ACPD 2009

Auxins and Cytokinins in Plant Development
International Symposium
July 10-14, 2009, Prague, Czech Republic

PROGRAMME

BOOK OF ABSTRACTS

LIST OF PARTICIPANTS

ACPD 2009

Auxins and Cytokinins in Plant Development
International Symposium
July 10-14, 2009, Prague, Czech Republic

Organized by

Institute of Experimental Botany of the AS CR, v. v. i.

in co-operation with

Organizing Committee

Alena Březinová, Institute of Experimental Botany AS CR, Prague
Břetislav Brzobohatý, Mendel University of Agriculture and Forestry and Institute of Biophysics AS CR, Brno
Alena Gaudinová, Institute of Experimental Botany AS CR, Prague
Jan Hejátko, Masaryk University, Brno
Václav Hejnák, Czech University of Life Sciences, Prague
Klára Hoyerová, Institute of Experimental Botany AS CR, Prague
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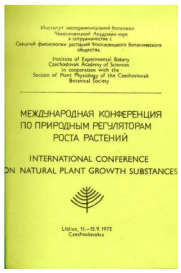
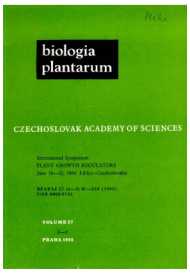
Miroslav Strnad, Institute of Experimental Botany AS CR, Prague
Radomíra Vaňková, **Vice-Chair**, Institute of Experimental Botany AS CR, Prague
Eva Zažímalová, **Chair**, Institute of Experimental Botany AS CR, Prague

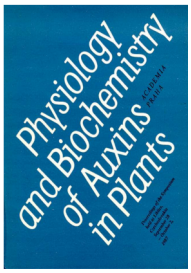
Past meetings



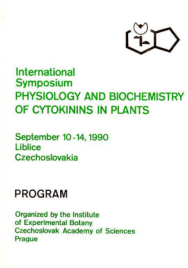
Liblice
September 11-15
1972



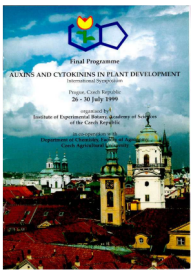
Liblice
June 18-22
1984



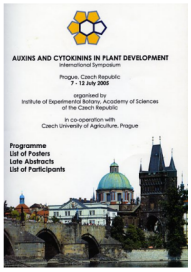
Liblice
September 28-October 2
1987



Liblice
September 10-14
1990



Prague
July 26-30
1999



Prague
July 7-12
2005

Table of Contents

The Institute of Experimental Botany of the Academy of Sciences of the Czech Republic obtained new equipment that will greatly facilitate its research in molecular and cellular plant biology. Instead of manually, routine laboratory work will now be performed by robots which are faster, more reliable, and more efficient. The equipment was financed by the European Fund for Regional Development via the Operational Programme Prague – Competitiveness.

The Institute purchased three automated systems for processing of plant samples. The systems will be used for isolation and analysis of nucleic acids, for proteomic analyses, and for *in situ* hybridisations and immunohistochemistry.

Programme At a Glance

Friday, July 10

16.00 - 20.00	Registration and poster mounting

Saturday, July 11

08.00 - 08.45	Registration and poster mounting
08.45 - 09.00	Opening of the Symposium
09.00 - 10.30	Plenary lectures
10.30 - 11.00	Coffee
11.00 - 12.35	Session 1: Biosynthesis and Metabolism
12.35 - 14.05	Lunch
14.05 - 15.40	Session 1: Biosynthesis and Metabolism
15.40 - 16.10	Coffee
16.10 - 17.25	Session 2: Signalling and Development
17.30 - 19.30	Poster Session I (P1, P2, P3), light refreshment
19.30 - 21.00	Get-together Party

Sunday, July 12

08.30 - 10.15	Session 2: Signalling and Development
10.15 - 10.45	Coffee
10.45 - 12.00	Session 2: Signalling and Development
12.00 - 13.30	Lunch
13.30 - 15.25	Session 3: Pattern Formation and Development
15.25 - 15.55	Coffee
15.55 - 17.50	Session 3: Pattern Formation and Development
18.00 - 20:00	Poster Session II (P4, P5, P6, P7), light refreshment

Monday, July 13

08.30 - 10.15	Session 4: Transport and Development
10.15 - 10.45	Coffee
10.45 - 12.40	Session 4: Transport and Development
12.40 - 14.10	Lunch
14.10 – 15.55	Session 5: Hormone Interactions and Plant Architecture
15.55 - 16.25	Coffee
16.25 - 17.40	Session 5: Hormone Interactions and Plant Architecture
17.50	Departure to Liblice
19.00 - 23.00	Congress Dinner at Liblice Castle

Tuesday, July 14

08.30 - 10.05	Session 6: Hormones, Environment and Applications
10.05 - 10.35	Coffee
10.35 - 12.30	Session 6: Hormones, Environment and Applications
12.30 - 14.00	Lunch
14.00 - 15.50	Session 7: Modelling and Advanced Methods
15.50 - 16.20	Coffee
16.20 - 18.10	Session 7: Modelling and Advanced Methods
18.10 - 18.30	Closing of the Symposium
18.30 - 22.00	Farewell Party

ACPD 2009 Programme

Friday, July 10

16.00 - 20.00		Registration and poster mounting

Saturday, July 11

08.00 - 08.45		Registration and poster mounting
08.45 - 09.00		Opening of the Symposium
		Plenary lectures
		Chair: Miroslav Kamínek
09.00 - 09.45		**Auxin transport - connecting cell polarity and patterning** **Jiří Friml**, VIB, Ghent University, Belgium
09.45 - 10.30		**Cytokinin signaling: Two components and more** **Joseph Kieber**, University of North Carolina, Chapel Hill, NC, USA
10.30 - 11.00		Coffee
		Session 1: Biosynthesis and Metabolism
		Chair: Karin Ljung
11.00 - 11.35	O1-1	**Cytokinin biosynthesis pathway: not as simple as it looks** **Hitoshi Sakakibara**, RIKEN Plant Science Center, Yokohama, Japan OlChemIm Honorary Lecture
11.35 - 11.55	O1-2	**Cytokinin interconversion is disrupted by adenosine kinase deficiency** **Barbara Moffatt**, University of Waterloo, Canada
11.55 - 12.15	O1-3	**Structural characterization of cytokinin oxidase/dehydrogenase mutants** **David Kopečný**, INRA, France and Palacký University, Olomouc, Czech Republic
12.15 - 12.35	O1-4	**Cis-zeatins in plants: their distribution, bioactivities, transport and metabolism** **Václav Motyka**, Institute of Experimental Botany AS CR, Prague, Czech Republic
12.35 - 14.05		Lunch
		Chair: Hitoshi Sakakibara
14.05 - 14.40	O1-5	**Regulation of auxin and cytokinin metabolism during Arabidopsis root development** **Karin Ljung**, Umeå Plant Science Centre, Sweden OlChemIm Honorary Lecture
14.40 - 15.00	O1-6	**Proteomics and metabolomics of cytokinin-induced bud formation in Physcomitrella patens** **Anika Erxleben**, University of Freiburg, Germany
15.00 - 15.20	O1-7	**Oxylipins contribute to the transcriptional regulation of YUC8 and YUC9, thereby controlling local auxin biosynthesis in Arabidopsis thaliana** **Stephan Pollmann**, Ruhr-University Bochum, Bochum, Germany

15.20 - 15.40 O1-8 **Auxin amidohydrolases from Brassica rapa cleave conjugates of indole propionic and indole butyric acid as preferable substrates: a biochemical and modelling approach**
Jutta Ludwig-Müller, Technische Universität Dresden, Dresden, Germany

15.40 - 16.10 Coffee

Session 2: Signalling and Development

Chair: Tatsuo Kakimoto

16.10 - 16.45 O2-1 **Interpreting the tracks of cytokinin signaling during Arabidopsis gametophyte and embryo development**
Bruno Müller, Harvard Medical School, Boston, USA and University of Zürich, Switzerland

16.45 - 17.05 O2-2 **Analysis of cytokinin receptor specificity in Arabidopsis thaliana**
Michael Riefler, Freie Universität Berlin, Germany

17.05 - 17.25 O2-3 **Cytokinin response factors in Arabidopsis and tomato**
Aaron M. Rashotte, Auburn University, Auburn, AL, USA

17.30 - 19.30 **Poster Session I (P1, P2, P3)**, light refreshment. Posters with odd and even numbers should be presented from 17.30 to 18.30 and from 18.30 to 19.30, respectively.

19.30 - 21.00 Get-together Party

Sunday, July 12

Chair: Mark Estelle

08.30 - 09.05 O2-4 **The TAF-related protein CKH1 and the chromatin remodeling-factor CKH2 negatively regulate cytokinin-induced callus formation in Arabidopsis**
Tatsuo Kakimoto, Osaka University, Japan

09.05 - 09.35 O2-5 **Histidine kinases CKI1, AHK2 and AHK3 control vascular tissue development in Arabidopsis shoots**
Ildoo Hwang, Pohang University of Science and Technology, Pohang, Korea

09.35 - 09.55 O2-6 **Early cytokinin response proteins and phosphoproteins of Arabidopsis thaliana**
Martin Černý, Mendel University of Agriculture and Forestry & Institute of Biophysics AS CR, v.v.i., Brno, Czech Republic

09.55 - 10.15 O2-7 **The Arabidopsis cytokinin reponse is mediated by tissue-specific transcriptional cascades**
Eric G. Schaller, Dartmouth College, Hanover, NH, USA

10.15 - 10.45 Coffee

Chair: Bruno Müller

10.45 - 11.20 O2-8 **Auxin signaling: A short (but complex) pathway**
Mark Estelle, The University of California, CA, USA

11.20 - 11.40 O2-9 **A cellular expression map of the auxin response factor family reveals cell type-specific auxin responses**
Barbara Möller, Wageningen University, Wageningen, The Netherlands

11.40 - 12.00	O2-10	**Activation mechanism of patatin-related phospholipase A by phosphorylation and function of phospholipases A in auxin and light signaling** **Günther F.E. Scherer**, University Hannover, Hannover, Germany
12.00 - 13.30		Lunch

Session 3: Pattern Formation and Development

Chair: Sabrina Sabatini

13.30 - 14.05	O3-1	**Integration of hormonal and genetic regulation during vascular morphogenesis in Arabidopsis** **Ykä Helariutta**, University of Helsinki, Finland
14.05 - 14.25	O3-2	**Molecular analysis of auxin regulation of wood formation** **Rishikesh P. Bhalerao**, Umeå Plant Science Center, Umeå, Sweden
14.25 - 14.45	O3-3	**DORNROESCHEN and DORNROESCHEN-LIKE function with the CUC genes and MP to modulate embryo symmetry via auxin-dependent pathways** **John W. Chandler**, Cologne, Germany
14.45 - 15.05	O3-4	**Multiple monopteros-dependent pathways are involved in leaf initiation** **Jim Mattsson**, Simon Fraser University, Canada
15.05 - 15.25	O3-5	**Auto- regulated expression of cytokinin biosynthesis confers drought tolerance in plants** **Shimon Gepstein**, Faculty of Biology, Technion, Haifa, Israel
15.25 - 15.55		Coffee

Chair: Ykä Helariutta

15.55 - 16.30	O3-6	**A genetic framework for the auxin/cytokinin control of cell division and differentiation in the root meristem** **Sabrina Sabatini**, Sapienza University of Rome, Italy
16.30 - 16.50	O3-7	**The role of cytokinin response factors during lateral root initiation** **Giel van Noorden**, VIB, Ghent University, Belgium
16.50 - 17.10	O3-8	**KNOXI genes and cytokinin regulate leaf development** **Naomi Ori**, The Hebrew University of Jerusalem, Rehovot, Israel
17.10 - 17.30	O3-9	**Small RNAs facilitate polarity and laminar growth of tomato leaves** **Tamar Yifhar**, The Weizmann Institute of Science, Rehovot, Israel
17.30 - 17.50	O3-10	**Cytokinins can stimulate Arabidopsis hypocotyl elongation at decreased light intensity** **Alena Reková**, Mendel University of Agriculture and Forestry and Institute of Biophysics AS CR, v.v.i., Brno, Czech Republic
18.00 - 20:00		**Poster Session II (P4, P5, P6, P7)**, light refreshment. Posters with odd and even numbers should be presented from 17.30 to 18.30 and from 18.30 to 19.30, respectively.

Monday, July 13

Session 4: Transport and Development

Chair: René Benjamins

08.30 - 09.05	O4-1	**Lateral root development: an emerging story…** **Malcolm J. Bennett**, University of Nottingham, UK
09.05 - 09.35	O4-2	**Comparison of transport activity and interactions of ABCB, AUX1, and PIN auxin transporters** **Angus S. Murphy**, Purdue University, West Lafayette IN, USA
09.35 - 09.55	O4-3	**Auxin influx carriers are involved in regulating apical hook development of Arabidopsis** **Filip Vandenbussche**, Ghent University, Ghent, Belgium
09.55 - 10.15	O4-4	**PINOID controls PIN1 polar targeting through evolutionarily conserved phosphoserines** **Fang Huang**, Institute of Biology, Leiden University, Leiden, The Netherlands
10.15 - 10.45		Coffee

Chair: Malcolm J. Bennett

10.45 - 11.20	O4-5	**Up and down and all around: PIN polarity regulation in Arabidopsis** **René Benjamins**, University of Utrecht, Utrecht, The Netherlands
11.20 - 11.40	O4-6	**Mechanistic framework for polar PIN targeting** **Jürgen Kleine-Vehn**, VIB , University Gent, Gent, Belgium
11.40 - 12.00	O4-7	**The NPA-binding protein TWISTED DWARF1 controls ABCB-mediated auxin transport** **Hanna Valpuri Sovero**, University of Zurich and Zurich-Basel Plant Science Center, Zurich, Switzerland
12.00 - 12.20	O4-8	**Post-transcriptional control of PIN expression by an Arabidopsis thaliana elongator complex** **Johannes Leitner**, University of Natural Resources and Applied Life Sciences, Vienna, Austria
12.20 - 12.40	O4-9	**ROCK1 encodes a putative transport protein of unknown function** **Tomáš Werner**, Free University of Berlin, Germany
12.40 - 14.10		Lunch

Session 5: Hormone Interactions and Plant Architecture

Chair: Christine A. Beveridge

14.10 – 14.45	O5-1	**Long range signalling in the control of shoot branching** **Ottoline Leyser**, University of York, York, UK
14.45 – 15.15	O5-2	**Auxin - cytokinin interaction shaping root architecture** **Eva Benková**, VIB, University Gent, Belgium

15.15 - 15.35	O5-3	**Apical dominance is controlled by interaction between cytokinin biosynthesis/degradation and auxin in stem** **Hitoshi Mori**, Nagoya University, Nagoya, Japan
15.35 – 15.55	O5-4	**Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux** **Markéta Pernisová**, Masaryk University, Brno, Czech Republic
15.55 - 16.25		Coffee
		Chair: Ottoline Leyser
16.25 - 17.00	O5-5	**Regulation of axillary bud outgrowth by strigolactones** **Christine A. Beveridge**, University of Queensland, Brisbane, Australia
17.00 - 17.20	O5-6	**Spatial and temporal regulation of auxin and cytokinin gene expression and responses in pea ramosus mutants** **Colin Turnbull**, Imperial College London, London, UK and University of Massachusetts, Amherst, MA, USA
17.20 - 17.40	O5-7	**Competitive canalization of PIN-dependent auxin flow from axillary buds controls apical dominance in pea** **Jozef Balla**, Mendel University of Agriculture and Forestry, Brno, Czech Republic
17.50		Departure to Liblice
19.00 - 23.00		Congress Dinner at Liblice Castle

Tuesday, July 14

Session 6: Hormones, Environment and Applications

Chair: Thomas Schmülling

08.30 - 09.05	O6-1	**The importance of plant biotechnology for society and environment** **Marc Van Montagu**, Ghent University, Ghent, Belgium
09.05 - 09.25	O6-2	**Characterization and biological activity of novel purine-derived inhibitor of cytokinin oxidase/dehydrogenase INCYDE and its potential use for in vivo studies** **Lukáš Spíchal**, IEB AS CR & Palacký University, Olomouc, Czech Republic
09.25 - 09.45	O6-3	**Light/PHOT1-dependent polar translocation of PIN3 auxin carrier during phototropisms in Arabidopsis** **Zhaojun Ding**, VIB, Ghent University, Ghent, Belgium
09.45 - 10.05	O6-4	**Cytokinin regulates sodium homeostasis** **Michael Mason**, University of Queensland, Australia
10.05 - 10.35		Coffee
		Chair: Marc Van Montagu
10.35 - 11.10	O6-5	**Applied perspective of cytokinin-mediated growth modulation in crop plants** **Thomas Schmülling**, Free University of Berlin, Germany

11.10 - 11.30	O6-6	**Molecular and functional analyses of changes in the pedicel abscission zone transcriptome following auxin depletion** **Shimon Meir**, ARO, The Volcani Center, Bet-Dagan, Israel
11.30 - 11.50	O6-7	**Cytokinin signalling in Medicago truncatula root and nodule organogenesis** **Florian Frugier**, Institut des Sciences du Végétal, CNRS, Gif-sur-Yvette, France
11.50 - 12.10	O6-8	**Comparison of cytokinin role in drought and heat stress response of tobacco plants** **Radomíra Vaňková**, Institute of Experimental Botany AS CR, Prague, Czech Republic
12.10 - 12.30	O6-9	**Metabolism and possible function of cytokinin during abiotic stress in maize** **Petr Galuszka**, Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic
12.30 - 14.00		Lunch

Session 7: Modelling and Advanced Methods

Chair: Przemek Prusinkiewicz

14.00 - 14.35	O7-1	**Quantitative approaches to plant development** **Cris Kuhlemeier**, University of Bern, Switzerland
14.35 - 15.10	O7-2	**A computational model of phyllotaxis in Costus** **Przemek Prusinkiewicz**, University of Calgary, Canada
15.10 - 15.30	O7-3	**Agent based modelling of auxin transport canalisation** **Philip Garnett**, University of York, York, UK
15.30 - 15.50	O7-4	**Towards a model of auxin response in root epidermis** **Martin Kieffer**, University of Leeds, UK
15.50 - 16.20		Coffee

Chair: Cris Kuhlemeier

16.20 - 16.50	O7-5	**Modelling of auxin transport processes on a single cell level** **Klára Hoyerová**, Institute of Experimental Botany AS CR, Prague, Czech Republic
16.50 - 17.10	O7-6	**Modelling of positive-feedback mechanism for auxin carrier polarization during auxin-dependent plant development** **Krzysztof Wabnik**, VIB, Ghent University, Ghent, Belgium
17.10 - 17.30	O7-7	**Developing a real-time, quantitative biosensor for auxin and ABA** **Richard Napier**, University of Warwick, UK
17.30 - 17.50	O7-8	**Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and UPLC-ESI-qMS/MS: an application for hormone profiling in Oryza sativa** **Hitoshi Sakakibara**, RIKEN Plant Science Center, Yokohama, Japan
17.50 - 18.10	O7-9	**New purification and mass spectrometric approach for cytokinin analysis** **Ondřej Novák**, Palacký University & Institute of Experimental Botany, Olomouc, Czech Republic
18.10 - 18.30		Closing of the Symposium
18.30 - 22.00		Farewell Party

AUXIN TRANSPORT – CONNECTING CELL POLARITY AND PATTERNING

Jiří Friml

Department of Plant Systems Biology, VIB, and Department of Plant Biotechnology and Genetics, Ghent University, 9052 Gent, Belgium

Auxin is a prominent intercellular signal in plants and acts as a versatile trigger of developmental change. Directional, active, cell-to-cell transport over short distances mediates differential auxin distributions within tissues (auxin gradients) that are required for various patterning processes, including apical-basal axis formation, organogenesis and tropisms. Various environmental and endogenous signals can be integrated into changes in auxin distribution through their effects on intercellular auxin transport. Differentially expressed auxin transporters of the PIN family, each with specific polar, subcellular localization form a network for directional 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. PIN proteins undergo cycles of a clathrin-dependent endocytosis and ARF GEF-dependent recycling that serves to feed-back regulate throughput and directionality of intercellular auxin flow. Thus, the PIN-dependent auxin transport network, whose directional throughput is modulated by both endogenous and exogenous signals, provides one of the mechanisms underlying the plasticity and adaptability of plant development.

CYTOKININ SIGNALING: TWO COMPONENTS AND MORE

Cristiana Argueso, Jayson Punwani, Fernando Ferreira, Jenn To, and Joseph Kieber

Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280

Cytokinins have been implicated a wide variety of plant growth and development processes and have been shown to interact with various other signals. Recent studies have demonstrated that cytokinin signal transduction occurs through a classic bacterial two-component signaling system, in which signal propagation relies on the transfer of phosphates between alternating histidine and aspartic acid residues. Genes encoding proteins corresponding to each of these two-component elements have been identified in Arabidopsis. Using molecular, genetic and biochemical approaches, the roles of the Arabidopsis two-component genes in plant growth and development have been defined. There is extensive functional redundancy in these gene families. Analysis of lines harboring multiple disruptions in multiple two-component genes has indicated that these elements play roles in various signaling pathways. The effect of these loss-of-function mutations on various aspects of growth and development and on the response to environmental interactions will be presented. We have also identified a number of transcription factors that are regulated by cytokinin using a variety of approaches. The analysis of the role of these transcription factors in cytokinin function will be discussed.

O1-1 CYTOKININ BIOSYNTHESIS PATHWAY: NOT AS SIMPLE AS IT LOOKS

Hitoshi Sakakibara

RIKEN Plant Science Center, 1-7-22, Suehiro, Tsurumi, Yokohama 230-0045, Japan

Cytokinins play a crucial role in various aspects of plant growth and development. Spatiotemporal distribution of bioactive cytokinins is finely controlled by the metabolic enzymes. Cytokinin in plants is first synthesized as N^6-(Δ^2-isopentenyl)adenine (iP) riboside phosphate by adenosine phosphate-isopentenyltransferase (IPT) and then hydroxylated to *trans*-zeatin (tZ) riboside phosphate by a cytochrome P450 monooxygenase, CYP735A. An activation step of cytokinin is catalyzed by LOG, a cytokinin-specific phosphoribohydrolase, which directly converts the cytokinin-nucleotide to the biologically active free-base form. Our recent studies in *Arabidopsis* and rice have demonstrated that *IPTs*, *CYP735As*, and *LOGs* are expressed in various parts during growth and development, and differentially regulate the synthesis of iP- and tZ-type cytokinins in plant body. Multiple mutants of *AtLOGs* showed a lower sensitivity to iP riboside in terms of lateral root formation and altered root and shoot morphology. As is the case with rice, our recent results strongly suggest that direct activation pathway via AtLOGs play a pivotal role for regulating cytokinin activity in normal growth and development in *Arabidopsis*. We will outline recent progress in the regulation of cytokinin biosynthesis, especially focusing on the activation step.

O1-2 CYTOKININ INTERCONVERSION IS DISRUPTED BY ADENOSINE KINASE DEFICIENCY

B. Moffatt[1], S. Schoor[1], S. Farrow[2], N. Emery[2]

[1]University of Waterloo, Waterloo, Canada [2]Trent University, Peterborough, Canada

Biosynthesis, degradation and interconversion all contribute to defining the cytokinin content of a cell. Purine salvage enzymes have long been implicated in catalyzing several of the interconversions related to reducing cytokinin activity. We are using molecular genetics to investigate the involvement of adenosine kinase (ADK) in the inactivation of cytokinin ribosides. Arabidopsis ADK-deficient lines were generated by both sense ADK1 cDNA and artificial microRNA over-expression. These ADK-deficient lines have altered branching, delayed senescence and a decreased rosette leaf and cell size. Closer examination of leaf sections shows they contain an increased number of cells and reduced intercellular space relative to the wild type. To determine whether or not this phenotype is associated with abnormal cytokinin profiles, we are using LC/MS/MS analysis on leaves from 4-week-old plants. Preliminary results show ADK-deficient leaves to have a significant increase in zeatin riboside levels. These analyses are being complemented by an analysis of the expression of cell division and cytokinin- responsive reporters in an ADK-deficient background. Dissecting the phenotype of these transgenic lines should reveal whether ADK contributes to the regulation of intracellular cytokinin homeostasis.

O1-3 STRUCTURAL CHARACTERIZATION OF CYTOKININ OXIDASE/DEHYDROGENASE MUTANTS

David Kopečný[1,2], Hana Popelková[3], Catherine Madzak[4], Pierre Briozzo[5], Marek Šebela[2], Ivo Frébort[2], Amel Majira[1], Michel Laloue[1] and Nicole Houba-Hérin[1]

[1]Laboratoire de Biologie Cellulaire, INRA, Route de Saint-Cyr, F-78026 Versailles Cedex, France. [2]Department of Biochemistry, Faculty of Science, Palacký University Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic. [3]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA. [4]Laboratoire de Microbiologie et Génétique Moléculaire, INRA-CNRS-INAPG, F-78850 Thiverval-Grignon, France. eUMR INRA-INAPG 206 de Chimie Biologique, Institut National Agronomique Paris-Grignon, 78850 Thiverval-Grignon, France.

FAD-containing cytokinin oxidases/dehydrogenases (CKO) catalyze the oxidative breakdown of isoprenoid cytokinins to adenine/adenosine and the corresponding unsaturated aldehydes. Oxygen as well as quinones can re-oxidize FAD reduced during catalytic reaction. While cytokinin substrate binds at the active site, the binding of electron acceptor is not fully understood. Site-directed mutagenesis with subsequent X-ray analysis provides a powerful tool to clarify the function of amino acid residues of interest. A CKO from *Zea mays* (ZmCKO1) was cloned and expressed in the yeast *Yarrowia lipolytica*. Several ZmCKO1 mutants were constructed and their kinetics was analyzed. Active-site mutants pointed out the importance of D169 as a crucial catalytic residue. Mutation of residues located at the entrance or inside the active site like E381, P427 or V378, strongly affected the substrate specificity, sensitivity to inhibition by *N*-phenyl-*N´*-pyridylurea derivatives as well as reaction rates with various electron acceptors. Mutant H105A containing noncovalent FAD was active and showed both reduced reaction rates and affinity to natural substrates. Several mutants were crystallized, crystals infiltrated with substrates or inhibitors and then X-ray data were collected up to 1.8 Å resolution. Refinement of the structures is underway.

