in the div mutant compared to wild type corolla tissue. Within the AmMYBML1 promoter is an I-box motif; the recognition sequence for transcription factors related to DIV. We show that DIV protein binds the promoter of AmMYBML1 and suggest that AmMYBML1 is transcriptionally regulated by DIV and, consequently, controls cell specification within the ventral petal.

T12-006

A population genomic search for maize domestication genes

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Levels and patterns of SNP variation provide insights into the historical process of selection and demography. Currently, large-scale measurement of SNP variation is ongoing in only two plant species: Arabidopsis and maize (Zea mays ssp. mays). Our studies in maize are designed explicitly to identify genes that were selected during the domestication process. Here I detail our analysis of 599 gene regions that were sampled for SNP diversity in a sample of 14 maize inbred lines and in a sample of 16 individuals representing the wild ancestor of maize. SNP variation in maize is roughly 60% of the variation found in its wild ancestor; this decrease in genetic diversity is consistent with a population bottleneck during domestication. However, genes should fall into two historical classes: those that were bottlenecked (without artificial selection) and those that were also selected during domestication and subsequent breeding. We used the SNP data both to estimate the proportion of genes that were selected in maize and to generate a list of candidate selected genes. To do this, we fit the SNP data to population models, using extensive coalescent simulations of genetic bottlenecks, and we also devised a maximum likelihood estimator. We estimate that 7% of maize genes were under selection similar in strength to that experienced by tb1, a well-known domestication gene. We have also generated a prioritized list of candidate genes, many of which may have agronomic importance. Our results illustrate the power of population genomics, and our analytical approaches can be modified for wide variety of applications.

T12-007

Wox gene phylogeny and expression in Maize and Arabidopsis: A comparison of embryonic pattern formation

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Although plant morphology exhibits tremendous variability, the basic principles of development, e.g. those based on meristem function, are conserved between plant species. Comparative approaches should therefore allow the discrimination of common mechanisms from species-specific pecularities. Within the angiosperms, the divergence of monocots and dicots is estimated to have occurred over 150 million years ago (1). The difference giving rise to the name, single (mono) or two (di) cotyledons is already manifested during early embryogenesis and is related to the formation of the anlage of the shoot apical meristem (SAM). In contrast to an apical position in the Arabidopsis globular embryo, the SAM anlage is established at a lateral position of the transition stage maize embryo. Therefore, genes associated with SAM function are attractive candidates as molecular markers to compare patterning between monocot and dicot embryos. One of the earliest markers associated with the Arabidopsis SAM anlage is the WUSCHEL (WUS) gene, which promotes stem cell fate and antagonizes the CLAVATA signalling pathway restricting the number of pluripotent stem cells in the SAM. WUS encodes a homeodomain transcription factor and is a member of a subfamily consisting of about 20 genes in Arabidopsis. Many of these so-called WOX genes have been isolated recently and show informative expression patterns during embryogenesis (2). Another member of this gene family is PRESSED FLOWER (PRS; 3).

The availability of the Arabidopsis genome sequence allows a phylogenetic approach to be taken to directly compare sequence homologies between maize and Arabidopsis and search for orthologous gene functions. Using this approach, we have isolated and characterized several WUS-like homeobox genes in maize. A comparison of the expression patterns of these genes reveal informative similarities and differences during embryonic patterning between maize and Arabidopsis.

For example, Narrow sheath1 and 2 (NS1, NS2) both represent maize orthologues of PRS (Nardmann et al., in press). A comparison of the prs and ns mutant phenotypes and the expression patterns of the corresponding genes indicates that the development of the maize coleoptile and not of the scutellum is similar to that of the Arabidopsis cotelydons. The isolation and characterization of two putative WUS-orthologues from maize and their implication on the conservation of the WUSCHEL-CLAVATA pathway in monocots will also be discussed

(1) Wikstrom N. and Kenrick P. (2001)
(2) Haecker et al. (2004)
(3) Matsumoto N. and Okada K. (2001)

T12-008

Tobacco functional genomics: Uncovering the importance of "-like" and "unknown" genes for plant fitness

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The sequencing of full genomes has revealed that we know little or nothing about the function of the majority of genes that they contain. One of the largest challenges for post-genomics research is to improve our understanding of genes whose function is poorly understood or completely unknown. Here we describe the identification of genes that play a key role in plants by using an unbiased high-throughput random post-transcriptional gene silencing (PTGS)-approach. The advantage of this technology is that there is typically a residual expression of 5-40% at the protein level when gene expression is inhibited by either “sense”- or “antisense”-constructs. This is a more rigorous way to identify key genes than the prevalent paradigm of knock-out mutants, which may miss some absolutely essential genes because knock-outs in them are lethal. At present, more then 90 cDNAs have already passed the validation process (a second independent transformation). This number can be predicted to rise to >120 (corresponds to 0,6% of the 20.000 genes tested). Moreover, the ongoing work uncovers a substantial subset of poorly annotated genes, for which quantitative changes in their expression levels have major impact on plant health and performance.

T12-009

Cold-induced pollen sterility in rice is associated with a disruption in sugar metabolism and increase in ABA levels

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Cold-induced pollen sterility is a problem that greatly affects the rice crops grown in Australia and other temperate-climate regions of the world. Male gametophyte development in rice is highly sensitive to abiotic stresses such as drought, cold, heat, and salinity. Cold temperatures at the young microspore stage of pollen development cause a reduction in pollen fertility, which ultimately results in large reductions in grain yield. We are studying the effect of cold temperature on sugar metabolism in rice anthers. We have found that pollen sterility induced by cold is associated with an accumulation of sucrose and decreased cell wall invertase activity in anthers at the young microspore stage, and cold-affected pollen lacks starch at maturity. We have identified a cell wall invertase gene (OSINV4) that is expressed in rice anthers and is down-regulated by cold treatment. Expression analysis of monosaccharide transporter genes in rice anthers indicates that a sugar transport pathway involving OSINV4 could be blocked by cold. These results suggest that cold temperatures may induce a re-direction of sugars away from the developing microspores. Cold-induced pollen sterility is also associated with an increase in the hormone ABA within 5 hours of cold treatment at the young microspore stage of pollen development. These results indicate that ABA might be involved in signalling events that cause pollen sterility, possibly interacting with sugar signalling pathways.

T12-010

Molecular and Genetic Analysis of rough endosperm Mutants in Maize

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The Arabidopsis endosperm is largely subsumed in the mature seed with only a persistent single cell layer that is comparable to a cereal aleurone. Due to the reduced size of the Arabidopsis endosperm, it is difficult to identify genes required for cereal grain development from Arabidopsis mutants. In contrast, the maize endosperm is an expanded and differentiated organ in mature kernels, and thus, is an accessible genetic model for plant development. As a direct product of fertilization, the endosperm does not require a functional meristem for organ formation or cell type differentiation. A molecular understanding of endosperm development mutants should provide a basis for targeted seed improvement and will answer basic questions about organ formation in maize. The rough endosperm (rgh) class of seed mutants disrupts normal endosperm and embryo development and is characterized by seeds with a pitted or etched surface. We have identified 140 rgh mutant isolates from the UniformMu population. UniformMu is a Robertson's Mutator, transposon-active population that is introgressed into the W22 color-converted inbred. We used SSR and B-A translocation mapping to locate 17 of these rgh isolates to the long arm of chromosome 5. Mature kernel sections of the 5L rghs showed several distinct endosperm and embryo defects suggesting possible allelic groups. Preliminary complementation tests based on these phenotypic groups indicate at least 4 loci. Furthermore, the complementation tests identified multiple alleles for 3 of the 5L rgh loci To clone the rgh genes, we are utilizing MuTAIL-PCR to amplify Mu-flanking sequences from selected mutants.

T12-011

Drought Stress Tolerance: from gene discovery in Arabidopsis to an application in Crops

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Efficient use of water in agricultural production will be one of the great challenges during the 21st Century, with agriculture currently being responsible for ~70% of freshwater withdrawal. As such, yield improvement through tolerance to water deficits that occur routinely in the Central Corn Belt and frequently in western states are an important challenge in the coming decade. Benefits of improving water utilization efficiency, in addition to higher yield, are expected to include reduced water consumption and environmental sustainability. Genetic approaches using model systems are adding to our understanding of plant pathways that are important to water stress tolerance. Transgenic approaches in model systems such as Arabidopsis have identified genes that effectively confer drought tolerance in both Arabidopsis and crops such as soybean and corn. This presentation will illustrate our ability to uncover novel drought protection mechanisms using genomics data and will highlight data from Arabidopsis, corn and soy transgenics that demonstrate the enormous opportunity that exists for the application of genomics to product development in crops.

T12-012

Subcellular analysis of carbon partitioning and regulation of ADP-Glucose-pyrophosphorylase in rice endosperm

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ADP-Glucose-pyrophosphorylase (AGPase) is catalysing the conversion of glucose-1-phosphate and ATP to pyrophosphate and ADP-glucose, the latter being the direct precursor for starch synthesis in plants. Most of the previous studies on the regulation of this enzyme were performed in potato tubers (1) and Arabidopsis leaves (2) leading to the recent finding that the activity of this enzyme is regulated by post-translational redox-modification. Reduction leads to activation of the enzyme, involving breakage of a cystein bridge which is formed between the two catalytic subunits (AGPB) of the hetero-tetrameric holoenzyme. In Arabidopsis and potato, AGPase is localised exclusively in the plastids where starch synthesis occurs. In contrast, graminaceous endosperm contains a second isoform of AGPase, which is found to be abundantly present in the cytosol. Little is known concerning the regulation of this AGPase isoform and its role for carbon partitioning between the sub-cellular compartments. To investigate this, we used developing rice seeds as a model system. The activity of AGPase extracted from rice endosperm was found to be allosterically activated by 3PGA and inhibited by Pi and fructose-1,6-bisphosphate. Activation by 3PGA occurred at physiological concentrations (500 µM) and was strongest at low ADP-glucose concentrations (200µM). Further increase of the AGPase activity was obtained by adding the reducing agent dithiothreitol (DTT), indicating that AGPase in rice endosperm is redox-regulated in a similar way as in Arabidopsis and potato. Using non-reductive SDS-PAGE and detection of AGPB by Western blotting we investigated whether enzyme activation was accompanied by changes in the dimerisation state of AGPB. A small but significant amount of AGPB was present as a dimer, which was monomerised after adding DTT. Similar results were obtained with AGPase from maize endosperm. The physiological relevance of this mechanism is currently under investigation. Furthermore, non-aqueous fractionation of rice endosperm is being used to separate cytosolic and plastidial AGPase isoforms and analyse their individual regulatory properties. Metabolic profiling of the different sub-cellular fractions is currently underway to resolve the sub-cellular distribution of metabolites and the thermodynamic structure of the starch synthesis pathway in rice endosperm in vivo.

(1) Tiessen et al. (2002) Plant Cell 14, 2191-2213
(2) Hendriks et al. (2003) Plant Physiol 133, 838-849

T12-013

Two Zn-responsive metallothionein genes from Thlaspi caerulescens

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Thlaspi caerulescens is a small plant with high capacity to accumulate zinc, cadmium and nickel to normally toxic amounts in its shoots. The molecular mechanisms of heavy metal accumulation are largely unexplored. To find active, Zn-responsive genes from T. caerulescens, differential display analysis was made. With this approach, ca. 20 cDNA fragments were found which are differentially expressed in Zn exposure.

Among the Zn-responsive genes were two metallothioneins (MTs). Based on the arrangement of cysteine-rich domains, one of the MTs (TcMT2) could be classified as type 2, having a high homology with A. thaliana MT2a. The second MT (TcMT3) has one amino acid difference compared to domain classification of MT3 but, based on sequence homology, it was classified as MT3.

The accessions showed differences at the MT mRNA levels: TcMT2 was induced by elevated Zn concentrations in calaminous population, whereas TcMT3 was induced by Zn in both non-calaminous and calaminous populations.

To study if TcMTs are able to increase Cd or Cu tolerance, the genes were transferred into yeast. In metal exposure, yeasts expressing TcMT2 or TcMT3 gene were able to grow at higher Cu or Cd concentrations. Of the two genes, the TcMT2 increased metal tolerance more than TcMT3 did.

In this study, it was shown that TcMT2 and TcMT3 are capable of conferring Cu and Cd tolerance in vivo, suggesting that the TcMTs are active proteins which directly bind metals or indirectly protect against metal toxicity and are involved in metal tolerance.

Acknowledgements: EU project PHYTAC contract nº: QLRT-2001-00429; Academy of Finland project nº: 53885; The Finnish Graduate School in Environmental Science and Technology

New Phytologist, 159; 411-419, 2003

T12-014

Ecological Genomics of Glucosinolates in Boechera (Brassicaceae)

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Plants must defend themselves against a variety of herbivorous insects. For example, the Brassicaceae have evolved a two-component defense system based upon a diverse class of amino acid-derived secondary metabolites, the glucosinolates (GSL's), and the hydrolysis of these compounds to toxic products by endogenous myrosinase enzymes. In the annual, Arabidopsis thaliana, aliphatic GSL's derived from methionine (Met) display a high level structural variability resulting from chain-elongation of Met and subsequent side-chain modification of the assembled GSL. Quantitative genetic studies implicate loci associated with both processes in insect resistance. We are developing Boechera drummondii and Boechera holboellii as models to investigate natural variation and GSL function / diversity. These perennial species are subject to a diverse herbivore community and, thus, the evolution of resistance traits is expected to reflect multiple selective episodes throughout the multiyear lifecycle of the plants.

In a survey of ten B. drummondii populations and 11 B. holboellii popluations, we have found that GSL's with aliphatic side-chains are the major constituents of the GSL pool. Met-derived GSL's are present in both species with carbon-chain elongation forming the basis of GSL structural diversity. Variation in GSL phenotype, both side-chain length and secondary side-chain modification, is observed in both inter- and intraspecific comparisons. Quantitative genetic analyses reveal that resistance of these species to the generalist herbivore, Trichoplusia ni, has a genetic basis and is significantly positively correlated to GSL quantity. We have constructed genomic libraries from six distinct Boechera genotypes. Sequence data obtained from these libraries is being used in the functional analysis of GSL diversity in B. drummondii and B. holboellii, and to identify the evolutionary forces shaping this diversity. The candidate locus for the Met-chain-elongation function associated with aliphatic-GSL's is the first target of our study.

T12-015

PROTEIN-PROTEIN INTERACTIONS BETWEEN MADS BOX TRANSCRIPTION FACTORS DIRECTING FLOWER DEVELOPMENT IN SUNFLOWER AND CHRYSANTHEMUM

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MADS-box genes are components of complex networks of genes that play important role in flower development and tend to share similar developmental functions. Their products have been shown to act in combination to determine meristem identity and to specify the fate of floral organ primordia. Quartet model suggests that MADS-box proteins regulate transcription of target genes as quarternary complexes. In model plant A. thaliana the petal identity is determined by quartet of MADS-box proteins of А, В and Е groups; the stamen identity ¯ quartet of B, C and E MADS-box proteins; the carpel identity ¯ quartet of C and E MADS-box proteins (1).
Our research deals with the investigation of inflorescence development in sunflower Helianthus annuus and chrysanthemum Dendrathema grandiflora (2) ¯ the characteristic members of the Compositae - the most evolutionary advanced family of flowering plants.
Here we present the results of comparative functional analysis of protein-protein interactions between MADS-box transcription factors of A. thaliana and Compositae¯ sunflower H. annuus (HAM proteins) and chrysanthemum D. grandirlora (CDM proteins). The chrysanthemum and sunflower cDNA libraries were assayed using the yeast two-hybrid GAL4 system. Analysis of the sunflower cDNA library with HAM31 as bait resulted in the isolation of two full-length cDNA copies of MADS-box genes HAM65 and HAM31. Analysis of the chrysanthemum cDNA library with CDM86 as bait resulted in the isolation of two full-length cDNA copies of MADS-box genes CDM19 and CDM115. Two-hybrid analyses of the chrysanthemum cDNA library with CDM111 and HAM75 as baits identified in both cases cDNA of MADS-box gene CDM36. CDM and HAM proteins interactions were assayed in the yeast two- and three-hybrid GAL4 system. The network of CDM/HAM proteins interactions allowed us to suggest possible roles for CDM and HAM transcription factors, respectively, in chrysanthemum and sunflower inflorescence development. Existence of several paralogues of CDM and HAM transcription factors allowed us to suggest that floral organ identity can be determined by several quartets composed from different paralogues. Some of HAM and CDM proteins interactions have no literature analogues yet and suppose an existing of additional quarternary complexes participating in floral differentiation.
This study was supported by the Netherlands¯Russia Foundation NWO (project no. 047-007-016).

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T12-016

Proteomic profiling of Thlaspi caerulescens populations

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Thlaspi caerulescens is one of the most interesting metal hyperaccumulator plants, which belongs to the same taxonomic family (Cruciferae) as does Arabidopsis thaliana. Even though the phenomenon of hyperaccumulation has been widely explored, the molecular mechanism of metal accumulation is largely unknown. The main objectives of the present study were to characterise proteins and protein networks related to metal exposure and accumulation by comparing protein patterns of various Thlaspi populations and identifying metal-responsive proteins putatively related to the hyperaccumulation.
T. caerulescens populations Monte Prinzera, Lellingen and La Calamine with different characteristics of metal transport and accumulation were exposed to Zn deprivation, 500 µM and 1000 µM Zn and 60 μM Cd in hydroponics. Total soluble proteins were extracted from the leaves and roots. A 2-dimensional electrophoresis was run and gels were stained with Sypro orange fluorescent stain. The protein pattern analysis was done with PDQuest software and using statistical analysis: analysis of variance, Kruskal-Wallis and principal component analysis. Proteins of interest were identified with HPLC-ESI-MS/MS mass spectrometry.
Statistical approach showed that greatest differences in protein expression mainly among populations whereas the effects of different exposure levels were less pronounced. On the basis of the statistical analysis the most interesting proteins were chosen for mass spectrometric analysis. Among the identified proteins were many proteins related to photosynthesis, carbohydrate metabolism and general stress response. The proteins were identified mainly based on homologous genes and proteins in Arabidopsis, as expected. It is possible that the differences between the populations seen in this study represent allelic differences or post-translational modifications of the proteins but may also indicate functional variation. In addition, the hyperaccumulator plant Thlaspi provides a good model since many of the proteins can be identified based on homology to Arabidopsis. However, many proteins remained unidentified. These proteins may be of particular interest for further studies of metal tolerance, uptake and accumulation. This study showed that proteomic approach combined with advanced statistical analyses offers great possibilities for a large-scale comparison of protein patterns in various plant populations.

Assunção A et al. (2003) New Phytol. 159: 411-419.
Koistinen K et al. (2002) New Phytol. 155: 381-391.

T12-017

Cytokinin signaling in secondary vascular development

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The secondary (cambial) phase of plant development is initiated through the activation of a lateral meristem, the vascular cambium. Secondary vascular tissues are produced via cell divisions taking place in the cambium. We have previously shown that a mutation, wooden leg (wol), in the CRE1/WOL/AHK4 gene coding for a cytokinin receptor, results in reduced cell proliferation during primary (procambial) development of the Arabidopsis root (Mähönen et al. 2000: Genes & Dev. 14:2938-2943).
To examine, if cytokinin signaling is involved in the regulation of cell proliferation also during the secondary development, we have investigated the role of CRE1-like receptors in the regulation of cambial activity in two tree species, silver birch and poplar. We have been able to identify from birch three genes (BHK4, BHK2, BHK3) highly homologous for three Arabidopsis CRE1-like genes. The BHK genes are active in the cambial zone of the birch stem, and the putative birch ortholog of CRE1, BHK4, is functional as a cytokinin receptor in Arabidopsis. In addition to the BHKs, we have been able to identify several other birch sequences homologous for genes encoding components of the Arabidopsis cytokinin signaling pathway. To demonstrate the role of cytokinin signaling during secondary vascular development we are producing transgenic trees with reduced cytokinin signaling.

T12-018

Altered apyrase activity influences potato (Solanum tuberosum) plant development and tuber yield

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Apyrases hydrolyze nucleoside triphosphates and diphosphates and are found in all eucaryotes, where they have essential functions in platelet aggregation and neuronal signaltermination. Although their biochemical properties have been characterized in detail, only little is known about their physiological function in plants. In addition to the insoluble membrane-localized apyrase which appears ubiquitous in plants, S. tuberosum features a soluble apyrase with high activity. By using apyrase-GFP-fusion proteins and in vivo-activity measurements we were able to provide evidence for apoplastic localization of this soluble apyrase. Silencing of the apyrase gene family with RNAi-constructs under the control of the constitutive 35S-promotor led to drastic phenotypic changes in potato plants such as growth retardation, necrosis of the leaves and morphological changes of the tubers, while suppression under control of the tuber-specific B33-patatin promoter resulted in increased tuber yield. Reduction of apyrase led to higher growth rates of potato tuber tissue in response to external supply of auxin and cytokinins and to higher rates of 14C-trans-zeatin uptake. Apyrase overexpressing plants showed no phenotypic changes, but potato tuber tissue from these plants revealed slower growth rates or died in response to external supply of these phytohormones. The results suggest a crucial role of apoplastic apyrase in transport processes and phytohormon partitioning in potato plants.

T12-019

Linkage Disequilibrium and Potential Demographic Factors Shaping Genetic Variation in Arabidopsis lyrata subsp. petraea

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The relationship between linkage disequilibrium and effective population size reflects the potential impact of selection on genetic variation at the population level. Furthermore, this tight relationship suggests that the partitioning of genetic variation attributable to demographic forces versus natural selection is complex. An important question then arises: Can we distinguish between the relative effects of demographic forces and natural selection on genetic variation? To investigate the relationships among linkage disequilibrium, demographic forces, and natural selection, we are conducting a multilocus comparative survey of Arabidopsis thaliana and A. lyrata subsp. petraea (hereafter A. petraea). These two species differ in several aspects of life history and population structure. A. thaliana is an annual, inbreeding, self-compatible species while A. petraea is a perennial, outcrossing, self-incompatible species. Studies further indicate that A. thaliana is a pandemic species that expanded post-glacially and exhibits disturbance-associated population dynamics (Sharbel, T.F. et al. 2000). In contrast, A. petraea is a species that has persisted in restricted, disjunct European localities that are possible remnants of glacial refugia and exhibits patterns consistent with an extensive metapopulation network. These contrasting population structures appear to have significant effects on nucleotide polymorphism (Clauss, M.J. et al. 2002). Furthermore, recent results using a limited dataset indicate that A. petraea has reduced linkage disequilibrium (LD) versus A. thaliana that appears to be greater than expected in an outcrossing species (Wright, S.I. et al. 2003). To assess the range of effective recombination (LD) and potential population structure effects on genetic variation in A. petraea, we are currently examining ~ 20 single copy nuclear loci in several populations from different potential glacial refugia. Because the outcrossing A. petraea exhibits high genetic variation and heterozygosity, we have developed a new experimental and analytical approach to capture both alleles that avoids error-prone cloning-based issues. We present an outline of this approach, preliminary results employing this method, and future research directions.