Supported by grants MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic and 522/08/P113 from the Czech Science Foundation.

O1-4 CIS-ZEATINS IN PLANTS: THEIR DISTRIBUTION, BIOACTIVITIES, TRANSPORT AND METABOLISM

S. Gajdošová, M. Kamínek, K. Hoyerová, P. Klíma, P. I. Dobrev, A. Gaudinová, E. Žižková, V. Motyka

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Cis-zeatin (*cis*Z)-type cytokinins (CKs) have been for years considered inactive or weakly active adjunct to their corresponding *trans* isomers. Despite it, thanks to the recent impressive progress of sophisticated methods for CK analysis, the *cis*Z derivatives have been revealed to occur in high concentrations exceeding those of *trans*-zeatin in many plant species. Moreover, enzymes catalyzing specifically *cis*Z metabolic reactions as well as the signal perception by histidine kinases responsive to *cis*Z and/or its riboside were reported. All these findings suggest that *cis*Z derivatives are more prevalent and relevant to CK biology than previously considered, having probably unique functions in plants and being synthetized in a distinct way compared to their *trans* counterparts. The data concerning abundance and distribution of *cis*Z and its derivatives in plant kingdom, their activities in different bioassays as well as their transport and metabolism in mono- and dicotyledonous plant species will be presented. The role of *cis*Z-type CKs in maintenance of CK homeostasis and in control of plant development will be discussed.

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O1-5 REGULATION OF AUXIN AND CYTOKININ METABOLISM DURING ARABIDOPSIS ROOT DEVELOPMENT

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We have recently developed methods to study IAA distribution and metabolism with cellular resolution, using a combination of Fluorescence Activated Cell Sorting (FACS) of protoplasts from specific cell types and mass spectrometry, in order to produce a high resolution IAA distribution map over the Arabidopsis root apex. The map shows the presence of IAA concentration gradients, with a clear IAA maximum in the QC. We can also show that IAA is rapidly catabolised to oxIAA and oxIAA-glucose in the columella cells of the root apex. Our data indicates that IAA biosynthesis and catabolism are important homeostatic mechanisms that most likely act in concert with IAA transport to regulate root development. Using in vivo labelling we have also demonstrated that Arabidopsis plants have multiple sites of synthesis for auxin and cytokinins, and that the two hormones can interact on the metabolic level in controlling plant development. We have demonstrated that there is an important auxin source within the meristematic region of the primary root tip, and that this synthesis site is under rapid postive control of cytokinins. We are currently trying to identify the signalling and synthesis pathways involved, in order to get a better understanding how interactions between auxin and cytokinin metabolism could influence root development.

O1-6 PROTEOMICS AND METABOLOMICS OF CYTOKININ-INDUCED BUD FORMATION IN PHYSCOMITRELLA PATENS

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Cytokinin plays a major role in many developmental processes in plants. Within three days exogenously applied cytokinin induces bud formation in *Physcomitrella patens*. These buds give rise to leafy gametophores. This dramatic morphological change in the phenotype of the moss goes along with changes in protein synthesis and metabolite composition. Proteomics and metabolic profiling were used to correlate changes on the protein and metabolite level with bud development in response to cytokinin treatment. Moss was harvested and analysed at different time points between 0 and 72 hours after stimulation. Based on the proteome analysis many biological processes (e.g. cell differentiation, reproduction, nitrogen and sugar metabolism) are affected during bud formation. This information is used to map the proteins specific for each developmental stage and to unravel the complexity of cytokinin action in *Physcomitrella*. Results from metabolic profiling also allows an assignment of metabolites (mainly sugars and amino acids) to define developmental stages in the process of bud formation.

(The work was supported by the Deutsche Forschungsgemeinschaft, GRK 1305).

O1-7 OXYLIPINS CONTRIBUTE TO THE TRANSCRIPTIONAL REGULATION OF YUC8 AND YUC9, THEREBY CONTROLLING LOCAL AUXIN BIOSYNTHESIS IN ARABIDOPSIS THALIANA

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Auxin plays a pivotal role in the regulation of virtually every aspect of plant growth and development as well as in responses to altered environmental cues. Most growth effects are mediated by local auxin gradients that are established by a complex interplay of site-specific *de-novo* biosynthesis and auxin transport. In a survey for genes that are specifically regulated by either OPDA or JA, we found two members of the *YUCCA* gene family, *YUC8* and *YUC9*, substantially induced by those bioactive compounds, suggesting a direct connection between oxylipin signaling and auxin synthesis. Here we present first molecular and genetic evidence for an oxylipin-dependent induction of the two *YUCCA* isogenes that efficiently contribute to auxin formation when expressed *in planta*. Besides elevated auxin contents and corresponding phenotypic alterations, overexpression lines of these two genes exhibit additional ethylene related phenotypes and an increased resistance towards ethylene biosynthesis inhibitors. In conclusion, our data unveil the existence of a so far undiscovered oxylipin-mediated induction of an auxin-ethylene loop which is seemingly involved in plant stress responses and perhaps the regulation of root development.

O1-8 AUXIN AMIDOHYDROLASES FROM BRASSICA RAPA CLEAVE CONJUGATES OF INDOLE PROPIONIC AND INDOLE BUTYRIC ACID AS PREFERABLE SUBSTRATES: A BIOCHEMICAL AND MODELING APPROACH

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Two auxin-amidohydrolases, BrIAR3 and BrILL2, from Chinese cabbage (*Brassica rapa* ssp. pekinensis) were produced by heterologous expression of the respective genes in *E. coli*, purified, and screened for activity towards different amino acid (alanine, beta-alanine, aspartate) conjugates with the auxins indole-3-acetic acid (IAA), indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA). IPA-Ala was shown to be the favored substrate of both enzymes, but BrILL2 was approximately 10 times more active than BrIAR3. Both enzymes cleaved IBA-Ala and IAA-Ala to a lesser extent. The enzyme kinetics was measured for BrILL2 and the obtained parameters suggested similar binding affinities for long-chained auxin-amino acid conjugates (IPA-Ala and IBA-Ala). The velocity of the hydrolyzing reaction decreased in the order IPA-Ala > IBA-Ala > IAA-Ala. The two conjugates IPA-Ala and IBA-Ala showed higher root growth inhibition of *Brassica* seedlings in comparison to IAA-Ala, indicating cleavage of these conjugates also in vivo. A model of BrILL2 was generated using the X-ray structure of *Arabidopsis thaliana* IAA-amino acid conjugate hydrolase as a template. The metal binding and substrate binding sites are proposed. This is the first report on auxin amino acid conjugate hydrolases from a dicotyledonous plant species, which cleaves longer chain auxins with preference over IAA conjugates. Because of the high activities found for these enzymes, some other possible functions will be discussed.

P1-1 HIGH ALKALOIDS PROMISING INDUCED MUTANTS BY GAMMA RAYS AND THEIR MOLECULAR MARKERS IN ATROPA BELLADONNA L.

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This investigation was carried out to induce gamma rays mutants in Atropa belladonna L. possessing high alkaloids contents. The used gamma rays doses were 50, 80, 110 and 150 Gy. The mutants had apparent morphological changes in plant height, no. of leaves and flowers as well as large leaf area. Three promising high alkaloids mutants were selected from M2, M-11-1, M-11-2 and M-15-1. These promising mutants seemed to be a very important for their high alkaloids content, they possessed twice values than the control.These high alkaloids mutants, possessed stable morphological criteria at M3 generation. Molecular studies on these mutants were done for identification of them by ISSR technique confirming the difference between these mutants and control. The three mutants were distinguished by unique molecular markers, i.e. Mutant M-11-1 distinguished by three molecular markers with molecular sizes 1397, 1149 and 874 bp (base pair). Mutant M-11-2 distinguished by four molecular markers with molecular sizes 1537, 1075, 839 and 510 bp. Mutant M-15-1 distinguished by three molecular markers with molecular sizes 1749, 817and 756 bp. These findings drew the attention to the importance of genetic variation between these mutants and mother genotype, as well as, it considered a primary study to finger printing them. Key words: Atropa belladonna L., gamma rays, molecular markers, total alkaloids.

P1-2 THE ROLE OF META-TOPOLINS ON THE MICROPROPAGATION OF SELECTED PLANTS

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For many years, the development of tissue culture protocols relied on certain types of cytokinins such as benzyladenine, kinetin, isopentenyladenine, thidiazuron and zeatin to mention the main ones. The search for new cytokinins continues due to the widening scope of cytokinin research, its application and the apparent limitations of the existing cytokinins. Topolins, a class of aromatic cytokinins, are one of the products of this cytokinin research. Research findings in the past decade or so witnessed the emergence of *meta*-Topolins as genuine alternatives in plant tissue culture. We tested the role of different types of *meta*-Topolins on tissue cultures of a wide range of plant species representing herbaceous, woody, succulent, monocotyledons and dicotyledons. In addition to their superior shoot multiplication potential, they help alleviate and/or control tissue culture related problems such as hyperhydricity and shoot necrosis on different species. Rooting and acclimatization was better when plants were cultured in *meta*-Topolin-containing media. Their potent activity, broad spectrum of action and ability to minimize or control some tissue culture related problems are therefore, worth pursuing. This paper will discuss some of the results of our comparative studies.

P1-3 THE EFFECT OF HEAT STRESS ON CYTOKININ POOL, ENZYME ACTIVITIES AND EXPRESSION OF GENES ASSOCIATED WITH CYTOKININ METABOLISM IN ARABIDOPSIS PLANTS

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Cytokinins (CKs) exhibit multiple physiological functions, e.g. positive regulation of photosynthesis and elevation of sink strength, which might enhance plant stress tolerance. Heat stress (HS; 40°C) response was investigated in hydroponically grown Arabidopsis plants. Dynamics of CK pool was followed in apex, leaves and roots at the early phase of HS by evaluation of the expression profiles of genes coding for CK biosynthetic enzymes (isopentenyltransferases; IPTs) and degradation enzymes (cytokinin oxidase/dehydrogenases; CKXs) and by determination of CKX activity and CK levels. HS was associated with almost immediate decrease (within 15 minutes) of the expression of IPTs followed by decline of CKXs. Reduced CKX expression was accompanied by the decrease in total CKX activity. Down-regulation of CK degradation contributed to the delay in the decrease of CK levels, which indicated the tendency to maintain CK homeostasis.

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P1-4 THE POTENTIAL OF DEVELOPING WHEAT GRAINS TO BIOSYNTHESIZE PLANT HORMONES

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There is distinct and reproducible patterning of hormones' content during the grain development in wheat. A transient massive accumulation of cytokinins (CKs) occurs 3-6 days after anthesis (DAA) while auxin levels remain low until 15-20 DAA, when they gradually increase and stay high until 40-50 DAA. To test the capability of developing wheat grains to biosynthesize *de novo* CKs we isolated wheat grains at 3-4 DAA and 22 DAA, corresponding to the high and low cytokinin/auxin concentration ratios, respectively. Grains were incubated with ^2H_2O and the levels of isotope enrichment of CKs which are proportional to their metabolic activity were determined. We found that wheat grains are capable of *de novo* CK biosynthesis with highest label incorporation early after anthesis mainly into trans-zeatin and isopentenyladenine type nucleotides and ribosides. Interestingly no incorporation was found into the bioactive trans-zeatin. The label incorporation at 22 DAA was much reduced but still present mainly into CK nucleotides. Studies of *de novo* biosynthesis of auxin in wheat grains are in progress.

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P1-5 THE OCCURRENCE OF CIS- AND TRANS-ISOMER CYTOKININ SYSTEMS AMONG SEEDS OF LEGUME SPECIES AND THEIR RELATIONSHIPS TO GROWTH AND PHYLOGENY

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Two distinct forms are possible for CKs with unsaturated isopentenyl side chains: the cis- and trans-isomers. It is now known that the isomers are derived from separate pathways and there is unlikely any isomerization once formed. Moreover CK receptors can be specific to one isomer or respond to both. Still, we have no idea of the functional significance of these two pathways or the relative predominance of the two isomer systems among organs or species. Cytokinins are implicated in fruit set and seed development of several species, including a few legumes. Their presence is transient and peaks at early development while the endosperm remains liquid and precedes rapid embryo growth. At this stage, by HPLC-electrospray tandem mass spectrometry (LC-(ESI)MS/MS), we have profiled the cytokinin complement in early endospermic seeds of 10 legume species representing the Genistoid, Hologalegina, and Phaseoloid-Milletiod legume groups. The survey of seeds also represented a thousand fold range of final seed mass. Results showed species with extremely high and low CK content and a strong contrast in isomer systems among the two model genetic legumes, *Medicago trucatula* and *Lotus japonicus*.

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P1-6 ANALYSIS OF NATURAL ELECTRON ACCEPTOR OF MAIZE CYTOKININ DEHYDROGENASE

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The activity of cytokinin dehydrogenase (CKX) depends on the presence of a suitable electron acceptor capable of effective reoxidation of cytokinin-reduced flavin cofactor. We have previously detected an unknown compound in the maize phloem sap enhancing the activity of ZmCKX1 when converted by peroxidase (POX) or polyphenol oxidases (PPO). The active precursor compounds were isolated from the maize phloem sap using HPLC fractionation and their structure was determined by quadrupole time-of-flight (Q-TOF) mass spectrometry. Identified compounds and particularly their PPO and POX oxidation products functioned as effective electron acceptors in the catalytic reaction of CKX. The identity of authentic electron acceptors was determined by Q-TOF mass spectrometry, accompanied by UV/VIS spectrophotometry and HPLC. The isolated compounds belong to a group of well-known secondary metabolites specific to maize and other *Poaceae* plants that however have never been studied in context of cytokinin metabolism or activity. Their concentration in the phloem sap of maize seedlings increased after exogenous application of cytokinin.

P1-7 CHANGES IN THE DISTRIBUTION OF ARABINOGALACTAN PROTEIN GAL4 DURING SOMATIC EMBRYOGENESIS AT THE DROSERA SPECIES

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Pretreatment with 2,4-D (10nM) applied on the leaves of sundew with the folloving cultivation on MS medium supplemented with 10nM NAA and BAP induced in epidermal and subepidermal cells somatic embryogenesis. Leaf cells are strolling complicated molecular and biochamical reconstruction which are projected into the deep ultrastructural and morphological changes. The aim of our work was to concern our attention on the synthesis and distribution of 1,6-beta-galactactan (GAL4) during somatic embryogenesis of *Drosera* species. Obtained results showed that GAL4 was synthesized in higher concentration in proembryogenic and young embryogenic cells. It was found in the epidermal and nearest subepidermal cells of tentaculas. Cells from the deeper layers had a weak signal, what indicated on the cell specific expression. Its extracellular moleculs can affect a rate of somatic embryogenesis.

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P1-8 REVERSIBLE GLUCOSYLATION IN THE HOMEOSTASIS OF ZEATIN-TYPE CYTOKININS – THE ROLE OF SUBCELLULAR COMPARTMENTATION

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The maize beta-glucosidase Zm-p60.1 catalyzes the only known deglycosylation of cytokinin (CK) glucosides, releasing free CK from O- and N3-glucosides. Reversible glucosylation is important in the homeostasis of active CK forms in the cell and the sub-cellular location of this conversion is proposed to affect final CK levels. We have used Zm-p60.1 expressed in different subcellular compartments as a molecular tool to address this question. We have shown that over-expression of Zm-p60.1 disrupts the zeatin metabolic network during early seedling development that the vacuole is indeed the storage organelle for ZOG. We have investigated the phenotypes of the progeny of crosses with plants over-expressing the glucosyltransferase *ZOG1*, and the progeny of crosses between a couple of the subcellular variants of Zm-p60.1. The molecular and physiological characterization of the phenotypes will be presented.

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P1-9 METABOLISM OF BENZYLAMINOPURINE IN INDUCTION OF SHOOT ORGANOGENESIS ON PETUNIA LEAF SEGMENTS

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The metabolism of ^{3}H-6-benzylaminopurine (BA) and the changes in the levels of endogenous cytokinins during induction of shoot organogenesis on petunia leaf segments in relation to the dynamics of regenerant formation was studied. The explants were cultivated on induction medium (MS) with a 3 μM BA and 0.5 μM NAA. The effect of BA and metabolites on the frequency of shoot organogenesis *in vitro* was observed. Microscopic analyses indicated the presence of organised meristems and leaf primordia already after 10 days of cultivation. The highest frequency of shoot regeneration was observed on induction medium, the transfer of segments after 5 and 7 days on regulator-free MS medium evidently decreased the frequency of shoot regeneration, whereas subculture on fresh induction medium increased the number of regenerated shoots. The presence of BA in the induction medium did not affect the level of endogenous cytokinins, only low levels of endogenous cytokinins (iP and their riboside) were determined. 3H-benzylaminopurine was intensively metabolised to ^{3}H-BA7G and ^{3}H-BAR, and ^{3}H-BARMP as a minor metabolite. In further experiments BA riboside and ribotide induced shoot formation on petunia leaves *in vitro*, whereas BA7G did not.

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P 1-10 FUNCTIONAL ANALYSIS OF THE CYTOSOLIC CYTOKININ-DEGRADING CKX7 ENZYME OF ARABIDOPSIS

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Cytokinin oxidase/dehydrogenases (CKX) are cytokinin-degrading enzymes. The seven CKX enzymes of Arabidopsis differ in their substrate specificities and subcellular localisations. Plants overexpressing individual *CKX* genes show partially different phenotypes indicating that the subcellular compartmentation of cytokinins and the type of cytokinin metabolite are relevant in determining the developmental activities of the hormone. Here we present the functional analysis of *CKX7*. A promoter:GUS gene showed expression in the vasculature of young seedlings and in the female gametophyte. The CKX7-GFP fusion protein was localized in the cytosol. Arabidopsis plants overexpressing *CKX7* showed a particularly low content of specific cytokinin metabolites. The primary roots of these plants were extremely short due to reduced meristem activity and contained only protoxylem, thus resembling the *wol* mutants of the *CRE1/AHK4* receptor gene. CRE1/AHK4 activity was required to establish the *CKX7* overexpression phenotype. It is speculated that *c*Z, *c*Z9G and iP9G which were strongly and specifically reduced in *CKX7* overexpressing seedlings are important for the correct differentiation of the vascular tissues.

P1-11 LIGHT-DEPENDENT EXPRESSION OF CYTOKININ METABOLISM GENES IN ARABIDOPSIS THALIANA SEEDLINGS

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Many processes in plants are regulated in concordance with diurnal rhythms. Some of them could be linked to changes in actual hormone levels. In previous years it has been shown that hormone metabolite levels alter depending on the time of day. Diurnal variation of cytokinin metabolism gene expression was determined in 10 day-old *A. thaliana* seedlings. We have shown that the expression level of the analyzed cytokinin oxidase/dehydrogenases is stable during the whole day and the expression pattern of isopentenyltransferase *AtIPT1* and glucosyltransferases *At1g22400* and *At5g05860* undergo significant changes. Additional experiments confirmed that the expression of these genes is not regulated by the cellular clock but directly by light. It remains to be elucidated what physiological or developmental processes could be linked with this phenomenon. Therefore insertion mutants in *AtIPT1*, *At1g22400* and *At5g05860* were investigated but the disruption of diurnal regulation caused by these mutations did not result in readily observable changes in seedling development.

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P1-12 OCCURRENCE AND POSSIBLE FUNCTION OF CYTOKININS IN CHLORELLA MINUTISSIMA (CHLOROPHYTA)

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Little is known about plant hormones in microalgae. Endogenous cytokinin conjugates were detected in axenic cultures of *Chlorella minutissima* (MACC 361). These included isopentenyladenine (iP), *cis*-zeatin (*c*Z) and *trans*-zeatin (*t*Z), benzyladenine (BA) and topolin conjugates with free bases and O-glucosides occurring in the highest concentrations. No dihydrozeatin (DHZ) conjugates and no N-glusosides were detected. In synchronous *C. minutissima* cultures, cell division occurred at the start of the dark phase and cells increased in size during the following light phase. Only *c*Z, iP and BA conjugates were detected in these cultures. All cytokinin levels were low during the dark phase and increased during the light phase. Free base, riboside and O-glucoside forms occurred in low concentrations and peaked at the end of the light phase (24 h). Ribotides (iPRMP and *c*ZRMP) occurred in higher concentrations and peaked earlier in the light phase (18-21 h), suggesting *de novo* cytokinin biosynthesis with the ribotides being the first cytokinins formed. These results suggest that cytokinin levels may be regulated by light and/or cell size in *C. minutissima*. Exogenously applied *c*Z (10^{-6} M), *meta*-topolin (10^{-8} M) and kinetin (10^{-8} M and 10^{-6} M) significantly inhibited the growth rate of *C. minutissima* grown in batch culture while iP, *t*Z and BA had no effect. The results suggest that like in vascular plants, cytokinins play an important role in regulating cell division in *C. minutissima*. However, unlike many vascular plants, *c*Z conjugates occur in high concentrations and appear to be biologically active. This needs to be further investigated.

P1-13 CHARACTERISATION OF NON-SECRETED CYTOKININ DEHYDROGENASE FROM MAIZE - COMPARISON WITH APOPLASTIC ISOFORM

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Plant hormones cytokinins are degraded by a cytokinin dehydrogenase (CKX, EC 1.5.99.12). There are several isoforms of CKX in each plant targeted differentially within cell compartments. This compartmentation enable to achieve specific regulatory functions of each isoform. Most of the CKX isoforms are localized in apoplast or vacuole; there is generally only one CKX isoform per plant per species that lacks a translocation signal and presumably functions in cytosol. The best characterized CKX from maize is ZmCKX1, known to be secreted into the apoplast, while a gene encoding the nonsecreted CKX has not been cloned before. In this work, the gene for ZmCKX10 was isolated (GenBank FJ269181) and the protein was characterized and compared to ZmCKX1. A subcellular localization was determined using confocal microscopy of fluorescently labeled proteins overexpressed in tomato hairy roots. While ZmCKX1-GFP signal was confirmed in apoplast, the signal of ZmCKX10-GFP was found in cytosol as predicted. The phenotype of the transgenic roots was assessed. For further biochemical characterization, the recombinant ZmCKX10 protein was prepared using *Pichia pastoris* expression system.

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P1-14 INFLUENCE OF 6-BENZYLAMINOPURINE ON RATIO OF PHOSPHOLIPIDS (PA, PC, PE) AND FATTY ACID COMPOSITION PHOSPHOLIPIDS FROM ROOTS AND COLEOPTILES MAIZE

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Cytokinins are plant hormones that play a central role in the regulation of the cell cycle and plant development. However, the mechanisms of their action are still not available. Phospholipids were separated by TLC, and its fatty acid composition was analyzed by gas chromatography. The analysis of 6-benzylaminopurine (BAP) (10^{-5}mM) action in maize roots was shown that phosphatidylcholine (PC) content increased and phosphatidylethanolamine (PE) level decreased, but phosphatidic acid (PA) level was not changed. That means that BAP activate transmethylation PE in PC. Another effect was revealed after treatment coleoptiles BAP. We have shown that PA content increased and the level PE decreased. The contents of PC remained unchanged. Consequently, the obtained results testify that the BAP probably activates of a phospholipase D which hydrolyze PE as substrate in maize coleoptiles. Also BAP action induced changes of fatty acid composition of phospholipids. Analysis of the fatty acid composition of PA from the microsomal fraction coleoptiles showed increase in stearic acid and linolenic acid and decrease palmitic, oleic and linoleic acids 30 min after BAP treatment. It was reported that the levels of palmitate increased and level of linoleic acid decreased in PC and PE coleoptiles maize after BAP action. These results strongly suggest that only in the coleoptiles of maize, cytokinin activates the PLD, which leads to the production of PA.

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P1-15 CYTOKININ PROFILE, METABOLISM AND BIOSYNTHESIS IN THE MOSS PHYSCOMITRELLA PATENS

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We have undertaken a LC-MS based approach to determine the extractable cytokinins in *Physcomitrella patens* (Hedw.) B.S.G. and detected 23 different forms including members of the *cis*-zeatin- (cZ), *trans*-zeatin- (tZ), isopentenyladenine- (iP) and aromatic (BA, T) groups. In addition 2-methylthio cytokinins of cZ- and iP-type were found. In a budding bioassay using Physcomitrella iP, tZ and BA exhibited the strongest activity. 2MeS-iP had only a very weak bud inducing capacity, whereas 2-MeS-tZ and 2-MeS-cZ as well as cZ showed no activity at all. Studies of *in vivo* metabolism with ^{3}H-cytokinin ribosides have shown a strong riboside-base conversion in Physcomitrella. We currently analyse three ribohydrolase isoforms in order to determine their contribution to the release of cytokinin bases. Adenylate *IPT* genes being absent the genome sequence of Physcomitrella, we hypothesise that for the biosynthesis of isoprenoid cytokinins the tRNA pathway is of great importance. Conclusions on the evolution of cytokinin biosynthesis and metabolism are presented.

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P1-16 THE CYTOKININ METABOLISM IN MAIZE

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Cytokinins (CKs) are plant hormones which widely affect the growth. Based on the expression pattern of CK biosynthetic genes in Arabidopsis it was assumed that the CKs are produced in a wide range of organs in plants (Miyawaki *et al.*, 2004). To get more insight into CK metabolism during the maize development we explored the expression profiles of genes involved in CK degradation, synthesis and inactivation. Interestingly, the expression of adenylate-isopentenyltransferases (IPT) was weak until the fifth day. Since then, IPT genes were up-regulated and the total CK turnover uprose. Simultaneously, the fluctuations in endogenous CK levels were quantified. During the imbibition and germination, dihydrozeatin (DHZ) and its derivatives reached the highest levels from all the metabolites measured. However, as the seedling developed the levels of DHZ types decreased rapidly. We supposed that during the early development, seedling used its stored DHZ forms to produce the active CK necessary for its growth until the IPT genes start to be expressed. To confirm our hypothesis we are looking for potential enzymes converting zeatin forms.

P1-17 POLYAMINE AND CYTOKININ INDUCE NITRIC OXIDE BIOSYNTHESIS IN ARABIDOPSIS

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Cytokinin induces NO biosynthesis in *Arabidopsis* (Tun et al., 2008). Some overlapping functions of cytokinin and polyamines (PAs) led to investigating potential role of PAs in regulating NO biosynthesis. When *Arabidopsis* seedlings were treated with PAs putrescine, spermidine and spermine, increased extracellular accumulation of NO was observed by fluorimetry using NO binding dye DAR-4M. Fluorescence microscopic observations of PA treated seedlings using DAR-4M-AM, revealed enhanced NO biosynthesis in elongation zone of the root tips and primary leaves especially in the veins and trichomes. The tissue specific NO distribution is similar to the cytokinin-induced NO distribution pattern. Enhancement of NO mediated posttranslational protein modification through S-nitrosylation was observed in the PA treated seedlings by biotin switch method. Compared to wild type, a T-DNA insertional knockout defect in polyamine oxidase showed relatively lower NO release, lesser NO production in root tips in response to PAs, and altered root morphology to ABA induced stress. The results suggest that involvement of PAs and polyamine oxidases in regulation of NO biosynthesis and in root development.

P 1-18 CYTOKININ N-GLUCOSIDES: NOVEL DATA CONCERNING THEIR (IN)ACTIVITIES AND METABOLISM IN PLANTS

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Cytokinin (CK)-*N*-glucoconjugates have been repeatedly found to occur in high concentrations exceeding those of other CK derivatives in many plant species. Since their discovery in plants, CK-*N7*- and *N9*-glucosides have been viewed as inactive or weakly active deactivation CK products resistant to hydrolysis by β-glucosidase. In contrast to literature data, we have detected antisenescent activities of *N9*-glucosides of trans-zeatin, dihydrozeatin (DHZ), isopentenyladenine and benzyladenine (applied at 10^{-4} M and $5x10^{-4}$ M) on chlorophyll retention in detached oat leaf segments in the dark. CK-*N7*-glucosides were essentially inactive in the chlorophyll retention bioassay, however, DHZ-*N7*-glucoside (10^{-5} M) was found to exhibit stimulatory cell division effects in tobacco calli. We have also revealed a diurnal rhytmicity of CK- *N7*- and *N9*-glucosides and their resistence against the cytokinin oxidase/dehydrogenase attack in *Arabidopsis* plants indicating a potential existence of a unique metabolic pathway responsible for their down-regulation.

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O2-1 INTERPRETING THE TRACKS OF CYTOKININ SIGNALING DURING ARABIDOPSIS GAMETOPHYTE AND EMBRYO DEVELOPMENT

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Similar to animals, plants use a small number of signaling systems, such as the auxin or cytokinin signaling circuitry, for many and diverse developmental processes. Plant hormones are small organic molecules, elusive to *in situ* detection. Therefore, reporter genes, which label all signal-receiving cells in vivo, have proved powerful tools to dissect fundamental developmental processes. To analyze a long-postulated function of cytokinin during embryonic pattern formation, I have constructed a novel synthetic sensor, TCS (two-component signaling sensor) that labels the cytokinin-signaling cells *in planta*. Combined with targeted genetic manipulations I have discovered an antagonistic relation between auxin and cytokinin that is required for embryonic stem-cell specification. The critical but transient interaction between auxin and cytokinin depends on a novel and conserved *cis*-regulatory DNA motif that directs expression of cytokinin repressor genes to embryonic stem cells. I will report on these findings. In addition, I will talk about the design of an updated cytokinin sensor, TCSv2 that exhibits higher sensitivity and reduced tendency to become silenced compared to TCS. Genetic evidence indicate an essential role for cytokinin signaling during reproductive development. Cytokinin output visualized by TCSv2-GFP appears specific and dynamic throughout female gametophyte development.

O2-2 ANALYSIS OF CYTOKININ RECEPTOR SPECIFICITY IN ARABIDOPSIS THALIANA

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Genetic analysis of the three cytokinin receptors of Arabidopsis has shown their functional redundancy, but also a certain degree of specificity. We have explored which parameters contribute to signal specification. In a bacterial assay the CHASE domain of AHK2 has a similar ligand binding spectrum as CRE1/AHK4. Consistently, promoter-swap experiments demonstrated that CRE1/AHK4 can functionally replace AHK2 but not AHK3. Activation of immediate early cytokinin response genes by *t*Z or iP cytokinin in different double receptor mutant plants was tested by qRT-PCR and an introgressed *ARR5:GUS* reporter. Results confirmed a lower sensitivity of AHK3 to iP compared to *t*Z. The relevance of its ligandbinding CHASE domain was further supported by the finding that expression of a chimeric receptor containing the AHK3 CHASE domain coupled to the CRE1/AHK4 His kinase and response regulator domains was able to complement the *ahk2 ahk3* loss of function phenotype. Together the results show that gene regulation and ligand recognition contribute to the functional specificity of cytokinin receptors.

O2-3 CYTOKININ RESPONSE FACTORS IN ARABIDOPSIS AND TOMATO

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Cytokinin is an essential plant hormone affecting numerous aspects of plant growth and development. Our work focuses on a series of transcription factor genes that are a side branch of the cytokinin signaling pathway known as Cytokinin Response Factors (CRFs). We have taken multiple approaches to determine the involvement of CRFs in regulating cytokinin responses. Expression analyses show that three of the eight *CRF* genes in *Arabidopsis* are transcriptionally induced by cytokinin. Interestingly at the protein level localization of CRFs to the nucleus appears to be controlled by cytokinin in a rapid manner for most CRFs. We are also examining the interaction of CRF proteins with each other and with the different parts of the cytokinin signaling pathway using Y2H and spilt YFP analyses. A mutational based approach in combination with reporter gene analyses have revealed that CRFs function redundantly to regulate the development of embryos, cotyledons, leaves, and likely lateral root development. Initial examination of CRF gene expression during development supports the findings of these analyses suggesting roles in both roots and leaves. We have recently broadened our analyses of CRFs beyond *Arabidopsis* to include Tomato, which contains a non-simple leaf structure whose complexity has been shown to be affected by cytokinin. Initial results will be presented of our identification of several CRFs in Tomato along with their expression and cytokinin regulation.

O2-4 THE TAF-RELATED PROTEIN CKH1 AND THE CHROMATIN REMODELING-FACTOR CKH2 NEGATIVELY REGULATE CYTOKININ-INDUCED CALLUS FORMATION IN ARABIDOPSIS

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We previously isolated two *Arabidopsis* mutants, *ckh1* (*cytokinin-hypersensitive1*) and *ckh2*, which were hypersensitive to cytokinins for callus growth and chloroplast development. *CKH1* encoded a protein resembling TAF12b, which is a histone-fold domain-containing TATA-box binding protein associated factor. *CKH2* encoded PKL, a CHD3 class of SWI/SNF2 family chromatin remodeling factor. CHD3 is a component of the Mi-2/NuRD nucleosome remodeling histone deacetylation complex in animals and yeast. A microarray experiment revealed that *ckh1* and *ckh2* are hypersensitive to cytokinins also in terms of gene expression. The *ckh1ckh2* double mutant produced calli independently of cytokinins. This synergistic effect of two mutations suggests that CKH1 and CKH2 may function in the same pathway. A yeast two-Hybrid assay and BiFC assay in tobacco indicated protein interaction between CKH1 and CKH2. We also show that HDA6, a histone deacetylase, also interacts CKH1. Thus it is possible that CKH1 and CKH2 are involved in the same complex that regulates target gene expression through modulation of the acetylation status of histones.

O2-5 HISTIDINE KINASES CKI1, AHK2 AND AHK3 CONTROL VASCULAR TISSUE DEVELOPMENT IN ARABIDOPSIS SHOOTS

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The development and activity of the procambium and cambium, which ensure vascular tissue formation, is critical for overall plant architecture and growth. However, little is known about the molecular factors affecting the activity of vascular meristems and vascular tissue formation. Here we show that the histidine kinase CKI1 and the cytokinin receptors AHK2 and AHK3 are important regulators of vascular tissue development in *Arabidopsis* shoots. Genetic modifications of CKI1 activity in *Arabidopsis* causes dysfunction of the two-component signaling pathway and defects in procambial cell maintenance. *CKI1* overexpression in protoplasts leads to cytokinin-independent activation of the two-component phosphorelay, and intracellular domains are responsible for cytokinin-independent activity of CKI1. *CKI1* expression is restricted to vascular tissues in inflorescence stems, and CKI1 forms homodimers both *in vitro* and *in planta*. Loss-of-function *ahk2* and *ahk3* mutants and plants with reduced levels of endogenous cytokinins show defects in procambium proliferation and an absence of secondary growth. CKI1 partially rescues *ahk2 ahk3* phenotypes in vascular tissue, while the negative mutation CKI1H405Q further accentuates mutant phenotypes. These results indicate that the cytokinin-independent activity of CKI1 and cytokinin-induced AHK2 and AHK3 is important for vascular bundle formation in *Arabidopsis*.

02-6 EARLY CYTOKININ RESPONSE PROTEINS AND PHOSPHOPROTEINS OF ARABIDOPSIS THALIANA IDENTIFIED BY PROTEOME AND PHOSPHOPROTEOME PROFILING

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Cytokinins (CKs) regulate diverse developmental processes in plants. To get an insight into CK regulated molecular events at the proteome level, we employed 2-DE followed by image analysis and MALDI-TOF-TOF MS to analyze early changes in steady-state protein levels and phosphorylation status of the proteome in CK treated *Arabidopsis* seedlings. Effects of four principal CKs, *t*-Z, iP, BA and TDZ were compared. We observed over 160 and 90 differently expressed proteins in proteome and phosphoproteome maps, respectively, which represent about 20% of detected protein spots. Out of them, 102 proteins were identified. They represent a snapshot of early links involved in CK regulated signaling circuits and cellular processes including light signaling and photosynthesis, nitrogen metabolism, ethylene biosynthesis, CLAVATA pathway, and protein and gene expression regulation which are in line with previously described CK functions. Furthermore, our results point to a link between temperature and CK signaling.

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O2-7 THE ARABIDOPSIS CYTOKININ RESPONSE IS MEDIATED BY TISSUE-SPECIFIC TRANSCRIPTIONAL CASCADES

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Cytokinins regulate many developmental and physiological processes in plants, such as cell division, root and shoot growth, chloroplast development, and leaf senescence. Cytokinin signal transduction is mediated by a two-component signaling pathway that culminates in regulation of the type-B response regulators (type-B ARR family). Mutational analysis indicates that the type-B ARRs are the primary transcription factors regulating the plant's response to cytokinin. Functional analysis of the type-B ARRs supports subfamily-specific roles in signaling and also suggests that the type-B ARRs may cooperatively regulate transcription. Among the primary response genes regulated by the type-B ARR's are additional families of transcription factors, many of which are differentially expressed in the root and shoot. Our results support a model in which the type-B ARRs control the cytokinin response through tissue-specific transcriptional cascades.

O2-8 AUXIN SIGNALING: A SHORT (BUT COMPLEX) PATHWAY

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Auxin regulates a bewildering array of processes during plant growth and development. This complexity is belied by the apparent simplicity of the auxin-signaling pathway. Auxin regulates transcription via the TIR1/AFB-Aux/IAA-ARF pathway. The hormone directly promotes Aux/IAA degradation through the action of $SCF^{TIR1/AFB}$ thus permitting ARF-dependent transcription. In the case of the TIR1/AFB proteins, recent results indicate that different members of the family have distinct activities both with respect to auxin binding and Aux/IAA interaction. We are currently exploring the possibility that these differences contribute to the complexity of auxin response.

O2-9 A CELLULAR EXPRESSION MAP OF THE AUXIN RESPONSE FACTOR FAMILY REVEALS CELL TYPE-SPECIFIC AUXIN RESPONSES

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The plant hormone auxin influences many processes in plant development, such as embryogenesis, root and shoot patterning, growth, branching and organogenesis. Importantly, different cell types execute distinct cellular responses to auxin. This poses the question how auxin can generate so many different cellular outputs. Changes in the transcriptome of cells upon auxin signaling are mediated by the AUXIN RESPONSE FACTOR (ARF) family of transcription factors.

Up to now, investigation of *arf* mutants revealed a number of single and double *arf* mutant combinations with auxin-related developmental defects. However, the proportion of *arf* single and double mutants with a phenotype is small and there are many auxin-regulated processes that are not linked to an ARF. This suggests that many auxin responses are mediated by combinations of ARFs that are not yet investigated. We have seen that misexpression of ARFs causes severe developmental phenotypes. Hence ARF expression patterns are likely to contribute to distinct cellular auxin responses.

To establish an expression map at cellular resolution, we fused all 23 Arabidopsis ARF promoters to a sensitive nuclear GFP reporter. Investigations of embryos and root meristems showed that ARFs generally have specific, localized expression patterns. Furthermore the overlay of these expression patterns revealed that every cell type has its own specific subset of ARFs. This implies that the huge variety of auxin responses is achieved by combining different ARF activities within a cell. The expression map has guided the selection of novel multiple mutant combinations not previously selected by their homology. We will present our analysis of *arf* multiple mutants and of cell-type specific ARF misexpression.

O2-10 ACTIVATION MECHANISM OF PATATIN-RELATED PHOSPHOLIPASE A BY PHOSPHORYLATION AND FUNCTION OF PHOSPHOLIPASES A IN AUXIN AND LIGHT SIGNALING

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In *Arabidopsis*, a family of ten phospholipase A (PLA) genes has been identified. Gene *PLAIVA* is expressed strongly and exclusively in roots and *plaIVA* knockouts have reduced lateral root num-bers, characteristic of an impaired auxin response. *PLAIVB* is expressed weakly in roots, cotyledons and leaves &transcription slowly induced by auxin. *plaIVB* knockouts are slightly smaller in blue light. *PLAIVC* expression is in the gynaecium and induced in roots by abscisic acid (ABA) or Pi de-ficiency. *plaIVC* knockouts exhibit an impaired response to Pi deficiency, have long hypocotyls, and flower early in SD. Recombinant proteins ex-pressed in *Escherichia coli* areactive on galac-tolipids and phospholipids, but not triacylglycerol. PLAIVA and PLAIVB, but not PLAIVC, are phosphorylated by calcium-dependent protein kinases *in vitro* at a C-terminal serine. This en-hances their phospholipase A activities pointing to a mechanism of receptor-initiated posttranslational stimulation of PLA activities in the plant.

P2-1 DRM AND ITS RELATION WITH AUXIN DURING BUD BURST IN ROSA X HYBRIDA L.

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As sessile organisms, plants have evolved strategies for surviving natural unfavorable growing conditions. Production of axillary buds ensures growth and reproduction in the case that environmental conditions trigger the death of the active apical bud. Because uncontrolled growth of axillary buds will have consequence on plant architecture, reproduction and survival, plants possess finely regulated mechanisms to control bud growth and development. Shoot branching depends on a key decision for the axillary bud: growing to give a new branch or remaining in the dormant state. Studies conducted in *Arabidopsis*, pea and petunia to understand the control of branching have lead to identify the MAX/RMS/DAD signaling pathway in these species. Here we studied the *RhDRM* gene in roses and its involvement during the release of dormancy and its relation with auxin. Growth of preformed leaves and meristem organogenesis in decapited plants was analyzed during bud burst under different environmental factors (light/dark, low temperature, N nutrition). *RhDRM* expression was investigated in relation to bud dormancy, environmental factors and application of exogenous auxin. Implication of *RhDRM* in dormancy is discussed.

The work was supported by grant from Region Pays de la Loire (COSAVE program)

Key-words: DRM, auxin, Rosa x hybrida L., environmental factors

P2-2 ROLE OF CYTOKININ PERCEPTION IN LATERAL ROOT ORGANOGENESIS

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Lateral root (LR) organogenesis is hormonally and environmentally regulated developmental process. It has been shown that plant hormones auxin and cytokinin are key regulators of LR organogenesis and they exhibit antagonistic mode of interaction. Stimulatory effect of auxin on LR organogenesis is counteracted by cytokinin.

To investigate the role of cytokinin perception pathway in LR organogenesis and in the control of cytokinin interaction with auxin a detailed phenotype characterization of cytokinin receptor mutants *ahk2-2*(*arabidopsis histidine kinase2*), *ahk3-3*, *ahk4/cre1-12* and their double mutant combinations was performed. Our analyses revealed specific and partially overlapping function of cytokinin receptors in LR organogenesis. While *AHK2* and *AHK4* positively control LR initiation, *ahk3* lack of function mutant phenotype suggests *AHK3* acting rather as a negative regulator of LR initiation. In addition, *AHK4* seems to have an important role in control of LR organogenesis at increased cytokinin levels. All cytokinin receptors mutants exhibited changed auxin sensitivity thus pointing to the function of cytokinin receptors in control of cytokinin interaction with auxin.

P2-3 MOLECULAR CHARACTERISATION OF DOMINANT REPRESSORS OF THE CYTOKININ DEFICIENCY SYNDROME

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Cytokinin oxidase/dehydrogenase enzymes (CKX) catalyze the metabolic inactivation of cytokinins. *35S:CKX* transgenic plants show a cytokinin-deficiency phenotype, which mainly consists of a dwarfed shoot with a smaller apical meristem and an enhanced root system. We carried out mutagenesis of *CKX1* overexpressing transgenic *Arabidopsis* plants and screened for mutants that showed a reversion of this phenotype.

We isolated second site suppressor mutations termed *rock* (*repressor of cytokinin deficiency*), which are being characterised. using map-based cloning we identified the dominant *rock2* and *rock3* mutant alleles as missense mutations in the *AHK2* and *AHK3* cytokinin receptor genes. These gain-of-function cytokinin receptor alleles caused an almost complete suppression of the cytokinin-deficiency syndrome. Detailed phenotypic analysis of *rock2* and *rock3* mutants in wild type and *35S:CKX1* background revealed partially specific growth and developmental changes throughout the plant life cycle. A detailed analysis of the consequences of an enhanced cytokinin status due to the *rock2* and *rock3* mutations will be reported.

P2-4 INVOLVEMENT OF AUXIN-BINDING PROTEINS AND AUXIN IN RESPONSE OF MAIZE SEEDLING TO BLUE LIGHT

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Auxin-Binding Protein 1 (ABP1), a putative auxin receptor has been extensively studied. It was found that in *Arabidopsis*, ABP1 is essential for embryo development, and it participates in auxin-mediated cell elongation in different species. In maize, several ABPs have been identified but their roles are still not understood. The aim of this study is to contribute to the understanding of the role of maize ABP1 and ABP4 during growth and development, with special reference to seedlings developed in blue light (BL). We have observed that BL decreases the level of free IAA in maize aerial organs. Using maize *abp1* and *abp4* single mutants, and the *abp1abp4* double mutant we have found that ABP1 and/or ABP4 regulate this BL-induced response. However, extent of the elongation of coleoptile and mesocotyl in BL does not correlate with the levels of free IAA. Interestingly, we observed that BL inhibits root elongation in WT plants, but not in *abp1*, *abp4* single and double mutants. Our results indicate that in maize, ABPs positively influence elongation growth of etiolated seedlings, and that ABP1 and ABP4 are involved in BL-signaling pathway that regulates auxin accumulation. Additionally, the data suggest that ABP1 and ABP4 are engaged in BL-induced inhibition of root elongation.

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P 2-5 AUXIN-BINDING PROTEIN1 (ABP1), THE SECOND AUXIN RECEPTOR

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Despite of knowing the 3-dimensional structure ABP1 is not fully acknowledged as an auxin receptor. We used the ABP1 insertional mutant (Chen et al. 2001). It is lethal when homozygous but viable in the hemizygous *abp1/ABP1* state. Hemizygous plants produce 2:1 resistant:wild type progeny on kanamycin agar due to the T-DNA. Seedlings from *abp1/ABP1* plants are defect in phototropism and gravitropism of roots and shoots. Those populations are composed of a major slow reacting and a minor normal reacting group. *abp1/ABP1* seedlings show strong root slanting, longer hypocotyls, and slightly increased lateral root number. Root auxin responses in *abp1/ABP1* seedlings are slightly less sensitive than in wt. In short days and long days *abp1/ABP1* plants flower earlier. They have more branches and decreased main stem diameter, indicating decreased apical dominance. Auxin-induced genes (qPCR: *IAA2, IAA11, IAA13, IAA14, IAA19, IAA20*) respond to auxin (0.1µM/1µM/10µM) 2-10 fold stronger in wt than in *abp1/ABP1* seedlings (30 & 60 min). Thus ABP1 is a receptor with probable functions in auxin transport and gene regulation. The apparent functional link to TIR1-linked gene regulation could be provided by phospholipase A (Scherer et al., 2007)

P2-6 LIGHT ALTERS PLANT ELONGATION RESPONSES TO EXOGENOUS AUXIN

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Many fundamental issues of interaction between light and hormone signaling pathways involved in plant growth remain to be uncovered. In model plants *Arabidopsis*, tomato and maize we investigated effects of light on plant growth responses to exogenous auxins. In dark, blue light (BL) and red light (RL), exogenous auxin inhibits long-term growth in intact *Arabidopsis* and tomato hypocotyl, and in corn coleoptile. Compared to dark- and RL-grown plants, inhibitory effect of auxin on elongation of *Arabidopsis* hypocotyl developed in BL is weak. Mutant analyses revealed that CRY1 mediates Blinduced reduction of hypocotyl sensitivity to exogenous auxin, and that ZTL1 is required for maintenance of hypocotyl response to exogenous auxin in BL and RL. Photoreceptor CRY1 is also involved in BL- and RL-induced reduction of hypocotyl sensitivity to NAA in tomato. Data indicate that tomato hypocotyl responses to the inhibitory effects of NAA and 2,4-D are regulated by light via different mechanisms. Analysis of *elm1* mutant in corn indicated that phytochromes mediate the Bland RL-induced decline in coleoptile response to exogenous auxin. Our results confirmed the existence of interaction between light and auxin signaling in plant growth. Analyses also suggest the existence of diverse mechanisms of the cross-talk between light and auxin in different plant species.

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P2-7 THE ROLE OF FUSICOCCINE-LIKE SECONDARY HORMONE IN THE CYTOKININE SIGNAL TRANSDUCTION.

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It was shown that cytokinine causes the formation of cytokinine secondary hormone (CSH) in embryos of germinating wheat seeds. CSH was purified by chromatography on nanostructured carbon sorbent "Nanocarbosorb". It was established by mass-spectra that CSH related to fusicoccine. The CSH showed the typically cytokinine activities such as: the derepression of apical dominance, the greening of yellow leaves and synthesis of amaranthin. CSH was active at concentration 1000 times less at 2-3 times quicker, than cytokinine. The one of interesting property of CSH is its ability to increase the tolerance of germinating wheat seeds to salt stress. We developed very interesting enzyme sensor model for investigation of signal transduction of cytokinine. It was established that cytokinine causes the formation of NADP-GDh in aleurone layer of wheat seeds. We suggest the next scheme of signal transduction of cytokinine. First step is the formation of CSH. Then molecules of CSH are binded with fusicoccine receptors of plasmatic membrane. This let to increase the level cytosolic Ca^{2+}. The last step of signal tranduction is switching on the activity of protein kinase C. But this process demands the present of another low molecular regulator which is formed under the effect of CSH on wheat seeds embryos.

P2-8 UNCOVERING THE DISTINCT ROLES OF AUXIN SIGNALING F-BOX (AFB) 4 AND 5 AS AUXIN RECEPTORS

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The auxin receptor family is comprised of six members; TIR1 and AFB1-5. Phylogenetic analysis reveals that the AFB4/5 clade diverged from the other members before seed plant radiation whereas the TIR1/AFB1 and AFB2/3 clades diverged within the angiosperm lineage (Prigge & Estelle, unpublished). The conservation of these receptors across seed plants suggests that they maintain a distinct function. Our goal is to understand the diverse functions of AFB4/5 in relation to other members of the family. Preliminary studies reveal that picloram works specifically through AFB4/5. The *afb4-2* and *afb5-5* single mutants are resistant to picloram compared to wild type while *tir1-1* is not. Biochemical analysis confirms that picloram enhances the affinity of Aux/IAA with AFB4/5 but not with TIR1. The basis for this specificity remains unknown. The *afb4-2afb5-5* double displays hypersensitivity to root growth at 29C, a condition that results in increased levels of auxin biosynthesis. In contrast, the *tir1-1afb2-3* mutants are resistant at 29C. To improve our understanding of TIR1/AFB function we are generating and characterizing all mutant combinations in conjunction with an investigation of the biochemical properties of AFB4/5 compared to TIR1.

P2-9 UNRAVELING THE EVOLUTION OF CYTOKININ SIGNAL TRANSDUCTION

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The conquest of the land by plants required dramatic morphological and metabolic adaptations. During this process, complex developmental programs under tight regulation evolved. One class of regulators of plant development are the phytohormones, such as cytokinins. The cytokinin signal transduction system, which is mediated via a multi-step variant of the bacterial twocomponent signaling system, is well characterized in the model plant *Arabidopsis thaliana*. In order to understand the origin and evolutionary pattern of this signaling pathway, we surveyed the genomes of several sequenced key plant species ranging from unicellular algae, moss and lycophytes, to higher land plants, including *Arabidopsis* and rice, for the presence of proteins involved in the signal transduction of cytokinin. Phylogenetic analysis revealed that the hormone-binding receptor and a class of negative regulators first appeared in land plants. Other components of the signaling pathway were present in all species investigated. Furthermore, we found that the receptors evolved under different evolutionary constraints from the other components of the pathway: the number of receptors remained fairly constant, while the other protein families expanded.