Sharbel et al. 2000 Mol Ecol 9:2109-18;
Clauss et al. 2002 Mol Ecol 11:591-601;
Wright et al. 2003 Mol Ecol 12:1247-63

T12-020

Changes in the modifications of core histone H3 after salt stress in the BY-2 tobacco cell line and Arabidopsis thaliana cells

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Regulated gene expression is required for a variety of developmental and physiological processes in all organisms. Transcriptional regulation is dependent not only on the specific interactions between transcription factors and the promoter elements to which they bind, but also on the local and regional chromatin environment. The N-terminal tails of core histones, the building blocks of nucleosomes, contain residues that can be posttranslationally modified. Over recent years, considerable information has been accumulated concerning histone H3 and its function in gene regulation, which shows that covalent modifications of this protein are involved in response to environmental changes.

Stimulation of mammalian cells with various factors causes rapid and transient activation of phosphorylation of residue S10 and also acetylation of K9 and K14 in the H3 tail. These modifications occur concurrently with the transcriptional induction of immediate-early (IE) response genes. Further investigations using animal cells suggest that H3 phosphorylation is the result of MAP kinase activity (reviewed in 1).

Here, we provide evidence that H3 modifications are also involved in the response to environmental changes in plant cells. Time-course analysis of salt-treated BY-2 cells shows rapid induction of phosphorylation at S10 and S28 and phosphoacetylation of H3, which is decreased during further treatment. Currently we perform similar studies on Arabidopsis thaliana cell culture to examine if such response to stress is common in plants. Similarities in the scheme of MAP kinase cascades which are found between plants and animals, highly conserved core histones N-tails and our initial results indicate that histone modifications participate in signal transduction and response to stimuli in plants as they do in animals.

T12-021

Population biology of the other Arabidopsis: life history, ecology and population dynamics of A. lyrata, a close relative of A. thaliana.

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Arabidopsis lyrata is increasingly compared to the model Arabidopsis thaliana in population genetic and functional investigations. While growth habit (perennial versus annual) and breeding system (inbreeding versus out-crossing) are oft cited differences between the species, we know surprisingly little about even the basic population biology of A. lyrata. For two years, we have followed the fate of individuals in permanently marked patches in a single focal population of A. lyrata subspp. petraea, in central Europe. Concurrently, we are conducting quantitative genetic and molecular genetic studies using genotypes collected within this same population. Here, we report on the basic population structure, germination and reproductive phenology, pollination biology, and sources of mortality in the A. lyrata Plech population in 2002-03. Results from a quantitative genetics study in laboratory and common garden conditions supplement our knowledge of plant growth and development in this long-lived perennial. We have observed significant loss of leaf biomass to herbivory, extensive vegetative propagation via ramets, a large mortality event due to drought, and recruitment from the seed bank. The allocation of meristems to reproduction can occur under a range of photoperiods (between April and October), yet seed production is sensitive to environmental conditions relating to pollinator and water availability. Understanding the differences in phenotype and population structure among Arabidopsis species can suggest hypotheses concerning function and genetics that will allow us to move beyond the model organism.

T12-022

Use of halophytic Arabidopsis relative model systems (ARMS) to reveal unique genetic components of salt tolerance

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The use of the glycophyte Arabidopsis thaliana as a genetic model system for salt tolerance studies has dramatically altered research capabilities in plant biology, but there is a critical need for a halophytic system with molecular genetic attributes that compare to Arabidopsis. The lack of a such model system has limited progress towards the understanding of salt tolerance in halophytes. Thellungiella halophila and Thellungiella parvula are both halophytic close relatives of Arabidopsis that can be used as molecular genetic models in salt tolerance studies. Several features make them attractive as genetic models in salt stress research; they are small winter annuals, with short life cycles, high seed yield, and small genomes. T. halophila and T. parvula accumulate high levels of proline and they can reproduce after exposure to extreme salinity. However despite their salt tolerance, they are not exceptionally tolerant to soil desiccation. Descurainia pinnata, another close halophytic relative of Arabidopsis has a high tolerance to desiccation, and is a promising model for studies of the osmotic components of salt tolerance. In this research we present a detailed physiological and genetic characterization of these model plants focusing on their responses to salt and other environmental stresses.

T12-023

Modification of Flower Color in Dianthus caryophyllus by Genetic Transformation

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The unusual mosaic appearance of flower petal color has long been attractive to geneticists. Some of these variegations are produced by viral infections and others are caused by periclinal or mericlinal chimeras. The most interesting instance of unstable anthocyanin expression is caused by mobility of transposable elements. Carnation (D. caryophyllus) basically has three kinds of flower color, white, yellow, and red. Most structural genes encoding the enzymes involved in anthocyanin synthesis have been isolated, and those functional effects to the flower colors have been confirmed in the mosaic flower. The recent research suggests that in the 'Rhapsody' cultivars of the carnation, which bears white flowers variegated with red flecks and sectors, a transposable element, dTdic1, belonging to the Ac/Ds superfamily, was found within the dihydroflavonol-4-reductase (DFR) gene, therefore the red flecks and sector of 'Rhapsody' may be attributable to a reversion to DFR activity after the excision of dTdic1. On the basis of these results. we modified the color of the carnation by transferring the chalcone synthase (CHS), the dihydroflavonol-4-reductase (DFR), or the anthocyanidin synthase (ANS) gene through a revised Agrobacterium-mediated gene transfer methods. In this study, we have cloned and sequenced gene fragments from the three anthocyanin biosynthetic genes, CHS, DFR, and ANS. Using quantitative reverse-transcriptase-polymerase chain reaction (RT-PCR), we have determined their expressional levels during the flower development. The cellular localization of mRNA were analysed with in situ hybridization and assay. After expressional profiles of the anthocyanin biosynthetic genes were characterized, we introduced the CHS, DFR, or ANS gene into the carnation in the sense orientation to induce over-expression or suppress the expression with dsRNA vector in order to modify the flower color. We regard this system to be a useful method for creating new flower color in the carnation and for studying gene expressions.

T12-024

Analysis of reproductive mode, ploidy, and flowering time in Boechera (Brassicaceae) populations

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The cruciferous genus Boechera contains an array of species found mostly in montane and dry environments of western North America. The life-history strategies of Boechera differ significantly from its close relative Arabidopsis thaliana. Whereas A. thaliana is a self-pollinating diploid annual species that grows in disturbed ecological sites, Boechera species, which tend to grow in pristine areas, can be either diploid or triploid, sexual or apomictic, and biennial or perennial. Thus, Boechera provides the opportunity to ask important evolutionary questions that cannot be addressed using A. thaliana. We are examining the mode of reproduction, ploidy, and flowering time variation in 90 lines (or "ecotypes") of 17 species of Boechera, with a large sample from the B. holbollii/ B. drummondii complex.

To study the mode of reproduction and ploidy, individuals from each line were reciprocally crossed to a common diploid sexual B. drummondii tester. Flow cytometry and microsatellite analyses of the parental lines and their F1 progeny showed an array of outcomes including diploid apomicts, partially sexual triploids, and diploids producing 2n pollen grains. The analysis of ploidy confirms that triploid or tetraploid lines can be established via hybridization events.

To study the variation in flowering time, replicates of each line were grown in both non-vernalizing and vernalizing conditions. Flowering time varied widely among the non-vernalized plants. However, the vernalized plants all flowered shortly after removal from the cold. Based on these findings, we have selected 20 lines representing the variation in flowering time to examine sequence diversity and expression levels of the key regulatory gene, Flowering Locus C (FLC). Our results suggest that vernalization can be an important flowering time cue for perennial species.

T12-025

Dissecting symbiotic nitrogen fixation in legumes

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Legumes and a few close relatives are unique among plants because they establish a symbiotic relationship with soil bacteria termed "rhizobia". The product of this cross-kingdom collaboration is the conversion of atmospheric di-nitrogen to ammonia. Symbiosis is carried out within a novel plant organ, the "nodule", where an environment of low oxygen tension and energy from photosynthate provide conditions for operation of the bacterial nitrogenase enzyme.

Symbiotic development can be divided into two broad processes: infection of the plant by the bacterium and development of the nodule organ. Both infection and development are controlled by the perception of chitin-based lipooligosaccharide signals (called "Nod factors") of bacterial origin. Remarkably, components of this signaling pathway are also essential for mycorrhizal symbiosis in legumes. Pharmacological and cell biological assays implicate a calcium-sensitive pathway in early Nod factor signaling, while molecular-genetic characterization reveals a system of receptor-like kinases, a putative cation channel, and a putative calcium-responsive kinase, each of which are required for symbiotic development and infection. In all cases, orthologous proteins are either absent from Arabidopsis or present in Arabidopsis but not yet characterized. By contrast, other aspects of symbiotic establishment are derived from pathways where the antecedents are well characterized in Arabidopsis. Thus, the ortholog of AtEin2 negatively regulates Nod factor signaling and alters both infection and developmental phenotypes, while a paralog of clavata 1 regulates the initiation of nodule meristems.

Symbiosis research has been aided by the parallel development of data and tools related to the structure and function of legume genomes. Medicago truncatula and Lotus japonicus are the subjects of international genomics initiatives, with ongoing projects at the whole genome, transcriptome, proteome and metabolome levels. Hundreds of genes are candidate for nodulation based on inferences from gene structure and expression studies. Renewed effort, employing a combination of traditional forward genetics, reverse genetics, cell biology and biochemistry, will be required to unravel the network of interacting elements that comprise symbiotic nitrogen fixation.

This presentation will provide an overview of recent progress and current challenges of nodulation research.

Ané, Science 2004 303:1364
Endre, Nature 2002 417:962
Lévy, Science 2004 303:1361
Limpens, Science 2003 302:630

T12-026

What plant research will be like 10 years from now.

Steve Briggs(1)

Scientific originality arises from access to technology (the first to be able to do an experiment) and conception (the first to combine existing ideas in a particular way). Mainstream plant research has evolved from physiology, “the effect of this treatment upon that response”, to the integration of physiology with molecular biology and genetics, “this gene causes that trait”. This trend of integration will continue to add new technologies from other disciplines

for measuring effects of genes and the environment on plant molecules and behavior. We will soon be able to identify and quantify all non-polymeric molecules in a plant sample and this will enable the circuitry of plants to be described. I will illustrate using our efforts to map genetic networks by integrating quantitative genetics with genomic mRNA profiling. Application breakthroughs over the next 10 years will come from emerging concepts at the interfaces between plant research and the disciplines of microbiology, ecology, nutrition, and medicine. I will illustrate using microbial genes for crop processing and improvement. Plant research in 10 years will be led by scientists with early access to technology and ideas from outside plant biology focused on solving problems in health and the environment.

T12-027

Zooming-in on a Tomato Yield Quantitative Trait Nucleotide (QTN) with Wild Species Introgression Lines

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To explore natural biodiversity we developed and examined introgression lines (ILs) containing wild species chromosome segments in the background of the cultivated tomato. We identified Brix9-2-5, which is a minor Solanum pennellii QTL that increases sugar yield of tomatoes and was mapped within a fruit-specific invertase (LIN5), which is highly expressed in flowers. QTL analysis representing five different tomato species delimited a Brix9-2-5 QTN to a unique amino acid in an uncharacterized LIN5 domain modulating enzyme kinetics and fruit sink-strength. These results underline the power of diverse ILs for high-resolution perspectives on complex phenotypes.

T12-028

Model Systems, Plant Sciences, and the Shift to Horizontal Biology

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The past decades have seen an intense development of organismic biology and genomics of individual species – e.g. Arabidopsis, humans, Caenorhabditis elegans on one hand and population biology and evolutionary ecology on the other hand. While the great discoveries fueled by these systems will continue over the next decades, more and more discoveries will occur through comparative biology/genomics and in the interface between different biological disciplines. It is through such integrative approaches that the fuel and mechanisms of evolution and adaptation will be revealed. Currently, the genomes of at least 10 additional plant species are being fully or partially sequenced through a consortium of worldwide scientists and complement the well established existing model systems. We will outline some of the key questions likely to be addressed as we shift from “vertical” biology (studies within one species) to “horizontal biology” (comparisons between genotypes or species and their interactions with the environment), with examples from a new project called the Solanaceae Genome Initiative, which involves more than 30 countries and 10 plant species.

T13 Others

T13-001

Expression Profiles of Arabidopsis thaliana During Natural Leaf Senescence

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Leaf senescence is the final stage of leaf development. It occurs in response to aging; however, certain stresses and hormones can promote or repress senescence. During leaf senescence, the most visible symptom is leaf yellowing; that is due to the chloroplast breakdown and chlorophyll loss. Hydrolysis of macromolecules, such as proteins and lipids, occurs concomitantly. We divided the leaves of Arabidopsis thaliana into 5 stages, namely G, Y1, Y2, Y3 and Y4, according to the degree of yellowing. The fully green leaf is assigned as G stage and the leaf with more than 90% yellow area is assigned as Y4 stage. Total RNA from each stage was extracted for probe preparation. Affymatrix Arabidopsis ATH1 GeneChips containing 22810 probe sets were employed. Up to thousands of genes were up-regulated from stages Y2 to Y4 and more than one thousand genes were down-regulated at stages Y3 and Y4 based on the G as a control. Genes involved in photosynthesis, RNA and protein synthesis were more active in the green leaves. Some well-known senescence-associated genes (SAGs) were also identified at various stages of senescence. In Y3 and Y4 leaves, numerous genes encoding antioxidative enzymes were induced. Genes of nitrilases (NITs) and IAA-amino acid hydrolase (ILR1), which are responsible for the increase of the active IAA level were up-regulated. Increased IAA level may play a role in the regulation of nutrient partitioning. Moreover, IAA can induce ethylene production and accelerate leaf senescence. NIT4, one of the NIT members, may involve in cyanide detoxification during ethylene biosynthesis. The ribosomal protein S6 kinases (S6Ks) control in part the initiation step in translation. They were induced significantly in Y3 and Y4 leaves. Global and specific mRNA translation may be regulated by the changes in the activity of S6Ks. The prominent kinase induced during senescence is calcium-dependent kinase (CDPK) family. There are 46 CDPKs in Arabidopsis genome according to the gene annotation. Among them, 16 genes were induced at various yellowing stages, 3 genes were suppressed, and 12 genes were not expressed at all stages. There were 9 transcription factors with at least 2-fold increase of transcription level from Y1 to Y4 stages as compared to the G stage. The WRKY family is the major transcription factor family induced from the very early of leaf senescence. Relationship between some selected gene candidates and senescence is currently carrying out.

T13-002

Exploring a new detergent inducible promoter active in higher plants and its potential biotechnological application

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For the biotechnological use of higher plants, it is necessary to ensure the formation of the desired recombinant product in the right organ at the right time, and in the right cellular location, in large quantities and without exerting toxic effects in the cell. For many purposes, it may be desirable to exert exogenous control over the production of recombinant proteins using chemically controllable promoters. At the Department of Plant Physiology of the Ruhr-University Bochum the promoters of 12-oxophytodienoic acid re-ductases (OPR1 and OPR2) have been isolated in order to investigate jasmonic acid biosynthesis. Al-though OPR 1 is not the isoform that is important for jasmonate production, the OPR 1 gene has a very sensitive promoter and can be activated by mechanical and chemical inducers (Biesgen and Weiler, 1999; Yuehui and Susheng, 2001). One of those chemical inducers is the non-ionic detergent Tween 20. A number of deletions of the OPR 1 promoter will be designed and tested for their properties and range of applications using reporter gene analysis (ß-glucuronidase) to assay for promoter strengths and characteristics by Northern Blot and GUS fluorometric assays. In order to know which genes are induced in the ap-plication of Tween 20 detergent, microarray analysis will be done. Promoter functionality will be tested in a range of agricultural plants. The aim of the project is to advance our understanding of application-oriented promoter-design in the biotechnological use of higher plants.

T13-003

SH2 proteins in plants : The story is just beginning

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The protein modules known as SH2 (Src-homology-2) domains are key players in the signal transduction of animals. Two questions arise: Do such modules exist in plants, and when did SH2 domains evolve? Here I show that the Arabidopsis genome contains three strong candidates for plant SH2 proteins (referred to as PASTA1, 2 and 3 : GI:25513455, At1g78540, At1g17040 respectively) with homology to the SH2 domains and the adjacent linker region of STAT proteins (Signal Transducer and Activator of Transcription). The three characteristics features of a STAT protein sequence1, namely, (i) the SH2 domain with a conserved arginine residue crucial for binding to a phospho-tyrosine residue (ii) a tyrosine residue outside the C-terminus of the SH2-domain for phosphorylation during signalling and (iii) a DNA-binding domain are conserved in the PASTA3 protein. However, PASTA 1 and 2 proteins lack a tyrosine in a similar position.

PASTA proteins are not homologous to STAT proteins outside the SH2 and linker regions. The three PASTA proteins are 70 to 80 % identical to one another.

Gene expression studies with PASTA2 reveal that it is expressed in all the plant tissues tested. Preliminary indications are that plants homozygous for PASTA2 do not have any obvious phenotype, most likely due to redundancies. An antisense experiment with PASTA3 antisense DNA in PASTA2 mutant background is being carried out presently.

STAT proteins need to be phosphorylated at tyrosine residues for their function. Since no candidate gene for a plant protein tyrosine kinase has been identified so far, it has been argued that plants are not likely to have STAT proteins. Moreover, the current hypothesis is that tyrosine phosphorylation is linked to metazoan evolution2. Based on the sequence homology of the PASTA proteins to STAT proteins I propose the following. (1) The new proteins PASTA1, 2 and 3 are Plant Analogues of the animal STAT proteins (hence the name PASTA); (ii) the PASTA proteins function by a currently unknown mechanism, which may not involve a tyrosine phosphorylation. These observations suggest in turn that (iii) STAT-related proteins already existed in the common ancestor of plants and animals, and (iv) the mechanism of STAT-mediated signalling in plants and animals has subsequently diverged.

(1) Levy, D.E. and Darnell Jr, J.E. Nat. Rev. (2002) 3, 651 (2) Darnell Jr, J.E. Proc. Natl. Acad. Sci. (1997) 94, 11767

T13-004

Autophosphorylation Activity of the Arabidopsis Ethylene Receptor Multigene Family

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Plant receptors for the gaseous hormone ethylene show sequence similarity to bacterial two-component histidine kinases. These receptors are encoded by a multigene family that can be divided into subfamilies 1 and 2. It has been previously shown that a subfamily 1 Arabidopsis ethylene receptor, ETR1, autophosphorylates in vitro on a conserved histidine residue (1). However, sequence comparisons between the five ethylene receptor family members suggest that subfamily 2 members do not have the motifs necessary for histidine kinase activity. It has been reported that a tobacco subfamily 2 receptor, NTHK1, autophosphorylates on serines and threonines in vitro (2). Here we show that all five Arabidopsis ethylene receptor proteins autophosphorylate in vitro. We analyzed the nature of the phosphorylated amino acids by acid/base stability and bi-dimensional thin layer electrophoresis, and demonstrated that unlike ETR1 all other ethylene receptors autophosphorylate predominantly on serine residues. ERS1, the only other subfamily 1 receptor, is able to phosphorylate on both histidine and serine residues in the presence of Mn2+. However, this activity is lost when ERS1 is assayed in the presence of both Mg2+ and Mn2+, suggesting that histidine autophosphorylation may not occur in vivo unless a Mn2+ donor is present. Furthermore, mutation of the histidine residue conserved in two-component systems does not abolish serine autophosphorylation, discarding the possibility of a histidine to serine phosphotransfer. Our biochemical observations complement the recently published genetic data that histidine kinase activity is not necessary for ethylene receptor function in plants and suggest that ethylene signal transduction does not occur through a phosphorelay mechanism.

1) Gamble et al. (1998) Proc Natl Acad Sci USA. 95, 7825-7829.
2) Xie et al. (2003) Plant J. 33, 385-393.

T13-005

Protein-protein interactions in the cytokinin signal transduction pathways of Arabidopsis thaliana

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In recent years it has been shown that the hormone cytokinin (Ck) is recognized in Arabidopsis by three histidine kinases and that the signal is transmitted through the two component signalling system (TCS). Analysis of the Arabidopsis genome reveals, in addition to the three cytokinin binding histidine kinase receptors, five phosphotransmitter proteins and 22 response regulators. It is known that the Ck-signal is transduced in the TCS via phospho-relay involving protein-protein interaction, and a number of these interactions have been identified. However, so far no comprehensive interaction network, necessary for understanding this signalling pathway, has been established. The primary aim of our project is to test by means of the yeast two-hybrid system the interaction between the ca. 30 TCS proteins in order to elucidate the signalling network. In a second approach, we are attempting to extend the signalling network beyond the TCS by screening for their interacting partners in the Arabidopsis proteome in vivo and by combining co-affinity purification and mass spectrometry. First results of these experiments will be presented.

T13-006

Characterization of loss-of-function mutants of cytokinin oxidase/dehydrogenase genes and suppressor-mutagenesis of transgenic plants with reduced cytokinin content

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The catabolic inactivation of cytokinins is catalyzed by cytokinin oxidases/dehydrogenases (CKX). To assign functions to each individual AtCKX gene of Arabidopsis, we isolated 12 homozygous insertion lines in six of the seven genes. Knockouts of single AtCKX genes have minimal consequences for plant development. To overcome the problem of functional redundancy, insertion lines have been systematically crossed to produce double and multiple knockout mutants. In addition, transgenic Arabidopsis plants expressing RNAi-constructs of individual AtCKX genes as well as regions common to all genes have been generated.
We carried out supressor mutagenesis of AtCKX1-overexpressing transgenic Arabidopsis plants which display a strong cytokinin-deficient phenotype. By this approach we aim to identify genes which are necessary for the establishment of the cytokinin-deficiency syndrome, e.g. negative control elements of cytokinin signal transduction or of cytokinin synthesis. We identified several recessive and dominant second site mutations which are being characterised. Intragenic suppressor mutations in the AtCKX1 transgene identified several amino acids essential for the activity of the CKX enzyme.

T13-007

Characterization of the Cullins AtCUL3a and AtCUL3b in Arabidopsis thaliana

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The ubiquitin proteasome pathway is highly conserved among all eukaryotes. We are working on a family of proteins called cullins that participate as subunits of E3 ligases. Here we describe expression patterns of the cullins AtCUL3a and AtCUL3b in Arabidopsis. In addition, analysis of null mutants for changed sensitivities against different growth conditions revealed increased tolerance of the mutants towards stress. The data presented here give evidence for the two cullins to be involved in stress regulation.