P2-10 AUXIN SIGNALING COMPONENTS INVOLVED IN ARABIDOPSIS HYPOCOTYL RESPONSE TO AUXIN

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We are using Arabidopsis hypocotyls to examine the role of auxin signaling pathways in cell elongation and growth, and to explore potential interactions among hormone and light perception and other transcriptional programs. Analysis of genome-wide auxin-regulated gene expression in hypocotyls revealed significant overlap with genes responsive to auxin in roots and whole seedlings, particularly Aux/IAA, SAUR, and GH3 genes. However, overlap with dark-regulated and other hormone-regulated gene sets was minimal, suggesting that a specific auxin-regulated transcriptional program acts in hypocotyl elongation. Transcript profiles, mutant phenotypes, and expression profiles of promoter-reporter fusion constructs were analyzed to identify components of TIR1/AFB-Aux/IAA-ARF pathways likely involved in hypocotyl elongation. Results suggest that auxin signaling components may have overlapping functions in the hypocotyl auxin response.

P2-11 CLONING, PURIFICATION, CRYSTALLIZATION AND X-RAY ANALYSIS OF THE RECEIVER DOMAIN OF THE HISTIDINE KINASE CKI1 FROM ARABIDOPSIS THALIANA

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The receiver domain (RD) of a sensor histidine kinases (HKs) catalyses transphosphorylation reaction during the action of HKs in hormonal and abiotic signalling in plants. Crystals of the recombinant RD of the *Arabidopsis* HK CYTOKININ-INDEPENDENT1 ($CKI1_{RD}$) have been obtained by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant and glycerol as a cryoprotectant. The crystals diffracted at beamline BW7B of the DORIS-III storage ring to approx. 2.4Å. The diffraction has been improved significantly - to at least 2.0Å - after applying of a non-water cryoprotectant. The crystals belong to space group $C222_1$ with unit-cell parameters a=54.46, b=99.82, c=79.94 Å, the asymmetric unit contains one molecule of the protein. The structure of $CKI1_{RD}$ had been solved by a molecular-replacement. $CKI1_{RD}$ is a single domain protein folded in a (β/α)5 manner with a central β-sheet formed from five β-strands and surrounded by sides by two and three α-helices.

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P2-12 IDENTIFICATION OF GENES PARTICIPATING IN THE REGULATION OF CKI1 EXPRESSION USING FORWARD GENETIC APPROACH

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Overexpression of a hybrid histidine kinase *CKI1* results into cytokinin-independent activation of two-component signaling pathway and cytokinin-like response in *Arabidopsis* hypocotyl explants. *CKI1* was found to be involved in the female gametophyte development and procambium activity during vascular tissue formation. Recent data suggest that regulation of *CKI1* expression might contribute to the regulation of two-component signaling. We used forward genetics approach to identify the factors regulating the activity of CKI1 promoter.

Stable transgenic lines carrying *ProCKI1:uidA* construct were mutated using EMS and M2 seedlings were screened for changes in the GUS pattern. To eliminate constraints of the rate-limiting factor that is a microscopy of the screened mutant lines, we have adopted automated microscopy using the dotSlide system. The level of *CKI1* gene expression in mutant lines was tested using qRT-PCR and the more detailed analysis of selected mutants is in the progress. Phenotype of selected mutant lines and the influence of spatiotemporal changes in *CKI1* expression on the two-component signalling will be analyzed and affected genes will be identified using map-based cloning.

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P2-13 IAA- INDUCED ACTIVATION OF PLASMALEMMA H^+-PUMPING ATPASE UNDERLINES THE HORMONE EFFECTS ON GERMINATING PETUNIA MALE GAMETOPHYTE

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Our previous studies have shown that exogenous auxin (IAA) is able to stimulate petunia pollen germination and this effect is accompanied by orthovanadate-sensitive alkaline shift of intercellular pH (pH_c increase), suggesting an involvement in of plasmalemma P-type H^+-ATPase in the hormone action. Here, we report that IAA action on germinating petunia pollen grains is also coupled with pronounced hyperpolarization of plasma membrane monitored by DiS-C_3-(5) and safranin O. A membrane hyperpolarization of pollen cells similar to that triggered by IAA was found to be also observed under the action of both fusicoccin and 2, 4-D, a well-known stimulator of plasmalemma H^+-ATPase and active synthetic IAA analogue, respectively. The hormone-induced increase in the membrane potential has been found to be completely abolished in the presence of orthovanadate and inhibited by EGTA as well as verapamil. These finding provide independent validation that the IAA-induced effects in germinating pollen grains are due to activation by the hormone of H^+-pumping ATPase. The data obtained also suggest that the IAA-triggered hyperpolarization of the pollen grain cells is Ca^{2+}-dependent and requires external Ca^{2+} entry. These results suggest that the target of IAA leading to the plasma membrane hyperpolarization in germinating pollen grains is most likely a certain signal pathway controlling the activity of the ATP-dependent proton pump.

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P2-14 MOSS DIAGEOTROPICA MUTANTS ARE AUXIN INSENSITIVE

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The tomato *Diageotropica* (*DGT*) gene encodes a peptidyl-prolyl cis-trans isomerase (PPIase) cyclophilin A-type. Ledgt mutants lack a number of typical auxin responses, including absence of lateral roots, reduced apical dominance, altered gravitropic response and fruit development. We sequenced auxin isensitive-P*hyscomitrella patens* mutants and identified five different mutants in the moss ortholog of *DGT*. Knockout of *PpDGT* results in a reduction in auxin sensitivity and typical auxin response phenotypes, suggesting a conserved ancestral function of DGT in auxin perception. *PpDGT* is expressed in outgrowing protonemal cells, in the shoot apex of leafy gametophores and rhizoids. It encodes an active PPIase protein with a typical cytoplasmic localization. The activity of SCFTIR1/AFB is likely to function normally in *Ppdgt* mutants, while the induction of the three *PpAux/IAA* genes is blocked. Taken together, we propose a role for DGT in auxin-regulated gene expression.

P2-15 CYTOKININ RECEPTORS FROM ZEA MAYS DIFFER IN LIGAND BINDING AND OTHER PROPERTIES

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Ligand binding properties of maize cytokinin receptors were studied by means of radioligand method. Receptors showed high affinity to main cytokinins (Kd at nanomolar levels) but had different preferences to various cytokinin versions. Receptor ZmHK2 displayed high affinity to *trans*-zeatin, thidiazuron and *meta*-topolin. ZmHK1 and ZmHK3 tightly bound isopentenyladenine, ZmHK1 also benzyladenine. ZmHK1 interacted weaker with *trans*-zeatin and bound *trans*- and *cis*-zeatin with similar affinities. Cytokinin receptors were able to interact with bacterial components of signal transduction and activate genes containing *cps* promoter. Activation of *cps::LacZ* showed correlation with binding data. In addition, three other receptors were isolated, ZmHK1a2 (homologous to ZmHK1), ZmHK1b1 and ZmHK1b2 (both homologous to ZmHK2). These receptors were similar to their homologues in the ligand dependence of *cps::LacZ* activation. Mono- and divalent cations did not influence the binding. Cytokinin binding to receptor ZmHK1, but not ZmHK2, appeared to be pH-sensitive. Western-blot and data on the cytokinin binding by different membrane fractions indicate the difference in the localization of receptors of ZmHK1-type and ZmHK1b-type. Data obtained corroborated the hypothesis on the role of cytokinin receptor heterogeneity in the communication between root and shoot.

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P2-16 RAPID AUXIN-INDUCED GROWTH WITHOUT "THE" AUXIN RECEPTOR?

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The classical auxin effect, auxin-induced growth, is a very rapid response. Historically there has been a long debate if it is brought about by rapid expression of growth limiting genes or by more direct mechanisms. Recently TIR1/AFBs have been identified as the receptors for auxin-induced gene expression. In order to check the relevance of these receptors for growth induction, we recorded time courses of auxin-induced elongation in the tiny hypocotyl segments of *Arabidopsis* using a CCD auxanometer at a very high time resolution. The general level of auxin-induced gene expression was monitored through the dr5::GFP reporter system. Auxin mediated GFP expression was found to be drastically reduced in the *tir1-afb2-3 afb3-4* triple receptor mutant background. IAA-induced growth responses were wild-type-like, but delayed, in the triple and the *tir1-afb 1-3 afb2-3 afb3-4* quadruple mutants. Growth induced by the synthetic auxin 2,4-D was much more strongly affected by the receptor mutations. We will discuss the nature of the delay and the reason for the strong effects of the mutations on 2,4-D-induced growth in order to figure out if auxin-induced gene expression sets the stage for the growth process or if it actually constitutes the trigger for this important element of plant development.

P2-17 NOVEL REGULATORS AND MECHANISMS IN AUXIN RESPONSE

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A diverse number of developmental events are intricately coordinated by auxin-regulated gene expression. There are several auxin responses which remain to be explained by current models. With the aim of discovering novel regulators of auxin signalling, several lines of research have been instigated. Firstly, a gain-of-function screen is being carried out whereby 12,000 Full-length cDNA Over-eXpression (FOX) lines of *A.thaliana*, each over-expressing, on average, 2.6 different cDNAs are screened for resistance to auxin. Secondly, two Auxin-Related Transcription Factors (ARFs), ARF7 and ARF1, have been used as bait to screen a Yeast-two Hybrid (Y2H) library. Results from both experiments are discussed.

The precise mechanism by which auxin-regulated gene expression is controlled remains unclear. Aux/IAA repressors have been shown to interact with the TOPLESS family of co-repressors, however the agravitropic phenotype of the *axr3-1* mutant, expressing a stabilized version of the axr3 repressor, is only partially rescued when *axr3-1* is engineered to contain a second mutation in the LxLxL domain. Data presented here shows that mutation of the LxLxL EAR-like motif of AXR3 is sufficient to abolish the interaction with TOPLESS suggesting that Aux/IAAs may possess other activities within this signalling network.

P2-18 CYTOKININ RECEPTOR ANTAGONISTS DERIVED FROM 6-BENZYLAMINOPURINE

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Recently we published 6-(2-hydroxy-3-methylbenzylamino)purine (PI-55) as the first molecule antagonizing cytokinin activity at receptor level. Here we present synthesis and *in vitro* biological testing of seven PI-55 derivatives with various substitution in C2, N7 and N9 positions at the purine ring and six new BAP analogues substituted in the benzyl ring. The ability of the compounds to interact with *Arabidopsis* cytokinin receptors AHK3 and CRE1/AHK4 was tested in bacterial receptor and live cell binding assays, and in *Arabidopsis* ARR5:GUS reporter gene assay, respectivelly. The cytokinin activity was further assayed in classical cytokinin biotests (tobacco callus, wheat leaf senescence and *Amaranthus* bioassay). None of the PI-55 modifications led to the improvement of antagonistic properties, however 6-(2,5-dihydroxybenzylamino)purine (LGR-991) was identified as a new cytokinin perception antagonist. At the molecular level LGR-991 blocks cytokinin receptor CRE1/AHK4 with the same potency as PI-55. Moreover, LGR-991 acts as competitive inhibitor of receptor AHK3, and importantly shows reduced agonistic effects in comparison to PI-55 in ARR5:GUS and cytokinin bioassays. LGR-991 causes more rapid germination of *Arabidopsis* seeds which is a characteristic of seeds from plants with a reduced cytokinin status. To conclude, LGR-991 shows new structural motive that can lead to preparation of cytokinin antagonists with broader activity and reduced agonistic properties.

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P2-19 NMR ANALYSES OF THE RECEIVER DOMAIN OF CKI1 HISTIDINE KINASE AND ITS INTERACTION WITH AHP PROTEINS

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In a multistep phosphorelay, the C-terminal receiver domain of sensor histidine kinases is supposed to be involved in protein-protein interactions with its downstream signalling partners, the AHP proteins. Here we show that $CKI1_{RD}$ interacts *in vivo* and *in vitro* with AHP2, 3, and 5 with different affinities. To understand protein-protein interactions on the molecular level, the structure of $CKI1_{RD}$ in solution has been studied in details by nuclear magnetic resonance (NMR). Effects of magnesium ions (Mg^{2+}) and phosphate analogue beryllium fluoride on chemical shift changes of $CKI1_{RD}$ have been studied in a series of titration experiments and the most significantly affected residues were identified. Observed chemical shift changes were mapped on a solved crystallographic structure of the protein. Molecular motions were investigated by NMR relaxation experiments with free, Mg2+-bound, and beryllofluorinated $CKI1_{RD}$. Based on these data and in combination with bioinformatics approach, we determined four regions that might be responsible for the observed specificity of protein-protein interactions between $CKI1_{RD}$ and individual AHP proteins.

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P2-20 CA^{2+} SIGNALING IN ABNORMAL AUXIN PERCEPTION

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Variety of auxin physiological responses might base on the differences in perception and transduction of hormone signal. Role of TIR1 receptor, requiring ubiquitin system of protein degradation, was proved clearly. Perception of auxin at the plant cell surface is thought to involve ABP1. In this investigation we fulfill a comparison of primary auxin sensitivity of *tir1* and *axr1 Arabidopsis* mutants and By2 tobacco transgenic line with lower ABP1 concentration (NAS). The primary universal auxin-triggered rise in the free Ca^{2+} cytosolic concentration was used as test reaction sensitivity to hormone and measured by fluorescent microscopy. Both *Arabidopsis* mutants developed elevation in Ca^{2+} concentration, which did not differ in amplitude and velocity from thus reaction of wild type cells. On the contrary cells of NAS line was characterized by elimination of hormone-triggered Ca^{2+} shift. It can be concluded that TIR1-related hormone perception and transduction pathway does not include Ca^{2+} signaling. While ABP1, shown to be important in cell elongation, according to our data is strongly required Ca^{2+} ion transport through plasma membrane. This coincides with early suggested model of plasmalemma receptor, consisted of ABP1 (associative domain) and Ca^{2+}-channel (transmembrane domain).

P2-21 WOX4 IS A POTENTIAL MEDIATOR BETWEEN AUXIN SIGNALING AND SECONDARY GROWTH IN ARABIDOPSIS THALIANA

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Lateral expansion of growth axes is essential for land plants for creating extended shoot and root systems. Lateral or secondary growth is mediated by the cambium, a two-dimensional meristematic tissue, which is organised as a cylinder enclosing the centre of growth axes. The essential role of the basipetal transport of auxin along the shoot in promoting cambial activity has been reported. Since secondary growth has also been described to take place in *Arabidopsis thaliana* stems, it is used as a model for analysing the process. Still, knowledge about the molecular control of secondary growth initiation and cambial activity is very limited and direct cambium-specific targets of auxin signaling have not been identified.

Here, we analyse the interaction of the transcription factor *WUSCHEL-RELATED HOMEOBOX 4* (*WOX4*) in *Arabidopsis* with auxin signaling with respect to secondary growth regulation. *WOX4* is specifically expressed in the vascular cambium and *wox4* mutants display a dramatic reduction of cambial activity in the shoot demonstrating that *WOX4* is an essential positive regulator. *WOX4* expression co-localizes with increased auxin signaling and auxin-responsive elements (AuxREs) in the *WOX4* promoter argue for a direct regulation of *WOX4* by auxin signaling. By analysing the effect of site-directed mutagenesis of AuxREs on the expression of *WOX4:GUS* reporters accompanied with auxin inducibility assays we address the direct regulation of *WOX4* expression by auxin.

P2-22 PHYSIOLOGICAL EFFECT OF NEBULARINE - DOES IT WORKS AS CYTOKININ ANTAGONIST?

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Nebularine is known for the cytotoxicity in animals caused by triphosphate accumulation and inhibition of a variety of enzymes. In plants, nebularine is not included among herbicides, but supposed to be an anticytokinin as detected previously using cytokinin bioassays.

In this study, nebularine antagonized the effect of cytokinins in senescence and callus test, but not in *Amaranthus* bioassay. Cytotoxicity of nebularine in the tobacco BY-2 cells was about 10 times lower than in mammalian cell lines. Spraying of *A. thaliana* plants with nebularine led to yellowing and purpuring followed by local drying or withering. Younger plants that generally contain higher level of cytokinins, showed an increased resilience. With *A. thaliana* seedlings, nebularine caused bold lateral root formation and shortening of the main root. When the plants were treated together with nebularine and cytokinin, nebularine reversed the inhibitory effect of cytokinin on lateral root formation. On the other hand, direct binding of nebularine to the cytokinin receptors AHK3 and CRE1/AHK4 was not observed. In the Arabidopsis *ARR5:GUS* reporter gene assay, nebularine blocked the cytokinin perception and signaling pathway in plants. The same effect was observed for *DR5:GUS*, an auxin signaling reporter gene.

Together with results obtained in receptor assays this finding indicates that the effect is not specific to cytokinin signaling and thus nebularine cannot be classified as cytokinin antagonist. The physiological effect of nebularine seems to be rather a combined function of inhibition of various processes as described for animal systems.

P2-23 SILENCING OF THE TIR1 AUXIN RECEPTORS BY ARTIFICIAL MICRORNAS IN PHYSCOMITRELLA PATENS

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The moss *Physcomitrella patens* has a simple morphology, with a defined number of differentiation steps that therefore is suitable for the analysis of developmental processes. In *Physcomitrella* the differentiation of the filamentous tissue (protonema) and the elongation of the adult gametophores is regulated by auxin. In 2005, the first auxin receptor (TIR1) was found in *Arabidopsis* (Dharmasiri et al., 2005, Kepinski and Leyser, 2005). TIR1 is a component of the SCF^{TIR1} ubiquitin ligase complex. The substrates for TIR1, Aux/IAA repressors, are recruited to the receptor in an auxin dependent manner and, after binding to TIR1, are degraded. In *Physcomitrella* four TIR1 homologs (PpTIR1) were found which cluster in two subgroups. All homologs show high identity in their sequence. Using an artificial microRNA (amiRNA) approach (Khraiwesh et al., 2008) all four homologs were silenced to study the effect of a down-regulation of the PpTIR1 transcript level in moss. Three different artificial miRNAs were designed: one to target all four homologs and two specific for each subgroup. AmiTIR1 was overexpressed in wild type and in GH3::GUS plants (Bierfreund et al., 2003) containing an auxin-inducible promoter. The reduction of PpTIR1 transcripts led to strong effects on the auxin distribution pattern after GUS staining indicating auxin distribution. Results from phenotypic analysis with regard to protonema development and the putative role of TIR1 in *Physcomitrella* are discussed.

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P2-24 A ROLE FOR CYTOKININ IN REGULATING SEED GERMINATION OF ARABIDOPSIS THALIANA

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Cytokinin plays diverse roles in plant growth and development. In *Arabidopsis thaliana* three cytokinin receptors (AHK2, AHK3, AHK4/CRE1) perceive the hormone signal and induce downstream signalling through a His-to-Asp phospho-relay. Phenotypical analysis of loss-of-function receptor mutants revealed an altered germination phenotype, especially an altered light sensitivity of the mutant seeds, indicating that cytokinin might be involved in regulating seed germination (Riefler *et al.*, Plant Cell, 2006). Using an extended experimental setup we refined the analysis and investigated this phenotype in more detail. One focus of the study was to clarify if the phenotype can be attributed to a single receptor or a distinct receptor combination. Our data shed new light on the cross-talk between light and cytokinin and the interaction of cytokinin and other phytohormones in the germination process.

P2-25 CYTOKININ REGULATION OF AUXIN BIOSYNTHESIS IN ARABIDOPSIS INVOLVES AUXIN SIGNALING

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The plant hormones auxin and cytokinin are both essential plant growth regulators and are known to influence cell division, cell differentiation and cell elongation in various developmental contexts. Whereas molecular mechanisms underlying the hormonal crosstalk are still poorly understood, recent discoveries have been adding pieces to the puzzle (Dello Ioio *et al.*, 2008; Ruzicka *et al.*, 2009). Several labs have shown interactions at the level of signal transduction pathways (Rashotte *et al.* 2003), and we have now shown clearly that there is another level of interactions, the biosynthesis of the hormones themselves. Our data has revealed that cytokinins up-regulate auxin biosynthesis. This effect is accompanied by changes in the level of transcripts for several putative auxin biosynthesis genes. We have also shown that cytokinin regulation of auxin synthesis occurs through auxin signaling, and that the TIR1-AXR3/IAA17 network is a central component of the crosstalk between cytokinin and auxin at the metabolic level. Because, there are reports that AXR3/IAA17 interacts with the response to several other hormones, ie brassinosteroids, ethylene and gibberellic acid, we are currently investigating the potential role of these hormones in the observed auxin/cytokinin biosynthesis crosstalk.

Dello Ioio et al., 2008. Science. 322: 1380

Rashotte et al., 2003. Plant Physiology. 132:1998

Ruzicka et al., 2009. PNAS. 106(11): 4284

P2-26 CYTOKININS PARTICIPATE IN THE REGULATION OF CHLOROPLAST GENE TRANSCRIPTION.

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Chloroplasts are among the main targets of cytokinin action in the plant cell. We here report on the activation of transcription by cytokinin as detected by run-on assays with chloroplasts isolated from apical parts of first leaves detached from 9-day-old barley (*Hordeum vulgare* L.) seedlings. Leaves were pre-incubated on water for 24h and incubated for 3h on a 2.2 x 10^{-5}M solution of benzyladenine (BA). Cytokinin enhanced the transcription of several chloroplast genes even above initial levels measured before BA treatment, however, only in apical (oldest) segment of primary detached leaves (but not in middle and basal) cytokinin efficiently enhanced chloroplast transcription. BA-induced stimulation of transcription of *trnEY, rps14, rpl16, matK, petD* and *petLG* required light during the period of incubation on BA, whereas the activation of transcription of *rrn16, rrn23, rps4, rps16, rbcL, atpB* and *ndhC* were fully light-independent. Northern analysis detected also a BA-induced increase in the accumulation of chloroplast mRNAs. Our data revealed positive and differential effects of cytokinin on the transcription of chloroplast genes that were dependent on light and on the age (developmental stage) of cells and leaves.

O3-1 INTEGRATION OF HORMONAL AND GENETIC REGULATION DURING VASCULAR MORPHOGENESIS IN ARABIDOPSIS

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Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. The cell lineages of the root vascular cylinder harboring phloem and xylem and the intervening procambial tissue originate from stem cells near the root tip. We and others have taken a combination of genetic and genomic approaches to understand how the specification of vascular cell lineages is determined at a molecular level. We have recently demonstrated that in *Arabidopsis*, cytokinin phytohormones negatively regulate protoxylem specification, a “default” identity. AHP6, an inhibitory pseudophosphotransfer protein, counteracts cytokinin signaling in a spatially specific manner, allowing protoxylem formation in this domain. On the other hand, APL, a MYB coiled-coil-type transcription factor has a dual role in promoting phloem differentiation and in repressing protoxylem differentiation. Recent progress in understanding the molecular control of vascular tissue specification by cytokinins and APL together with other regulatory pathways will be presented.

O3-2 MOLECULAR ANALYSIS OF AUXIN REGULATION OF WOOD FORMATION

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Phytohormone indole-acetic acid (auxin) is a key regulator of vascular development. In vascular tissue of woody plants, a concentration gradient of auxin overlaps the developmental gradient of secondary xylem. This overlap between auxin gradient and developing secondary xylem has lead to the suggestion that auxin gradient could act like a morphogen in regulating secondary xylem development. In addition auxin also plays a key role in environmental control of vascular cell division activity in wood plants. I will discuss our results based on analysis of auxin responsive transcriptome in woody tissues and the effect of modulation of auxin responsiveness on secondary xylem development in model plant hybrid aspen to explain the role of auxin in regulation of vascular development in woody plants. We have also analysed the mechanism underlying day length regulation of vascular meristem activity and I will discuss our results that show that modulation of auxin signaling by short day signal is important for seasonal control of the vascular meristem activity.

O3-3 DORNROESCHEN AND DORNROESCHEN-LIKE FUNCTION WITH THE CUC GENES AND MP TO MODULATE EMBRYO SYMMETRY VIA AUXIN-DEPENDENT PATHWAYS

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The AP2 domain paralogues *DORNROESCHEN* (*DRN*) and *DORNROESCHEN-LIKE* (*DRNL*) redundantly control embryo, cotyledon and floral development. The fundamental transition from radial to bilateral symmetry during embryogenesis is wrought by cotyledon growth and boundary separation, involving auxin signalling and response and the redundant *CUP-SHAPED COTYLEDON* (*CUC*) genes. Double or triple mutants between *drn*, *drnl* and individual *cuc* mutants show large increases in cotyledon defect penetrance, implying that these genes additively or redundantly control the majority of cotyledon specification pathways. Asymmetric *CUC* and *STM* gene expression in *drn* and *drn drnl* mutants shows that *DRN* and *DRNL* regulate the spatial expression of these genes.

DRN is downstream of auxin responses involving *MONOPTEROS* (MP) in cotyledon tips, shown by mutation of auxin response elements in the *DRN* promoter, expression of *DRN::GFP* in a *mp* background and by ChIP. *DRN* also acts upstream of auxin signalling/perception/response as shown by *DR5::GFP* and *PIN1* expression.

The basis of redundancy between *DRN* and *DRNL* has been addressed via their frequent differential interactions with genes involved in local auxin biosynthesis and polar auxin transport and also via *DRN/DRNL* promoter swap experiments followed by mutant complementation.

O3-4 MULTIPLE MONOPTEROS-DEPENDENT PATHWAYS ARE INVOLVED IN LEAF INITIATION

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Initiation of leaves at the flanks of the shoot apical meristem occurs at sites of auxin accumulation and pronounced expression of auxin-inducible PIN genes, suggesting a feedback loop to progressively focus auxin in concrete spots. Since PIN expression is regulated by Auxin Response Factor (ARF) activity, including MONOPTEROS (MP), it appeared possible that MP affects leaf formation as a positive regulator of PIN genes and auxin transport. Here we analyze a novel, completely leafless phenotype arising from simultaneous interference with both auxin signaling and auxin transport. We show that *mp pin1* double mutants, as well as *mp* mutants treated with auxin-efflux inhibitors, display synergistic abnormalities, not seen in wild type regardless of how strongly auxin transport was reduced. The synergism of abnormalities indicates that the role of MP in shoot meristem organization is not limited to auxin transport regulation. In *mp* mutant background, auxin transport inhibition abolishes leaf formation. Instead of forming leaves, the abnormal shoot meristems dramatically increase in size harboring enlarged expression domains of *CLV3* and *STM*. The observed synergism under conditions of auxin efflux inhibition was further supported by an unrestricted PIN1 expression in *mp* meristems, as compared to a partial restriction in wild type meristems. Auxin transport-inhibited *mp* meristems also lacked detectable auxin maxima. We conclude that MP promotes the focusing of auxin and leaf initiation in part through pathways not affected by auxin efflux inhibitors.

O3-5 AUTO- REGULATED EXPRESSION OF CYTOKININ BIOSYNTHESIS CONFERS DROUGHT TOLERANCE IN PLANTS

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One of the prominent symptoms of plants under drought stress is the appearance of premature leaf senescence. However, the senescence program could be unnecessarily activated during drought and leads to premature death. We hypothesized the possibility of enhancing plant drought-tolerance by delaying the drought-induced leaves' senescence by cytokinins.

We created transgenic tobacco plants carrying the autoregulatory system of cytokinins synthesis directed by an early senescence promoter of the senescence SARK gene. The promoter was fused to *IPT*, the key gene of cytokinins synthesis. The transgenic plants displayed a dramatic delay of the senescence symptoms and extend the shelf life of detached leaves. Most surprisingly, the tobacco transgenic plants also displayed water stress tolerance as reflected by vigorous growth after a severe drought (14 days without watering), as well as minimal yield loss when watered with only 30% of the amount of water used under controlled conditions. The transgenic plants retained photosynthetic activity, an improved water use efficiency and substantially higher water content in leaves. After rewatering, the plants recovered photosynthetic activity and active growth. Macroarray analysis of stress-dependent genes showed under strong drought stress elevated expression of reactive oxygen scavenging mechanisms, indicating a stress protection mechanism as a contributor to the resistance phenotype.

O3-6 A GENETIC FRAMEWORK FOR THE AUXIN/CYTOKININ CONTROL OF CELL DIVISION AND DIFFERENTIATION IN THE ROOT MERISTEM

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Plant post-embryonic development takes place in the meristems. In the root meristem a stem cell niche generate transit-amplifying cells, which undergo additional division in the proximal meristem, and differentiate in the distal meristem transition zone that encompasses the boundary between dividing and expanding (differentiating) cells in the different cell files. For meristem maintenance, and therefore continuous root growth, the rate of cell differentiation must equal the rate of generation of new cells: how this balance is achieved is a central question in plant development.

While the molecular mechanisms involved in stem cell positioning and activity are partially comprehended, the regulatory networks controlling the shift from transit-amplifying identity to differentiation are still poorly understood.

We have previously shown that in the *Arabidopsis* root meristem the hormone cytokinin controls the differentiation rate of transit-amplifying cells by antagonizing the activities of a diffusible input in the vascular tissue of the transition zone. Here we demonstrate that this diffusible input is auxin, and that the balance between cell differentiation and cell division, necessary for controlling root meristem size and root growth is the result of the interaction between cytokinin and auxin through a simple regulatory circuit converging on the *SHY2* gene. In particular, in the vascular tissue of the transition zone, a primary cytokinin-response transcription factor, ARR1, activates the gene *SHY2*, a repressor of auxin signaling that negatively regulates the *PIN* genes that encode auxin transport facilitators. Thus, cytokinin causes redistribution of auxin, prompting cell differentiation. Conversely, auxin mediates degradation of the SHY2 protein, sustaining the activity of the *PIN* genes and prompting cell division.

O3-7 THE ROLE OF CYTOKININ RESPONSE FACTORS DURING LATERAL ROOT INITIATION

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Roots are essential for the growth and development of most plants. One of the strategies the plant uses to cope with its dynamic environment is the continual initiation of new lateral roots (LRs). Once fully developed, LRs have the same cell pattern as the main root, including a functional meristem. Because, the LRs are initiated post-embryonically, the study into LR initiation (LRI) is not only of agricultural importance but provides an excellent model to study meristem initiation and development.

Auxin and cytokinins have always been seen as opposite regulators of root development, auxin promotes the initiation of lateral roots whereas cytokinins are considered negative regulators of LRI. Transcript profiling, aimed at identifying auxin related genes during early LRI, revealed the differential regulation of a set of transcription factors belonging to the group of Cytokinin Response Factors (CRFs). This was confirmed with QPCR, mutant analysis and the spatiotemporal expression pattern of several of the CRFs suggest their potential role in root development. The functional characterization of these potential key-regulators of lateral root initiation is currently ongoing and examples will be discussed.

O3-8 KNOXI GENES AND CYTOKININ REGULATE LEAF DEVELOPMENT

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Plant meristems retain morphogenetic capacity throughout life. *Class I homeobox* (*KNOXI*) genes are expressed in the shoot apical meristem (SAM), and play central roles in SAM function and leaf patterning. KNOXI proteins act in part through the regulation of hormone levels. We found that KNOXI proteins positively regulate cytokinin accumulation by activating the transcription of the Arabidopsis cytokinin biosynthesis gene *isopentenyl transferase 7* (*AtIPT7*). Down-regulation of the Arabidopsis *KNOXI* genes *BP* and *STM* by expression of a synthetic microRNA (*miRSTM/BP*) through the *STM* promoter phenocopied the *stm* phenotype. Strikingly, co-expression of *miRSTM/BP* and *IPT* through the same promoter resulted in a suppression of the *miRSTM/BP* phenotype. Overexpression of *IPT* or *KNOXI* genes in tomato leaves resulted in partially overlapping phenotypes that included reiteration of leaflet formation. The phenotypes were strongly affected by the precise developmental timing of the ectopic expression, suggesting that the developmental context strongly influences KNOXI and CK function. Down-regulating *KNOXI* gene targets, as well as degradation of CK by expressing *CKX3* in tomato leaves resulted in a dramatic reduction in leaf complexity, revealing a novel role of CK.

O3-9 SMALL RNAS FACILITATE POLARITY AND LAMINAR GROWTH OF TOMATO LEAVES

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The current models for leaf initiation suggest that the partitioning of the leaf primordia to adaxial and abaxial domains requires positional information coming from the adjacent meristem. This model can explain leaf primordia initiation, but leaflets are initiated remotely from the meristem, and yet exhibit adaxial-abaxial asymmetry. To better understand the abaxial/adaxial patterning of leaflets in the compound tomato leaves, we have identified 4 mutants, corresponding to the classical, disease mimic, wiry syndrome. In these mutants, first 3-5 formed leaves have bifacial lamina made many primary, but almost no secondary leaflets, occasionally radial leaflets. Later formed leaves nearly entirely lack, and appear abaxialy unifacial. The four WIRY genes encode enzymes involved in siRNA and trans-acting siRNA biogenesis pathways and in their mutants, the level of the tasi-ARF decreases dramatically while the RNA level of its target SlARF4 is increased. Moreover, the abaxial SlARF4 expression expands to the adaxial side of mutant leaf primordia and missexpression of a tasi-ARF insensitive form of it, phenocopy wiry mutants. In agreement, overexpression of amiR-ARF, that targets ARF2, ARF3 and ARF4 rescue the lamina of later formed wiry leaves. Moreover, amiR-ARF overexpression could rescue the radial leaves of tomato lacking SlPHAN or SlAS2 activities. These mutants also produced radial leaves in an age dependent manner, but maintained tasiR-ARF production. Indeed, cotyledons and all leaves of plants mutant for slphan or slas2 and a tasiRNA-biogenesis wiry were perfectly radial. In contrast, Arabidopsis “wiry” mutants, even strong ones such as as2 rdr6 double mutants have a weak phenotype, a reflection of reduced responsiveness of its leaves to altered levels of the two ARFs, either from self or tomato origin. Likewise, overexpression of At ARF3 or AtARF4 in tomato was as effective as the tomato genes in stimulating the wiry syndrome. Surprisingly, similar manipulations in simple leaved Solanaceae tobacco also did not change their basic structure. Thus, differential responses to ETT and ARF4 may depend on the basic form of the leaves, where simple leaves are less ‘responsive’ to the two ARFs then compound leaves.

O3-10 CYTOKININS CAN STIMULATE ARABIDOPSIS HYPOCOTYL ELONGATION AT DECREASED LIGHT INTENSITY

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Cytokinins (CKs) were reported not to affect hypocotyl elongation in *Arabidopsis* seedlings grown in the light, and CK reportedly promoted hypocotyl elongation in the white light when ethylene action or auxin transport was blocked. Here we re-investigated the effects of CKs on hypocotyl elongation in *Arabidopsis*. While only a marginal effect of CKs on hypocotyl length was observed at a standard white light intensity (80 μmol m^{-2} s^{-1}), a pronounced stimulation of hypocotyl elongation by CKs was found when the seedlings were cultivated at a decreased white light intensity (20 μmol m^{-2} s^{-1}). The CK effect on hypocotyl length was due to cell elongation. The stimulation of hypocotyl elongation was observed for all four principal CKs (*t*-Z, iP, BA, TDZ) and was dose-dependent in the nanomolar range. The stimulatory effect of the CKs was antagonized by PI-55. Mutant and transgenic plant analysis indicated that the canonical two-component response pathway is necessary for this process with prevailing contribution of AHK2 and AHK3 receptors. This CK action was independent of ethylene signaling and partially inhibited by IAA.

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P3-1 CKX GENES REGULATING GENERATIVE MERISTEM ACTIVITY IN ARABIDOPSIS

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To assign the function to different members of the *cytokinin oxidase/dehydrogenase* (*CKX*) gene family of *Arabidopsis* we analyzed individual knockout mutants. Knockouts of single *CKX* genes had minimal consequences for the plant development, indicating functional redundancy. Nevertheless, double and multiple knockout mutants revealed specific processes in which different *CKX* genes are involved. Here we show that one specific double knockout combination developed larger and more active inflorescence meristems, resulting in an increased rate of flower primordia initiation. An enhanced activity of floral meristems was indicated by an increased number of floral organs, which were bigger than in wild type. The mutant gynoecia were strongly enlarged and contained more ovules, which suggest a role of cytokinin in regulating the proliferative activity of the placenta. These results indicate that a sub-group of *CKX* genes probably acts as a negative regulator of cell division activity during developmental processes linked to generative development.

P3-2 THE CYTOKININ-REGULATED TRANSCRIPTOME OF ARABIDOPSIS ROOTS AND SHOOTS

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Cytokinin affects root and shoot growth differentially: In shoots it is a positive growth regulator, in roots it inhibits growth. It is to be assumed that organ-specific regulation of gene expression is involved in these differential activities, but little is known about it. To get insight into the transcriptional events in roots and shoots triggered by cytokinin, we studied genome-wide gene expression in cytokinin-treated and cytokinin-deficient roots and shoots.

We could assign a root or shoot-specific expression to a number of genes previously known to react to a cytokinin signal. We identified novel organ-specific cytokinin-regulated genes, which had escaped previous discovery most probably due to unspecific sampling. Contrasting with the dramatic difference of the developmental response, we found that the vast majority of the cytokinin-regulated transcriptome shows similar expression patterns in roots and shoots.

By global analysis of the transcriptomic data, we found that the immediate-early response to a cytokinin stimulus differs from the later response, and that the transcriptome of cytokinin-deficient plants is different from both the early and the late cytokinin induction response.

P3-3 AUXIN DEFINES DEVELOPMENTAL WINDOW FOR LATERAL ROOT INITIATION

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Auxin is a key regulator of lateral root organogenesis. Recently, it has been shown that auxin regulates early lateral root initiation (LRI) acting as a morphogenetic trigger for specification of founder cells. However, it is not known whether auxin might regulate position of new founder cells and thus it is involved also in the control of the overall root system architecture. We show that developmental window where LRI takes place is determined by a threshold level of auxin gradient built in the root central cylinder including pericycle and that the pericycle cells within the developmental window exhibit highest competence to acquire founder cell fate upon auxin stimulus. When auxin gradient or auxin response along the root are altered genetically or pharmacologically, regular acropetal pattern of lateral root initiation no longer can be maintained. Our data suggest that auxin distribution along the parent root creates a morphogenetic zone where founder cells for lateral root primordia acquire their identity upon auxin signal.

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P3-4 THE MAB4/ENP FAMILY GENES INVOLVED IN AUXIN-REGULATED MORPHOGENESIS

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Polar auxin transport is mainly dependent on the activity of auxin efflux carriers, PIN-FORMED (PIN) proteins, localized in the plasma membrane with polarity. Recently, MACCHI-BOU 4/ENHANCER OF PINOID (MAB4/ENP), a NONPHOTOTROPIC HYPOCOTYL 3 (NPH3)-like protein, has been reported to control subcellular localization of PIN proteins. However, the contribution of *MAB4/ENP* to polar auxin transport is restricted because the *mab4/enp* station causes mild defects only in aerial organ formation, suggesting that redundant factors may function in polar auxin transport with *MAB4/ENP.*

Four NPH3 members are highly homologous to MAB4/ENP, named as MAB4/ENP-LIKE (MEL) 1-4. To examine the function of *MEL* genes, we first analyzed expression pattern of *MEL* genes and subcellular localization of MEL proteins. *MEL* genes displayed different expression pattern from *MAB4/ENP*, but, in part, overlapping with *MAB4/ENP.* In their expression domains, MAB4/ENP and MEL proteins were localized nearby the plasma membrane with polarity, almost identical to that of PIN proteins there. Next, genetic interactions between *MEL* genes and/or *MAB4/ENP* were examined. Both *mel1* and *mel2* mutations enhanced *mab4* mutant phenotypes as *mab4 mel1 mel2* triple mutants developer pin-like inflorescences. Surprisingly, *mel* quadruple mutants displayed severe defects in the root gravitropism. These results indicate that *MEL* genes function in various auxin-dependent morphogenesis, not only organ formation but also root gravitropism.

P3-5 WORK IN PROGRESS: EFFECTS OF THE CYTOKININ ANTAGONIST PI-55 ON THE NODULATION PHENOTYPE OF PEA (PISUM SATIVUM L.)

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PI-55 is an antagonist of benzyl-aminopurine (BAP), an active plant cytokinin, which targets CRE1/AHK4, one of 3 known cytokinin receptors. Homologues of this receptor have been found to play a critical role in nodule development. Therefore, we were interested in studying PI-55 effects on pea nodulation. To assess these, we inoculated 3 d-old seedlings with *Rhizobium leguminosarum* bv. *viciae* and treated them exogenously 1, 3 & 5 days after inoculation with PI-55. Nodule numbers on 14-d-old plants were similar to those of non-treated controls up to 10 µM, concentration at which inhibition occurred. These preliminary results have stimulated us to pursue this line of study. To ensure that PI-55 acts as a BAP competitor and not as an agonist, inoculated seedlings need to be treated now with both PI-55 & BAP. Because BAP-treated plants were shown earlier to exhibit nodule inhibition on their distal lateral roots, maps displaying nodule spatial arrangement will be compared between control plants and plants treated with various concentrations of PI-55, BAP and PI-55 & BAP. Finally, PI-55 treated plants will be inoculated with lacZ rhizobia so that infection threads can be observed and compared to the twisted ITs of BAP-treated plants. We will assess the nodulation phenotype of PI-55-treated plants by comparing it to that of R50 (*sym16*), a nodulation mutant the cytokinin oxidase of which is defective and which accumulates large amounts of cytokinins.

P 3-6 CYTOKININ DEFICIENCY CAUSES DISTINCT CHANGES OF SINK AND SOURCE PARAMETERS IN SHOOTS AND ROOTS

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Cytokinin deficiency causes pleiotropic changes during plant development, such as retarded shoot and enhanced root growth. We have investigated whether cytokinin-deficient tobacco plants exhibit changes of different sink and source parameters, which could be causally linked to these growth alterations. We studied cellular ultrastructure, examined the cell cycle and determined several photosynthetic parameters, contents of carbohydrates and adenylates, as well as activities of enzymes of carbon metabolism. Our results strongly support a regulatory function of cytokinin concerning shoot sink strength and its reduction may be a reason for the altered shoot phenotype. However, cytokinins appear to regulate sink activity in shoot and root in a different fashion.

P3-7 MODULATION OF PROKARYOTIC TZS GENE EXPRESSION IN TRANSGENIC TREES

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We modulated the level of a hormone gene expression in poplars using either 35S promoter (p35S) of Cauliflower mosaic virus or *aux* promoter (pAUX) of *A. rhizogenes*. Northern blot analysis of the transgenic poplars probed with *tzs* coding region showed that the total *tzs* mRNA expression by p35S was approximately 200 to 300 folds higher than that driven by pAUX. Cellular cytokinin level of p35S-*tzs* and pAUX-tzs poplars were 600 pg/g and 118 pg/g respectively. The p35S-*tzs* transgenic poplars showed severe phenotypic changes including spontaneous regeneration of multiple adventitious shoots on hormone free media, thick stems, small leaves, indistinguishable petioles, and suppression of both shoot elongation and root formation. In contrast, the pAUX-*tzs* plants showed close-to-normal phenotype. Compared with nontransgenic control, the pAUX-*tzs* trees had higher photosynthetic rate, less height growth and larger biomass resulting from multiple branches. Other morphological and developmental effects by the expression of the *tzs* will be discussed in detail.

P3-8 THE AS2 REGULATES LEAF POLARITY BY REPRESSING ETT/ARF3 IN ARABIDOPSIS

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The *ASYMMETRIC LEAVES2* (*AS2*) and *ASYMMETRIC LEAVES1* (*AS1*) genes of Arabidopsis are required for symmetrical and flat lamina expansion. *AS2* encodes a plant specific protein that contains AS2/LOB domain[1)], and *AS1* encodes a myb (SANT) domain protein. *AS2* and *AS1* are thought to act as a transcriptional regulator of certain genes including class 1 *KNOX* genes. We have previously shown that *AS1* and *AS2* regulate leaf polarity by repressing *ETT/ARF3, KAN2, YAB5*, which are abaxial determinants[2)]. *ETT/ARF3* and *ARF4* are closely related members of ARF family, and known to be targets of trans-acting siRNA, tasiR-ARF, which in turn regulated by miR390. *ett* and *arf4* mutations suppressed the asymmetrical and downward curling leaf phenotype of *as2*, suggesting that *AS2* involves both *ETT/ARF3* and *ARF4* repression. We report how *AS2* regulates *ETT/ARF3* and *ARF4* in the process of leaf development.

1) Matsumira et al., Plant J. (2009)

2) Iwakawa et al., Plant J. (2007)

P3-9 ROLE OF AUXIN, AUXIN-BINDING PROTEINS AND LIGHT IN THE DEVELOPMENT OF MAIZE SEEDLINGS

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Modern maize (*Zea mays* L) hybrids developing erect leaves show less sensitivity to dense planting and reduced responsiveness to auxin and light in comparison to older, density sensitive varieties. In addition, in modern hybrid, the expression of *Auxin-Binding Protein 4* (*ABP4*), a hypothetical auxin receptor, is impaired. In this study we investigated the role of auxin, ABPs and light in maize growth and development. *In vitro*, etiolated maize mutants affected in *ABP1* and/or *ABP4* genes showed less sensitivity to auxin in comparison to wild type (WT). We found that in *abp4* and *abp1/abp4* double mutant red light (RL) promoted elongation of coleoptile whereas in WT and *abp1* RL did not have any effect on coleoptile elongation. In the greenhouse (*in vivo*), *abp1* and *abp4* single mutants developed more and less erect leaves, respectively, while *abp1/abp4* double mutant had leaves less vertical than WT. *abp4* plants developed the tallest stature with longer leaves than WT, although *abp1* mutant developed the longest and the widest leaves. Our data suggest that auxin regulates development of maize seedlings through ABPs and that *ABP4* mediates RL-induced inhibition of coleoptile elongation. The data support the existence of interaction between auxin and light in maize growth and development. In addition, these findings suggest the involvement of *ABPs* in leaf angle development, as well as an important role of *ABP1* in maize leaf morphology.

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P3-10 THE UNIVERSAL GROWTH HORMONE FLORIGEN COORDINATES MORPHOGENETIC GRADIENTS OF AUXIN IN THE SAMS, COMPOUND LEAVES AND ABSCISSION ZONES OF TOMATO

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Florigen was recently established as the first plant protein functioning as a general growth hormone (Shalit et al., PNAS, in Press). The mobile Florigen (Lifschitz et al., PNAS, 2006) regulates growth by modulating organ-specific balances between SINGLE FLOWER TRUSS (SFT), the tomato precursor of florigen, and SELF PRUNING (SP), a potent SFT-dependent SFT inhibitor. The graft-transmissible impacts of florigen on organ-specific traits in tomato show that in addition to import by shoot apical meristems (SAM), florigen is imported by organs where *SFT* is already expressed. By modulating local SFT/SP balances, florigen confers differential flowering responses of primary and secondary apical meristems, regulates the reiterative growth and termination cycles typical of perennial plants, accelerates leaf maturation and determines the patterning of leaflets in the compound leaves and the formation of abscission zones in the floral pedicles. Possible ways in which florigen interacts with auxin to regulate the morphogenetic gradients in sympodial meristems and compound leaves will be discussed.

P3-11 THE EFFECT OF DIFFERENT AROMATIC CYTOKININS ON ORGANOGENESIS OF SORBUS TORMINALIS

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The influence of three different aromatic cytokinin derivatives (6-benzylaminopurine, *meta*-topolin and 6-(3-methoxybenzylamino) purine-9-ß-D-ribofuranoside (MeOBAPR)) on *in-vitro* multiplication and rhizogenesis of the wild service tree (*Sorbus torminalis* (L.) Crantz) were compared. The highest micropropagation rate was achieved on media containing BAP. On the other hand, the best rooting microcuttings were those multiplied on a medium containing MeoBAPR. To compare these results with the levels of endogenous cytokinins in multiplied explants, a newly developed UPLC-ESI(+)-MS/MS method was used to determine levels of 50 cytokinin metabolites in explants cultivated 12 weeks on media supplemented by BAP and the other cytokinin analogues used. Several significant differences among the levels of endogenous cytokinins, extracted from the explants, were found. Concentration of BAP9G, an important metabolite, suspected to be responsible for rooting and acclimatization of newly formed plantlets, was found to be the highest in microcuttings grown on media supplemented with BAP. This agrees well with the results of our rooting experiments; the lowest percentages of rooted plantlets 6 weeks after transferring shoots on rooting medium were present on explants multiplied on BAP. In contrast, the BAP was still the most effective on the induction of bud formation on primary explants. Levels of the most active endogenous isoprenoid cytokinins, tZ, tZR and iPR as well as O-glucosides were also suppressed in explants grown on BAP, compared to explants treated with other cytokinin derivatives. This may be the result of a very high BAP uptake into the explants grown on this cytokinin. On the other hand, endogenous concentrations of *cis*-zeatin derivatives as well as dihydrozeatin derivatives were not affected. The highest ethylene levels were detected in the vessels containing media supplemented with mT. They were 2-4 times higher in comparison with the production by the *S. torminalis* explants cultivated on other media used. The results about optimal plant hormone concentrations, found in this study, may be used to improve *in-vitro* rooting efficiency of the wild service tree, and possibly also of other plant species.

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P3-12 ROLE OF AUXIN EFFLUX IN APICAL HOOK DEVELOPMENT

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Apical hook of dark-grown seedlings is a simple structure, whose differential growth is regulated by hormones. Apical hook develops soon after germination and is maintained until the seedling emerges from the soil, and the hook opens upon its exposure to the light. We observed that progress of apical hook through individual developmental phases depends on dynamic and asymmetric distribution of auxin regulated by auxin efflux carriers of *PIN* family. Several *PIN* genes exhibit specific and only partially overlapping expression patterns. Genetic manipulation of individual *PIN*s activities differentially interferes with particular stages of the hook development. Thus, specific combinations of different *PIN*s are required for the progress of the apical hook through developmental phase. Detailed analysis of the ethylene regulated apical hook development suggests that at least part of the ethylene effect might be mediated through interaction with auxin and ethylene-dependent regulation of the auxin transport.

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P3-13 DISSECTING THE GENETIC REGULATION OF BARLEY (HORDEUM VULGARE) ROOT ARCHITECTURE

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Little is known about the genetic basis of root system formation and architecture in cereals. Based on *Arabidopsis thaliana* data, effective auxin uptake by the AtAUX1 influx carrier is an important process underlying the patterning of root architecture. Sequences similar to auxin influx carriers have been found in *Oryza sativa*, yet its existence in barley has not been confirmed. From *in silico* search for barley ESTs matching the *AtAUX1* nucleotide sequence, we have assembled a putative *HvAUX1* and we have confirmed its expression in root tissues of the Optic barley variety. Sequence and phylogenetic analyses of the amino acid/ auxin permease (AAAP) family, which includes AtAUX1 prote, revealed that HvAUX1 is the most similar (90% of similarity) to Os01g63770 (Os Like AUX1) and shares 81% similarity with AtAUX1. This *O. sativa* protein sequence is also the most similar to AtAUX1 (82% of similarity). Taken together, we have identified a putative *HvAUX1*, which is a potential ortholog of *A. thaliana AUX1*. Functional analysis is underway to check this hypothesis.

P3-14 SCREENING AND CHARACTERIZATION OF SAR (SUPPRESSORS OF ADVENTITIOUS ROOTING)

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Adventitious root formation allows clonal propagation and rapid fixation of superior genotypes prior to their introduction into production or breeding programs. This strategy is often used for long-lived woody species. Nevertheless the inability to initiate adventitious roots remains an obstacle for elite genotypes of many crop and woody species and the regulatory mechanisms are still not well understood. The hormone auxin is one of the main endogenous factors controlling adventitious root formation and the superroot (sur1 and 2) mutants over-produce auxin and spontaneously make adventitious roots. In an attempt to identify new genes involved in the regulation of adventitious rooting we performed a screen for suppressors of the sur2 mutation. 2345 independent M2 families derived from sur2-1 homozygote seeds mutagenized with EMS were screened for the suppression of the adventitious root phenotype. 38 suppressors were confirmed in the M3 progeny. F2 mapping populations after crossing with atr4-1, a sur2 allele in Col-0 background, have been produced for all of these suppressors. A more detailed characterization was performed for the first eleven suppressors. Complementation tests indicate that they belong to six groups of complementation. Their position on the chromosomes has been established and the map based cloning for these six genes is in progress. For selected mutants, the free and conjugated IAA levels were quantified. In suppressors sar1 and sar2 the total auxin content is back to the WT level, while in the suppressors sar3, sar4, sar5 and sar6 the free IAA level is similar as in sur2-1. These preliminary data show that these mutants will allow in the future to identify new genes that not only regulate adventitious root formation but most likely also auxin homeostasis.