T13-008

The Arabidopsis Biological Resource Center ¯ 2003-2004 Activities; Resource Acquisitions and Stock Distribution

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The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis. ABRC stocks can be ordered through the TAIR database Web site maintained by the Carnegie Institution of Washington (http://arabidopsis.org), with informatics support from the National Center for Genomic Resources (NCGR).

Seed stocks have been added to our collections in the past year, including: A) 50,000 flank-tagged T-DNA lines from Syngenta Biotechnology, Inc. ("SAIL" collection); B. 2,000 additional lines from the R. Martienssen laboratory; C) mutant and transgenic lines, and D) natural accessions, including 97 new lines from J. Bergelson, M. Kreitman and M. Nordborg and 42 from M. Koornneef. The T-DNA insertions of the SALK and SAIL collections provide insertions in 23,000+ different Arabidopsis genes, with 15,000+ genes being represented by at least two independent insertions.

New DNA stocks added to the collection include sequence-validated open Reading Frame (ORF) clones from the SSP Consortium, so that there are now 11,361 of these clones being distributed. The destination vectors for the pUNI system used for most of the SSP cDNA clones have been received from S. Elledge and are being distributed. A plant expression destination vector for the pUNI system, from J. Ecker, is also being distributed. A number of other clones, with diverse functions have been received.

We are pleased to be able to distribute cDNA clones in the Gateway™ entry and expression vectors. 1,051 transcription factor cDNA ORF clones in Gateway™ have been received from a consortium at Yale University and Peking, PRC.

During the past year, ABRC distributed 71,000 seed and 35,000 DNA stocks to researchers. We are pleased to announce that the seed facility has moved from its original location in Jennings Hall to newly renovated space at 1060 Carmack Rd. The new location include state-of-the-art dry seed handling and dry/cold seed storage facilities. This is the same location at which the DNA facility is located.

ABRC is supported by the National Science Foundation.

T13-009

Genomics-Related Stocks Distributed by ABRC

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The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration functional genomics, including many received from NSF 2010 Project grant recipients.

Several flank-tagged insertion collections are distributed by ABRC. The 152,000 SALK lines from J. Ecker contain insertions in 21,000+ genes. Syngenta Biotechnology, Inc. has donated 50,000 lines of its "SAIL" collection. This material contributes insertions in 2,500 additional genes plus many second alleles. These lines are useful not only for reverse genetics, but also as an adjunct to identification of target genes in positional cloning and for other functional genomics investigations.

Full length cDNA clones from the SSP Consortium and Salk are still being received. 11,631 of these fully sequence-validated clones are now being distributed. The Cre-Lox system, employed for many clones of this collection, enables recombination of these entry clones with destination vectors that enable expression in E. coli, yeast and plants. ABRC distributes these vectors.

ABRC is now able to distribute cDNA clones in the versatile Gateway™ system. We can accept both entry clones and expression clones. Many novel clones arising from 2010 Project research will be received. We are already distributing a number of full length cDNA clones in the pENTR™ vector.

We are formatting some of the new cDNA clone resources into plates. For example, 10,000 SSP clones are presently available in this format. We plan to distribute other similar large collections in this way.

Additional genome-related stocks received or to be received include: a) 2000 full length cDNA clones of hypothetical genes; b) 1,051 transcription-factor full length cDNA clones; c) expression constructs that have combination translational fusion-promoter reporter function; and d) transposon insertion lines.

In addition to the above, we have available large numbers of T-DNA lines that are organized as pools for forward genetic screening, the BAC genomic clones utilized for the AGI sequencing project and EST collections representing ca. 10,000 Arabidopsis genes. The T-DNAs employed to generate our lines include enhancer trap, activation tagging and over-expression constructs, as well as simple insertions.

ABRC is supported by the National Science Foundation.

T13-010

Cytokinin oxidases/dehydrogenases (CKX) of Arabidopsis as a tool to study cytokinin functions in shoot and root development

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Cytokinin oxidases/dehydrogenases (CKX) catalyze the degradation of cytokinins (CKs) and in Arabidopsis they are encoded by a small AtCKX gene family. These genes were used to engineer transgenic tobacco and Arabidopsis plants with a reduced CK content in order to study for which processes CKs are rate-limiting and possibly regulatory molecules. CK-deficient plants have a severely retarded shoot development and an enlarged root system, suggesting that the hormone regulates shoot and root meristematic activity and morphogenesis in opposite modes. A root-specific cytokinin reduction enhanced root growth significantly and specifically, indicating that the consequences of CK-deficiency are to a large extent tissue-autonomous and opening thus the possibility to modulate organ growth in a targeted fashion. Analysis of promoter::GUS fusions indicated that individual AtCKX genes are differentially expressed in distinct, almost non-overlapping domains. CK treatment up-regulated several AtCKX genes suggesting a positive feed back control of CK abundance in planta. Additional functional divergence of individual AtCKX family members, as revealed by CKX::GFP fusion proteins, is presumably realized by their different subcellular targeting to the apoplast and vacuoles.

T13-011

Cell wall polysaccharide analysis of tomato fruits and quantitative trait loci (QTL) mapping of responsible regions in the tomato genome

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Tomato is an important crop plant. Fruits are not only used directly from the plant but also processed to pastes and sauces. The consistency of these products is largely determined by the cell wall composition of the used tomato fruits.
Cell walls consist of cellulose microfibrils embedded in a matrix of complex heteropolysaccharides and glycoproteins. Although much is known about the structure of cell wall polysaccharides present in tomato fruits and their partially controlled disassembly during fruit ripening, little is known about the enzymes involved in their biosynthesis and particularly their regulation.
To gain further insights into wall biosynthesis and its regulation we used quantitative trait loci (QTL) mapping of chromosomal regions responsible for cell wall polysaccharide abundance.
This approach was carried out on two sets of 76 tomato introgression lines (ILs) harvested in field trials in 2000 and 2001 originating from a cross between the cultivated tomato Lycopersicon esculentum (cv M82) and the green fruited wild relative Lycopersicon pennellii. Each of the lines contains a single marker-defined homozygous L. pennellii chromosomal segment and together the ILs provide complete coverage of the tomato genome (Zamir, 2001). Cell wall material was prepared from each line and characterized in terms of wall mass, its monosaccharide composition, crystalline cellulose content, degree of methylesterification, degree of O-acetylation and xyloglucan oligosaccharide abundance.
These cell wall data were linked to the genetic map and chromosomal regions involved in the control of the regulation of wall biosynthesis were identified. Of special interest are several genomic regions which influence the concentration of pectin, the major cell wall component of tomato fruits and therefore important for mechanical properties.

Zamir, D. (2001). "Improving plant breeding with exotic genetic libraries." Nature Reviews Genetics 2(12): 983-989.

T13-012

Genetic mapping and characterization of the Arabidopsis trichome birefringence (tbr) mutant.

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Trichomes exhibit strong birefringence under polarized light, a characteristic of cell walls containing large amounts of highly ordered cellulose microfibrils. The tbr mutant of Arabidopsis lacks trichome birefringence and is deficient in secondary cell wall cellulose synthesis (Potikha and Delmer, 1995).
We have identified the TBR gene by recombinational mapping, candidate gene sequencing and molecular complementation using genomic cosmid clones as well as a p35S:TBR cDNA construct, fully rescuing the trichome phenotype in both cases. The only mutant allele (tbr-1) available carries a substitution (G to E) in a conserved amino acid stretch of the protein. Homozygous lines of T-DNA insertions in exon sequence of the annotated TBR gene have been isolated using PCR, but lack a phenotype for reasons that are not understood yet. TBR encodes a plant-specific protein with predicted plasmamembrane-localization, therefore fitting the idea that it is required for or is a novel component of a functional cellulose synthase complex. TBR is part of an Arabidopsis gene family, which, depending on homology, comprises up to 20 members, none of which has a biological or biochemical function attributed. Promoter-GUS lines were produced for TBR, as well as for its two closest homologs (one being a segmentally duplicated gene), using 1.6-2kb of sequence upstream of the annotated start codons. The TBR promoter was the only one of the three that yielded trichome expression, thus probably explaining the phenotype of the tbr mutant. Moreover TBR is expressed in leaves, in growing lateral roots, and in vascular tissues of young Arabidopsis seedlings and plantlets. Later on, the expression appears in inflorescence stems, flowers and green siliques. This expression pattern is largely overlapping with those of the two analyzed homologs. We’re currently investigating i) the subcellular localization of TBR, ii) the interacting protein partners using immunopulldown assays and mass spectrometry, as well as iii) the changes in cell wall structure and composition in mutant trichomes.

Potikha T and Delmer DP (1995) The Plant Journal 7(3), 453-460.

T13-013

Genetic and molecular dissection of the role of CPD steroid hydroxylase in regulation of brassinosteroid hormone biosynthesis and signalling in Arabidopsis

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The Arabidopsis CPD (Constitutive Photomorphogenic Dwarf) locus encodes a cytochrome P450 steroid C23-hydroxylase enzyme, CYP90A1, implicated in both early and late C6-oxidation pathways of brassinosteroid (BR) hormone biosynthesis. We found that overexpression of CYP90A1 by the mannopine synthase promoter in the cpd knockout mutant results in activation of pathogenesis related PR genes, and that CYP90A1 shows interaction with several signaling factors, including a MAP kinase (MAPK), an oxysterol-binding (OBP) and an IAP (inhibitor of apoptosis-like) RING protein in the yeast two-hybrid system. To explore the expression pattern, regulation, cellular localization, and in vivo interactions of the CPD gene product, we expressed GFP and epitope tagged forms of CYP90A1 in the cpd mutant. CYP90A1 was localized in the plasma membrane, ER and nuclear envelope. In addition to using the native CPD promoter, we expressed CYP90A1 with various tissue specific promoters, as well as using a chemically inducible system, in order to obtain tools for studying the temporal and spatial control of BR biosynthesis and transport. Whereas several P450 enzymes in BR biosynthesis seem to utilize NADPH P450 reductases as electron donors, our data indicate that these enzymes fail to interact with and donate electrons to CYP90A1. Therefore, biochemical and cross-linking studies with complemented cpd knockout lines, producing GFP or epitope labeled CYP90A1, were initiated to identify the yet unknown CYP90A1 reductase and confirm in vivo interaction of CYP90A1 with the MAPK, OBP and IAP proteins. This biochemical approach is supported by genetic studies, in which we use T-DNA insertion mutations, as well as inducible overexpression and siRNA constructs, to examine the functions of CYP90A1-interacting MAPK, OBP and IAP proteins. This approach is supplemented by similar functional analysis of other members of small OBP and IAP families, which code for additional steroid carrier proteins and putative E3 ubiquitin ligase enzymes, respectively.

T13-014

Functional Analysis of Arabidopsis At4g23740 GeneF

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In this study, Arabidopsis gene F9D16.210 (At4g23740), a putative member of the LRR RLK gene family, is selected to characterize its possible biological function. The LRR RLK protein coded by this gene contains all three characteristic functional domains – an extracellular domain, a transmembrane domain, and an intracellular kinase domain. Its extracellular domain contains seven leucine-rich repeats. We found that this gene is expressed in root, stem, leaf, flower, and silique of Arabidopsis plants grown under normal conditions by using RT-PCR. Five mutant lines in this gene have been identified from the available T-DNA mutagenized Arabidopsis lines created by Salk Institute Genomic Laboratory (San Diego, CA). Two lines, Salk_004412 and Salk_092792, carry a T-DNA insertion in the promoter region; one line Salk_005132 contains an insert into the second exon in the coding region, and other two lines, Salk_096386 and Salk_047106 lines, contain an insert in 3’ untranslated region (3’UTR). These lines were verified by polymerase chain reaction (PCR) using the appropriated combination of a gene-specific primer and a T-DNA–specific primer. Both heterozygotes and homozygotes for the T-DNA insert were found from each insertion line. Currently, these verified homozygotes are being systematically screened for phenotypes to determine the consequences of the mutations on growth and development relative to the wild type that does not contain the insertion.

T13-015

Dynamics of protein complexes through the cell cycle: a proteome technical approach

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A lot of physiological processes are carried out by protein complexes. Complex formation between cyclin dependent kinases with varying regulatory cyclins is considered the major driving forces of the cell cycle. The formation and activity of such complexes is strongly regulated by different parameters such as the participating components, phosphorylation/dephosphorylation events, interaction with inhibitory (eg. KIP-related protein) and activating (CDK subunit protein) proteins. The goal of this study is to optimise a bioanalytical method for the purification and isolation of protein complexes in order to study their dynamics during the cell cycle. This can reveal new complexes or constituents, active during a certain phase of the cell cycle.

As model species, the highly synchronisable Nicotiana tabacum cv. Bright Yellow-2 (BY-2) cell suspension culture is used. Samples are ground in liquid nitrogen or sonicated, and subjected to non-denaturing 'Blue native polyacrylamide gelelectrophoresis (BN-PAGE)'(Schägger et al., 1991). In this first dimension, protein complexes are separated on the basis of their size. Samples are subsequently subjected to a denaturing second dimension, SDS-PAGE, in which the complexes will break up into their constituents. Protein identification is done by mass spectrometric analysis and based on the existing BY-2 proteome database, developed in our laboratory With this strategy, the dynamics of the complexes can by followed accurately during their progression through the cell cycle. (Laukens et al., 2004; http://tby-2www.uia.ac.be/tby2/;). This report will elaborate on our results and discuss the tricks and pitfalls of this approach.

Schagger H & von Jagow G. (1991) Anal. Biochem. 199(2):223-231.
Laukens et al. (2004) Proteomics 4(3):720-727.

T13-016

Localisation of the Agrobacterium oncoprotein A4-Orf8 and its constituent parts.

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The A4-orf8 gene is part of the pRiA4 T-DNA of the plant pathogen Agrobacterium rhizogenes, which causes hairy root disease in infected plants. A4-orf8 can be separated in two distinct parts. The 5' part (A4-Norf8) belongs to the rolB family. So far, rolB-like genes have only been found on T-DNAs of different agrobacteria. Their function is still unknown, but they are involved in cell proliferation in tumor growth or root development. The 3' part of A4-orf8 (A4-Corf8) has homologies with the tryptophan monooxygenase iaaM gene of Pseudomonas savastanoi, but in spite of this, lacks enzymatic activity (Otten and helfer, 2001).
To understand the function of these two domains and their interaction, both domains and the entire gene were expressed in Nicotiana tabacum plants. We obtained three different phenotypes. 2*35S-A4-Norf8 tobacco plants display reduced growth and chlorosis between leaf veins. We showed that this phenotype is due to an inhibition of sucrose export from source leaves (Umber et al., 2002). 2*35S-A4-Corf8 plants have no particular phenotype and 2*35S-A4-orf8 plants show blisters at the leaf surface, later they develop dark green leaves and swollen veins. Chlorosis appears at the leaf apex in mature plants.
Each of the three sequences was fused to the gfp3 gene and the localisation of the fusion proteins was studied first in infiltrated leaves of Nicotiana rustica. A4-NOrf8/GFP3 fusion proteins are localised in small dots at the surface of epidermal cells. Transgenic plants expressing 2*35S-A4-Norf8-gfp3 showed the same phenotype as 2*35S-A4-Norf8 plants and the protein was found associated with the cell wall between mesophyll cells in a punctate pattern. We postulate that A4-Norf8 localises to plasmodesmata and that this blocks the loading of sucrose to the phloem. A4-Orf8/GFP3 proteins display a similar pattern but are also found in the nucleus. Finally, A4-COrf8/GFP3 proteins are localised in the nucleus and diffusely in the cytoplasm.
Our results show that the dual localisation of A4-Orf8 is due to the localisation properties of its two constituent parts. First at the cell surface, due to the N-terminal part, A4-NOrf8, which blocks the sucrose export in tobacco leaves, and then in the nucleus, thanks to the C-terminal part, A4-COrf8, which contains a NLS-like sequence. Current research in our laboratory focuses on a more detailed in vivo localisation of A4-NOrf8 and an investigation of the role of a putative NLS sequence in A4-COrf8.

Otten, L. and Helfer, A. (2001)
Umber, M., Voll, L., Weber, A., Michler, P. and Otten, L. (2002)

T13-017

Molecular analysis of xyloglucan-specific galactosyltransferases in Arabidopsis

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Xyloglucans (XyGs) are abundant plant cell wall polysaccharides that bind to and cross-link cellulose microfibrils. The tight association between these two cell wall polymers leads to the establishment of a three-dimensional load-bearing network, which is embedded in a matrix of pectic components. Continuous remodeling of the XyG-cellulose network by wall-resident enzymes is believed to aid in the maintenance of a pliable but mechanically strong cell wall during expansion growth.
XyGs consist of a (1,4)-β-linked glucan backbone, which is substituted by xylose residues in an "XXXG" repeat pattern. Some of the second and third xylosyl residues within this core structure are known to carry galactosyl and fucosyl-galactosyl side chains, respectively, but the functional significance of these modifications is poorly understood. XyGs of the mur3 mutant of Arabidopsis lack the fucosyl-galactosyl side chain because of a defect in XyG galactosyltransferase I, which converts XXXG to its galactosylated derivative XXLG. Although XyG binding to cellulose is unaffected, mur3 hypocotyls exhibit abnormal cell swelling and greatly reduced tensile strength. Shoots of chemically induced mur3 alleles do not show obvious visible phenotypes; however, three independent T-DNA insertion lines are slightly stunted with reduced cellulose content, decreased tensile strength of elongating inflorescence stems, wrinkled leaves, and short filaments. These phenotypes suggest defects in wall assembly and cell elongation.
MUR3 belongs to glycosyltransferase family 47, which encompasses 39 members in Arabidopsis (http://afmb.cnrs-mrs.fr/CAZY/; Li et al. (2004) Plant Physiol. 134, 940-950). The recent isolation and characterization of a knockout mutant in the MUR3 paralog AtGT18 revealed only small amounts of galactose on the central xylose residue within the XyG repeat unit, suggesting that this gene encodes XyG galactosyltransferase II, which converts XXXG to XLXG. The atgt18 plants show a "droopy" phenotype with decreased cellulose content and abnormal patterns of lignin deposition in the inflorescence stems. Preliminary results on the F2 population from a mur3 x atgt18 cross indicate segregation for plants with severe growth defects, which appear to be double mutants. Comparative analysis of the various mutant lines should lead to interesting insights into the functional significance of xyloglucan galactosylation. Supported by grants from NSF-IBN and the Plant Genome Research Program.

T13-018

Autophosphorylation sites of AtCPK5

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AtCPK5 (At4g35310) is an isoform of CDPK (calcium-dependent protein kinase) that is localized with the plasma membrane. CDPKs are directly activated by Ca2+, and they play roles as calcium sensors in cellular responses stimuli. To better understand the function of CPK5, we used mass spectrometry to determine the sites on which it is autophosphorylated in vitro. CPK5 was fused N-terminally to glutathione S-transferase, expressed in Escherichia coli, and purified by chromatography on glutathione-agarose. The kinase was autophosphorylated by incubation for one hour at room temperature in a buffer containing MgCl2, EGTA, CaCl2, molybdate, vanadate and 1 mM ATP. For some studies the protein was further purified by denaturing electrophoresis. GST-CPK5 was digested with trypsin, and phospopeptides were identified by a variety of methods: 1) immobilized metal ion affinity chromatography followed by neutral fragment scanning on an LCQ-Deca ion trap mass spectrometer; 2) Comparison of untreated and beta-eliminated samples by anlaysis on a MALDI-TOF mass spectrometer; 3) precursor scanning on a hybrid quadrupole time-of-flight mass spectrometer (Q-Star or Q-TOF); or 4) mass labeling by 18O. Three confirmed and 14 unconfirmed phosphorylation sites located throughout the protein were identified, five of which were observed in at least two of the analyses. These results show that no single method may efficiently identify all phosporylation sites in a protein. Knowledge of these phosphorylation sites will facilitate future studies of CPK5 by supporting development of phosphorylation site-specific constructs and reagents, and by allowing directed mass spectrometric analysis of CPK5 isolated from plants. Studies are underway of CPK5 fused C-terminally to a modified TAP (tandem affinity purification) tag consisting of green fluorescent protein, a 6-His sequence, a TEV cleavage site, and protein A and expressed in plants under the control of the CMV-35S promoter. This work was support by a grant from the National Science Foundation (MCB0114769).

T13-019

DIURNAL CHANGES IN CYTOKININ LEVELS IN ARABIDOPSIS PLANTS

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Changes of cytokinin/auxin ratio are decisive for induction of specific developmental processes in plant cells. Cytokinin levels with respect to auxin in aerial parts of plants can be affected by diurnal changes in rates of their biosynthesis and metabolism and also by the rate of their import from roots which represent the main site of cytokinin biosynthesis. The rate of cytokinin transport to shoots via xylem sap flow is influenced by environmental factors affecting transpiration namely by light and temperature. The aim of our research has been to determine the changes in cytokinin levels during the day/night cycle.
Plants (Arabidopsis thaliana L. Columbia) were grown in hydroponics under 16h/8h day light) at 25 oC. Cultivation of plants under two different levels of relative air humidity (60% and 90%) allowed us to determine the effect of transpiration on cytokinin levels in aerial organs during the day/night cycle. Another set of plants was grown under green house condition in soil. 55 days old plants were harvested in 3 h intervals during one day/night cycle. Cytokinins were extracted separately from different plant organs using dual-mode solid phase extraction (Dobrev 2002) and determined by HPLC/MSn. Sixteen different cytokinin bases, ribosides and glucosides and four different cytokinin ribotides were identified and determined. Formation of isopentenyl-7-glucoside appeared to be the main way of down-regulation of physiologically active cytokinins in response to the temporal accumulation isoprenoid cytokinins. The importance of the day/night period for sampling of plant material for cytokinin analysis, effects of air humidity on cytokinin levels and differences in cytokinins in different plant organs will be discussed.
References: Dobrev P., Kamínek M.: Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid-phase extraction, Journal of Chromatography A, 950 (2002) 21-29.

Acknowledgements: Supported by the Grant Agency of the Czech Republic (grant No. 206/02/0967).