P3-15 AUXIN RESPONSES IN THE TOMATO MUTANTS ENTIRE AND POTATO LEAF

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Auxin controls many developmental responses such as, root induction, fruit set and leaf architecture. Here we tested whether the tomato (cv Micro-Tom) leaf-architecture mutants *entire* (*e*), a putative AUX/IAA, and *potato leaf* (*c*), a MYB protein, were altered in other auxin responses. The mutant *c* did not differ from Micro-Tom (MT) in any auxin response tested. However, *e* presented a high parthenocarpy in unpollinated ovaries, compared to MT. The *e* mutant also had a higher NAA-induced root formation in cotyledons, compared to MT. Root induction was suppressed in the auxin-insensitive *diageotropica* (*dgt*) mutant, but the double *dgt e* showed an intermediary (additive) response. The elongation of hypocotyls, upon exogenous IAA, showed no differences comparing MT and *e*, but both *dgt* and the double *dgt e* were auxin-insensitive. The double *dgt e* also showed the same agravitropic response of *dgt* shoots after 5 h of observation. Our results indicate that *e* may increase auxin sensitivity in some organs (e.g. ovaries and cotyledons) and the double mutant analysis suggests that *E* has a little participation, if any, in processes controlled by *DGT* (e.g. hypocotyl elongation and gravitropism). Double mutants also indicated that *E* and *DGT* act in different pathways controlling root induction.

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P3-16 UREA DERIVATIVE BIOLOGICAL ACTIVITIES: STATE OF THE ART

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Urea derivatives are synthetic compounds, some of which have proved to be positive regulators of cell division and differentiation. N-phenyl-N'-(2-chloro-4-pyridyl)urea (CPPU) and N-phenyl-N'-(1,2,3-thiadiazol-5-yl)urea (TDZ) are extensively used in *in vitro* plant morphogenesis studies, as they show cytokinin-like activity often exceeding that of naturally occurring adenine compounds. For years, in our work on structure–activity relationship, we synthesized a large number of urea derivatives and tested their biological activities by different bioassays. These studies allowed the identification of some compounds that could be used to induce *in vitro* morphogenesis in distantly related plants. Here we collect and summarize these data. New insights are also reported.

P3-17 IDENTIFICATION OF NOVEL COMPONENTS DEFINING XYLEM IDENTITY

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Despite the progress that has been recently made in understanding hormonal pathways, we know still very poorly how they regulate cell fate and tissue specification. Our group has made recently a breakthrough in understanding of cytokinin-based specification of protoxylem and protein AHP6, an inhibitor of cytokinin hormone signaling pathway in Arabidopsis, was proposed to be a key regulator of xylem specification.

In order to further study early molecular processes of cytokinin mediated of xylem formation, we have done a genetic screen aiming at uncovering factors involved in specifying of expression pattern of the earliest known marker of protoxylem formation, AHP6. Several novel mutant lines were identified based on altered *pAHP6::GFP* expression. One of these mutants, *px1*, non-allelic to the *ahp6* mutant, displays erratic protoxylem specification phenotype, similar to *ahp6*, which suggests that the *PX1* gene might control a similar aspect of protoxylem specification. Morphological phenotype of *px1* includes defects in gravitropism, lateral root formation, cotyledonal fusions and apical dominance, thus resembling auxin-like defects. The similarity of *px1* to auxin related mutants thus suggests a cytokinin-auxin interaction during early steps of vascular formation with crucial role of the *PX1* gene product.

P3-18 MOLECULAR MECHANISM OF THE AUXIN-DEPENDENT PIN LATERALIZATION

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Plant development is characterized by a profound ability to regenerate and form tissues with new axes of polarity. An unsolved question concerns how the position within a tissue and cues from neighboring cells are integrated to specify the polarity of individual cells. The canalization hypothesis proposes a feedback effect of the phytohormone auxin on the directionality of intercellular auxin flow as a means to polarize tissues. In a previous work, we addressed a cellular and molecular mechanism for canalization. Exogenous auxin application leads to a lateralization of the auxin transport proteins PIN in cortex and endodermis root cells, process which is cell type-specific, does not depend on PIN transcription, and involves the Aux/IAA-signaling pathway. In order to identify the molecular mechanism behind the auxin-dependent PIN lateralization, we perform microarray experiments using the HS::*axr3-2*, and we pick the genes that respond to auxin and which are differentially regulated in the HS::*axr3-2* compared to wt plants. This approach provided first insights into the molecular mechanism governing this process. A brief description of the characterization of some candidate gene families is presented.

P3-19 IDENTIFICATION OF ROOT VASCULAR PATTERNING MUTANTS

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The vasculature of the plant functions as a long distance transport system for water, nutrients, sugars and hormones. In the root the two conducting tissue types, phloem and xylem are arrangement in a highly ordered pattern. To identify new genes involved in vascular tissue development, two different genetic screens based on ethyl methane sulfonate (EMS) mutagenesis have been performed. To identify genes involved in phloem development, mutants with alterd AtSUC2::GFP (a phloem marker) expression pattern were identified. The second screen was designed to isolate genes acting up- or downstream of AHP6, an inhibitory pseudophosphotransfer protein that counteracts cytokinin signaling thereby allowing protoxylem formation. Several mutants with AHP6::GFP misexpression were isolated.

Here we describe the isolation and characterization of three mutants with similar phenotypes. The mutants have a short root with a disorganized stele pattern and an increased number of phloem cells. The similar stele phenotypes strongly suggest that the three genes acting on the same patterning process within the stele.

P3-20 CYTOKININ COMPOSITION IN BUD DETERMINATION OF A CONIFER TREE

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Recent research has implicated cytokinins in bud determination. Our studies have shown that not only total cytokinin concentration but also cytokinin profile faithfully characterize buds, suggesting that cytokinin metabolism differs according to bud type. The conifer tree *Abies nordmanniana* produces a hierarchy of overwintering buds with predetermined growth potentials and developmental fates. Four buds type, i.e., leader bud, subapical main branch bud (whorl buds), terminal and subterminal buds on upper branch whorl were com-pared while the primordial shoot was developing within the confines of the bud scales (preformed growth), and up to the time before bud break the following year. The leader bud produces an orthotropic shoot, all other buds plagiotropic shoots.

A positive role of high total cytokinin concentration for the immediate and future growth potential is indicated, the leader bud accumu¬lating about two times as high concentrations (per g FW) as the neighbouring, and morphologically very similar, whorl buds. Cytokinin concentrations of branch buds were correspondingly lower, in accordance with their growth potential and position. The leader bud was distinguished by high transhydration ratio and low O-conjugation ratio compared to other bud types. All bud types showed about the same IAA levels and the same distinctive pattern of changes over time; most markedly a minimum during early needle production in midsummer. Auxin is thus not a distinguishing factor for bud determination.

P3-21 BARLEY (HORDEUM VULGARE L.) TRANSFORMATION WITH CONSTITUTIVE AND ROOT-SPECIFIC PROMOTERS CONTROLLING CYTOKININ DEHYDROGENASE EXPRESSION

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Cytokinin dehydrogenase (CKX) is an enzyme responsible for the irreversible degradation of plant growth regulators - cytokinins. Transgenic tobacco carrying barley gene *gHvCKX2* (AF490591) under 35S promoter showed distinctive cytokinin deficient phenotype with dwarf aerial part and enormously enhanced root system. Unfortunately, regeneration frequency of barley calli was very low after the transformation with *gHvCKX2* gene under the ubiquitin promoter. Finally from more than 2,000 transformation events, the only one transgenic barley plant overexpressing *gHvCKX2* was recovered. The plant was distinguished by smaller culms and less tillering compared to control plants. Root system was not appreciably enhanced. Onset of flowering was not observed and the plant died untimely. Transgenic barley plants transformed with maize gene *CKX1* (AF044603) under the ubiquitin promoter showed enhanced root system and reduced growth of aerial part. More then ten transgenic plants were regenerated, however all of them died in the early stage of development probably as a result of cytokinin depletion.

Root-specific barley promoters for genes encoded phosphate transporter (*HvPHT1*) and root abundant factor (*HvRAF*) were cloned and functionally tested in *Arabidopsis thaliana* using *GUS* reporter gene. Barley plants transformed with *HvPHT1::ZmCKX1* construct showed more branching of the root system whereas aerial part was unchanged in comparison with the control plants.

O4-1 LATERAL ROOT DEVELOPMENT: AN EMERGING STORY…

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Lateral roots originate deep within the parental root from a small number of founder cells at the periphery of the vascular tissues that must emerge through intervening layers of tissues. Despite its importance to the integrity of the root system, little is known about the regulation of lateral root emergence. Our experimental studies have recently revealed that lateral root emergence is a highly regulated process involving the active participation of cells in both new lateral root primordia and the parental root. The hormone auxin originating from the developing lateral root appears to act as a local inductive signal which reprograms adjacent cells. Auxin induces the expression of a previously uncharacterized auxin influx carrier LAX3 in cortical and epidermal cells directly overlaying new primordia. Increased LAX3 activity reinforces the auxin-dependent induction of a selection of cell wall remodelling enzymes, promoting cell separation in advance of developing lateral root primordia. Auxin therefore appears to act as a common signal that synchronizes lateral root primordium initiation[2], patterning[3] and emergence[1] processes.

I will describe recent efforts to model the auxin response pathway through which lateral root emergence is coordinated with the aim to exemplify how theoretical work informs us about the biological system and aids making testable predictions.

1. Swarup et al (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nature Cell Biology, 10, 946-954

2. de Smet et al (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. Development 134, 681-90

3. Benkova et al (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115, 591-602

O4-2 COMPARISON OF TRANSPORT ACTIVITY AND INTERACTIONS OF ABCB, AUX1, AND PIN AUXIN TRANSPORTERS

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PIN, AUX/LAX, and ABCB/PGP auxin transporters define independent, but coordinated, transport systems. Specific PINs and ABCBs (PIN1/ABCB19, PIN2/ABCB1) function in synergistic pairs, and loss of multiple transporters results in supra-additive phenotypes. Loss of ABCBs from discrete membrane subdomains results in decreased PIN stability and activity. Elucidation of ABCB impact on PIN function has been impeded by the lack of a consensus heterologous expression system. We have implemented an expression system in *S. pombe* which has plant-like polar sterol-rich membrane domains and a more plant-like N-glycosylation mechanism. The system includes knockouts of all ABC and putative auxin transport genes and vectors for functional analysis and localization of recombinant proteins. We expressed ABCB1 and ABCB19 in *mam1pdr1* lines under the inducible nmt41 promoter. ABCB19 showed higher 3H-IAA export activity than ABCB1. PIN proteins were expressed in an *auxin effluxer like 1* (*AEL1*) line. PIN1 showed higher activity than PIN2. AUX1 expressed in a *vat3* line resulted in increased net auxin uptake. Finally, ABCB4 expressed in *mam1pdr1* displayed a concentration-dependent reversal of 3H-IAA transport that is consistent with its observed activity *in planta*. Structural modelling suggests that ABCB4 has three substrate interaction sites rather than the two found in ABCB1/19, thus providing a rationale for the observed substrate activation.

O4-3 AUXIN INFLUX CARRIERS ARE INVOLVED IN REGULATING APICAL HOOK DEVELOPMENT OF ARABIDOPSIS.

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Development of the apical hook in dark grown seedlings is dependent on auxin and ethylene pathways. Hook establishment is based on a gradient of auxin signaling, while hook exaggeration is part of the triple response provoked by ethylene in *Arabidopsis*. The molecular mechanisms which lead to the initial installation of the auxin gradient are still poorly understood. We investigated the cross-talk of auxin and ethylene in the apical hook. Auxin measurements, the expression pattern of the auxin reporter DR5::GUS and the localization of expression of auxin biosynthesis enzymes and influx carriers, collectively indicate the necessity for auxin biosynthesis and efficient auxin translocation from the cotyledons and meristem into the hypocotyl, in order to support proper hook development. Auxin accumulation, both in the meristem and cotyledons and in the hypocotyl is increased 2-fold upon treatment with ethylene. In addition, a strong ethylene signal leads to enhanced auxin biosynthesis at the concave side of the hook. Furthermore, mutant analysis shows that the auxin influx carrier LAX3 is indispensable for proper hook establishment and that the auxin influx carrier AUX1 is mainly involved in hook exaggeration caused by ethylene.

O4-4 PINOID CONTROLS PIN1 POLAR TARGETING THROUGH EVOLUTIONARILY CONSERVED PHOSPHOSERINES.

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Polar transport of the plant hormone auxin generates maxima and gradients that control many aspects of plant development. The direction of transport is determined by the PIN-FORMED (PIN) auxin efflux carriers through their asymmetric subcellular distributions. Previously, we have shown that PIN polar targeting is controlled by reversible phosphorylation of the PIN hydrophilic loop (PINHL), through the antagonistic action of the PINOID (PID) AGC kinase and PP2A phosphatases. Here, we mapped PID-dependent phosphorylation to the evolutionarily conserved serine residues present in all HL-containing PIN proteins. Substitution of the serines for alanines in PIN1:GFP resulted in embryo and inflorescence defects typical for *pid* mutants, correlating with a basal PIN1:GFP polar localization. The mutant versions of PIN1:GFP were insensitive to PID overexpression in the root, and phosphomimic *PIN1:GFP* mutants were able to complement *pin1* mutant phenotypes. Our results show that simultaneous PID phosphorylation of the conserved serines is required and sufficient for PIN1 apical targeting, and thus for proper plant development. The data imply that AGC kinase regulation of PIN polarity arose as a conserved mechanism to orient plant development around the time when plants conquered the land.

O4-5 UP AND DOWN AND ALL AROUND: PIN POLARITY REGULATION IN ARABIDOPSIS

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

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

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

O4-6 MECHANISTIC FRAMEWORK FOR POLAR PIN TARGETING

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The directional transit of the phyto-hormone auxin from cell to cell plays a decisive role in determining and redefining the polarity of plant tissues (Scarpella et al., 2006). Moreover, spatial and temporal auxin accumulations (auxin gradients) determine positional cues for the presumptive sites of primordial development (Benkova et al., 2003; Friml et al., 2003; Reinhardt et al., 2003). Hence, mechanisms that guide the auxin distribution and signaling represent a key to understand plant growth. The directional distribution of the phytohormone auxin depends largely on the PIN-FORMED (PIN) auxin efflux carriers that catalyze auxin transport from cell-to-cell. The coordinated polar localisation of PIN proteins at different cell sides determines the direction and rate of auxin flux (Wisniewska et al., 2006; Petrasek et al., 2006). Thus, the dynamic nature of the flexible polar PIN localisation regulates plant development by redefining the directional output of the auxin flux (Benkova et al., 2003; Kleine-Vehn et al., 2008). Here we present novel insight into polar PIN targeting. We used high end microscopy to address fundamental mechanism of polar exo-, endocytosis, and reduced lateral PIN diffusion within the plasma membrane. Our data indicates that these processes jointly work to establish and maintain the robust, polar PIN deposition.

O4-7 THE NPA-BINDING PROTEIN TWISTED DWARF1 CONTROLS ABCB-MEDIATED AUXIN TRANSPORT

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Cellular efflux by the independent and interactive action of ABCB/PGP- and PIN catalysts is the rate-limiting step of polar auxin transport. Here, we summarize recent progress in ABCB interaction with immunophilinlike FKBP42, TWISTED DWARF1 (TWD1). TWD1 co-localizes partially with

ABCB1 and ABCB19 in the root epidermis and stele, respectively. Using yeast and *in planta* BRET (bioluminescent resonance energy transfer) assays, we show that ABCB1-TWD1 interaction is disrupted by auxin transport inhibitors, leading to inactivation of ABCB1 activity. NPA binds to ABCBs but surprisingly also to the N-terminus of TWD1. As a consequence, auxin fluxes and gravitropism of *twd1* roots are less NPA sensitive while gain-of-function alleles display faster bending kinetics.

Our data demonstrate that TWD1 and ABCB1 are key components of the NPA-binding protein complex building the basis for the establishment and control of plastic asymmetric auxin fluxes.

O4-8 POST-TRANSCRIPTIONAL CONTROL OF PIN EXPRESSION BY AN ARABIDOPSIS THALIANA ELONGATOR COMPLEX

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Tight transcriptional and post-transcriptional regulation of PIN auxin efflux carriers is a prerequisite for their concise spatial and temporal expression patterns, which is essential for a coordinated distribution of the growth regulator.

In a mutant screen aimed at the characterization of modifiers of PIN expression we identified the *modulator of pin* (*mop*) mutants that exhibit significantly reduced PIN protein levels. We now have identified *MOP2* and *MOP3* as subunits of a putative *Arabidopsis* Elongator Complex.

The characterization of Elongator in non-plant eukaryotes suggested its involvement in various fundamental cellular processes, amongst which deficiencies in translational control might account for the additional defects associated with Elongator mutants. We initiated a functional characterization of subunits of the *Arabidopsis* Elongator Complex and found evidence for an important role of translational control in the model plant. We demonstrate that *Arabidopsis mop* mutants seemingly lack 5-methoxycarbonylmethyl (mcm^5) groups at wobble uridines of certain tRNA species, similar to the situation in yeast. Moreover, targeted degradation of mcm^5–modified tRNAs in *Arabidopsis*, results in a further accentuation of auxin-related *mop* phenotypes. These and additional findings support the view that diminished PIN protein levels in *mop* mutants are a consequence of translation defects due to aberrant maturation of tRNAs.

Collectively, our results support a scenario, in which codon usage and tRNA availability represent another means for adjustments in auxin distribution. A possible role of these quite general mechanisms in the control of PIN protein availability will be discussed.

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O4-9 ROCK1 ENCODES A PUTATIVE TRANSPORT PROTEIN OF UNKNOWN FUNCTION

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Cytokinin deficiency causes complex phenotypic changes such as retarded shoot and enhanced root growth. To identify new functional elements of the cytokinin pathway, we carried out suppressor mutagenesis of *35S:AtCKX1*-overexpressing *Arabidopsis* plants and screened for reversion of the cytokinin deficiency phenotype. We isolated a recessive second-site suppressor allele termed *rock1 (repressor of cytokinin deficiency* 1), which caused a prominent shoot reversion associated with increased concentration of specific cytokinin metabolites. The corresponding gene was cloned by a map-based approach. A missense mutation was localized in a locus being predicted to code for a putative membrane transport protein with a low sequence homology to nucleotide-sugar transporters. This gene has no paralogs in *Arabidopsi*s and other plant species contain presumably only one gene of this kind. We show that the *ROCK1* expression was predominantly associated with the vascular system, root meristem and shoot apex. ROCK1-GFP localized to Golgi vesicles. A strong over-expression caused lethality during the early seedling stage. We will discuss the possible role of ROCK1 in maintaining cellular cytokinin homeostasis.

P4-1 ROLE OF THE AUXIN BINDING PROTEIN 1 (ABP1) IN INTRACELLULAR AUXIN MANAGEMENT

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The intercellular flow of auxin and availability of physiologically active free auxin indole-3-acetic acid (IAA) is important for plant development. On cellular level, essential participation of ABP1 has been proved for control of cell division and of some plasma-membrane (PM)-residing processes. In the experimental system of suspension-cultured BY-2 cells (*Nicotiana tabacum* L., cv. Bright Yellow 2), a function of ABP1 becomes evident in case of changed auxin transport and/or availability of free auxin. The increase of cellular auxin efflux caused by overproduction of the auxin-efflux carriers ('long' PINs residing on the PM, typically PIN7) makes cells auxin-depleted, and thus elongated and of low cell division frequency. Under these conditions the overexpression of ABP1s rescues the proper cell shape and size, cell division frequency as well as intracellular IAA content. In the cells overexpressing PIN5, the representative of the endoplasmic-reticulum (ER)-residing 'short' PINs, all auxin is directed to the ER where IAA is converted to its metabolites. In the cell line overproducing PIN5 together with ABP1, the auxin binding protein 1 can bind and preserve some non-metabolized IAA for immediate usage. Thereby, ABP1 contributes to maintenance of auxin homeostasis within plant cells.

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P4-2 EXOCYST IS INVOLVED IN THE REGULATION OF POLARIZED PIN AUXIN EFFLUX CARRIER LOCALIZATION

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The exocyst, an octameric vesicle tethering complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, Exo84), localizes to specific domains of the plasma membrane and facilitates early events of polarized secretion in yeast and animals. Recently we proved that the exocyst functions as a complex also in plants and is involved in the regulation of plant cell polarity and morphogenesis (Hála et al. 2008).

Here we show that auxin related phenotype of Arabidopsis *exo70A1* mutants (Synek et al. 2006) could be virtually explained by reduced polar auxin transport (PAT) due to slowdown in targeting/cycling of auxin efflux carrier proteins PIN1 and PIN2. Reestablishment of polarized PINs localization after the BFA wash off is largely delayed in *exo70a1* seedlings roots. Transcytosis of PIN1 in root stele cells and PIN2 in root cortical cells caused by prolonged BFA treatment is inhibited in *exo70A1* seedlings. Immunolocalization of Exo70A1, Sec6 and Sec8 in the roots showed preferential localization at the transversal plasma membrane and there were no changes in localization of these proteins after 50μM BFA treatment. We conclude that phenotypes of *exo70A1* mutants are at least in part consequence of the defect in polar auxin transport and disturbed hormonal homeostasis. We assume that Exo70A1 functions as an exocytosis landmark and along with other exocyst subunits in tethering of membrane vesicles carrying PIN1 and PIN2 proteins to the plasma membrane.

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P4-3 A NOVEL ARABIDOPSIS FORWARD GENETICS SCREENING LEADS TO IDENTIFICATION OF PIN POLAR TARGETING REGULATORS

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Auxin is the hormone that coordinates plant development and more, by a directional flow through the plant tissues, it mediates the polarity of tissues and organs. The directional auxin flow (known as polar auxin transport; PAT) is achieved by the subcellular localization of auxin influx and efflux carriers. Plasma membrane localized PIN proteins have been identified as auxin efflux carriers (Petrášek et al., 2006) and their polar localization determines the direction of auxin flow (Wiśniewska et al., 2006). In order to understand how PIN proteins are delivered to the correct side of the cells and to identify new regulators of PIN polarity we EMS-mutagenized transgenic PIN2:PIN1:HA, an agravitropic line showing basal localization of PIN1:HA in the epidermal and cortical cells. We identified 5 novel *Arabidopsis* mutants displaying positive gravitropic response and changed localization of PIN1:HA in the epidermal cells. The screening and some data characterizing the mutants will be presented.

P4-4 INTERACTOR OF CONSTITUTIVE ACTIVE ROP(ICR1) TAKE PART IN REGULATION OF POLAR AUXIN TRANSPORT.

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The phytohormone auxin functions as a morphogen and is required for almost every aspect of plant development. Auxin flow is polar and is regulated by asymmetric distribution of PIN family efflux carriers. The plant Rho GTPases (ROPs) are master regulators of cell polarity, however their role in regulating polar protein trafficking and polar auxin transport has not been established. We show that the ROP interactor scaffold protein ICR1is required for recruitment of PIN proteins to the polar domains at the plasma membrane. ICR1 functions at the plasma membrane, but does not recycle together with PINs. DR5, a synthetic auxin response element in *icr1* mutant background, shows defects in auxin dispersing: auxin maxima in root meristem is moved or disappear. ICR1 expression is quickly induced by auxin but is suppressed at the positions of stable auxin maxima in the hypophysis and later in the embryonic and mature root meristems. Our results imply that ICR1 is part of an auxin regulated positive feedback loop realized by a unique integration of auxin-dependent transcriptional regulation into ROP-mediated modulation of cell polarity.

P4-5 PGP1 AND PGP19 SHOW SIGNIFICANT NAA EFFLUX IN ARABIDOPSIS THALIANA PLANTS

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P-glycoproteins (PGPs) are well-known as transporters of a wide group of chemical compounds in plants. PGP1 and PGP19 mediate auxin efflux in plant, yeast and animal cells. Recently, the data on the interaction of PGPs and PINs in the local auxin distribution in plant tissues was presented. However, the relevance of PGPs´ activity in auxin transport in intact plants and/or their organs has not been demonstrated yet. Therefore, we have used *Arabidopsis thaliana* plants expressing *AtPGP1* and *AtPGP19* under inducible promoters to characterize the auxin transport activity of PGPs in stem segments. Experiments with radiolabeled NAA showed significant differences in accumulation levels between non-induced and induced plants. The effects of inhibitors NPA and Gravacine on NAA accumulation levels were also investigated.

We show that PGP1 and PGP19 are important for long-distance auxin transport in stems, and the overexpression of *PGP1* and *PGP19* genes in *Arabidopsis* plants results in enhanced acropetal auxin transport in stem segments.

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P4-6 DIFFERENTIAL EFFECTS OF AUXIN INFLUX INHIBITORS 1-NOA AND 2-NOA ON THE ACTIVITY OF AUXIN CARRIERS

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Auxin is the phytohormone transported through plant body typically by specialized polar transport machinery. This machinery consists of a balanced system of passive diffusion combined with the activities of the auxin influx and efflux carriers. We have characterized the sensitivity of auxin carriers towards putative auxin influx inhibitors 1-naphthoxyacetic acid (1-NOA), 2-naphthoxyacetic acid (2-NOA) and 3-chloro-4-hydroxyphenylacetic acid (CHPAA). Using assay based on measurement of radioactively labelled auxin accumulation in BY-2 tobacco cells we have revealed that 1-NOA exhibits a mode of action different from the other auxin influx inhibitors 2-NOA or CHPAA. In addition, 1-NOA involves additional auxin efflux-blocking activity that is independent on the vesicle trafficking processes. 1-NOA inhibits cell division intensity in suspension-cultured BY-2 tobacco cells. Altogether, these findings demonstrate that 1-NOA, in contrast to 2-NOA and CHPAA, is not a suitable tool for the specific inhibition of the auxin influx carrier(s), and its action involves also the inhibition of the active auxin efflux.