Authors Index

A

Aalen, Reidunn B. T10-021
Aarts, Mark G.M. T04-069, T06-016
Abbott, Richard T12-003
Abe, Hiroshi T04-055, T10-013
Abe, Kiyomi T09-007
Abe, Mitsutomo T01-033, T01-044, T01-045
Abe, Tatsuya T03-041
Abel, Christian T07-079
Abel, Steffen T04-088, T07-086
Abelenda, José Antonio T03-084
Ábrahám, Edit T10-050
Adam, Gerhard T05-005
Adam, Zach T04-012, T04-025
Adamik, Monika T07-075
Adams, Nicolette T05-075
Adams, Tom T12-011
Adamska, Iwona T03-011, T04-041, T11-013
Ade, Jules T05-035
Affenzeller, Matthias T04-105
Afsar, K. T09-008
Agrawal, Vikas T10-007
Aguirrezabal, L. T04-043, T04-097, T10-022
Ahlfors, Reetta T04-044
Ahn, Il-Pyung T10-024, T10-046
Ahn, Ji Hoon T01-050, T01-089
Aida, Mitsuhiro T02-095
Airoldi, Chiara A. T09-039
Akinci, U. T09-062
Akiyama, K. T10-005
Albinsky, Doris T07-042
Albrieux, Catherine T03-015
Alcázar, Rubén T04-064
Al-Daoude, Antonious T05-055, T05-077
Ali, Jehad Shaikh T04-011
Ali, Shahjahan T07-009
Allegra, Domenico T02-026, T04-058
Allen, Ed T09-030
Allen, Rebecca T05-082
Almeida Engler, Janice de T02-014
Almeida, Aurélie De T04-021
Aloe, Amy E. T01-080
Alonso, Jose M. T01-013, T02-035, T04-022, T04-083
Alonso-Peral, M. M. T02-085
Al-Sady, Bassem T04-024, T04-051
Altabella, Teresa T04-064
Altamura, M.M. T01-055, T02-026
Altmann, Thomas T01-062, T02-040, T04-046, T06-014, T06-017, T08-008, T10-041, T10-048
Alvarado, Martha T10-050
Alvarez, Nena T02-071
Alves-Ferreira, Marcio T01-085
Al-Zwi, Ali T04-018
Amasino, Richard M. T01-049, T02-095, T04-034
An, Chung Sun T07-077
An, Hailong T01-048
Ana, Pop T06-012
Anders, Nadine T03-074
Andersen, Claus.H. T01-074
Anderson, Garrett H. T02-071, T02-119
Anderson, Lisa K. T05-064, T05-083
Andersson, Derek T05-047, T05-054
André, Bruno T10-004
Ané, Jean-Michel T12-025
Angelis, Karel J. T09-020
Angell, Sue T05-056, T05-059
Angelovici, Ruthie T10-016
Angenent, Gerco T01-041, T01-074
Aoki, Koh T02-007
Aono, Naoki T01-021
Apel, Klaus T04-051
Araki, Takashi T01-033, T01-044, T01-045
Aranzana, Maria Jose T06-030
Argyros, Aaron T13-018
Arias, Jonathan T10-043
Ariza, Rafael R. T09-032
Arlt, Matthias T09-059
Arroyo, Ma. Analilia T07-095
Arsovski, Andrej T03-025
Asami, Tadao T04-020
Asard, Han T03-021
Ascenzi, Robert T07-006
Asif, Mohmmad T05-001
Asnaghi, Carole T05-041
Assaad, Farhah T05-027, T05-073
Assael-David, Ora T08-012
Assie, Jean-Maurice T11-010
Atanassova, Ana T02-014
Atta, R. T02-064
Attia, Hounaïda T04-092
Aux, George T01-083
Aviezer-Hagai, Keren T03-033
Avramova, Z. T03-085

B

Babujee, Lavanya T03-054, T03-057
Bachmair, Andreas T03-004, T03-005
Bachmann, Konrad T06-008
Backhausen, Jan E. T09-040
Bae, Yun Soo T05-025
Baek, Ji-Sun T12-023
Baessler, Olivia T07-045
Baginsky, Sacha T10-027, T10-044
Bagnall, David T10-020
Bährecke, Annegret T04-093
Baier, Margarete T04-011
Bainbridge, Kath T02-094
Bairoch, Amos T11-004
Baker, A. T04-094
Baker, Neil R. T03-026
Balasubramanian, Dheepa T09-013
Balasubramanian, Sureshkumar T01-059, T01-066, T06-009
Balková, Simona T01-038

Ballesteros Maria, L.. . . . . T04-029
Baltensperger, Kurt T08-003
Baluska, Frantisek. T03-085, T03-086, T03-067
Bancroft, Ian T06-033, T12-004
Band, Mark. T07-009
Bangham, J.A. T02-096
Barber, Christine T07-028
Bardet, C. T09-034
Bari, Rajendra P. T04-077, T04-107, T10-023
Barny, Marie-Anne T05-026
Baroux, C T06-032
Barratt, Lynne T09-022
Barratt, Paul T07-036
Barrero, J. M. T02-102, T02-103, T09-051
Bartel, Bonnie T03-066, T03-071, T09-057
Bartel, David T09-057
Bartels, Dorothea T04-028
Bartetzko, Linda T07-070
Barth, Carina T05-003
Barthakur, Sharmistha T04-052
Barton, M. Kathryn T01-088
Bartrina, Isabel T13-006
Bartsch, Michael T05-089, T05-043
Bastow, Ruth M. T04-091
Bate, Nicholas. T09-018
Batistic, Oliver. T03-020, T04-023, T06-006, T06-007, T10-009
Batoux, Martine T02-076
Bauch, Marion T02-081
Baudry, A T09-011
Bauer, Petra T04-005
Bauer, Zsuzsa T05-086
Bauke, Antje T13-011
Baulcombe, David T02-117
Baumann, Alexander T04-067
Baumann, Nadine T07-047
Baumann, U.. . . . . T01-002
Baumberger, Nicolas T02-093
Bäumlein, Helmut T04-005, T09-041
Bäurle, Isabel T02-018
Bauwe, H.. . . . . T07-063, T07-065
Bauwe, U.. . . . . T07-063
Baxter, C. T01-009
Baxter, Catherine T09-022
Becher, Martina T04-046, T06-014, T06-017, T06-031
Beck, Andreas T04-076
Becker, Beril T09-040
Becker, Dirk T08-004
Becker, Douglas T11-022
Beckers, Gerold J.M.. . . . . T05-067
Beeckman, Tom T02-039
Beemster, Gerrit T02-014
Beis, Dimitris T02-095
Belbarhi, Lassaad T04-108, T07-090
Belles-Boix, Enric T04-044
Bellin, Diana T12-002
Bellmann, B. T01-002
Belzile, Francois T10-051
Ben-Ari, Giora T04-012
Bendahmane, M.. . . . . T01-095
Benderoth, Markus T06-004
Benfey, Philip N. T02-107, T02-108, T10-001
BENHAMED, M.. . . . . T09-049
Benito, María Jesús. T04-102
Benková, Eva T08-014
Bennett, Kristen T07-092
Bennett, Malcolm J. T02-003, T08-003
Bensen, Bob T12-011
Bensmihen, S. T02-096
Bentsink, Leónie T07-062
Benveniste, Pierre T07-094
Berardini, Tanya T10-054, T11-022
Berczi, Alajos T03-021
Berendzen, Kenneth T01-015, T07-010, T09-014, T11-003
Berezin, Irina T08-012
Berg, Michael T10-042
Bergelson, Joy T06-026
Berger, Bettina T07-034
Berger, Frédéric T01-097
Berger, Susanne T05-012
Bergman, Per T01-058
Bergmüller, Eveline T04-007, T09-044
Bernd, Weisshaar T09-011
Bernhardt, Christine T02-110
Berretti, Matteo. T01-069
Berthiaume, Chris T. T02-012
BERTRAND, C. T09-049
Beswick, Nicola. T01-103
Betsuyaku, Shigeyuki T05-045
Betts, Laurie T05-018
Bevan, Michael W.. . . . . T07-026, T07-044
Beynon, Jim T05-082, T10-041
Bhat, Riyaz A. T05-050
Bhatt, Anuj M.. . . . . T02-053
Bicker, Frank. T05-062
Biehl, Alexander T09-003
Bieniawska, Zuzanna. T07-036
Billington, Joanne T10-049, T10-053
Binder, Stefan T03-012, T07-004, T09-016
Birkemeyer, Claudia T04-050, T04-071, T05-040, T11-016
Birnbaum, Kenneth D. T02-107, T02-108, T10-001
Bishopp, Anthony T01-101
Bittel, Pascal. T05-061, T05-086
Bittner, Florian T04-015
Bittner-Eddy, Peter T05-082
Blacha, Anna T01-068, T06-017
Blanchard, Chris L. T12-009
Blanchoin, Laurent T01-097
Bläsing, Oliver. T07-070, T07-096, T11-008, T11-018
Blazevic, Dragica T03-020
Blilou, Ikram T02-095, T02-118
Bloch, Daria T03-003

Block, Maryse A. T03-015
Bocian, Edyta T03-012
Boer, Gert-Jan de T07-067
Böhlenius, Henrik T01-079
Böhm, Alexander T03-011
Böhme, Karen T02-001
Bohnert, Hans J. T05-084, T12-022
Boldt, Ralf T07-019, T07-063, T07-065, T07-083
Boles, Eckhard T10-004
Bolle, Cordelia T04-110
Boller, Thomas T05-061, T05-086
Bones, Atle M. T03-082, T03-083
Boniotti, M. Beatrice T03-051
Bonke, Martin T02-088
Boos, Winfried T03-011
Booth, Adam J. T01-080
Bordji, K. T01-095
Borevitz, Justin O T10-052
Borgen, Birgit H. T03-083
Borghi, Lorenzo T02-045
Börner, Thomas T09-046, T09-047
Börnke, Frederik T12-008
Bosmans, Stefan T06-015
Botella, Dr Jimmy T02-017
Böttcher, Christine T07-020
Bouchard-Kerr, Phoenix T03-025
Bouchier, S. T04-043
boudolf, Véronique T02-014
Bouget, François-Yves T03-022
Bourdon, Valerie T01-012
Boutsika, Konstantina T09-002
Bouveret, Romaric T09-056
Bouvier-Navé, Pierrette T07-094
Bouyer, Daniel T02-054, T02-061
Bovet, Lucien T04-105
Bowman, John L. T01-001
Bowman, Lewis T09-054
Bozo, Patrick T05-058
Bozovic, Vladan T04-036
Bracha-Drori, Keren T03-003, T10-016
BRADLEY, DESMOND T02-036
Braun, Hans-Peter T07-024
Brearley, Charles A. T04-002
Breiman, Adina T03-033
Brenner, Wolfram T02-051
Bressan, Ray A. T12-022
briat, Jean-François T04-006
Brice, David T02-117
Briggs, Georgette C. T02-002
Briggs, Steve T12-026
Brini, Faiçal T04-040
Brockmann, Regina T09-040
Broekhof, José T07-062
Brook, Emil T08-012
Brosche, Mikael T04-044
Brown, Matt T03-075
Brümmer, Janna T05-020
Bruno, Leonardo T02-021
Brüx, Angela T03-038, T03-060
Bryan, Gregory T01-103
Brzeski, Jan T09-024
Büchel, K T04-072
Buckhout, Thomas J. T02-109
Budziszewski, Gregory J. T07-006
Buettner, Michael T07-056
Bujarski, Jozef J. T05-010
Bulankova, Petra T03-036
Bülow, Leif T04-100
Bülow, Lorenz T11-005, T11-009
Bundock, Paul T09-037
Bürkle, Lukas T02-051, T13-005
Busetti, Alessandro T02-020
Bush, Daniel R. T07-088
Busó, Enrique T04-064
Busscher-Lange, Jacqueline T01-074
Buszewska, Malgorzata E. T04-026
Butaye, Katleen M.J. T10-028
Butcher, Sue T04-080
Butelli, Eugenio T12-005

C

CA, Raines T07-060
Caboche, Michel T02-116, T07-002, T07-014, T09-011
Cahoon, Edgar B T04-007
Cairns, Narelle G. T07-084
Calderón V., Luz Irina A. T03-079
Calikowski, Tomasz T10-008
Callis, Judy T03-080
Calonje, Myriam T01-029
Calvenzani, V. T02-026
Calvert, Charlene T04-080
Calvo, Vanessa T02-003
Cameron, Robin K T08-001
Cammue, Bruno P.A. T10-028
Campilho, Ana T02-081
Cande, W. Zacheus T09-006
Candela, H. T02-085, T02-102, T02-105
Caniard, Anne T05-061, T05-086
Cantero–Garcia, Araceli T04-052
Cao, Xiaofeng T09-026
Cappellaro, Corinna T10-004
Carbonero, Pilar T09-050
Carlberg, Inger T04-037
Carlsbecker, Annelie T02-088
Carlsson, Jenny T01-096
Caro, Elena T03-051
Carol, Rachel T02-065
Carpita, Nicholas T13-017
Carr, John T05-069
Carrari, Fernando T12-027
Carrasco, Pedro T04-064
Carrington, James C. T09-030, T09-058
Carstens, Maryke T05-072
Casanova, Sandrine T01-083

Case, Natalie T13-008, T13-009
Casimiro, Ilda T02-003
Casson, Stuart T10-038
Castellano, M. Mar T03-051, T03-064
Castiglioni, Paolo T12-011
Catarecha, Pablo T04-102
Cazale, Anne-Claire T07-046
Celaya, Brandon T05-088
Cerutti, Heriberto T09-021
Chaban, Christina T05-015, T09-014, T10-009
Chae, Lee T04-086
Chae, Min-Ju T04-087
Chakim, Ofir T09-031
Chakrabarty, Romit T05-047, T05-054
Chandler, John T02-049, T02-050, T02-052
Chang, Jeffrey H. T05-018
Chanvivattana, Yindee T01-101
Charlot, Florence T02-001
Charlton, W. L. T04-094
Chaudhury, Abed T01-046, T01-047
Chen, Feng T07-079
Chen, I-Peng T09-020
Chen, Jianxin T08-012
Chen, Lingjing T01-029
Chen, Meng T06-023
Chen, Xuemei T09-054
Chen, Z. Jeffrey T06-023
Chenu, K. T04-043, T10-022, T11-014, T11-015
Cheong, Jong-Joo T07-076
Chetty, C. T13-014
Chevalier, David T02-076
Chevone, Boris I. T07-038, T07-039
Chew, Orinda T03-001, T03-052, T04-001
Chi-Chen, Chang T04-078
Chinchilla, Delphine T05-061, T05-086
Chisholm, Stephen T05-085
Chiu, Wei-Hsin T02-049
Cho, Euna T01-075
Cho, Hyung-Taeg T02-025
Choe, Sunghwa T02-072, T02-074, T07-077
Choi, Hoonsung T02-059
Choi, J. T13-014
Choi, Yang Do T07-076
Choi, Yeonhee T09-026
Choi, Yoojin T05-038
Choi, Youn-Hee T12-023
Chong, Julie T04-108, T07-090
Chory, Joanne T01-066
Chriqui, D. T02-064
Christensen, Sioux T02-099
Christmann, Alexander T04-056, T04-076
Christophe, A. T11-014
Christopher, Wayne T10-027
Chua, Nam-Hai T02-069, T02-104, T04-048
Ciannamea, Stefano T01-074
Ciolkowski, Ingo T01-015, T09-014
Civjan, Natanya T07-009, T07-013
Clarenz, Oliver T02-080
Clark, Greg T04-052
Clark, Jennifer T11-010
Clark, Jeremy T12-018
Clark, Nicholas T02-092
Clark, Terry T11-024
Clarke, Jonathan T02-098
Clauss, MJ T12-021
Clemens, Stephan T04-093, T04-095
Clément, B. T13-016
Clemente, Thomas T09-021
Clifton, Rachel T03-001, T03-052, T04-053
Clissold, Leah T10-039
Clover, Charles C. T05-017
Cnudde, Filip T10-037
Cobbett, Christopher S. T04-010, T07-084
Coen, Enrico T01-009, T02-096, T11-012, T11-029, T12-003
Cohen, Jerry T01-054
Cole, Melanie T02-052, T02-078
Coleman, Mark T04-080
Coles, Jeremy P. T02-092
Colinas, Juliette T02-107
Collado, María Teresa T04-064
Coll-Garcia, Danahe T02-040
Colombo, Bilitis T09-039
Colombo, Lucia T09-039
Comai, Luca T06-001, T06-023
Cominelli, E T04-017
Compagnon, Vincent T07-094
Consonni, Chiara T05-029
Conti, L T04-017
CONTI, LUCIO T02-036
Cook, Douglas T12-025
Cookson, Sarah T02-011
Cordero, Israel T13-017
Cork, Jennifer M. T06-019
Corke, Fiona T07-026, T07-044
Corsar, J. T10-014
Cortés, Elena T07-095
Cortes-Valle, Enrique T02-117
Coruzzi, Gloria M. T07-041, T07-082, T11-023
Corvisy, Rachel T01-046
Costa, M.M.R. T01-009
Costa, Vanessa T09-037
Costantino, Paolo T01-055, T01-069, T02-020, T03-010
Coughlan, Sean J T04-007, T10-007
Coupland, George T01-018, T01-019, T01-031, T01-032, T01-035, T01-042, T01-048, T01-056, T01-057, T01-071
Coutts, Katherine T05-059
Cox, M. T04-061
Cramer, R.A. T05-087
Craven, K.D. T05-087
Crawford, B. T10-014
Crawford, Katrina T01-075

Creelman, Bob T12-011
Crist, Deborah. T13-008, T13-009
Cross, Joanna. T07-070
Cruikshank, A. T07-041
Cubas, Pilar T01-009, T02-015, T12-003
Cui, HongChang T02-108
Cuire, Catherine T04-006
Curaba, Julien T01-040
Cushman, John C.. . . . . T04-104
Cuzick, Alayne T05-068
Cvrckova, F. T03-040
Cvrckova, Fatima T03-043, T06-003
Cybelle, Smyczynski T03-017
CZECHOWSKI, T T10-023
Czechowski, Tomasz T01-068, T07-052

D

D`Angelo, Cecilia. T04-023, T06-006
Dabos, P. T09-034
Dahlbeck, Douglas T05-085
Dai, Liangying. T08-010
Daimon, Yasufumi T01-033
D'Angeli, S. T01-055
D'Angelo, Cecilia. T03-020, T06-007
Dangl, Jeffery L. T03-008, T04-004, T05-015, T05-017, T05-018, T05-019, T05-021, T05-068, T05-090
Danielsson, Jesper T05-047, T05-048
Darnielle, Lalitree T01-087
Darrah, Chiarina T06-005
Das, Sandip T06-018
Das, Taspos K. T05-001
Datta, Sourav T04-073
Dauzat, M. T04-097, T10-022
Davière, Jean-Michel T02-027
Davis, J.W. T05-087
Davis, R.W.. . . . . T10-005
Davis, Seth J. T01-049, T04-034, T04-050, T04-091
Davison, Timothy. T10-018
Davydov, Olga. T04-012
Day, David T04-053
Day, Irene S.. . . . . T03-016
De Bolle, Miguel F.C. T05-042, T10-028
de Koster, Chris G. T05-028
de Vries, Prof. Dr. Sacco C. T03-056
Deak, Karen T02-111
Dean, Caroline T01-060, T01-072, T06-030, T09-022
Debeaujon, Isabelle. T07-002, T07-014
Deckers, Peter T13-015
Degenkolbe, Thomas. T10-012
Dekker, Henk L.. . . . . T05-028
del Pozo, J. C.. . . . . T02-085
DELARUE, M. T02-097, T09-049
Delatorre, Carla. T04-088
Delauré, Stijn L. T10-028
Dellaporta, S. T04-017
Delmer, Deborah P. T13-012
Demar, Monika T01-016
Demidov, Dmitri T09-012
Demura, Taku T02-082, T02-083
den Ackerveken, Guido Van T05-006
Denby, Katherine. T05-072, T05-075
Deng, Xing-Wang T03-010, T04-073, T10-040
Dennis, Elizabeth S.. . . . . T01-011, T01-046, T09-036, T12-009
Denslow, Nancy D. T13-018
Derbyshire, P. T10-014
DERKSEN, Jan T03-078
Desbrosses-Fonrouge, Anne-Garlonn T06-031
Desimone, Marcelo T07-047
Desprez, Thierry T03-009
Dessi, Patrick T03-011
Desveaux, Darrell T05-018, T05-021
Desvoyes, Bénédicte T05-033
Dettmer, Jan. T01-036
Deuschle, Karen T07-029
Devic, Martine T07-014
Dewald, Miriam. T03-034
Dharmasiri, Nihal T02-120, T03-081
Dharmasiri, Sunethra T02-120, T03-081
Diamond, Chloe C. T01-008
Diaz-Triviño, Sara T03-064
Dickerman, Allan. T01-083
Dickinson, Hugh G. T01-012, T02-053
Diet, Anouck T02-093
Dieterich, Jens-Holger. T07-052
Dieterle, Monika T03-019
Dietz, Karl-Josef T04-011, T09-040
Dietzmann, Angela T09-003
Dijk, Karin van T09-021
Dijkhuis, Paul T01-027
Dill, Alyssa T01-013
Dinesh-Kumar, Savithramma P. T10-040
Ding, Derong T05-047
Dinneny, Jose R.. . . . . T01-098
Disch, Sabine T01-063
Dittgen, Jan T05-015, T05-022, T05-053
Ditzer, Andrea T04-028
Dixelius, Christina T05-048
Doczi, Robert T04-004
Doebley, John. T12-006
Doerge, R. W. T06-001, T06-023
Doerner, Peter. T02-046, T08-013
Dolan, Liam T02-065, T02-073, T02-079, T02-106, T10-014
Dolferus, Rudy T12-009
Dolniak, Blazej T01-052
Domagalska, Malgorzata T01-049, T04-050
Domenech, J.. . . . . T05-004
Dong, Xinnian T05-052, T05-064, T05-067, T05-083
Dongen, Joost Van T12-009
Doonan, John T03-023, T10-014
Dörmann, Peter T04-007, T07-073

Dorota, Konopka T04-026, T05-009
Dorta, Fernando T07-091
Dortay, H. T13-005
Dotson, Stan T12-011
Douglas, C. J. T09-053
Doutriaux, MP T09-001
Dowding, Joanne L. T02-053
Doyle, Mark R. T04-034
Dräger, Dörthe B. T06-031
Dray, E T09-001
Drdova, E T03-040
Drea, S. T10-014
Dresselhaus, T. T01-002, T01-064
Dröge-Laser, Wolfgang T09-050
Duan, Hui T07-009, T07-013, T11-002
Dubiella, Ullrich T05-060
Dubois, Annick T01-085
Dubreucq, B T09-011
Dubuis, Pierre-Henri T04-105
Dudova, Marketa T01-037
Dugas, Diana T09-057
Duk-Ju, Hwang T05-071
Dumas, C. T01-095
Dunoyer, Patrice T08-018, T09-025
Duroux, Meg T09-042
DUVAL, Frédéric D. T05-049
Dzianot, Aleksandra T05-010

E

Ebert, Berit T01-062, T10-029, T10-036
Ecker, Joseph R. T01-013, T01-087, T10-005, T10-052, T10-055
Eckermann, Nora T07-053
Edge, Dave T05-056
Edwards, Herb T05-073
Eggeling, Ferdinand von T10-036
Ehlert, Andrea T09-050
Ehlting, J. T09-053
Ehrhardt, David T03-046, T05-027, T05-073
Ehrhardt, Thomas T12-008
Ehrismann, Jasmin T02-008
Eicker, Andrea T02-087
Eide, D.J. T07-097
Eijk, Michiel van T07-062
Einset, John T04-003
Ejdebäck, Mikael T01-058, T04-081
Eklund, Magnus T01-065, T01-093
El-Bakkoury, Mohamed T10-004
Elbaz, Benayahu T08-012
Elhafez, Dina T04-053
Elias, M T03-040
Ellerström, Mats T09-041
El-Lithy, Mohamed E. T06-016, T07-062
Elzbieta, Kraszewska T07-001
Elzinga, N T07-003
Emans, Neil T08-015
Emanuel, Carola T09-047
Emilie, Vaillant T03-017
Endo, Masaki T09-007
Endres, Mathew T09-054
Enju, A. T10-005
Epp, Delwin T07-015
Epple, Petra T05-017
Erasmus, Yvette T11-012
Ergen, Neslihan T07-057
Eriksson, Maria M. T04-091
Eriksson, Sven T01-079
Ernst, Dieter T04-105
Ernst, W.H.O. T06-016
Esaka, Muneharu T09-038
Escobar, JM T06-032
Eshed, Ravit T13-012
Espinoza, Valeria T07-091
Estelle, Mark T02-120, T03-081
Estevez, Juan M. T07-042
Etchells, J. Peter T02-053
Etheridge, Naomi T02-022
Eubel, Holger T07-024
Eulgem, Thomas T04-004, T05-068
Exner, Vivien T09-056
Expert, Dominique T05-026

F

Facette Michelle, R. T07-074
Fagard, Mathilde T05-026
Fahnenstich, Holger T07-017
Fajardo, Diego T12-010
Falasca, G. T02-026
Falk, Stefanie T02-063
Falkenhahn, Doris T03-070
Fankhauser, Christian T04-109
Farchi-Pisanty, Odelia T03-033
Farmer, Edward E. T05-063
Farrona, Sara T01-001
Faure, Jean-Denis T03-017
Faust, Andrea T03-005
Fauth, Markus T03-068
Feher, Balazs T04-091
Fehr, Marcus T07-029
Feldmann, Kenneth A. T07-077
Felix, Georg T05-086
Felix, Jürg T05-061
Feng, Teng-Yung T05-013
Fernie, Alisdair T12-027
Ferrandiz, Cristina T01-039
Fesler, Amy T01-083
Fettke, Joerg T07-053
Feussner, Ivo T05-012
Feyereisen, René T05-041, T07-077
Feys, Bart J. T05-089, T05-050
Fiechter, Vincent T04-109
Fiehn, Oliver T06-014, T06-017, T10-017, T11-006
Fiene, Gabi T02-019
Fiers, Martijn T01-034
Filleur, S T04-063

Finzi, Laura T09-039
Fisahn, Joachim T06-014, T10-029, T10-033, T10-036
Fischer, Karsten T07-030, T07-033, T07-049, T07-050
Fischer, Robert L. T09-026
Fischer, Ute T09-028
Fittinghoff, Kirsten T04-057
Fitz Gerald, Jonathan N. T01-097, T06-024
Fitz, Joffrey T06-027
Fitz, Michael T07-047
Fitzgerald, Heather A. T09-058
Fleischer, Carola T10-004
Fletcher, Jennifer C. T01-063, T02-099
Flier, Annegret T02-052
Flörchinger, Martin T07-021
Flors, Víctor T05-044
Fluck, Juliane T11-018
Flügge, Ulf-Ingo T03-032, T07-017, T07-025, T07-030, T07-031, T07-033, T07-034, T07-049, T07-050, T08-005, T11-020
Folter, Stefan de T01-041
Forde, BG T04-063
Foreman, J. T02-046
Formanová, Nata_a T12-014
Forner, Joachim T09-016
Forsbach, Alexandra T09-061
Fox, S. T01-009
Francesca, Sparla T07-064
Franck, N. T11-015
Frank, Greg T05-068
Fransz, P T06-032
Frédéric, Cremer T01-071
Frédérique, Bitton T02-116
Fredricksen, Mark T12-022
Freixes, S T04-042
Frelet, Annie T04-027
Frémont, Nathalie T04-049
Freund, Susanne T10-012
Fridborg, Ingela T05-059
Fridman, Eyal T12-027
Frietsch, S. T01-020
Friml, Jiri T02-118, T08-003, T08-014, T08-015, T08-016, T08-017, T09-042
Froehlich, Anja T07-048
Frommer, Wolf B. T07-029, T07-047, T10-004
Frugis, Giovanna T04-048
Fründt, Corinne T09-023
Fu, Yong-Fu T01-019
Fuchs, Jörg T09-027
Fujioka, Shozo T02-059, T02-072
Fujita, Miki T10-005, T10-025
Fujita, Yasunari T04-009
Fujiwara, Makoto T. T03-024, T03-026
Fujiwara, Sumire T01-031, T01-032
Fujiwara, Toru T07-005, T07-023
Fukaki, Hidehiro T02-048
Fukuda, Hiroo T02-082, T02-083
Fulton, Lynette T02-076
Funck, Dietmar T04-075
Funke, Silke T03-037

G

Gabbert, Karen T12-011
Gabriele, Stefano T01-069
Gaëtan, Lemonnier T02-116
Gagliano, Wendy B. T01-076
Gahrtz, Manfred T08-004
Gakière, Bertrand T07-075
Galbiati, M T04-017
Galbraith, David W. T10-001
Gallagher, Kim T02-108
Galligan, Meghan T12-011
Gallois, Jean-Luc T02-009
Galuschka, Claudia T11-005, T11-009
Gampala, Srinivas S.L. T04-085
Gang, S. T13-016
Ganguli, Arnab T05-062
Garcia-Hernández, Margarita T11-011, T11-022
Garcia-Moreno, Jaime T11-013
García-Ortiz, Maria Victoria T09-032
Garzon, Marcus T03-004, T03-005
Gatz, Christiane T09-019
Gatzek, S. T11-006
Gaut, Brandon S. T12-006
Gebert, M. T01-002, T01-064
Geigenberger, Peter T07-008, T07-048, T07-068, T12-009, T12-012, T12-018
Geist, Birgit T07-087
Geldner, Niko T08-015
Genoud, Thierry T05-058
Genschik, Pascal T03-019
Gentilhomme, Jose T01-035
Geraldo, Nuno T01-060, T01-072, T09-022
Gerats, Tom T10-037
Gerco, Angenent T12-015
GERMAIN, Veronique T04-066
Gershenzon, Jonathan T05-040, T07-043, T07-079, T07-085
Geudens, Ilse T10-028
Gfeller, Aurélie T05-063
Ghandilyan, Artak T06-016
Ghazal, Hassan T10-007
Gheyselinck, J T06-032
Giacomo, Elisabetta Di T04-048
Giavalisco, Patrick T01-062, T08-007, T10-017
Gibon, Yves T01-068, T07-070, T07-096, T11-008, T11-018
Gierth, Markus T07-049
Gifford, Miriam L. T01-024
Gigolashvili, Tamara T07-034
Gilday, Alison D. T07-071, T07-072
Gillaspy, Glenda T07-078
Gillies, Amanda T12-003

Gil-Morrió, Mª José T05-034
Gils, Mario T09-061
GIRAUDAT, Jérôme T01-028
Giraudat-Pautot, V. T02-064
Gissot, Lionel T01-035, T01-056
Gittins, John T09-024
Glaser, Elisabeth T03-011
Glazebrook, J. T05-087
Glazov, Eugene T09-002
Glimelius, Kristina T01-096
Glinski, Mirko T07-045, T07-046, T10-035
Glombitza, Sabine T04-105
Glover, Beverley J. T02-044, T12-005
Goderis, Inge J.W.M. T10-028
Goel, Ajay Kumar T05-019
Goellner, Katharina T05-030
Goepfer, Simon T05-041
Goh, Chang-Hyo T07-077
Golan, Talila T04-068
Goldsmith, John T11-024
Golstein, Catherine T05-035
Golubovskaya, Inna N. T09-006
Gómez-Merino, Fernando C. T04-002
Gong, Lena X. T05-083
Gong, Qingqiu. T05-084, T12-022
Gonneau, Martine T03-009
Gonzalez, Antonio T02-110
Gonzalez, Tony T07-093
González-Bayón, Rebeca T02-058
Gonzalez-Carranza Zinnia, H. T10-031
González-Guzmán, M. T02-103
Goodrich, Justin T01-101, T09-029
Gor, Victoria T11-026
Gorecka, Karolina M. T04-026
Goritschnig, Sandra. T05-066
Goto, Koji T01-033, T01-053
Götz, Michael T04-105
Gourrierec, José Gentilhomme-Le T01-056
Goyal, Rana T06-030
Graaff, Eric van der T07-033, T08-005, T11-020
Grabowski, Janine. T07-018
Graf, Alexandra T08-004
Grafi, Gideon T09-017
Graham, Ian A. T04-094, T07-071, T07-072
Graham, Stuart T07-072
Grandjean, Olivier T07-014
Granier, C. T04-043, T04-097, T10-022
Granier, Christine T02-011
Grant, Murray T05-055, T05-077, T10-041
Grant, Sarah R. T05-018, T05-019
Grasser, Klaus D. T09-042
Grauer, Matthias T03-060
Greb, Thomas T01-060, T01-072, T02-080, T02-087, T09-022
Grec, Sebastien T07-058
Green, Pam T10-007
Green, Roland. T10-043
Grefen, Christopher T02-018, T09-015
Greiner, Steffen T07-055, T07-057, T07-059, T07-061
Gremski, Kristina. T01-043
Grenville, Laura. T05-082
Grewe, Britta T02-052
Grierson, Claire T02-001
Griesen, Dan. T03-021
Grigg, Stephen P. T02-099
Grill, Erwin T03-048, T04-056, T04-076
Grimsley, Nigel H. T05-024, T10-037
Grini, Paul T03-063
Grønseth, L. T03-083
Gropp, Gordon T07-015
Gross-Hardt, Rita T01-076
Grossniklaus, Ueli T01-076, T06-032, T09-045, T09-062
Grube, Esther T07-049
Gruissem, Wilhelm T09-044, T09-045, T09-056, T10-027, T10-044, T11-027
Guenther, Manuela T07-070
Guérin, Christophe T01-097
Guerineau, François T07-032
Guerinot, M.L. T07-097
Guevara, Arturo T07-095
Guivarc'h, A. T02-064
Gunesekera, Bhadra T07-078
Guo, Mengjuan T07-088
Gusmaroli, Giuliana T10-040
Gutierrez, Crisanto T03-051, T03-064, T05-033, T09-023
Gutierrez, Rodrigo A. T07-041, T07-082, T11-023
Gutierrez-Nava, María de la Luz T07-095
Gutterson, Neal T12-011
Guttman, David T06-021
GUYOMARC'H, S. T02-097
Gyula, Peter T04-091

H

Haasen, Dorothea T03-034
Hadfield, James T10-034, T10-039
Hahlbrock, Klaus T01-061
Haines, Karen T08-001
Hajji, Mokhtar T04-092, T04-099
Hala, Michal T03-040, T03-058
Halimaa, Pauliina T12-013
Hall, Anthony T04-091
Hall, D. T04-060
Hall, Dr Anthony T06-005
Hall, Sharon T05-082
Hamacher, Tanja T10-004
Hamard, P. T10-022
Hamberger, Björn T07-010
Hamilton, Alexandra A. T04-022, T04-083
Han, Hee T04-087
Hanano, Shigeru T04-050, T04-091
Hanin, Moez T04-040
Hanke, David E. T02-044
Hanna, Norén T04-059

Hannah, Matthew A. T04-047, T10-012
Hannemann, Jan. T11-008
Hannich, Barbara T07-056
Hannoufa, Abdelali T07-015, T09-018
Hano, Perdita T13-007
Hänsel, Ulrike T07-007
Hansen, Carsten H. T07-007, T10-010
Hanson, Maureen R. T02-071, T02-119
Hansson, Maria. T04-037
HANZAWA, YOSHIE T02-036
Hara-Nishimura, Ikuko. T03-062
Hardeman, Kris. T12-011
Hardtke, Christian S. T02-002
Harford, Jeff T10-002
Haring, Michel T07-067
Harmon, Alice C. T13-018
Harmon, Frank G. T10-052
Harper, Jeffrey F. T01-020, T07-097, T13-018
Harrison, Marcia A. T04-019
Harter, Klaus T02-016, T02-018, T02-019, T05-015, T09-014, T09-015, T10-009
Hartje, Stefanie T08-020
Hartmann, H. Andreas T05-029
Hartmann, Stefanie T11-019
Hartung, Frank T09-020
Hartwig, T. T07-063, T07-065
Hase, Shu. T05-074, T05-079
Hasebe, Mitsuyasu T01-021
Hasegawa, Paul M. T12-022
Haseloff, James P. T01-030, T02-081, T11-010
Hashimoto, Takashi T02-033, T03-041, T03-049
Hashimoto, Yukari T02-113
Hass, Claudia T02-016
Hassinen, Viivi. T12-013, T12-016
Hattori, Sayoko T02-068
Haughn, George W. T01-047, T03-025
Hauksdottir, Herborg T03-080
Hauser, Marie-Theres T03-006, T05-005
Haußühl, Kirsten T03-011
Hawkes, Valerie T08-002
Hay, Angela T02-027
Hayashi, Hiroaki T02-038, T07-023
Hayashi, Shinpei T04-020
Haydon, Michael J. T04-010
Hays, John B. T09-032
Hazen, Samuel P. T10-052
He, Sheng Yang T05-080
He, Zheng-Hui T04-086
Heard, Jacqueline T12-011
Heazlewood, Joshua L. T03-001, T03-002, T03-052, T04-001
Hedden, Peter. T02-027, T02-092
Hegedus, Dwayne T09-018
Hegeman, Adrian D. T02-037, T13-018
Hehl, Reinhard T11-005, T11-009
Hehn, Alain T05-041
Heiber, Isabelle T04-011
Heidstra, Renze. T02-084, T02-095, T02-118
Heim, M. A.. . . . . T09-011
Heinemeyer, Jesco T07-024
Heinz, J.. . . . . T13-014
Heisler, Marcus T11-026
Heitzeberg, Fabian T09-020
Helariutta, Ykä T02-088, T02-101, T02-113, T12-017
Helfer, A.. . . . . T13-016
Hell, Rüdiger T05-032
Hellens, Roger P. T01-090, T09-052
Helliwell, Chris T01-011, T10-020
Hellmann, Hanjo T13-007
Hemelrijck, Wendy Van T05-042
Hemmann, Georg T07-044
Hempel, Fred T01-075
Hendriks, Janneke H.M. T07-068
Hennig, Lars T09-045, T09-056, T11-027
Henz, Stefan T10-018
Herman, Höfte T02-116
Hermesdorf, Anne T03-038
Herschleb, Jill T03-080
Hervé, C. T09-034
Herve-Fleury, Delphine T02-021
Herzog, Michel T01-040
Hesberg, Christine. T04-015
Hesse, Holger T04-106, T07-075
Hewitt, Crystal T07-092
Heyer, Arnd G.. . . . . T04-047, T04-072, T10-011, T10-012
Heyl, A.. . . . . T13-005
Hibara, Ken-ichir. T02-032
Hibberd, Julian M.. . . . . T10-015
Hibberd, Victoria T04-091
Hibino, Takashi T03-029
Hicks, Karen A. T01-080, T01-081
Higginson, T.. . . . . T01-095
Higuchi, Masayuki. T02-113
Higuchi, Mieko T02-075
Hill, Emi J. T02-012
Hilliou, Frédérique T05-041
Hills, Melissa J.. . . . . T01-011
Hilpert, Melanie. T07-029
Hilson, Pierre T10-041
Himber, Christophe T09-025
Himbert, Sarah T03-013
Hincha, Dirk K. T04-047, T04-072, T10-012
Hinchey, Brendan T12-011
Hiratsu, Keiichiro. T02-112
Hiratsuka, Kazuyuki. T10-030
Hirayama, Takashi. T04-020, T07-080, T10-047
Hirsch-Hoffmann, Matthias T11-027
Hirt, Heribert T04-004, T04-038, T05-081
Hlavacka, A. T03-085
Hodge, Sarah T11-010
Hoecker, Ute T04-057
Hoefgen, Rainer T04-106, T07-075
Hoene, Melanie. T07-070

Hoffman, Peter D. T09-032
Hoffmann, Laurent T02-079
Hofmann, Christina T07-055
Höfte, Herman T03-009
Hogan, Justin D. T04-019
Hohn, Barbara T09-033, T10-037
Höhne, Melanie T11-008
Holk, André T02-057
Hollaender-Czytko, Heike T07-018
Holley, Clay T01-083
Holm, Magnus T04-073
Holman, Rachel T07-026, T07-044
Holmes, Andrew B. T05-028
Holmström, Kjell-Ove T04-081
Holt III, Ben F. T05-017
Holtgrefe, Simone T09-040
Holub, Eric B. T05-068
Holzinger, Susanna T07-087
Hong, Ji-Yeon T04-087
Hooykaas, Paul T09-037
Horackova, Martina T06-003
Horak, Jakub T02-018, T09-015
Horiguchi, Gorou T02-006, T02-114, T04-098
Horvath, ... T02-047
Hosy, E. T04-097
Hothorn, Michael T07-055, T07-061
Hou, Bi-Huei T05-023
Hou, Xuewen T04-086
Houben, Andreas T09-012, T09-042
Hövel, Klaus T07-069
Howard, T. T07-060
Howell, Katharine A. T12-001
Howell, Stephen H. T02-104
Hoyerová, K T13-019
Hoyt, Joyce M. T04-022, T04-083
Hrabak, Estelle M. T13-018
Hricova, Andrea T02-042
Hsian-En, Huang T05-013
Hu, H T06-032
Hu, Jianhong T01-013
Hu, Yuxin T02-069
Huala, Eva T11-022
Hudson, Matthew T04-051, T04-051
Huesgen, Pitter T03-011, T11-013
Hugueney, P. T01-095
Huijser, Peter T01-077
Hülskamp, Martin T02-054, T02-060, T02-061, T02-063, T03-028, T03-039, T03-042, T03-045, T03-075
Hummel, Florian T02-016
Hummel, Jan T11-007
Hundertmark, M T04-072
Hunger, Sandra T12-002
Hunzicker, Gretel M. T13-002
Huq, Enamul T04-024, T04-051
Hurban, Patrick T05-058
Hurtado, Lidia T01-001
Hüser, Anke T07-025
Hutchens, Steven T01-083
Huttlin, Edward L. T02-037
Hwang Seon, Hee T05-071
Hwang, Ildoo T02-016
Hwang, In-Sun T04-087

I

Iafrate, Silvia T03-010
Iannelli, Adelaide T04-048
Ichikawa, Hiroaki T09-007
Ichikawa, Takanari T04-054, T10-025
Ichimura, Kazuya T04-004
Ichinoki, Harutaka T01-033
Iglesias, Joaquín T04-102
Iida, K. T10-005
Iida, Ryusuke T02-048
Iizumi, Haruko T10-025
Ikeda, Akira T05-014
Ikeda, Yoko T01-033, T01-044
Ikeda, Yoshihisa T02-104
Ikegami, Masato T05-074, T05-079
Iki, Taichiro T05-007
Ilic, Katica T11-022
Im, Kyung-Hoan T05-038
Immanen, Juha T12-017
Immink, Richard T01-074
Imura, Yuko T10-047
Inada, Sayaka T04-016
Inan, Gunsu T12-022
Indorf, Martin T04-062
Ingelgem, Carl Van T03-072
Ingouff, Mathieu T01-097
Ingram, Gwyneth C. T01-024
Ingram, Paul T06-024
Innes, Roger T05-035
Inoue, Kayoko T02-038
Inzé, Dirk T02-014, T02-021, T02-039, T03-072, T03-075, T04-044
Ioio, Raffaele Dello T02-020
Irish, Vivian F. T01-008
Ishida, J. T10-005
Ishida, Tetsuya T02-068
Ishiguro, Sumie T01-094
Ishihara, Takeaki T05-074
Ishikawa, Akie T03-031, T04-054, T10-025
Ishikawa, Yuichi T09-007
Ito, Takuya T02-075, T04-055
Ito, Toshiro T01-006, T01-007
ito, Yuji T09-007
Itoh, Ryuuichi T03-024
Iuchi, Satoshi T10-013
Ivanov, Rumen T09-041
Iwata, Naoko T04-096
Iwona, Adamska T04-059

J

Jacek, Hennig T04-026, T05-009
Jach, Guido T01-015

Jackson, Angela T04-086
Jacob, Francis T05-051
Jacobsen, Steven E. T09-026, T09-027
Jaffe, Felix T12-005
Jaime, Elena Galiana T02-063
Jakab, Gábor T05-044
Jakobsson, Mattias T06-026
Jakoby, Marc T03-070, T04-005
James, Peacock W. T09-036
Jander, Georg T05-003, T07-035, T07-092
Jang, Seonghoe T01-057
Jang, Su Youn T02-074
Janowitz, Tim T07-012, T07-022
Jasencakova, Zuzana T09-027
Jasinski, Sophie T02-027
Jean, Finnegan E. T09-036
Jean, Marie Le T04-006
Jean, Martine T10-051
Jenik, Pablo D. T01-088
Jenkin, Linne E. T12-001
Jeong, Soon-Jae T05-036, T05-038
Jerzmanowski, Andrzej T09-005, T09-024, T09-055, T10-008, T12-020
Jian, Hu T05-031
Jiang, Hongmei T06-001, T06-023
Jiang, Lixi T01-073
Jin, Yongmei T04-090
Joanna, Bandorowicz-Pikula T05-009
Jocelyne, Olivier T05-031
Johns, Melloney K. T05-028
Johnson, B. T04-094
Johnson, Christopher T10-043
Joly, Robert J. T12-022
Jonak, Claudia T04-030, T04-031
Jones, Jonathan T03-008
Jones, Patrik. T07-087
Jong, Mark de T05-006
Jong, Yvonne de T06-015
Jönsson, Henrik T11-026
Joo, Jung Hee T05-025
Jorda, Lucia T05-020
Jordá, Lucía T05-034, T05-043
Joshi, Vijay T07-035
Jost, Ricarda T05-032
Jouannic, Stéphane. T02-073
Jover-Gil, S. T02-102, T02-105
Joy, Rebecca E. T01-088
Joyard, Jacques T03-015
Juenger, Thomas E. T04-101, T06-032
Juergens, Gerd T03-077, T08-015, T08-016
Juliette, de Meaux. T06-012
Jung, Eui-Hwan T05-071
Jung, Jee W. T10-001
Jung, Sabrina T09-040
Jünger, Melanie T07-020
Juraniec, Michel T03-009
Jürgens, Gerd T02-008, T03-073, T03-074
Justin, Samuel T01-099