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P4-7 FM DYES AFFECT THE LOCALIZATION AND ACTIVITY OF AUXIN TRANSPORTERS IN CELL CULTURE

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FM styryl dyes are widely used fluorescent probes marking processes of endocytosis and vesicle trafficking in eukaryotic cells. During our studies of proteins involved in the auxin efflux machinery we have observed yet unexplored effects of FM dyes. On the examples of plasma membrane-localized A.t. auxin transporters expressed in cell culture (BY2 or A.t.) we show, that routinely used concentrations of FM 4-64 and of FM 5-95 dye trigger transient internalization of these proteins. This process is active and seems to be cell culture specific because it was not observed in meristematic roots. In addition, FM1-43 severely affected both auxin efflux and influx as well as lipid metabolism. These results point to the necessary circumspection during in vivo studies of membrane proteins performed with simultaneous labelling with FM dyes.

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P4-8 TRANSPORT OF CYTOKININS IN BY-2 SUSPENSION-GROWN TOBACCO CELLS - TOWARDS A MATHEMATICAL MODEL

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Cytokinins (CK) are indispensable plant growth regulators with a broad range of effects on plant development. Their spatial distribution and thus the action are therefore precisely regulated. This control consists of metabolic regulation (synthesis, conjugation, degradation) and transport on the levels of the whole plant, its organs, tissues as well as cells. Recently, two groups of plant transmembrane transport facilitators were discovered to possibly play a role in CKs transport: purine permeases and equilibrative nucleoside transporters. Here we use tobacco BY-2 suspension-grown cells, a well-established auxin transport model, to demonstrate the characteristics of *trans*-zeatin (*t*Z) and *trans*-zeatinriboside (*t*ZR) uptake in the wild-type situation. We show the characteristics of ^{3}H-*t*Z and ^{3}H-*t*ZR accumulation and homologous and heterologous displacement studies with different types of CKs. Our data indicate distinct features of *t*Z and *t*ZR translocation and so they should serve as a basis for future mathematical characterization of CK transmembrane transport.

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P4-9 REGULATION OF AUXIN EFFLUX BY REACTIVE OXYGEN SPECIES

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Polar auxin transport is an important process influencing plant growth and development. However, the complex regulation of this process are still poorly understood. We have revealed that auxin efflux is inhibited by scavengers of reactive oxygen species (ROS). The effects of ROS scavengers on auxin transport were assessed by measurements of accumulation of radiolabelled compounds in cells in suspension culture (accumulation assay). Different radiolabelled compounds were employed to distinguish between effects on auxin efflux, auxin influx and on the transport of non-auxinic compounds. The effects of compounds influencing auxin efflux on redox potential in the cells were assessed by fluorometric measurements of redox-sensitive dyes. To study in more details the involvement of ROS in regulation of auxin efflux, we tested the effects of combination of modulation of ROS signalling with chemicals influencing auxin efflux or effects of ROS scavengers, together with genetic manipulations of auxin efflux machinery.

The scavengers of ROS inhibited auxin efflux specifically as they had no effect on uptake of auxins into cells or on the transport of other organic compounds.

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P4-10 EFFECT OF AUXIN ON PIN LOCALIZATION

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The plant signaling molecule auxin influences a remarkable variety of plant developmental processes. The current model proposes that plant cells integrate internal and external signals at the level of the polarity of auxin transport components (PIN proteins) and via the redirection of auxin fluxes which translate them into adaptive developmental changes. It has been shown that PIN proteins are not statically localized at their polar plasma membrane domains but show constitutive recycling between the plasma membrane and endosomes (Geldner et al., 2001). This cycling may enable rapid changes in subcellular PIN polarity (Benková et al., 2003; Friml et al., 2002; Reinhardt et al., 2003; Scarpella et al., 2006). Recent data showed that PIN2 degradation is also a key component of the regulation of development (Kleine-Vehn et al., 2008, Laxmi et al., 2008). Interestingly, auxin is able to repress quickly the endocytosis of PIN proteins and has also an influence on PIN protein stability. This provides a means for PIN accumulation at the plasma membrane and, thus, an essential feed-back regulation of auxin transport by auxin itself (Paciorek et al., 2005). The regulatory mechanisms of auxin itself on auxin transport components localization and stability will be discussed.

P4-11 TRANSPORT OF AUXINS IN ARABIDOPSIS CELL SUSPENSION

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Polar transport of plant hormone auxin is an important process determining many developmental processes. Most of the knowledge on genetic and developmental aspects of polar auxin transport comes from *Arabidopsis* plants and/or its organs and tissues. However, at the cellular level, kinetic parameters of auxin transport across membrane were described using predominantly suspension-cultured tobacco cells. It was shown there that naphthalene-1-acetic acid (NAA) penetrates into cells by passive diffusion and it is actively transported out of cells. On the contrary, another synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) enters cells only actively and it is a very weak substrate for efflux carriers. Native auxin, indole-3-acetic acid (IAA), is transported, depending on its dissociation form, both passively and actively into the cell and as an anion at intracellular ph 7.0 it is transported only actively via efflux carriers out of the cell.In *Arabidopsis* cell lines kinetic parameters of cellular auxin flow are completely missing. Here, we show the characteristics of auxin transport using *Arabidopsis* cell suspension. Our results on *Arabidopsis* cells prove higher rate of actively transported auxin molecules into and out of cells compared to tobacco cells. Moreover, not only rate of the active transport but also specificity towards particular auxins seems to be different between these two populations of cycling cells reflecting possibly species-specific differences.

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P4-12 SPECIFICITY OF AUXIN TRANSPORT AND PERCEPTION: A STUDY WITH AUXIN ANALOGUES

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Auxin structural analogues were screened to assess their physiological auxin activity, direct involvement in TIR1-mediated auxin signaling pathway, ability to inhibit endocytosis and their polar auxin transport potential in order to explore the specificity aspects of auxin mode of action. Physiological auxin activity of all compounds both on organ and cellular levels studied by *Arabidopsis* root growth assay and measurement of BY-2 (*Nicotiana tabacum* L., cv. Bright-Yellow 2) cell division activity, respectively. Their involvement in TIR1-mediated auxin signalling pathway was studied by assessing the induction of the auxin-responsive reporter DR5rev::GFP in the roots of *Arabidopsis* seedlings. Inhibitory effect on endocytic step of constitutive cycling was analyzed by tracking the FM4-64 labelled vesicle cycling. Transport kinetics of various auxin analogues has been studied in suspension-grown tobacco BY-2 and *Arabidopsis thaliana* ecotype Landsberg erecta cells on both influx and efflux levels using displacement of radio-labelled auxins by non-labelled auxin analogues. Our results demonstrate that, apart from few exceptions, physiological auxin activity, TIR1-mediated auxin signalling pathway, inhibition of endocytosis and polar auxin transport machinery share the same structure-activity relationships and they seem to be interlinked.

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P4-13 METABOLIC CHANGES ASSOCIATED WITH FUNCTION OF AUXIN CARRIER ATPIN5

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The PIN5 protein belongs to the PIN-FORMED (PIN) protein family, consisting of eight members of *Arabidopsis* transmembrane proteins with predicted transport function. Members of this family are generally recognized so far as auxin efflux carriers and acknowledged for its key role in polar auxin transport, the process by which plant control spatiotemporal distribution of phytohormone auxin and consequently control the overal body plan development.

In recent publication we demonstrated that *At*PIN5, one of three uncharacterised members of the family, localises unexpectedly in the membranes of endomembrane system, namely to the endoplasmic reticulum, and proved that the protein can execute its transport function there. To determine the cause of observed reduction of internal level of free IAA after *At*PIN5 overexpression, we used high-performance liquid chromatography (HPLC) to monitor products of metabolism of tritiated IAA in the BY-2 cells containing *At*PIN5 under control of glucocorticoid-inducible system. We demonstrated that change in the expression of *At*PIN5 proteins affects metabolic fate and consequently the homeostasis of the auxin, presumably due to its deposition to the compartment with different enzymatic system. New methodolical approach - HPLC profiling of ^{3}H-IAA metabolites allows us to monitor changes in metabolism of auxin and to check their potentiall to affect auxin transport.

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P4-14 THE ROLE OF THE PHOSPHORYLATION SITE IN THE PID-DEPENDENT PIN POLAR TARGETING

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Intercellular transport of the phytohormone auxin is essential for normal plant growth and development. Plant-specific PIN-FORMED (PIN) proteins mediate auxin efflux and determine the rate and direction of auxin flow on behalf of their polar plasma membrane localization. PID (Serine/threonine protein kinase) and PP2A (the protein phosphatase 2A) are the only documented molecular components directly involved in the regulation of intracellular polar delivery of PIN proteins. Here, we address that phosphorylations occur at specific Serine and Threonine residues in the central hydrophilic loop of PIN1 sequence are required for basal-apical PIN1 targeting through PID-dependent PIN phosphorylation.

P4-15 FORWARD GENETIC SCREEN TO IDENTIFY COMPONENTS OF AUXIN DEPENDENT INHIBITION OF ENDOCYTOSIS

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Local asymmetric distribution of auxin triggers a variety of developmental processes in plants, such as axis formation, meristem activity and maintenance, organ differentiation, and directional growth (tropisms). Auxin gradients are established by action of polarly localized auxin efflux carriers (PIN proteins) which enable the directional auxin flow between cells. PIN proteins constitutively cycle between the plasma membrane (PM) and endosomal compartments. Brefeldin A (BFA) inhibits PIN recycling from endosomes to the plasma membrane which leads to PIN internalization and accumulation in the so called 'BFA compartments'. Naturally occurring auxins such as IAA and its synthetic analogue naphthalene-1-acetic acid (NAA) efficiently inhibit the BFA-induced internalization of PIN proteins promoting their retention at the plasma membrane.

To understand the molecular mechanism, by which auxin inhibits endocytosis we performed a forward genetic screen on EMS mutagenised population of pPIN1::PIN1-GFP seeds. M2 seedlings were pre-treated with NAA and subsequently incubated in NAA/BFA mixture and screened for the presence of 'BFA compartments' in the cells, which indicate an alternation in the auxin effect on endocytosis. Here we present cellular and seedling phenotypes of mutants which show a defect in auxin-dependent endocytosis.

P4-16 POLAR AUXIN TRANSPORT: IS AUXIN EXOCYTOSIS INVOLVED?

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The most characteristic feature of auxin is its polar auxin transport (PAT) traversing the whole plant body. In the root apex, this PAT is driven by complex network of auxin-transporting proteins of PIN and ABC transporter families. Auxin, being a weak organic acid, is supposed to be transported by the mechanism described by chemiosmotic theory which considers only two relevant compartments: pH neutral cytoplasm and acidic cell walls. However, auxin transporters are recycling via endosomes and these are then equipped with transporters which should transport auxin into their acidic interior. In fact, this endosomal interior is topologically corresponding to the cellular exterior, so that the transport of auxin out of the cell across the plasma membrane and into endosomal vesicles and endosomes represent the same process. Until now, no study showed that these auxin transporters are inactive when inserted into endocytic vesicles and endosomes. We have taken advantage of the Arabidopsis PLD Zeta2 mutant lines to demonstrate that in cells of the root apex transition zone, up to 50% of cell-to-cell transport of auxin is possibly accomplished via exocytosis. This finding explains why there is close correlation between rates of the PAT and of the vesicular recycling driven by secretory endosomes. This model is also able to explain why and how is the PAT organized by mechanical forces imposed on plant cells via gravity. In conclusion, our data suggest that exocytosis of auxin is inherent part of the PAT in the root apex. This process is based on regulated secretion of auxin from recycling endosomes across the cross-walls (plant synapses) characterized via mechanical asymmetry of their two (cellular top and bottom with respect to the gravity vector) plasma membranes.

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O5-1 LONG RANGE SIGNALLING IN THE CONTROL OF SHOOT BRANCHING

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Plants continuously adjust their body plan to suit the environmental conditions in which they are growing. A good example of this is in the regulation of shoot branching. Axillary meristems, which are established in each leaf formed from the primary shoot apical meristem, can remain dormant or activate to produce a branch. The decision whether to activate an axillary meristem involves assessment of a wide range of external environmental, internal physiological and developmental factors. Much of this information is transmitted via a network of interacting hormonal signals that can integrate multiple inputs to generate a rich source of systemically transmitted information. We are studying this network, combining molecular biological, physiological and quantitative genetic approaches with computational simulation to try to understand how the component parts of the system are able to deliver environmentally responsive shoot branching patterns.

O5-2 AUXIN - CYTOKININ INTERACTION SHAPING ROOT ARCHITECTURE

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Plant development is characterized by a continuous growth and flexible adjustments of the plant architecture in response to the environment. Plants are able to maintain permanent stem cell populations, dedifferentiate already committed cells and, not least, to regenerate or form organs *de novo*. These developmental processes are governed and coordinated by signalling substances called plant hormones. Typically, in certain developmental processes more than one hormone is involved and, thus, coordination of their overlapping activities is crucial for correct plant development. Multiple hormones including cytokinin and auxin have been implicated in the regulation of root development however, molecular mechanisms underlying the hormonal cross-talk are only poorly understood. Our investigation of mechanisms of auxin-cytokinin revealed that CK regulates the cell-to-cell auxin transport by modulating expression of several PIN auxin efflux carriers. We propose a model for regulation of the auxin – cytokinin balance that is critical for root organogenesis. By modulating the auxin transport CK might control the auxin levels in cells and, thus, the ratio between auxin and CK. (The work was supported by the ERC starting independent research grant).

O5-3 APICAL DOMINANCE IS CONTROLLED BY INTERACTION BETWEEN CYTOKININ BIOSYNTHESIS/DEGRADATION AND AUXIN IN STEM.

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Apical dominance is a phenomenon in which a terminal bud inhibits the outgrowth of axillary buds. Although involvement of auxin, which represses axillary bud outgrowth, and cytokinin (CK), which promotes axillary bud outgrowth, has been proposed, little is known about the underlying molecular mechanisms. Firstly, we demonstrated that auxin negatively regulates local CK synthesis in the nodal stem by controlling the expression level of the gene pea *adenosine phosphate–isopentenyltransferase* (*PsIPT*), which encodes a key enzyme in CK biosynthesis. Before decapitation, *PsIPT1* and *PsIPT2* transcripts were undetectable; after decapitation, they were markedly induced in the nodal stem along with CK accumulation. *PsIPT* expression was repressed by the application of indole-3-acetic acid (IAA). In excised nodal stem, *PsIPT* expression and CK levels also increased under IAA-free conditions. Furthermore, *de novo*-synthesized IAA derived from a new shoot apex, which had previously been a dormant axillary bud, not only flowed to the stem 10 h after decapitation and again repressed *PsIPT* expression, but also induced gene expression of CK oxidase, which degrades CKs in the stem. As the result, CK levels in the stem were low again. These results indicate that, in apical dominance, one role of auxin is to control local CK level in the nodal stem.

CYTOKININS MODULATE AUXIN-INDUCED ORGANOGENESIS IN PLANTS VIA REGULATION OF THE AUXIN EFFLUX

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Cytokinins (CKs) and auxins were shown to be the principal regulators of plant organogenesis. However, the molecular mechanisms of the auxin/CKs interaction are still largely unknown. Here we show that auxin is able to induce organogenic response in hypocotyl explants, while CK modulates the auxin-induced organogenesis via regulation of intercellular auxin distribution. *De novo* organogenesis is accompanied with production of endogenous CKs and tissue-specific activation of CK signalling. The CK-mediated modulation of organogenesis could be simulated by the polar auxin transport inhibitor. CKs reduce auxin efflux from tobacco cells and regulate the expression of auxin efflux carriers from the *PIN* family. Finally, endogenous CKs are necessary to maintain proper auxin distribution and *PINs* expression in *Arabidopsis* roots. Based on these findings we propose a model, in which auxin acts as a trigger of the organogenic processes, whose output is modulated by the endogenously produced CKs via regulation of expression of auxin efflux carriers.

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O5-5 REGULATION OF AXILLARY BUD OUTGROWTH BY STRIGOLACTONES

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Strigolactones have recently been identified as the novel plant hormone regulating shoot branching. Mutants disrupted in *CAROTENOID CLEAVAGE DIOXYGENASE* genes, *CCD7* and *CCD8*, are deficient in strigolactones and exogenous strigolactones can restore branching inhibition to these plants. Strigolactones act at nM concentrations and in a dose-dependent manner. Decapitation, auxin transport and auxin-mutant studies show strigolactone functions down-stream of auxin. In this sense, strigolactones may not require auxin, or auxin signalling, to inhibit branching. It is not yet entirely clear whether strigolactones can act independently of cytokinin but preliminary evidence suggests that strigolactone deficiency alone is not sufficient to ensure outgrowth.

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Ferguson, B.J. and Beveridge, C.A. (2009) Roles for auxin, cytokinin and strigolactone in regulating shoot branching. Plant Physiol. 149: 1929-1944.

O5-5 SPATIAL AND TEMPORAL REGULATION OF AUXIN AND CYTOKININ GENE EXPRESSION AND RESPONSES IN PEA RAMOSUS MUTANTS

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We are examining cytokinin (CK) and auxin biology in pea *ramosus* (*rms*) mutants, now known to be defective in a third hormone, strigolactone. We measured transcripts and hormones following nodal isolation, decapitation and/or auxin treatment. Contrary to previous reports for decapitated plants, *rms* mutants retain near-normal auxin response in isolated nodes, based on inhibition of bud outgrowth and suppression of *PsIPT* expression required for CK biosynthesis. In decapitated or isolated stems of all genotypes, *PsIPT* and *PsPIN* transcripts are increased and reduced, respectively, but both are restored by auxin. In contrast, *PsAUX1* and *PsIPT* expression is substantially reduced in *rms* buds, but not stems. Cytokinin levels generally matched *IPT* expression in stems but not in buds. In *rms* buds, high CK levels and low *IPT* expression implicate a local feedback loop. Multiple, partially independent pathways appear to regulate hormone levels and bud outgrowth. Rapid decapitation-induced branching can be controlled largely by CK and auxin, independently of strigolactone signalling, whereas branching in intact plants involves interactions of all three hormones.

(The work was supported by BBSRC UK.)

O5-6 COMPETITIVE CANALIZATION OF PIN-DEPENDENT AUXIN FLOW FROM AXILLARY BUDS CONTROLS APICAL DOMINANCE IN PEA

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Shoot branching is one of the major determinants of plant architecture and for its control by a dominant apex transport of the phytohormone auxin in the stem is known to be necessary. Here we show that in pea (*Pisum sativum* L.) the axillary buds establish the directional auxin export by subcellular polarization of PIN auxin transporters following decapitation. Apical auxin application on the decapitated stem prevents this PIN polarization and canalization of laterally applied auxin. These results support a model in which the apical and lateral auxin sources compete for primary channels of auxin transport in the stem to control release of axillary buds from dormancy.

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P5-1 THE MUTUALLY INHIBITORY INTERACTION BETWEEN AUXIN AND CYTOKININ REGULATES PROTOXYLEM IDENTITY

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The protoxylem cell lineages are the first xylem cells to differentiate in the Arabidopsis root and offer the plant a transport channel with which to transport water and nutrients from root to shoot. We have previously shown that low cytokinin levels are a prerequisite to protoxylem differentiation and that AHP6 inhibits cytokinin in a spatially specific manner allowing protoxylem to differentiate. We now show that the interaction between cytokinin and auxin signalling patterns the xylem axis defining the location in which protoxylem cells differentiate.

P5-2 PHYTOHORMONE ANALYSIS AND EFFECTS OF INDOLE-3-ACETIC ACID ON TUBERIZATION IN TRANSGENIC IN VITRO CULTURED POTATO PLANTS EXPRESSING ATCKX GENES

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Auxins and cytokinins (CKs) interact in control of many developmental processes in plants. The CK pool in plant tissues is efficiently down-regulated by cytokinin oxidase/dehydrogenase (CKX) activity. Transgenic plants overexpressing *CKX* genes could be a valuable tool for investigation of auxin/CK interactions. With this regard, we analyzed contents of endogenous phytohormones (CKs, indole-3-acetic acid [IAA] and abscisic acid [ABA]), CKX activity and IAA effects in *AtCKX1* and *AtCKX2* transformed potato plants grown *in vitro*. For selected lines, the expression of *AtCKX* genes confirmed by RT-PCR in both roots and shoots was associated with an increase in CKX activity and with changes in CK, IAA and ABA levels. Exogenously applied IAA (0-10 μM) enhanced tuber formation in both transgenic and control plants. Unlike control, transformed plants showed presence of tubers even in tuber non-inducing conditions (long day).

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P5-3 BUD OUTGROWTH: STRIGOLACTONES, AUXIN & CYTOKININ

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Strigolactones were recently discovered as the long-sought-after shoot branching inhibitor. We tested its mode of action in relation to auxin and cytokinin-mediated control of branching. Auxin was previously shown to require the novel branching inhibitor to repress decapitation-induced bud outgrowth, and to regulate branching inhibitor biosynthesis gene expression. In agreement, we found that exogenous strigolactone completely inhibited pea bud outgrowth, even when auxin was depleted via decapitation. In addition, strigolactone treatment reduced branching in *Arabidopsis* auxin response mutants. This suggests that auxin mediates apical dominance via strigolactones. One hypothesis for strigolactone function is that it regulates polar auxin transport (PAT) properties to modulate bud outgrowth. However, compared to complete inhibition by strigolactone, application of the PAT inhibitor NPA to activated pea buds took several days to slow growth. Also, while strigolactone or NPA applied to larger buds reduced bud lengths, only NPA rapidly blocked PAT in buds. This highlights differences between strigolactone and PAT inhibitors, and also suggests that auxin transport out of buds is required for sustained, but not initial, bud growth. Recent results on interactions between strigolactone and cytokinin in bud outgrowth regulation will also be presented.

ARCANE CYTOKININ CIS-ZEATIN VS. TRANS-ZEATIN – STRUCTURAL TWINS YET DIFFERING IN ORGANOGENETIC MIGHT?

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Zeatin is isoprenoid cytokinin (CK) occurring in two isomers, *cis* and *trans,* referring to the position of terminal hydroxyl group at the isoprenoid moiety. *cis*-zeatin (*cis*Z) was identified in numerous plant species but to these days with unassigned specific function. To address its role we focused on organogenesis, which depends on auxin flow facilitated by auxin efflux carriers and modulated by CKs.

We found that *cis*Z riboside (*cis*ZR) increases auxin accumulation in the BY-2 cells with the same sensitivity as *trans*ZR probably due to similar impact on PIN expression and/or mode of activity. *cis*ZR also decreased DR5:GUS signal in *Arabidopsis* roots suggesting limited auxin transport. Furthermore a combination of auxin and *cis*ZR caused a slight additive effect on root growth inhibition though *trans*ZR with auxin showed a stronger reduction. The *de novo* organ formation in presence of auxin unveiled that both isomers induce root-like structures but unlike *trans*ZR, *cis*ZR was not able to provoke callus formation on whole hypocotyl just at the cut ends of the explants. Thus we conclude that dissimilarities in actions of both isomers might be uncovered on different levels of auxin transport and/or signaling.

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P5-5, A STRIGOLACTONE INSENSITIVE MUTANT OF RICE, SHOWS AN ACCELERATED OUTGROWTH OF TILLERS

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It has been long thought that auxins and cytokinins play major roles in the control of shoot branching. Recent studies usinghighly branched mutants of pea, *Arabidopsis* and rice have demonstrated that strigolactones, a group of terpenoid lactones, act as a new hormone class, or its biosynthetic precursors, in inhibiting shoot branching. Here, we provide evidence that *DWARF14* (*D14*) inhibits rice tillering and may act as a new component of the strigolactone-dependent branching inhibition pathway. The *d14* mutant exhibits increased shoot branching with reduced plant height as previously characterized strigolactone-deficient and -insensitive mutants, *d10* and *d3*, respectively. The phenotype of *d10-1 d14-1* double mutant indicates that *D10* and *D14* function in the same pathway. However unlike with *d10*, the *d14* branching phenotype could not be rescued by exogenous GR24, a synthetic strigolactone analogue. In addition, the *d14* mutant contained a higher level of 2'-epi-5-deoxystrigol than the wild type. Positional cloning revealed that *D14* encodes a protein of the α/β-fold hydrolase superfamily, some members of which play a role in metabolism or signaling of plant hormones. We propose that D14 functions downstream of strigolactone synthesis, as a component of hormone signaling or as an enzyme that participates in the conversion of strigolactones to the bioactive form.

P5-6 AUXIN-CYTOKININ CROSS-TALK IN SHOOT BRANCHING

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In seed plants, during postembryonic development secondary meristems are formed along the primary shoot axis. They are located between the stem and leaf primordia, i.e. in leaf axils. They develop into axillary buds, which can then either remain dormant or grow out to form side shoots. Thus the control of the activity of these axillary buds has a great impact on plant architecture, since it is determined by the degree of branching. The outgrowth of dormant buds is largely regulated by the plant hormones auxin and cytokinin. Whereas apically derived auxin exerts an inhibitory effect on bud activity, cytokinin promotes the outgrowth of buds into side shoots. Recently, strigolactones and/or derivatives were identified as a third hormone regulating branching. They move acropetally in the stems and repress bud outgrowth. In this study we try to investigate the mechanisms underlying the antagonistic effects of auxin and cytokinin as well as the role of strigolactones. Preliminary data of physiological and molecular genetics approaches are presented. Currently the functions of candidate genes from a transcriptional profiling of *Arabidopsis* buds are studied.