K

Kabayashi, Masatomo T01-091
Kadalayil, Latha T13-003
Kaden, Daniela T09-046
Kagochi, Trevor T06-001
Kakesova, H T03-040
Kakimoto, Tatsuo. T02-113
Kakutani, Tetsuji T09-026
Kalde, Monika. T05-073
Kaloudas, D T07-060
KAMADA, HIROSHI T01-031, T01-032
Kamei, A. T10-005
Kameya, Toshiaki T09-007
Kami, Chitose T04-096
Kamil, Olejnik T07-001
Kamil, Witek T05-009
Kaminaka, Hironori T05-015, T05-017
Kamínek, M. T13-019
Kamiya, Asako T10-047
Kamiya, Yuji T07-042
Kanayama, Yoshinori T05-079
Kandlbinder, Andrea T09-040
Kang, Mi-Young T04-085
Kangasjärvi, Jaakko T04-044, T04-074, T05-012
Kanwischer, Marion. T04-007, T07-073
Kapitza, Kristin T08-007
Kapri-Pardes, E. T04-025
Kärenlampi, Sirpa T12-013, T12-016
Karim, Sazzad. T01-058, T04-081
Karlowski, Wojciech M. T06-022
Karpinska, Barbara T04-078
Karpinski, Stanislaw T04-075, T04-078
Kasahara, Hiroyuki T07-042
Kasschau, Kristin D. T09-058
Katagiri, Takeshi T01-091
Kater, Martin M. T09-039
Kato, Naohiro T09-004
Kato, Takehide T04-070, T04-089
Kato, Tomohiko T02-038, T02-113
Katsura, Koji T04-009
Katz, Aviva T09-017, T09-031, T10-016
Kauppinen, Leila T12-017
Kaur, Hardip T02-027
Kawashima, Mika T04-054, T10-025
Kay, Steve A T10-052
Kazan, Kemal T09-035
Kearley, Mark T03-046
Kearsey, Mike T04-033, T04-045, T06-011, T10-049
Kehr, Julia T08-007, T10-017, T10-026
Keller, Beat T02-093
Keller, Ingo T05-011
Kelly, Amelie T07-073
Kemmerling, Birgit T05-060
Kempa, Stefan T04-106, T07-075
Kenyon, Janet. T01-012
Kepler, Thomas B T05-052

Kerekes, Irén T10-050
Kernebeck, Birgit T03-042
Kerzendorfer, Claudia T03-004
Keshvari, Nima T11-007
Kessler, Andre T12-028
Keurentjes, Joost T04-101
Kevei, Eva. T04-091
Khanna, Rajnish T04-024, T09-009
Kharel, Yugesh T07-081
Kiba, Takatoshi T02-007
Kieber, Joseph J.. . . . . T02-062, T07-040
Kikuchi, Jun T07-080
Kilian, Joachim T04-023, T06-007
Kim , Jee Eun T05-071
Kim, Doh-Hoon T05-036, T05-038
Kim, Dong Wook T02-025
Kim, Eun Hye T07-076
Kim, Gyung-Tae T02-006, T02-059, T04-098
Kim, Han Suk T05-021
Kim, Ho Bang T07-077
Kim, Insoon T01-075
Kim, Ju-Kon T07-076
Kim, Min Chul. T01-010, T01-059, T01-070
Kim, Minkyun T04-090
Kim, Minsung T12-003
Kim, Myeong Ae T07-076
Kim, Sang Hee T05-071
Kim, Sung T06-030
Kim, Sung-Jin. T12-023
Kim, Woe-Yeon T04-091
Kim, Yun Ju T05-071
Kim, Yunhee T01-089
Kinoshita, Tetsu. T09-026
Kinoshita, Yuki T09-026
Kinsman, Elizabeth A. T02-058
Kirch, Hans-Hubert T04-028
Kirik, Victor T02-054, T03-075
Kitahata, Nobutaka T03-026
Kiyose, Shinichiro T04-089
Klahre, Ueli T01-102, T09-002
Klasen, Rebecca T13-008, T13-009
Kleber, Regine. T10-026
Klee, Harry J. T13-004
Kleffmann, Torsten T10-027, T10-044
Klein, Marion T07-043
Klein, Markus T04-027
Kleine-Vehn, Juergen T08-015
Kleinow, Tatjana T03-013
Kley, Jeannette T07-068
Kliebenstein, Dan T05-075
Knappe, Silke T07-030, T07-050
Knee, Emma. T13-008, T13-009
Knill, Tanja T07-004
Ko, Jong-Hyun T05-055
Ko, Jun Ho T02-074
Kobayashi, Masaharu T07-005, T07-023
Kobayashi, Masatomo T02-113, T04-055, T10-013
Kobayashi, Toshihiro T10-013
Kobayashi, Yasushi T01-017, T01-045
Kocabek, Tomas T01-037, T04-018
Koehler, Sonja. T07-070
Kohchi, Takayuki T01-014, T04-096
Köhl, Karin I. T10-011, T10-012, T10-048
Köhler, Claudia T09-062, T09-045, T09-056
Köhn, Carsten A. T04-036
Kojima, Mikiko T08-006
Kolasa, Anna. T08-007
Kolbe, Anna T07-068, T12-012
Kollist, Hannes T04-044
Köllmer, Ireen T13-010
Kolmos, Elsebeth T04-034
Kolukisaoglu, Ü. T07-063, T07-065
Kombrink, Erich T07-069
Komiya, Yugo T03-049
Koncz, Csaba T03-013, T03-063, T04-103, T09-005, T10-050, T11-003, T13-013
Koncz, Zsuzsanna T13-013
Kondo, Maki T03-062
Kondou, Youichi T04-054, T10-025
Konstantin, Skryabin T12-015
Koo, Yeon Jong T07-076
Kooiker, Maarten. T09-039
Koornneef, Maarten T06-016, T07-062
Kopka, Claudia T07-083
Kopka, Joachim T04-007, T04-050, T04-106, T05-040, T07-091, T10-011, T10-045, T11-016
Koroleva, Olga T03-023
Koskull-Döring, Pascal von T05-062
Kosmehl, Tilbert T07-075
Kost, Benedikt T01-102
Kotak, Sachin T05-062
Kotchoni, Simeon T04-028
Kotlinski, Maciej T10-008
Kötting, Oliver T07-008
Kovács, Isabella T10-050
Kovalchuk, Igor T04-013
Koyama, Tanetoshi T07-081
Koyama, Tomotsugu T02-112
Kozbial, M. T09-005
Kozbial, P. T09-005
Kozma-Bognar, Laszlo T04-091
Kozuka, Toshiaki T02-059, T04-098
Krach, Christian T06-031
Kraft, Edward T03-080
Kraker, Jan-Willem de T07-085
Krämer, Ute T06-031, T11-019
Krasovskaja, Inga T10-050
Kratzin, Hartmut T03-057
Krause, Antje T11-019
Krauz, Achi T03-003
Kreitman, Martin T06-026
Kretsch, Thomas T03-019
Krishnamurthy, Nandini T05-085
Kronforst, John T09-013

Kroymann, Juergen T05-016, T06-004
Krupková, Eva T02-089
Kruse, Cordula T05-032
Ktistakis, Nicholas T. T05-028
Kubigsteltig, I. T05-039, T13-002
Kubo, Minoru T02-082, T02-083
Kudla, Jörg T03-020, T04-023, T06-006, T06-007, T10-009
Kuhla, Birte T09-046
Kuhlemeier, Cris T08-003
Kuhn, Josef M. T01-084
Kühn, Kristina T09-046
Kuhnle, Carola T03-079, T08-009
Kuijt, Suzanne T03-044
Kumar, Mukesh T04-008
Kumar, Prakash T01-007
Kunz, Sabine T05-011
Kunze, Gernot T05-061, T05-086
Kunze, Reinhard T08-005, T11-020
Kuramasu, Kiyoko T04-070
Kuras, M. T09-005
Kurata, Tetsuya T02-023
Kuroda, Hirofumi T03-031, T10-025
Kuromori, Takashi T02-034, T02-075, T04-020, T09-007, T10-047
Kursawe, Mandy T01-052
Kurup, Smita T11-010
Kusano, Tomonobu T05-079
Kuschinsky, Anja M. T10-010
Kuusela, Tiina T04-074
Kuusk, Sandra T01-093
Kwaaitaal, M.Sc. Mark T03-056
Kwak, June M. T03-008
Kwan, Waiking T09-006
Kwiatkowska, A. T09-005
Kwiatkowska, Dorota T11-001
Kwon, Mi T02-074
Kwon, Tack-Min T05-036

L

Lachaâl, Mokhtar T04-092, T04-099
Lafos, Marcel T03-063, T13-013
Lagarias, J. Clark T04-096
Lagercrantz, Ulf T01-096
Lagrange, Thierry T03-015
Lai, Fan T08-013
Lalonde, Sylvie T07-029
Laloue, Michel T02-001
Lam, Eric T03-018, T09-004
Lambert, Georgina M. T10-001
Lambie, Suzanne T02-071
Landtag, Jörn T05-022
Lång, Saara T04-044
Lange, Bernd Markus T10-006
Lange, Peter R. T07-019, T07-027, T07-083
Langer, Anke T07-048
Langridge, P. T01-002
Lanz, Christa T06-018
Lanzatella, Christina T09-009
Larkin, John C. T03-075
Larson, Tony R. T07-072
Last, Rob T07-092
Latshaw, Susan T12-010
Laubinger, Sascha T04-057
Laukens, Kris T13-015
Laurens, L. T02-064
Laurent, Corbesier T01-048
Laurent, Deslandes T05-031
Lavy, Meirav T03-003
Lawrence, C.B. T05-087
Lawson, Tracy T03-026
Lawton-Rauh, Amy T12-019
Laxell, Marjukka T12-017
Lebaudy, A T04-097
Lechtenberg, Berthold T09-061
Lecoeur, J. T11-014, T11-015
Lee, Horim T01-100
Lee, Hyeon-Se T06-023
Lee, Hyoungseok T07-077
Lee, Hyun-Kyung T02-072
Lee, Ilha T01-100
Lee, Jinsuk J. T06-023
Lee, Ji-Young T02-107, T02-108
Lee, Jong Seob T01-050, T01-089, T07-076
Lee, June Seung T05-025
Lee, Jung-Sook T05-071
Lee, Kwang-Woong T12-023
Lee, May-Nee T03-001, T03-052
Lee, Myeong Min T02-110
Lee, Sang Ho T02-025
Lee, Theresa T10-046
Leggewie, Georg T04-077
Legroune, Sadok T11-013
Lehesranta, Satu T12-016
Lehmann, Ute T07-045, T07-046, T10-029
Lehti-Shiu, Melissa T06-024
Lein, Wolfgang T12-008
Leino, Matti T01-096
Leister, Dario T09-003
Lejay, L. T07-041, T11-023
Lejay, Laurence V. T07-082
Lemke, Steffen T03-054
Lempe, Janne T01-010, T01-059, T01-066, T06-009
Lendzian, Klaus T04-076
Lenhard, Michael T01-063
León, Patricia T07-095
Leonhardt, N. T04-017
Leonhardt, Nathalie T03-008
Lepiniec, L T09-011
Lepiniec, Loïc T07-002, T07-014
Levesque, Mitch T02-108
Levy, Marganit T07-086
Leyser, Ottoline T02-077, T02-094, T08-019
Leyva, Antonio T04-102
Li, Hanbing T08-009

Li, Jingqiu T09-006
Li, Kunfeng T04-090
Li, Liang Liang T10-051
Li, Pinghua T05-084
Li, Xin T05-066
Li, Xuemei T13-017
Li, Yong T02-001
Liang, Lu T09-033
Lichtscheidl, Irene T03-067
Liechti, Robin T05-063
Liere, Karsten T09-046
Lijsebettens, Mieke Van T02-021
Liljegren, Sarah J. T01-039, T01-087, T03-014
Lim, Ze-Yi T05-028
Lindgren, Ove T02-088
Lindsey, Keith T02-024, T10-038
Link, Manuela T07-055, T07-059
Linke, Bettina T02-109
Linstead, Paul T02-065, T02-073, T02-079, T02-106
Lipka, Volker T05-022, T05-053, T05-057
Lisso, Janina T08-008
Lister, Clare T01-072, T06-030, T09-022
Lister, Ryan T03-001, T03-052, T04-053
Liu, Chun-ming T01-027
Liu, Fuquan T08-010, T09-022
Liu, Guoqin T05-076
Liu, Hongxia T05-076
Liu, Lei T07-009, T11-002
Liu, Yong-Sheng T12-027
Liu, Yule T10-040
Lízal, Pavel T01-038
Lloyd, Alan T02-110, T06-028, T07-093
Lloyd, Julie C. T07-037, T07-060
Loake, Gary J T05-078
Lobbes, Dajana T02-098
Loeffler, Christiane T05-012
Lohe, Allan T01-047
Lohmann, Christian T04-008
Lohmann, Jan U. T01-016, T10-018
Lohrmann, Jens T02-016
Lohse, Marc-André T08-020
López-Matas, Angeles T09-023
Lopukhina, Anna T07-022
Lorence, Argelia T07-038, T07-039
Lörz, Horst T08-004
Lu, Gui-Hua T01-061
Lu, Jun T05-052
Lucia, Marri T07-064
Lüdemann, Alexander T04-071, T10-011, T10-045, T11-016
Lüder, Franziska T03-057
Ludewig, Frank T07-025
Ludivine, Taconnat T02-116
Ludwig-Müller, Jutta T01-054, T05-011, T05-046, T05-051
Luijten, Marijn T02-084
Lundh, Dan T01-058, T04-081
Luo, Ming T01-046
Luschnig, Christian T02-091
Luz, Hannes T11-019
Ly, Eugene T11-025
Lynd, Kathryn E. T01-081

M

Ma, Changle T03-054, T03-055
Ma, Shisong T12-022
MacFarlane, Stuart T05-059
Machida, Chiyoko T02-090
Machida, Yasunori T02-090
Macknight, Richard T01-103
Maclean, Don T09-035
Madlung, Andreas T06-001, T06-023
Maes, Sara T02-014
Magnard, Jean-Louis T03-047
Magyar, Zoltan T02-014
Maher, Eileen T04-104
Mähönen, Ari Pekka T02-101, T02-113
Maizel, Alexis T01-005
Malamy, Jocelyn T02-111, T06-024
Malenica, Nenad T02-091
Mallory, Allison T09-057
Maloy, Michael A. T11-021
Mandal, Abul T01-058, T04-081
Mandel, Therese T08-003
Mang-Jye, Ger T05-013
Manikantan, Sankaraganesh T11-021
Mann, James T13-008, T13-009
Manners, John T09-035
Manzotti, Prescilla S. T09-039
Maple, Jodi T03-026
Marchese, Daniele T01-055
Marchfelder, Anita T03-012
Marco, Francisco T04-064
Marie-Laure, Martin-Magniette T02-116
Marion-Poll, Annie T04-021
Mariotti, Domenico T04-048
Marjoram, Paul T06-030
Markovitz, Inbal T01-008
Markus, Ralf T04-062
Marongiu, Barbara T04-058
Marquardt, Daniel T07-031
Marrocco, Katia T02-001
Martienssen, Robert T06-001
Martin, Cathie T02-098, T12-005
Martin, Seem T03-082
Martínez-Laborda, A. T02-105
Martinoia, Enrico T04-027
Martone, Julie T01-069
Maruyama, Kyonoshin T04-009
Maruyama, Nozomi T02-115
Maruyama-Nakashita, Akiko T07-051
Masmoudi, Khaled T04-040
Mason, Michael T02-067
Mate, Zoltan T04-067
Mateo, Alfonso T04-075, T04-078

Mathur, Jaideep T03-042
MATHUR, JAIDEEP T03-069
Mathur, Neeta T03-042
Matsubara, Kyoko T09-038
Matsui, K. T04-094
Matsui, Minami T03-031, T04-054, T10-025
Matsuo, Naoko T10-030
Matsuura, Hideyuki T05-014
Matthew, Louisa T10-020
Matthews, Dennis T02-067
Matthews, Ryan A. T05-019
Mattioli, Roberto T01-055
Matzke, Marjori T09-060
Mauch, Felix T04-105
Mauch-Mani, Brigitte T05-034, T05-044
Maurino, Verónica T07-017, T07-049
Mauro, M.L. T01-055
Maximova, Eugenia T06-017
May, Alexander G. T07-026
Mayama, Tomoko T04-016
Mayer, Klaus F.X. T06-022
Mayer, Ulrike T03-073
Mays, Charlotte T06-020
McCarty, Donald R. T12-010
McClung, C. Robertson T04-082
McCormick, Sheila T03-047
McDowell, John M. T05-068
McElver, John T01-083
McGrath, Ken T09-035
McKay, John T04-101
McMullen, Michael T12-006
McWatters, Dr Harriet T06-005
Medina, J. T04-029
Medina-Escobar, Nieves T05-089, T05-050
Meeley, Robert B. T09-006
Mehta, Monaz T05-077
Meier, Iris T03-076, T11-021
Meijer, Johan T05-047, T05-048, T05-049, T05-054
Meinel, Thomas T11-019
Meinke, David T01-082, T01-083, T10-042
Meins, Frederick T09-002
Meister, Armin T09-027
Meister, Robert T02-030
Meitz, Julia T05-082
Melle, Christian T10-036
Menand, Benoît T02-073, T02-079
Mendel, Ralf R. T04-015
Mendes, Pedro T07-038, T07-039
Menges, Margit T03-053
Mengiste, Tesfaye T05-081, T05-084
Menzel, D. T03-085, T03-086
Merkle, Thomas T03-034
Meskiene, Irute T04-038
Messner, Burkhard T07-087
Messutat, Sandra T07-019
Métraux, Jean-Pierre T05-058
Mette, M. Florian T09-028
Meulia, Tea T03-076
Mewett, Osman T05-056
Meyer, Andreas J T07-084
Meyer, Rhonda C. T04-046, T06-014, T06-017, T06-031
Meyer, Stefan T08-011
Meyer, Svenja T07-096, T11-017, T11-018
Meyerowitz, Elliot M. T01-006, T01-007, T01-085, T11-026
Meyers, Blake T10-007
Michael, Udvardi T10-023
Michl, K. T07-063, T07-065
Michler, P. T13-016
Michniewicz, Marta T08-016, T08-017
Micol, Jose Luis T02-042, T02-058, T02-085, T02-102, T02-103, T02-105, T09-051
Mikitova, Veronika T03-043
Millar, A. Harvey T03-001, T03-002, T03-050, T03-052, T04-001, T12-001
Millar, Andrew J. T01-049, T04-091
Millenaar, F.F T04-061
Miller, Julie T13-008, T13-009
Miller, Neil T11-011, T11-022
Mindrionos, Michael T01-075
Mira-Rodado, Virtudes T02-019
Mirko, Zaffagnini T07-064
Mirza, J. I. T01-086
Mishina, Tatiana T05-065
Mishra, Jibanananda T05-001
misson, ... T04-079
Miszalski, Zbigniew T04-078
Mitchell, T.K. T05-087
Mitchell-Olds, Thomas T05-016, T06-010, T06-012, T12-014, T12-019, T12-021, T12-024
Mittelsten Scheid, O. T09-008
Mittra, Bhabatosh T05-001
Miura, Asuka T09-026
Miwa, Kyoko T07-005, T07-023
Miyawaki, Kaori T02-113
Miyazaki, Saori T01-021
MIZOGUCHI, TSUYOSHI T01-031, T01-032, T01-042
Mizrachy-Dagri, Talya T08-012
Mizuno, Takeshi T02-007
Mjolsness, Eric T11-026
Mock, Hans-Peter T03-032
Mockaitis, Keithanne T02-120
Moeller, Claudia T09-048
Mohammed, Asif T08-001
Mohanty, Prasanna T05-001
Möhlmann, Torsten T07-021
Möhring, Silke T12-002
Moisan, Lisa J. T05-089, T05-050
Molinier, Jean T09-033
Molitor, John T06-030
Møller, Simon Geir T03-024, T03-026, T03-027
MONEY, TRACY T02-036

Monroe-Augustus, Melanie T03-066
Monte, Elena T04-024
Montgomery, Taiowa T09-058
Montoya, Mary T10-054, T11-011, T11-022
Moon, Jihyun T01-100
Moon, Yong Hwan T01-101
Mooney, Sutton T03-081
Moore, Ian T03-040, T10-020
Moore, James M. T01-076
Morcuende, Rosa T04-035, T07-052
Morgan, Colin T06-033
Morgenthal, ... T10-035
Mori, Izumi T03-008
Morita, Miyo T. T04-089
Morita, Miyo Terao. T04-032, T04-070, T04-084
Morita-Yamamuro, Chizuko T05-014
Moritz, Thomas T01-040
Morris, David T08-015
Moseyko, Nick T11-011, T11-022
Moskal, William. T10-019
Mosquna, Assaf T09-017
Mot, René De T05-042
Motohashi, Reiko T02-034, T02-075, T10-025
Mouchel, Céline F. T02-002
Mounia, Heddad T04-059
Mouradov, Aidyn T01-048
Moussatche, Patricia. T13-004
Moyle, Dr Richard T02-017
Mravec, Jozef T08-014
Mueller, Elisabeth T07-058
Mueller, Margarete T02-109
Mueller-Roeber, Bernd. T01-052, T04-002, T05-040, T07-089, T08-020
Muendermann, Lars T11-012
Mühlenbock, Per. T04-078
Mukougawa, Keiko T04-096
Müller, Axel. T04-056
Muller, B. T04-042, T10-022
Muller, Félix T07-094
Müller, Kai T01-015
Müller, Renate. T04-039
Müller, Sabine. T03-006
Müller, Steffen T07-019
Müller-Moule, Patricia T04-068
Mullineaux, Philip M. T04-075, T04-078
Mundodi, Suparna. T10-054, T11-011, T11-022
Munnik, Teun T05-028
Muramoto, Takuya. T04-096
Murayama, Maki. T04-020
Murcha, Monika T03-001
Murray, James A.H. T03-053
Murray, Shane T05-072, T05-075
Murtas, Giovanni. T01-019
Muskett, Paul T05-008
Muskett, Paul R. T05-045
Müssig, Carsten T02-040, T05-039
Mutlu, Aysegül T13-007
Muto, Shu T04-054
Mutondo, Moola T03-039, T03-045
Mylne, Joshua S. T01-060, T01-072, T09-022
Mylona, Panaglyota. T02-106
Myouga, Fumiyoshi T02-034, T02-075
Myrenås, Mattias T01-065, T01-093

N

Nagasaka, Atsushi T05-037
Nagasaka, Ryoko T02-038
Nagata, Noriko T02-034, T02-075, T10-025
Nagata, Toshifumi T05-037
Nagel, Axel T07-096, T11-017, T11-018
Nagel, Wolfgang T05-011
Nagy, Ferenc T01-049, T04-034, T04-067, T04-091
Nagy, István T05-042
Nagy, Réka T10-050
Nakabayashi, Kazumi T07-042
NAKAGAWA, MAYU T01-031, T01-042
Nakagawa, Yuko T07-005
Nakajima, Keiji T02-033
Nakajima, M. T10-005
Nakamoto, Shiho. T03-029
Nakamura, Ayako T03-024
Nakamura, Kenzo T01-094
Nakamura, Masayoshi T03-049
Nakamura, Yumiko T07-051
Nakano, Akihiko T03-035, T03-065
Nakao, Yoko T02-048
Nakashima, Kazuo T04-009, T04-014
Nakayama, Akira. T01-053
Nakazawa, Miki. T04-054, T10-025
Näke, Christian T05-015
Nam, Jaesung T05-036, T05-038
Nam, Kyoung Hee T05-025
Nandi, Ashis T05-074
Naoi, Kuniko T03-041
Nardmann, Judith T12-007
Narita, Noriyuki N. T02-114
Naudts, Mirande T02-039
Navaud, O. T09-034
Nelissen, Hilde T02-021
Nelson, Don T12-011
Nesi, Nathalie T07-014
Nessler, Craig L. T07-038, T07-039
Neu, Christina. T05-070
Neuffer, Barbara T06-025
Neuhaus, Gunther. T04-062
Neuhaus, H. Ekkehard T07-021
Neumann, Melody. T08-001
Newbury, H. John T04-060, T10-049, T10-053
Nguyen, Diana T06-022
Nickstadt, Anja T05-012
Nielsen, Tom Hamborg T04-039
Nieminen, Kaisa M. T12-017
Niewiadomski, Patrycja T07-030, T07-033, T08-005
Niihama, Mitsuru. T04-032
Nikiforova, Viktoria T04-071, T04-106

Nilsson, Lena T04-039
Nilsson, Ove T01-079
Nilsson, Robert T02-100
Nimchuk, Zachary T05-018
Nishal, Bindu T06-002
Nishimori, Yuka T01-094
Nishimura, Mikio T03-062
Nishimura, Noriyuki T04-020
Nita, Ana-Silvia T13-012
Niwa, Yasuo T10-044
Niyogi Krishna, K. T04-068
Noël, Laurent D. T05-045
Noguchi, Masahiro T02-023
Nogué, Fabien T02-001
Noh, Yoo-Sun T02-095
Nora, Fabiana R. T02-009, T02-086
Nordborg, Magnus T06-026, T06-030
North, Helen T04-021
Notaguchi, Michitaka. T01-033
Noutsos, Christos T09-003
Novotny, Marian T06-003
Nowack, Moritz T03-063
Nozawa, Akira T07-005
Nozomu, Koizumi T09-043
Nunan, Naoise T12-016
Nurmberg, Pedro L T05-078
Nürnberger, Thorsten. T05-060
Nussaume, Laurent T02-095, T04-079
Nutt, Pia T06-025

O

O. Manes, Carmem-Lara de. T02-039, T03-022
O´Neill, Carmel T06-033
Oakeley, Edward T04-067
Oberschall, Attila. T10-050
Obrdlik, Petr T10-004
Ochando, I. T02-105
ODA, ATSUSHI T01-031, T01-042
Oecking, Claudia. T07-022, T10-009
Offringa, Remko T08-017
Ogawa, Daisuke T05-014
Ohad, Nir T09-017, T09-031, T10-016
Ohgishi, Maki T04-016
Ohme-Takagi, Masaru T02-112, T04-094
Okada, Kiyotaka T01-092, T01-094, T02-001, T02-023, T02-038, T02-068, T02-115, T04-016, T06-013, T10-047
Ökrész, László T03-013
Okushima, Yoko T02-048
Olczak, Katarzyna T09-024
Oldacres, Angela. T10-049
Oldacres, Angela M. T10-053
Oldroyd, Giles T10-034
Olesen, Karen L. T05-027
Oliva, Moran T09-031, T10-016
Oliver, Sandra N. T12-009
Olivier1, Grandjean T03-017
Olsson, Peter T04-100
Oñate-Sánchez, Luis T03-050
Onckelen, Harry Van T03-072, T13-015
Oono, Y. T10-005
Oppermann, Esther T02-057
Oravecz, Attila. T04-067
Orel, Nadiya T09-020
Ortega-Martínez, Olga T02-065
Ortlip, Charles L. T07-006
Osakabe, Keishi T09-007
Osborn, Thomas C. T06-023
Østergaard, Lars T03-014
Osuna, Daniel T04-035
Otegui, Marisa T01-075
Otten, L. T13-016
Ouaked, Fatma T05-081
Ouerghi, Zeineb T04-099
Ovecka, Miroslav. T03-067
Oven, Matjaz. T04-076
Overmyer, Kirk T04-044
Oyaizu, Hiroshi T05-007

P

P.A.Cammue, Bruno T05-042
Paciorek, Tomasz T08-015, T08-016
Padhukasahasram, Badri. T06-026
Page, D T06-032
Paicu, Teodora T01-004
Palacios-Rojas, Natalia T07-089
Palatnik, Javier T02-031, T09-010, T09-030
Palatnik, Javier F. T02-015
Palenchar, P. T07-041
Palenchar, Peter M. T07-082
Palme, klaus T02-118
Palmgren, M.G. T01-020
Palva, Tapio T04-044
Panstruga, Ralph T05-029, T05-030
Paolo, Pupillo T07-064
Paolo, Trost. T07-064
Papanov, Ivan T02-118
Pape, Utz T10-018
Papenbrock, Jutta. T07-043
Paquette, Alice T02-108
PARCY, François T01-028
Parcy, François T01-040
Parizotto, Eneida Abreu T09-025
Park, Hyeong Cheol. T05-068
Park, Seijin T01-054
Parker, Jane E. T05-089, T04-078, T05-008, T05-043, T05-045, T05-050
Parker, Karen T04-053
Parker, Karen L. T03-052
Parmentier, Yves T03-019
Parniske, Martin T05-011
Pasch, Jan C. T03-037
Paszkowski, J. T09-008
Patel, Shalaka. T03-076
Pater, Sylvia de T09-037

Patton, David T01-083
Pauly, Markus T02-089, T07-007, T07-032, T10-010, T13-011
Pawlikowska, K. T09-005
Pawlowski, Wojtek P. T09-006
Paz-Ares, Javier T04-102, T10-041
Peacock, Jim T01-011
Peacock, WJ. T01-046
Peck, Scott T02-022, T05-073
Peeters, A.J.M. T04-061, T04-065, T06-015
Pei, Zhen-Ming T03-008
Pena, Andrea T04-011
Peña-Cortes, Hugo T07-091
Pena-Muralla, Rosanna T01-082
Penfield, Steven T07-072
Peng, Jinrong T01-007
Peng, Wen T08-010
Penmesta R., Varma. T12-025
Per, Winge T03-082
Perales, Mariano. T07-024
Perera, S A C N. T06-011
Perez, Pascual T07-014
Perez-Gomez, Jose T02-092
Perez-Rodriguez, Maria T12-005
Pernas, Monica. T02-106
Pesce, Eva-Rachele. T08-003
Pesch, Martina T02-060
Pestsova, Elena. T12-002
Peters, Janny L. T10-037
Petrasek, Jan T08-015
Petri, Jana T07-079
Petricka, Jalean T02-005
Petroni, K. T02-026
Petsch, Katherine T02-017
Petutschnig, Elena T04-030, T04-031
Pfalz, Marina T05-016
Phillips, Andrew T02-027
Phillips, Andrew L.. . . . . T02-092
Pia A, Stieger T02-055
Pichersky, Eran T07-079
Pickova, Denisa T06-003
Piening, Niklas T08-004
Piepenburg, Katrin T04-077
Pieterse, Corné M.J. T05-067
Pillitteri, Lynn J. T02-012
Pilon, J.L. T05-087
Pinfield-Wells, Helen T07-071
Piotrowski, Markus T07-011, T07-012, T07-022
Piqueras, P.. . . . . T02-103
Pirhonen, Minna T04-081
Pischke, Melissa S. T02-037, T02-113
Plagnol, Vincent T06-026
Plesch, Gunnar T08-020
Plume, Andrew T05-077
Poel, Hein van der T07-062
Poethig, Scott T02-066
Pogson, Barry T01-011
Poh, Huay Mei T02-069
Poirier, Yves T04-108, T07-090
Pollmann, Stephan T02-043, T07-020
Ponce, M. R.. . . . . T02-085, T02-102, T02-103, T02-105, T09-051
Ponce, María Rosa T02-058
Pontier, Dominique T03-015
Poorter, Hendrik T06-015
Popma, Theodore M. T03-025
Popp, Marianne T04-046
Poraty, Limor T03-003
Poree, Fabian T01-052
Porfirova, Svetlana T07-073
Port, Markus. T05-062
Porter, John E. T04-019
Posey, Garret T13-008, T13-009
Potnov, Irena. T03-003
Pott, Marcella B. T03-046
Pourcel, Lucille T07-002, T07-014
Prashar, Ankush T04-033
Preuss, Daphne T01-078, T11-024
PRIBAT, Anne T04-066
Pritchard, J. T04-033, T04-045, T04-060
Provart, Nicholas J. T06-021, T11-025
Pruneda-Paz, Jose L T10-052
Prusinkiewicz, Przemyslaw T11-012, T11-028
Prymakowska-Bosak, M. T12-020
Ptak, Maria. T03-012
Puah, Ching San T01-073
Puchta, Holger T09-020
Puddephat, Ian T10-049, T10-053
Pufky, John. T05-058
Pulkkinen, Pinja T04-044
Pullen, Margaret T02-024
Purugganan, Michael D. T06-013, T06-019, T06-020
Pyke, Kevin A.. . . . . T02-058

Q

Qiu, Jin-long. T05-027
Qiu, Jin-Long T05-073
Quail, Peter H. T04-024, T04-051, T09-009
Quentin, M.. . . . . T03-040, T03-078
Quesada, Victor. T02-042, T02-058

R

Raatz, Bodo T04-011
Ragone, Gianluca T01-069
Rahm, Nadia. T09-025
Rahmani, F. T07-003
Raman, Smita. T02-080
Ramirez, Ingrid T07-091
Ramírez-Parra, Elena T05-033, T09-023
Ramonell, Katrina T05-073
Ranavaya, B.. . . . . T04-045
Rao, Sheetal T06-023
Rashotte, Aaron M. T02-062
Rask, Lars T05-049
Raskind, Alexander T04-012
Ratcliffe, Oliver T02-036, T12-011

Rauh, Brad T06-029
Rausch, Thomas T03-059, T07-055, T07-057, T07-059
Rautengarten, Carsten. T01-062
Ravenscroft, Dean. T01-057
Raz, Vered T01-040
Reddy, A.S.N. T03-016
Reddy, G. Venugopala T11-026
Reddy, Vaka S. T03-016
Redestig, Henning. T11-018
Redman, Julia. T10-019, T10-043
Reeves, Paul H.. . . . . T01-019
Rehmany, Anne. T05-082
Reichelt, Michael T05-040, T07-043
Reidt, Wim T04-005, T09-041
Reiland, Sonja. T12-012
Reindl, Andreas. T12-008
Reinhardt, Didier. T08-003
Reisenbigler, Emily T12-011
Reiser, Leonore. T10-054, T11-022
Reiser, Verena. T04-059
Reiter, Wolf-Dieter. T13-017
Relichová, Jirina T01-038
Remmerie, Noor T13-015
Ren, Dongtao T05-076
Rengasamy, Ramamoorthy T02-010
Renou, Jean Pierre T02-116
Repkova, Jana T01-037
Reumann, Sigrun T03-054, T03-055, T03-057
Reva, Oleg T05-048
Revilla, Gloria T03-084
Revuelta, Jose L.. . . . . T10-004
Reyes, José Carlos T01-001
Reymond, P. T05-039
Rhee, Seung Yon. T10-054, T11-011, T11-022
Ribot, Cécile T04-108, T07-090
Ricaud, Lilian T11-010
Richards, Jim T04-101
Richaud, A T09-001
Richly, Erik T09-003
Richmond, Todd T10-043
Richter, A.. . . . . T07-063
Richter, Uwe T09-046
Riechmann, Jose Luis T01-085
Riefler, Michael T02-056, T04-049, T13-006
Riely, Brendan. T12-025
Riester, Markus. T09-010
Rietz, Steffen T02-057
Riewe, David. T12-018
Rigó, Gábor T10-050
Riha, Karel T03-036
Ringli, Christoph T02-093
Rios, G. T09-005
Ripoll, J. J. T02-105
Ritte, Gerhard T07-008
Ritu, Bhalla. T02-010
Rivero, Luz T13-008, T13-009
Robatzek, Silke T05-061, T05-086
Robert, Hélène T01-097
Robert, Stéphanie T03-009
Roberts, Jeremy A. T10-031
Roberts, Keith. T07-016
Robinson, Jon. T07-038
Robles, P. T02-102
Robles, Pedro T01-043
Rock, Christopher D. T04-085
Rodriguez Milla, Miguel A. T04-104
Rodríguez, P. L. T02-103
Rodriguez-Franco, Marta T04-062
Roeder, Adrienne. T01-039, T01-087
Roef, Luc T03-072
Roffey, Paul E.. . . . . T12-009
Rogers, Amber M. T07-039
Rogers, Rebecca. T01-082, T10-042
Rojas Stütz, Marc Christian T04-041
Roldán-Arjona, Teresa T09-032
Rolfe, Stephen T04-018
Rolland, G. T04-043, T04-097
Roman, Carolina San. T07-095
Romanov, Georgy T02-051
Romanowsky, S.M. T01-020
Romeis, Tina. T07-046
Roodbarkelari, Farshad T03-061
Rook, Fred T07-026
Rosahl, Sabine T05-022
Rose, Annkatrin. T03-076, T11-021
Rossiter, John T. T03-083
Roth, Braden T09-054
ROTHAN, Christophe T04-066
Roussot, Clotilde T01-048
Routaboul, Jean-Marc T07-002, T07-014
Roux, Camille T05-026
Roux, Stanley J T04-052
Rowe, Heather T05-075
Roxrud, Ingrid T02-028
Rozhon, Wilfried T04-030, T04-031
Rozilio, Tamar T01-008
Rubio, Vicente. T10-040
Rudge, Tim T11-010
Ruelland, Eric T04-112
Ruezinsky, Diane. T07-092
Ruiz-Rivero, Omar J. T02-092
Rupasinghe, Sanjeewa T07-009, T07-066
Russenberger, Doris T10-027
Russinova, Dr. Eugenia T03-056
Rustérucci, Christine T04-078
Ruthardt, Nadia. T08-015
Rutschow, Heidi T02-100
Ryan, Eoin T02-073, T02-106
Rylott, Elizabeth L.. . . . . T07-071
Rylott, Elizabeth R. T07-072

S

Saadaoui, Dhouha. T04-099
Sabatini, Sabrina. T02-020
Sablowski, Robert T02-009, T02-086

Sadot, Einat T03-003
Saedler, Rainer T03-039, T03-042, T03-045
Saether, Barbro E. T10-021
Sagasser, Martin T01-061
Saijo, Yusuke T10-040
Saini, Hargurdeep S. T12-009
Saito, Kazuki T07-054, T07-087
Sakai, Tatsuya T04-016
Sakakibara, Hitoshi T02-007, T08-006
Sakuma, Yoh T04-014
Sakurai, T. T10-005
Sala, T T04-017
Salamini, Francesco T01-015, T12-002
Salchert, Klaus T03-013
Saleeba, Jennifer A T02-070
Salinas, J.. . . . . T04-029
Salomé, Patrice A T04-082
Salt, D.E. T07-097
Samaj, J. T03-086
Samantha, Vernhettes T02-116
Samson, Teresa T09-037
Sanchez, Elizabeth T07-091
Sanchez, Mari-Paz T03-064
Sandorf, Iris T07-018
Sandra, Pelletier T02-116
Sang-ho, Kang T10-024, T10-046
Sano, Ryosuke T02-038
Santaella-Tenorio, Marcella T07-050
Santerre-Ayotte, Samuel T10-051
Santi, Luca T01-015
Sappl, Pia T03-052
Sappl, Pia G T03-050
Sarnowski, T.J. T09-005
Sarx, Johannes T05-011
Sato, Masa H. T03-035, T03-065
Sato, Masanao T05-014
Sato, Shigeru T03-029
Sato, Shirley T09-021
Sato, Shusei T02-038
Sato-Nara, Kumi T05-037
Satou, M. T10-005
Sauer, Michael. T08-016
Sauer, Norbert T08-004, T08-011
Saul, Helen T08-012
Saul, Vered T08-012
Saumitou-Laprade, Pierre T06-031
Sauviac, Laurent T05-024
SAVINO, Gil. T01-028
Sawai, Rie T01-014
Schad, Martina T10-017
Schaeffer, Aurélie T07-094
Schaeffner, Tony R. T04-105, T07-087
Schäfer, Eberhard T02-019, T04-067
Schäfer, Tina. T02-043
Schäfer, Ulrike T07-015, T09-018
Schaller, Eric. T02-067
Schaller, G. Eric. T02-022
Schaller, Hubert T07-077, T07-094
Schat, Henk T04-069, T06-016
Scheel, Dierk T05-012, T05-022
Scheffzek, Klaus T07-055, T07-061
Scheibe, Renate T09-040
Scheible, Wolf-Rüdiger T01-068, T04-035, T04-077, T04-107, T06-017, T07-052, T10-023, T13-012
Scheikl, Elisabeth T04-004
Schellmann, Swen T03-028
Schemenwitz, Andreas T07-022
Schenk, Peer T09-035
Scherer Günther, FE T02-057
Scheres, Ben T02-081, T02-084, T02-095, T02-118
Schiefelbein, John. T02-054, T02-110
Schikora, Adam. T04-006
Schindler, Martin. T11-005
Schiøtt, M. T01-020
Schlereth, Alexandra T02-008
Schlereth, Armin T01-068
Schliep, Martin T10-033
Schlingensiepen, Simone. T04-028
Schlögelhofer, Peter T03-004
Schmelzer, Elmon T01-061
Schmid, Karl. T06-029
Schmid, Markus T02-029, T09-010, T10-018
Schmidt, Anja T07-047
Schmidt, Ed D.L. T02-028
Schmidt, Oliver T07-004
Schmidt, Renate T01-036, T09-028, T09-059, T09-061
Schmidt, Wolfgang T02-109
Schmied, Katja T03-034
Schmiele, Yvonne T08-008
Schmitt, Johanna T06-020
Schmitt, Jürgen M. T04-036
Schmülling, Thomas T02-051, T02-056, T02-089, T04-049, T05-011, T13-005, T13-006, T13-010
Schmuths, Heike. T06-008
Schneider, Anja. T07-030, T07-031, T07-033, T08-005, T11-020
Schneider, Antje T08-020
Schneider, Katharina T12-002
Schneider, Katja T07-069
Schneider, Michel T11-004
Schneitz, Kay T02-076
Schnittger, Arp T02-061, T02-063, T03-044, T03-051, T03-061, T03-063, T03-070
Schnorr, Kirk M. T07-007, T10-010
Schöffl, Friedrich. T04-008
Schölkopf, Bernhard T10-018
Scholl, Randy T13-008, T13-009
Scholz, M. T11-006
Schomburg, Dietmar T07-069
Schomburg, Fritz M. T01-049
Schommer, Carla T02-015, T09-010, T09-030

Schönrock, Nicole T09-056
Schoofs, Geert J.A. T05-042
Schraegle, Shannon J. T11-021
Schranz, M. Eric T12-024
Schrick, Kathrin T06-022
Schroeder, Julian I. T07-097, T01-084, T03-008, T03-048
Schroeder, Steven T12-006
Schruff, Marie C. T01-025, T01-026
Schubert, Daniel T01-101, T09-029, T09-059, T09-061
Schubert, Ingo T09-027
Schuhmann, Holger T11-013
Schuler, Mary A. T07-009, T07-013, T07-066, T09-013, T11-002
Schuller, Astrid T05-011, T05-046
Schultz, Thomas F. T10-052
Schulz, Britta T12-002
Schulz, Burkhard T09-048
Schulze, Silke T02-080
Schulze-Lefert, Paul T05-022, T05-029, T05-053, T05-057, T05-070
Schumacher, Karin T01-036, T03-038, T03-060
Schünemann, Danja T03-037
Schuster, Joachim T07-004
Schuster, Stephan T06-018
Schütze, Katia T05-015, T09-014, T10-009
Schwab, Rebecca T09-010, T09-030
Schwacke, Rainer T08-005, T11-020
Schwandt, S. T05-039
Schwartz, Chris T01-066
Schwechheimer, Claus T03-079, T08-009
Schwedt, Anne T05-060
Schweighofer, Alois T04-038
Scott, Rod J. T01-025, T01-026
Seguela, M T04-042
Segura, María Dolores T04-102
Seidel, Claudia T01-054
Seidlitz, Harald K. T04-105
Seifert, Georg J. T07-016, T07-028
Seifertová, Daniela T08-014
Seki, Hikaru T05-020
Seki, Motoaki T02-113, T03-031, T04-009, T04-055, T05-037, T10-005, T10-025
Sekine, Ken-Taro T05-074
Selbig, Joachim T11-006, T11-007, T11-008, T11-016, T11-018, T11-019
Sels, Jan T05-042
Sentenac, H T04-097
Seok-cheol, Suh T10-024, T10-046
Serino, Giovanna T03-010
Serio, Francesco Di T09-002
Serrano, R. T02-103
Servomaa, Kristina T12-013
Settles, A. Mark T12-010
Shah, Jyoti T05-074, T05-079
Shapiro, Bruce E. T11-026
Shasha, Dennis E. T07-041, T07-082, T10-001, T11-023
Shaul, Orit T08-012
Shaw, Peter T03-023, T10-014
Shchennikova, Anna T12-015
Sheldon Candice, C. T09-036
Shen, Wen-Hui T03-019
Shen, Yunping T10-040
Sheridan, William F. T09-006
Sherman, Bjorg T01-046
Shi, Dong-Qiao T01-022, T01-023
Shi, Huazhong T12-022
Shi, Liang T09-006
Shibata, Daisuke T02-038
Shichrur, Keren T03-003, T10-016
Shimada, Tomoo T03-062
Shimada, Yukihisa T03-024
Shimizu, Kentar T06-013
Shimotohno, Akie T03-030
Shindo, Chikako T09-022
Shindo, Chikako T06-030
Shinomura, Tomoko T04-096
Shinozaki, Kazuo T01-091, T02-034, T02-075, T02-113, T03-031, T04-004, T04-009, T04-014, T04-020, T04-055, T05-037, T07-080, T09-007, T10-005, T10-025, T10-047
Shippen, Dorothy E. T03-036
Shirano, Yumiko T02-038
Shochat, Susana T09-017
Shpak, Elena D. T02-012
Shulga, Olga T12-015
Shumate, Alice M. T12-014
Siaud, N T09-001
Siddique, Asim T10-027
Siemens, Johannes T05-011
Siemsen, Tanja T09-019
Silva, Illeana T03-066
Simon, Marissa T02-054
Simon, Rüdiger T02-041, T02-045
Simonneau, T. T04-043, T04-097, T10-022
Simon-Rosin, Ulrike T10-033
Simons, Cas T09-052
Singer, Alex U. T05-018, T05-021
Singh, Anandita T01-010, T01-059
Singh, Bijay K. T07-006
Singh, Karam B. T03-050
Singh, P T07-060
Sinvany, Galit T04-012
Sivasankar, Shoba T02-030
Sjolander, Kimmen T05-085, T10-027
Skirycz, Aleksandra T05-040
Skovorodnikova, Julia T03-033
Slawomir, Pikula T04-026, T05-009
Sligar, Stephen G. T07-009, T07-013
Small, Ian T10-020, T10-041
Smeekens, S T07-003

Smith, Alison M. T07-036
Smith, Michael R. T02-066
Smith, Neil T10-020
Smith, Richard D. T10-017
Smyth, David R. T01-004
Snoek, Basten T04-065
Sofer, Luc T05-041
Sohlberg, JJ T01-065
Sohlberg, Joel T01-093
Sohn, Hwang Bae T07-076
Sokol, A. T12-020
Soltani, B. T09-053
Somers, David E. T04-091
Somerville, Chris R. T05-002, T05-073, T07-074
Somerville, Shauna C. T05-002, T05-023
Somssich, Imre E. T05-020, T11-003
Sondek, John T05-018, T05-021
Song, Ki-hong T02-072
Song, Sang Ik T07-076
Song, Yoon Jung T05-025
Sonnewald, Uwe T12-008
Sonoda, Yutaka T05-014
Sonoike, Kinntake T02-075
Soppe, Wim T01-018
Sorek, Nadav T03-003
Sørensen, Mikael Blom T01-097
Sorteberg, Hilde-Gunn Opsahl T02-028
Soukupova, Hana T03-040, T03-058
Southern, Megan M. T04-091
Speckhart, Michelle T03-075
Spencer, Matthew T10-038
Spielman, Melissa T01-025, T01-026
Spillane, C T06-032
Spitzer, Christoph T03-028
Spoel, Steven H. T05-067
Sprunck, S. T01-002, T01-064
Srinivas, Bhylahalli P. T03-042
Srinivasan, Ramachandran T02-010
Stadler, Ruth T08-004
Stahl, Yvonne T02-041
Stahlberg, Eric A. T11-021
Staskawicz, Brian T05-085
Staub, Eike T11-019
Stefanovic, Aleksandra T04-108, T07-090
Steffens, Nils Ole T11-005, T11-009
Stehling, Sandra T01-016
Stein, Monica T05-023
Steinborn, Katharina T03-073
Steinebrunner, Iris T01-051, T05-051
Steinhauser, Dirk T01-062, T04-106, T10-045
Stepanova, Anna N. T02-035, T04-022, T04-083
Sterling, A. T11-006
Steuer, ... T10-035
Steup, Martin T07-008, T07-053
Stieger, Pia T08-003
Stierhof, York D. T01-036
Stinchcombe, John R. T06-020
Stitt, Mark T01-068, T04-035, T04-072, T04-107, T07-052, T07-070, T07-096, T10-023, T11-008, T11-018, T12-008, T07-098
Stock, Christine T01-101
Stokes, David T06-033
Stone, Sophia T03-080
Stoutemyer, Mark T05-035
Strachan, Camille N. T13-018
Strenberg, Hasana T03-003
Ströher, Elke T04-011
Stüber, Kurt T01-015, T07-010, T11-003
Stuible, Hans-Peter T07-069
Suarez-Lopez, Paula T01-048
Suh, Seok-Cheol T04-087, T05-071
Suh, Su Jeoung T04-027
Sumi, Yoshinori T02-115
Sumikawa, Naomi T01-021
Sun, Qiang T05-037
Sun, Tai-ping T01-013
Sun, Yujing T05-076
Sundaresan, Venkatesan T01-073, T06-002
Sundberg, Eva T01-065, T01-093
Sunderhaus, Stefanie T07-024
Sung, Z. Renee T01-029, T01-101
Sureshkumar, Sridevi T01-066, T06-009
Sussman, Michael R. T02-037, T02-113
Suzuki, Hitoshi T05-037
Suzuki, Kumiko T04-054, T10-025
Svensson, Janne T04-036
Svensson, Maria T01-058, T04-081
Swarup, Kamal T02-003
Swarup, Ranjan T02-003
Sweeney, Colleen T01-083
Sweere, Uta T02-019
Sweetlove, Lee J. T04-001
Swiatecka, Monika T09-046
Swiezewska, Ewa T03-007
Swiezewski, S. T09-005
Symonds, Vaughan T06-028
Synek, Lukas T03-040, T03-058
Szabados, László T04-103, T10-050
Szakonyi, Dora T01-015
Szecsi, J. T01-095
Szittya, Gyorgy T09-022
Szopa, Jan T05-040

T

Tabata, Satoshi T02-038, T02-113
Tacklind, Julie T11-022
Tähtiharju, Sari T02-088, T12-017
TAJIMA, TAKEOMI T01-031
Takahashi, Hideki T05-074, T05-079, T07-051, T07-054
Takahashi, Seiji T07-081
Takahashi, Tetsuya T04-032
Takano, Junpei T07-005, T07-023
Takatsuto, Suguru T02-059, T02-072

Takeda, Seiji T01-092
Takei, Kentaro T08-006
Takemura, Miho T01-014
Takeyasu, Kunio T03-035
Talke, Ina N. T06-031
Tamaoki, Masanori T05-014
Tamara, Gigolashvili T03-032
Tamura, Kentaro T03-062
Tanaka, Hirokazu T02-090
Tanaka, Toshihiro T02-090
Tang, Chunlao T06-030
Taniguchi, Naohide T02-048
Tanksley, Steven D. T12-028
Tantikanjana, Titima T06-002
Tardieu, F. T04-043, T04-097, T10-022
Tasaka, Masao T02-032, T02-048, T04-032, T04-070, T04-084, T04-089
Tatiana, Kolesnik T02-010
Tauriainen, Airi T04-044
Tax, Frans E. T02-059
Teige, Markus T04-004
Teixeira, Rita T01-096
Tennstedt, Pierre T04-095
Tepperman, James T04-024
Terauchi, Daiju T07-081
Tervahauta, Arja T12-013, T12-016
Tessadori, F T06-032
Testerink, Christa T05-028
Textor, Susanne T06-004, T07-085
Thangstad Ole, P. T03-083
Theissen, Guenter T06-025
Theologis, A. T10-005
Theologis, Athanasios T02-048
Theres, Klaus T02-080, T02-087
Theresa, Lee T10-024
THIBAUD, ... T04-079
Thibaud, M-C T04-042
Thibaud-Nissen, Françoise T10-043
Thilmony, Roger T05-080
Thimm, Oliver T07-096, T10-045, T11-008, T11-018
Thimmapuram, Jyothi T07-009, T11-002
Thimoté, Masson T03-017
Thioux, J.J. T10-022
Thitamadee, Siripong T02-088
Thole, Vera T07-036
Tholl, Dorothea T07-079
Thomann, Alexis T03-019
Thomas, Stephen G. T01-013
Thomas, Tyler T03-016
Thomma, Bart T05-012
Thordal-Christensen, Hans T05-027, T05-073
Thulke, Oliver T04-105
Tian, Lu T06-023
Tiburcio, Antonio F. T04-064
Ticconi, Carla T04-088
Tiedemann, Jens T09-041
Tiessen, Axel T07-008, T07-053, T07-068, T12-012
Tippmann, Helge T05-027
Tiwari, Sushma T01-026
To, Alexandra T01-028
To, Kin-Ying T13-001
Tognolli, Michael T11-004
Toki, Seiichi T09-007
Tokuhisa, Jim T07-043, T07-085
Tomlinson, Matthew T03-023
Tomscha, Jennifer T07-040
Ton, Jurriaan T05-044
Tonelli, C. T02-026, T04-017
Tonelli, Chiara T04-058
Tong, Hongyun T04-086
Toomajian, Chris T06-026
Topping, Jennifer T02-024
Tore, Brembu T03-082
Torian, Udana T10-019
Torii, Keiko U. T02-012
Törjek, Otto. T06-014, T06-017
Torres Zabala, Marta de. T05-055, T05-077
Torres, Juan Antonio T02-039
Torres, Miguel A. T03-008
Torres-Galea, Patricia T04-110
Toth, Reka T04-091
Toufighi, Kiana T06-021, T11-025
Town, Christopher T10-019, T10-043
Townsend, Jared. T04-104
Traas, J. T02-064
Traas, Jan T02-039, T08-003
Trainor, Vernon T02-071
Trampczynska, Aleksandra. T04-093
Tran, Lam-Son Phan T04-014
Tranchina, Daniel T07-082
Traub, Michaela T07-021
Trémousaygue, D. T09-034
Trigalet, André T05-031
Trigalet, Danièle T05-031
Trovato, Maurizio. T01-055
Troy, Andy. T03-080
Truernit, Elisabeth B. T01-030, T10-015
Tsiantis, Miltos T02-027
Tsugeki, Ryuji T01-092, T02-115
Tsuhara, Yumi. T10-025
Tsukaya, Hirokazu T02-004, T02-006, T02-059, T02-114, T04-098
Tsutsui, Tomokazu. T05-014
Tuomainen, Marjo T12-016
Tuominen, Hannele T04-044
Turnbull, Colin. T01-099
Turner, John T08-002
Tyler, Ludmila T01-013
Tzafrir, Iris T01-083

U

Uchimiya, Hirofumi T03-030

Udvardi, Michael T01-068, T04-077, T06-017, T07-052
Ueda, Nanae T08-006
Ueda, Takashi T03-035, T03-065
Uemura, Tomohiro T03-035, T03-065
Uhrig, Joachim T04-034, T04-107, T05-057
Ülker, Bekir T05-020, T05-070
Ulm, Roman T04-067
Ulrike, Goebel T06-012
Umber, M.. . . . . T13-016
Umeda, Masaaki T03-030
Umemura, Yoshimi T09-038
Underwood, Beverly T10-019
Underwood, William. T05-080
Uno, Yuichi T04-104
Unte, Ulrike T05-057
Updegraff, Emily T01-078
URAO, Takeshi T04-055
Usadel, Björn T07-032, T10-045
Uwer, Ursula T08-008
Uyeda, Ichiro. T05-014

V

Vachon, Gilles T01-040
Valdes, Jorge T07-091
VALK, Henry van der T03-078
VALON, Christiane T01-028
Valverde, Federico. T01-018, T01-057
van de Mortel, Judith E.. . . . . T04-069
van Dongen, Joost T.. . . . . T07-048, T12-012
van Wijk, Klaas J. T02-100
Vance, Vicki T09-054
Vargas, Amy T03-021
VAULTIER, Marie-Noëlle T04-112
Vavasseur, A T04-017
Veerappan, Vijaykumar T04-085
Veit, Bruce T02-071
Velghe, Inge E. T05-042
Vener, Alexander V. T04-037
Vera, A. T02-105
Vera, Pablo T05-034
Vergne, P.. . . . . T01-095
Verica, Joseph T04-086
Vernhettes, Samantha T03-009
Vernoux, Teva T02-108
Veronese, Paola T05-081, T05-084
Veylder, Lieven De. T02-014, T02-039, T03-072, T03-075
Vicente-Carbajosa, Jesús T09-050
Viczian, Andras T04-034
Vidal-Rico, Ana M.. . . . . T02-092
Vierstra, Richard D.. . . . . T01-049
Vieten, Anne T08-014, T08-016
Vigeolas, Helene T07-048
Vilarino, Cristina T10-004
Vincent, Coral T01-048
Vingron, Martin T10-018, T11-019
Vittorioso, Paola T01-069
Vivian, Pamela T13-008, T13-009
Vizcay-Barrena, Gema. T01-067
Vlieghe, Kobe T02-014
Voesenek, L.A.C.J. T04-061
Voesenek, Laurentius T04-065
Vogel, Heiko T05-016
Voigt, B. T03-085, T03-086
Voigt, Katrin T06-031
Voinnet, Olivier T08-018, T09-025
Voitsekhovskaja, Olga V. T07-028
Volkmann, D. T03-085
Voll, L. T13-016
Volmer, Julia J. T07-011
Vorwerk, Sonja T05-002
Vreugdenhil, Dick T06-016, T07-062
Vroh, Irie Bi. T12-006
Vuylsteke, M T04-017
Vyskot, Boris. T03-036

W

Waadt, Rainer T07-025
Wachter, Andreas T03-059
Wada, Takuji T02-023, T02-038, T02-068, T10-047
Wahl, Vanessa T02-029
Walford, Sally-Ann T05-072
Walter, Achim T06-014
Walter, Michael T10-009
Walton, Eric T01-090
Walz, Alexander T01-054
Wang, Chen-Kuen. T13-001
Wang, Dong T05-052
Wang, Hong-yu. T04-005
Wang, Jean Y.. . . . . T10-001
Wang, Jianlin T06-023
Wang, Jiyuan J. T06-023
Wang, Ming-bo. T10-020
Wang, Qiaomei T07-086
Wang, Xiao-Jun T05-068
Wang, Xuan T09-050
Wang, Xun T09-006
Wang, Ying T05-076
Wang, Yixing. T03-047
Wang, Yong T04-108, T07-090
Wang, Yongsheng T05-047
Wang, Zhilong T08-010
Wanke, Dierk T01-015, T07-010, T09-014, T09-048, T11-003
Wanner, Gehard T05-073
Ward, J.M T07-097
Warthmann, Norman T06-027, T09-030
Watanabe, Koichi T09-004
Watanabe, Masaru T02-090
Watanabe, Naohide. T03-018
Waterhouse, Peter T10-020
Watson, Brian T06-001, T06-023
Watson, Matthew J. T03-036
Weaver, Natalie. T05-052

Weber, A. T13-016
Weber, Bärbel T09-016
Weber, Christian T03-068
Weber, Michael T04-093
Weckermann, Katrin T07-018
Weckwerth, Wolfram T07-045, T07-046, T10-017, T10-029, T10-032, T10-035, T10-036, T11-007
Weems, Dan T11-011, T11-022
Wei, Ning E. T06-023
Weicht, Daniel T11-016, T11-018
Weigel, Charlotte. T07-092
Weigel, Detlef T01-003, T01-005, T01-010, T01-016, T01-017, T01-059, T01-066, T01-070, T01-098, T02-015, T02-031, T06-009, T06-018, T06-027, T09-010, T09-030, T10-018
Weigel, Ralf T09-019
Weihe, Andreas. T09-046, T09-047
Weijers, Dolf T02-008, T08-017
Weiler, Elmar W. T02-043, T05-039, T07-018, T07-020, T07-022, T13-002
Weinand, Tanja T02-029
Weingartner, Magdalena T10-048
Weinig, Cynthia. T06-020
Weinl, Christina. T03-044
Weinl, Stefan T03-020, T04-023, T06-006, T06-007
Weiss, Christian T03-011
Weisshaar, Bernd T01-061, T03-034, T04-005
Wellmer, Frank T01-006, T01-085
Weltmeier, Fridtjof T09-050
Welzl, Gerhard T04-105
Wenkel, Stephan. T01-035, T01-056
Werck-Reichhart, Danièle T04-105, T05-041, T07-009, T07-058
Werner, Tomas T13-006, T13-010
Werner, Ulrike T05-005
Werr, Wolfgang T02-013, T02-049, T02-050, T02-052, T02-078, T10-009, T12-007
Wesley, Varsha T10-020
Western, Tamara L. T03-025
Westphal, Lore T05-022
Whelan, James. T03-001, T03-052, T04-053, T12-001
Whelan, Jim T03-002, T04-001
Whittam, Thomas T05-080
Whyte, Jennifer. T08-013
Wielopolska, Anna. T10-020
Wienkoop, Stefanie T07-045, T10-017, T10-029, T10-035, T10-036
Wiermer, Marcel T05-089, T05-050
Wierzbicki, Andrzej T.. . . . . T09-055
Wiese, A. T07-003
Wiethoelter, Caterina T09-016
Wigge, Philip A.. . . . . T01-070
Wijnhoven, Paul T10-037
Wildwater, Marjolein T02-118
Wiley, Jim. T10-003
Wilkes, T. M. T04-033, T04-045, T06-011, T10-049
Wilkins, Christopher G. T02-044
Willemsen, Viola T02-095, T02-118
Willett, Barbara T02-077
Williams, Alan T05-056, T05-059
Williams, Leor T02-099
Williams, Luis T03-080
Willmitzer, Lothar T07-091
Wilson, Kate T05-069
Wilson, Zoe T01-067
Windsor, Aaron J. T12-014
Winichayakul, Somrutai T01-103
Winter, Verena T03-006
Winters, Silke T02-045
Wirén, Nicolaus von T07-023
Wisniewska, Justyna T08-014, T08-016
Witt, Isabell. T05-040, T07-089
Witters, Erwin T13-015
Witucka-Wall, Hanna. T04-046, T06-014, T06-017
Wobbes, B T07-003
Wohlgemuth, Heike T10-002, T10-003
Wojtas, Magdalena T03-007
Wolf, Carolin T01-051
Wolf, Sebastian. T07-055, T07-061
Wolkenfelt, Harald. T02-081
Wollmann, Heike. T02-031
Wolschin, Florian. T10-032
Won, Soyun T04-090
Woodward, Andrew W.. . . . . T03-071
Woolley, Lindsey T02-092
Workum, Wilbert van T04-069
Wouters, Piet F.J. T10-028
Wright, Stephen T12-006
Wu, Hank T10-019
Wu, Jingrui. T12-011
Wu, Rong Mei. T01-090
Wu, Xuelin T09-030
Wurtz, Virginie T03-057

X

Xiao, Shi. T08-010
Xiao, Yongli T10-019
Xie, Conghua T01-027
Xie, Daoxin T08-010
Xie, Li-Fen T01-073
Xie, Mingtang T02-099
Xin, Feng Dong T05-031
Xin, Ge T09-054
Xiong, Yang. T08-017
Xu, Iris T10-054, T11-011, T11-022
Xu, Jianping T09-021
Xu, Jun T01-027
Xu, Xiang Ming T03-027

Y

Yadav, Ram Kishor T02-076
Yagi, Noriyoshi T01-092
Yalovsky, Shaul T03-003, T10-016
Yamada, Miho T01-094
Yamada, Nanae T03-029
Yamaguchi, Ayako T01-033, T01-045
Yamaguchi, Junji T05-014
Yamaguchi, Masatoshi T02-082, T02-083
Yamaguchi, Shinjiro T07-042
Yamaguchi-Shinozaki, Kazuko T04-009, T04-014, T04-055, T10-005
Yamamoto, Sumiko T01-033, T01-045
Yamasaki, Masanori T12-006
Yamasita, Hizuru T05-037
Yamaya, Tomoyuki T07-051, T07-054, T08-006
Yan, Thomas T11-022
Yang, Aidong T05-056, T05-059
Yang, Jinyoung T05-064
Yang, Ming T03-047
Yang, Shulan T01-073
Yang, Wei-Cai T01-022, T01-023, T01-073
Yannick, Bellec T03-017
Yano, Daisuke T04-084
Yanofsky, Martin T01-039, T01-087
Yanofsky, Martin F. T01-043, T01-098, T03-014
Yao, Youli T04-013
Ye, De T01-022, T01-023, T01-073
Yephremov, Alexander T03-005
Yeu, Song Yion T07-076
Yi, Young-Byung T05-036, T05-038
Yin, Xiao-jun T03-005
Yokota, Akiho T04-096
Yokouchi, Takuro T10-047
Yoneyama, Tadakatsu T07-023
Yong-hwan, Park T10-024, T10-046
Yoo, Daniel T10-054, T11-022
Yoo, Sang Dong T02-016
Yoo, Seung Kwan T01-050
Yoo, So Yeon T01-089
Yoon, In-Sun T04-087
Yoshida, Shigeo T02-059, T02-072, T03-024, T03-026
Yoshida, Tomo T04-020
Yoshihara, Teruhiko T05-014
Yoshimoto, Naoko T07-054
Youn, Ji-Young T01-087
Young, Jared T03-048
Young-mi, Kim T10-024, T10-046
Youngs, Heather T05-073
Ytterberg, Jimmy T02-100
Yu, Hao T01-006, T01-007
Yuguchi, Masahiro T10-047
Yuji, Iwata T09-043
Yves, Marco T05-031

Z

Zachowski, Alain T04-112
Zahkleniuk, O T07-060
Zainudin, Fadhilah T10-049, T10-053
Zakhleniuk, Oksana V T07-037
Zambryski, Patricia T01-075
Zamir, Dani T12-027, T13-011
Zanor, Maria Ines T04-002, T05-040, T07-089
Zarra, Ignacio T03-084
Zarsky, V T03-040
Zarsky, Viktor T03-043, T03-058, T06-003
Zazimalova, Eva T08-015
Zeier, Jürgen T05-012, T05-065
Zhang, Fan T02-110
Zhang, Haiying T01-027
Zhang, Jessie Cui T11-011, T11-022
Zhang, Jiaming T05-054
Zhang, Peifen T11-022
Zhang, Wenyan T07-039
Zhang, X. T13-014
Zhang, Yan T01-077
Zhang, Yuelin T05-066
Zhang, Zhen T13-008, T13-009
Zhao, Keyan T06-026, T06-030
Zhao, Yuanxiang T01-007
Zhao, Zhiying T08-001
Zheng, Honggang T06-030
Zheng, Ming Shu T05-079
Zheng, Wenming T04-077
ZHOU, D.-X. T02-097, T09-049
Zhou, Ling T13-008, T13-009
Zhou, Tianhong T05-076
Zhu, Jian-Kang T04-111, T12-022
Zhu, Tong T02-016
Ziak, D T03-040
Zik, Moriyah T01-008
Zimmerli, Laurent T05-073
Zimmermann, Ilona T03-039, T03-045
Zimmermann, Philip T10-044, T11-027
Zimmermann, Roman T02-013
Zimmermann, Sabine T08-020
Zipfel, Cyril T05-061, T05-086
Zoeckler, Brandon T11-022
Zoeller, Daniela T10-029, T10-033
Zolman, Bethany K. T03-066
Zourelidou, Melina T08-009
Zrenner, Rita T07-019, T07-027, T07-036, T07-083
Zsigmond, Laura T04-103, T10-050
Zuijlen, Lisette van T01-041
Zuther, E. T04-072
Zychlinski, Anne von T10-027, T10-044

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