P5-7 A GENETIC FRAMEWORK FOR THE AUXIN/CYTOKININ CONTROL OF CELL DIVISION AND DIFFERENTIATION IN THE ROOT MERISTEM

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Plant post-embryonic development takes place in the meristems. In the root meristem a stem cell niche generate transit-amplifying cells, which undergo additional division in the proximal meristem, and differentiate in the distal meristem transition zone that encompasses the boundary between dividing and expanding (differentiating) cells in the different cell files. For meristem maintenance, and therefore continuous root growth, the rate of cell differentiation must equal the rate of generation of new cells: how this balance is achieved is a central question in plant development. While the molecular mechanisms involved in stem cell positioning and activity are partially comprehended, the regulatory networks controlling the shift from transit-amplifying identity to differentiation are still poorly understood. We have previously shown that in the *Arabidopsis* root meristem the hormone cytokinin controls the differentiation rate of transit-amplifying cells by antagonizing the activities of a diffusible input in the vascular tissue of the transition zone. Here we demonstrate that this diffusible input is auxin, and that the balance between cell differentiation and cell division, necessary for controlling root meristem size and root growth is the result of the interaction between cytokinin and auxin through a simple regulatory circuit converging on the *SHY2* gene. In particular, in the vascular tissue of the transition zone, a primary cytokinin-response transcription factor, ARR1, activates the gene *SHY2*, a repressor of auxin signaling that negatively regulates the PIN genes that encode auxin transport facilitators. Thus, cytokinin causes redistribution of auxin, prompting cell differentiation. Conversely, auxin mediates degradation of the SHY2 protein, sustaining the activity of the PIN genes and prompting cell division.

P5-8 EFFECTS AND MODE OF ACTION OF CYTOKININS COMBINED WITH SUCROSE IN DELAYING SENESCENCE OF GREVILLEA 'SPIDERMAN' CUT FLOWERS

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Cytokinins, known as senescence retardants, are often used as a means to extend the vase life of various cut flowers. The cytokinin effect is improved by addition of sugars, and extracellular invertase has been found to be an essential component of the cytokinin-mediated delay of leaf senescence. The aim of the present study was to examine whether a similar mode of action operates in improving quality of cut *Grevillea* 'Spiderman' cut flowers, which responded positively to cytokinins and sugars. We hypothesized that cytokinins act by increasing invertase activity, thereby increasing the sink strength of the flower, and/or by improving sugar uptake from the vase solution. Our results show that *G.* 'Spiderman' cut flowers reacted positively to dipping the inflorescences in cytokinin solutions, with thidiazuron (TDZ) being more effective than bezyladenine (BA), and their positive effect increased by provision of sucrose in the vase solution. This combined treatment delayed flower senescence and pigment (chlorophyll and carotenoids) breakdown in the perianth, improved the water balance of the cut flowering branches, and prevented floret abscission. Consequently, the vase life of the cut flowers was significantly extended. Additionally, a combined treatment of TDZ and sucrose enhanced sucrose transport from the vase solution to the infloresences and increased sucrose hydrolysis in the florets and activity of the cell wall invertase. It seems, therefore, that the sink strength in *Grevillea* flowers is controlled by cell wall invertase. Our results suggest that the promising effect of the combined treatment of cytokinin and sucrose in delaying flower senescence of *G.* 'Spiderman' operates via the regulation of sink-source relationships.

P5-9 AUXIN AND OTHER PHYTOHORMONES INFLUENCE CHLOROPLAST TRANSCRIPTION AND STEADYSTATE RNA LEVELS IN BARLEY LEAVES

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We have investigated the effects of auxin and other phytohormones on the transcription of chloroplast genes and chloroplast RNA levels in apical and basal segments of 9-days old primary leaves of barley, *Hordeum vulgare, cv.* Luch. The leaf segments were incubated for 24 h on indole-3-acetic acid (IAA) solution or water (control) under continuous illumination. We observed effects of exogenously applied IAA on transcriptional activity and on steady-state RNAs levels of certain chloroplast genes. A concentration of 60nM IAA was found to most strongly influence chloroplast gene expression. IAA reduced the total transcriptional activity of chloroplasts isolated from basal segments to about 60 % of the water control. The most strongly repressed genes were *atpF, ndhC, psbK, rbcL, rps14, rps16* and *rrn23*. Effects of IAA on steady-state RNA levels were more pronounced in apical segments. The levels of *atpF, psbK, rps14* mRNAs were found to be the most reduced ones by IAA. Under identical experimental conditions, methyl-jasmonate (MeJa) was observed to inhibit chloroplast transcription and reduce transcript levels more drastically than auxin. Surprisingly, if applied together, auxin could partially reduce the effects of MeJa on chloroplast gene expression, an effect that was also observed for cytokinin.

P5-10 IAA AND FLAVONOIDS IN THE PROGAMIC PHASE OF FERTILIZATION

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The results concerning the IAA and flavonoid localization in the *in vitro* system (germinating pollen) and two *in vivo* systems (anther-male gametophyte and pollen-pistil) obtained in experiments with two petunia clones (self-compatible and self-incompatible) permit some suppositions about flavonoid role in the growth and development of the male gametophyte. Male gametophyte development was accompanied by increase in the contents of IAA and flavonoids in anther sporophyte tissue. During pollen grain germination and pollen tube growth both *in vitro* and *in vivo* (in stigma tissues), the levels of IAA and flavonoids rose as well. This kinetics was observed during the first 3-4 h after compatible and incompatible pollination. We could suppose that growth of the male gametophyte within style tissues is mainly determined by the reserved IAA and flavonoids in these tissues. The germinating male gametophyte of self-compatible clone contained more IAA and flavonoids than that of self-incompatible clone. It seems evident that the correlation between IAA and flavonoids contents we observed is an important factor in the development of male gametophyte fertility but is not related to the formation of the mechanism of self-incompatibility. We can suppose that flavonoids blocked auxin efflux from the germinating male gametophyte, thus rising its intracellular concentration, which in its turn facilitated pollen tube polar growth.

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O6-1 THE IMPORTANCE OF PLANT BIOTECHNOLOGY FOR SOCIETY AND ENVIRONMENT.

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A 35 years ago, when studying crown gall inductions by *Agrobacterium tumefacien*s, we became aware of the existence of many type of galls: teratoma crown galls, witches broom, leafy galls, genetic tumours, insect galls etc. This subject keeps fascinating me and I wonder if they indeed are all due to changes in the concentration and ratio of auxine/cytokine, as was thought in those days? As soon as we realised that crown gall induction was a natural genetic engineering event, attention focused on altering the Ti-plasmid in a way that it would become a vector for the introduction of novel genes into plants.

In view of the recent discoveries of many classes of small regulatory RNA's, often processed from large transcripts and of many hundreds of small peptides, some involved in auxine "potentiation", one may wonder if a deeper study of these T-DNA's should not merit better attention.

Agrobacterium mediated gene transfer became a potent method in fundamental plant molecular genetic research. It became also the base for engineering novel and important traits into crop plants.

Today more the hundred million hectares of transgenic crops are grown annually and the number steadily increases. Remarkably however the GM's commercialised limit themselves too a few crops (corn, soy, cotton and some rapeseed) and too two traits, insect tolerance and herbicide résistance. Is the pipeline clogged? Yes it is, hundreds of new prototype plants were constructed by public and private sector scientists in the developed and developing world. Few received authorisation for field trials, none for commercialisation. It is the duty of public sector scientist to explain why the GM technology can help the plant breeders to construct the novel plants which society and our environment badly need. With the ongoing population growth in the 3rd World countries. The knowledge that today already 1 Bi persons are undernourished and half of the world population has to live with less then 2 € a day, forces us to develop a high yielding agriculture. To double the output of our harvests, will have to be done on the same amount of arable land. For this we need higher yielding crops, with a better uptake of nutrients, with lower losses to biotic and abiotic stresses. On this same surface we also will also have to grow plants as raw material for industry, since our chemical end plastic industry cannot perform with the high petroleum prices experienced in 2008. Together with being high yielding our agriculture and industry will have to be less polluting, otherwise the loss of biodiversity will be irreversible. Plant biotechnologists do not only have the challenging task of engineering these plants, but thery also will have to explain to society, why this is needed and what the enormous benefits for environment can be.

O6-2 CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NOVEL PURINE-DERIVED INHIBITOR OF CYTOKININ OXIDASE/DEHYDROGENASE INCYDE AND ITS POTENTIAL USE FOR IN VIVO STUDIES

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Cytokinin oxidase/dehydrogenase (CKX) is a key enzyme of cytokinin degradation and is involved in the regulation of endogenous cytokinin levels in plants. Recently we have described substituted 6-anilinopurines as a new group of potent CKX inhibitors. Here we present biological characterization of compound INCYDE (Inhibitor of Cytokinin Degradation). INCYDE competitively inhibits activity of recombinant *Arabidopsis* CKX enzymes *in vitro* in dose-dependent manner. Co-crystallization with maize CKX1 confirmed the binding of the compound into the enzyme active site. INCYDE effectively inhibits degradation of exogenously applied radiolabelled isopentenyladenosine in intact *Arabidopsis* seedlings. *In vivo* function of the inhibitor was further confirmed by treatment of CKX overproducing transgenic plants. INCYDE application led to the release of the shoots of the treated plants from growth inhibition and complementation of wild-type phenotype. Targeted treatment of wild-type *Arabidopsis* plants resulted in altered development, increase of flower size and number, and consequent higher fruit yield. The results indicate that INCYDE modulates endogenous cytokinin level *in planta* and may have find interesting applications in studies of cytokinin action as well as a growth regulator for modifying the traits of crop plants.

O6-3 LIGHT/PHOT1-DEPENDENT POLAR TRANSLOCATION OF PIN3 AUXIN CARRIER DURING PHOTOTROPISMS IN ARABIDOPSIS

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Light induced lateral auxin transportation plays a critical role during phototropism. However, the molecular mechanism for this process is still not clear. Recently, we have reported that a member of the PIN family of putative polar auxin efflux carriers, PIN3, is a potential regulator for auxin lateral flow during phototropism. Here, we further characterized PIN3 functions during phototropic response. The PIN3:PIN3GFP fusion protein was also created for examining PIN3 cellular and subcellular localization in Arabidopsis etiolated and lateral light treated seedlings. Our studies indicated that PIN3 functions as a downstream of PHOT1 during phototropism. In hypocotyls of etiolated seedlings, PIN3GFP has a nopolar localization property in endodermal cells. However, this nopolar localization of PIN3GFP could be changed by lateral light treatment, showing reduced PIN3GFP signal at the outerside of endodermal cell of the illuminated side of hypocotyl. This light induced PIN3GFP translocation might be a PHOT1 dependent process since we could not observed this effects in *phot1* under the same conditions. We also found GNOM plays a very important role during phototropism. Both BFA treatment and in a weak allele of GNOM mutant R5 almost completely lost phototropic response, while in $GNOM^{M696L}$ line which is a BFA-resistant GNOM line, there was no effect on phototropic response with BFA treatment. Furthermore, the light induced PIN3GFP translocation was not observed in BFA treated seedling but we can see it in $GNOM^{M696L}$ line under the same treatments. In the epidermal cells of hypocotyl, we can see clear BFA compartments after BFA treatment, while light treatment could reduce these BFA compartments. The formation of BFA compartment might be a PHOT1 regulated process since BFA compartment looks normal in *phot1*. Our investigations indicated that light induced PIN3 translocation drives auxin lateral flow which results into asymmetric auxin gradient. GNOM-dependent protein trafficking involves into this process.

O6-4 CYTOKININ REGULATES SODIUM HOMEOSTASIS

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Cytokinin is an important signalling molecule that is involved in a variety of plant processes including: senescence, root and shoot development, and signalling nutrient status. Previous studies in Sorghum have found that cytokinin treatment raises sodium levels. In this study, we aimed to further investigate the association between cytokinin and sodium homeostasis by utilizing our large collection of Arabidopsis cytokinin signalling mutants. Analysis of Arabidopsis plants by inductively coupled plasma mass spectrometry confirmed that cytokinin treatment increases sodium levels in Arabidopsis plants. Several of the cytokinin mutants also displayed significantly altered levels of sodium. However, it does not appear that the level of cytokinin sensitivity of the mutants, based on root length assays, is proportional to its ability to exclude sodium. This suggests that cytokinin's effect on sodium homeostasis is mediated by a specific set of cytokinin response genes. Based on our data, we believe that cytokinin plays an important role in regulating sodium homeostasis and salt tolerance in plants.

O6-5 APPLIED PERSPECTIVE OF CYTOKININ-MEDIATED GROWTH MODULATION IN CROP PLANTS

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In the past, modification of plant growth and development achieved through an altered hormonal status has made important contributions to improve yield of crop plants. Increased knowledge about the biological functions and regulatory activities of cytokinin as well as the availability of various tools to decrease or increase the cytokinin status in a targeted manner enable new strategies to explore the consequences of an altered cytokinin status to achieve yield enhancement. Cytokinin is a positive regulator of shoot growth and a negative regulator of root growth and proof-of-concept has been obtained that these traits can be modulated in model plants. A significant example is the enhancement of the root system which may be useful to improve the access to limiting nutrient factors in the soil, including water and micro- and macronutrients. A larger root system was obtained in *Arabidopsis* and tobacco by a predominantly root-specific expression of *CKX* genes. These plants showed a significant increase in the content of several elements in the aerial plant parts, indicating that this approach could reduce the use of fertilizers and may support biofortification of crop plants. Root enhancement also caused improved drought resistance in tobacco plants. A subgroup of *CKX* genes was found to act in *Arabidopsis* as a negative regulator of cell division activity during developmental processes linked to reproductive development. Those genes are therefore being tested for their potential to achieve growth modulation during the reproductive phase. These and other examples of application-oriented research will be discussed.

O6-6 MOLECULAR AND FUNCTIONAL ANALYSES OF CHANGES IN THE PEDICEL ABSCISSION ZONE TRANSCRIPTOME FOLLOWING AUXIN DEPLETION

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Abscission of organs from the plant is initiated by changes in the auxin gradient across the abscission zone (AZ) which sensitizes the AZ to ethylene. Changes in gene expression have been correlated with the ethylene-mediated execution of abscission, but there has been little study of the molecular and biochemical basis of the role of auxin depletion. After excising flowers from tomato (*Solanum lycopersicum* Mill.) inflorescences, leading to rapid pedicel abscission, we examined transcriptome changes in the flower AZ. Microarray analysis using the Affymetrix Tomato GeneChip revealed changes in expression, occurring prior to and during pedicel abscission, of many genes with possible regulatory functions. They included a range of auxin-related transcription factors (TFs) such as seven *Aux/IAA* genes, supporting the suggestion that auxin depletion is an important mediator of the abscission response. In addition, several ethylene-related and other TFs showed transient up-regulation just after flower removal. A group of other structural and regulatory AZ-specific genes were strongly up-regulated shortly after flower removal. Virus-Induced Gene Silencing was used to test the effects of silencing AZ-specific genes with a potential regulatory role. A significant delay in pedicel abscission in response to flower removal was observed after silencing the AZ-specific TF genes encoding Knox-like homeodomain proteins (*TKN4* and *KD1*) and a gene encoding a proline-rich protein (*TPRP-F1*). The contribution of these results to our understanding of the role of auxin in regulating abscission and of auxin-ethylene cross talk during the abscission process will be discussed.

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06-7 CYTOKININ SIGNALLING IN MEDICAGO TRUNCATULA ROOT AND NODULE ORGANOGENESIS

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Legumes can develop two types of root lateral organs depending on environmental conditions: lateral roots and nitrogen-fixing symbiotic nodules. Genetic data indicate that their development involves common regulatory pathways, including phytohormonal controls. Cytokinin signalling mediated by the receptor MtCRE1 was indeed shown to be crucial for both organogeneses (Gonzalez-Rizzo et al., 2006).

Recently, we characterized root and nodule phenotypes of *M. truncatula* mutants affected in cytokinin signalling. In addition, a consensus sequence bound in vitro by the MtRR1 B-type Response Regulator was identified using a SELEX approach. This result, coupled with i) a transcriptomic analysis of early root apex responses to cytokinin and ii) a search for MtRR1-like boxes in their promoters, allowed identifying new cytokinin response genes able to act in root meristems. In addition, we identified cross-talk elements between nodulation and cytokinin signalling pathways, which are currently under validation *in planta*.

Overall, we could pinpoint specificities of cytokinin signalling pathways acting in legume root and nodule development.

O6-8 COMPARISON OF CYTOKININ ROLE IN DROUGHT AND HEAT STRESS RESPONSES OF TOBACCO PLANTS

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Phytohormones play an important role in plant stress responses. Apart from classical stress hormones, which stimulate defence pathways, also other hormones known as positive regulators of growth and development, cytokinins (CKs) and auxins, are involved. When drought and heat stress (HS) responses were followed, the initial stress phase was found associated with maintenance of CK homeostasis or even transient mild elevation of bioactive CK content, coincident with fast down-regulation of CK degradation by cytokinin oxidase/dehydrogenase (CKX). Prolonged stress was accompanied in both cases by a decrease of bioactive CKs. Unlike of relatively uniform HS response, drought was associated with preferential protection of upper leaves, coinciding with elevation of their sink strength by a gradient of bioactive CKs (due to stimulation of CKX activity in lower leaves). Both stresses were accompanied by similar changes in the activity of ascorbate peroxidase and catalase isoenzymes, the main difference being in abscisic acid/CK ratio, very high in drought and temporarily decreased in HS. Changes in CK levels reflected the actual physiological state of plants and were subjected to strict time- and space-specific regulation.

O6-9 METABOLISM AND POSSIBLE FUNCTION OF CYTOKININ DURING ABIOTIC STRESS IN MAIZE

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Cytokinins have been for a long time considered to be involved in plant responses to stress. Nevertheless their exact role in processes linked to stress signalization and acclimatization to adverse environmental conditions is unknown. Salt and osmotic stress accelerate CK metabolism in maize seedlings leading to a moderate increase of active CK forms lasting several days during acclimatization to stress. By enhanced CK levels, plants perhaps settle to a reduction of growth rates maintained by ABA accumulation in stressed tissues. Direct effect of CKs to mediate stress responses does not seem to be possible due to the slow changes in metabolite levels. However, CK transduction pathway is affected within 0.5h of stress induction by unknown mechanism resulting in down-regulation of primary CK response genes. A second role of CK receptors to respond besides CK stimuli as well to turgor changes is hypothesized. *cis*-Zeatin and its derivatives were found to be the most abundant CKs in maize seedlings. Thus, the crucial role of *c*Z is suggested as it originated independently to *de novo* biosynthesis in stressed tissues most probably by elevated RNA degradation.

P6-1 DISTRIBUTION OF 14C-INTO BIOCHEMICAL COMPONENTS OF NONNODULATING, NODULATING AND SUPERNODULATING SOYBEAN (GLYCINE MAX L.) GENOTYPES EXPOSED TO DROUGHT AND /OR POTASSIUM

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A pot experiment was conducted at the greenhouse of the National Research Centre, Cairo, Egypt, to examine the interactive effects of two levels of potassium and water stress on the distribution of ^{14}C- into biochemical components (solube carbohydrates, fat and protein) of three Japanese soybean genotyps (nonnodulating, normal nodulating and supernodulating). ^{14}C-fixation was superior in normal nodulation genotype followed by supernodulating and finally the lowest amount fixed was in the nonnodulating one. The distribution of ^{14}C- into different components was affected by the genotypes, water status and K levels. Leaf chlorophyll content (SPAD value) of the supernodulating plants was higher than that of the other genotypes under all experimental condition. K had no significant effect in the leaf chlorophyll content of the well-watered plants after 3 days of drought initiation in the three genotypes. In water stressed plants the high level of K resulted in significant increase in the chlorophyll content of nonnodulating and super nodulating plants.

P6-2 LOCAL INDUCTION OF SENESCENCE BY DARKNESS IN CUCURBITA PEPO (ZUCCHINI) COTYLEDONS AND PRIMARY LEAVES AFFECTS HORMONAL STATUS OF THE CORRESPONDING ORGANS WHICH REMAINED ILLUMINATED

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Local induction of senescence by darkness affected also the levels of plant hormones in the corresponding organs of zucchini plants which remained illuminated. The endogenous cytokinin (CK) as well as IAA levels were strongly reduced in both darkened cotyledons whereas in a single darkened cotyledon their content was less affected by darkness. Darkening of both cotyledons led to a slight decrease in the level of total CKs in the illuminated primary leaves mainly due to reduced bioactive CKs and the much less active *cis*-zeatins while ABA levels were increased. On the contrary, when the primary leaves were darkened, the levels of total CKs slightly increased in the illuminated cotyledons due to higher levels of bioactive CKs and *cis*-zeatins whereas ABA content was decreased. Alternations in the hormone levels are related to changes in the photosynthetic activity and the expression of the chloroplast-encoded genes *rbcL, psaB* and *psbA* in the darkened and illuminated leaf organs.

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P6-3 PHYSIOLOGICAL EFFECTS OF CADMIUM ON HORMONE TOMATO MUTANTS

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Cadmium (Cd) can negatively interfere with plant processes which are dependent upon metal concentration, plant species, organ/tissue and duration of exposure. Cd is also capable of inducing oxidative stress which in turn can result in a variety of antioxidant responses. Micro-Tom plants harbouring natural genetic variations and hormonal mutations have been produced, such as the *diageotropica* mutation that causes a loss of sensitivity to auxin, and the *Never ripe* mutation, which blocks ethylene perception in tomato leading to incomplete fruit ripening. The aim of this work was to observe alterations in lipid peroxidation, catalase, glutathione reductase and peroxidases activities induced by Cd in these two mutants and their wild-type Micro-Tom counterpart. Our results have indicated a toxic effect of Cd on all genotypes, but depending on the time length of metal exposure, concentration of the metal and mutant. The information available in this work is an important step towards obtaining a better understanding of the physiological changes caused by Cd and its effects on metabolic processes.

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P6-4 DO GROWTH REGULATORS PLAY A RADIOPROTECTIVE FUNCTION IN THE POST-RADIATION PERIOD?

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Irradiation with heavy ions induces secondary radiation which affected the overall effect in the irradiated cells. The aim of our work was to study postradiation effects in the peanut cells culture irradiated with different doses of non-relativistic particles 11B (E= 385 MeV, LET=55keV/μm). The results show that immediately after irradiation with doses D≥1Gy new proteins with Mr~84, 50, 25 and 24 kDa appeared and quantitative changes were observed for 68-70 kDa proteins. 28 days after irradiation the protein expression pattern changed and the amount of newly synthesized proteins decreased. For the highest doses, D=10-50 Gy, many proteins are absent in comparison to the control, and the expression level of many others decreased. The qualitative and quantitative content of the peroxidases strongly changed. For the highest doses, peroxidase activity in the cell was not detected still 28 days after irradiation. Dose LD50 was D=10±3Gy. Results indicated that extracellular GR affect rate of some reparation pathways.

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P6-5 MEDIATOR OF CYTOKININE-POWERFULL STIMULATOR FOR INCREASING OF PRODUCTIVITY AND STRESS TOLERANCE OF THE CROP PLANTS

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For first time from higher plant – wheat seedlings the mediator of cytokinine (MC) was isolated. It was developed the new effective method of preparative isolation of MC by using chromatography on nanostructured carbon sorbent – "Nanocarbosorb". For determination of chemical structure of MC the ion-absorber mass-spectrometer Agilent 1100 Esquire 3000 plus was used. It was established that MC by its structure relates to fusicoccin. By bio-tests it was established that MC shows the cytokinine activity. So MC derepresses the apical dominance and increases the content of amaranthin. It was shown that MC causes fast formation of basic and collateral roots and it's also causes fast growth of leaves and steames of plants shanks. MC shows its activity in nanogramm quantity. MC very effective for vegetative reproduction of forest and garden plants for example: (Syngonium auritum), (Euonymus verrucosa), (Malus silvestris), (Purus vulgaris), (Tamarix ramosissima), (Halimodendron halodendron), (Acacia farnesiana), (Eleaegnus argentea), (Ribes nigrum), (Lonicera olgae), (Spiraea lasiocarpa), (Hippophae rhamnoides), (Rhododendron hirsutum), (Roza acicularis). It were carried out the field experiments at scientific-industrial centre "KazAgroInnovation", at agricultural companies "Zher - Ana" (Taldykorgan) and at LTD "Bichkol". It was shown that the pretreatment of the seeds of winter wheat, rye and sugar beet before sewing gave the increasing of their productivity on 20-40% and acceleration the time of their maturing on 5-15 days. MC increases the tolerance of the plants to salt and cold stresses.

P6-6 THE ROLE OF SUGARS DURING HEAT STRESS RESPONSE OF TOBACCO PLANTS WITH MODULATED CYTOKININ CONTENT

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Abiotic stress is often associated with modulation of sugar accumulation. Sugars can exhibit role of osmolytes and regulate redox state and signalling. The complexity of sink-source relations involves responses to diverse sugar signals and metabolites. The signalling network is affected by phytohormones, nutrients as well as environmental conditions. The effect of cytokinins on heat stress (HS) tolerance was followed using tobacco plants over-expressing *trans*-zeatin-type and isopentenyladenine-type cytokinins under dexamethasone inducible promoter. During HS, the level of glucose and fructose was significantly increased in mature leaves with elevated content of *trans*-zeatin derivatives. Lower content of glucose and fructose was observed in mature leaves of plants with increased isopentenyladenine-type cytokinins. The sucrose content decreased during HS in WT plants and those with increased level of isopentenyladenine derivatives, while in plants with elevated content of *trans*-zeatin was invariable. The results indicate intensive cross-talk between sugars and specific cytokinins during HS response.

(The work was supported by GA CR 206/09/2062 and MEYS project LC06034.)

P6-7 MODULATION OF CYTOKININ ACTION BY DECREASED INTENSITY OF WHITE LIGHT IN ARABIDOPSIS – A PROTEOMIC ANALYSIS

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Light and cytokinin (CK) signaling are intertwined at several levels, and the underlying molecular mechanisms are being actively researched. To get an insight into the modulation of CK action by decreased light intensity at the proteomic level, we used 2-DE followed by image analysis and MALDI-TOF-TOF MS to analyze changes in steady-state protein levels in *Arabidopsis thaliana* seedlings with increased content of endogenous CKs cultivated at standard (90 μmol m^{-2} s^{-1}) and decreased (20 μmol m^{-2} s^{-1}) white light intensities. After activation of the CK-biosynthetic gene *ipt*, we observed about 75 differentially expressed protein spots (representing about 12% of detected spots). Out of the 75 protein spots, 22 were regulated in a comparable fashion at both light intensities, and 8 and 45 were differentially regulated at only standard or decreased light intensity, respectively. Till now more than 35 proteins have been identified, and can be classified as proteins involved in seed germination, photosynthesis, carbon and nitrogen metabolism and metabolism of xenobiotics.

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P6-8 A PUTATIVE ROLE OF CYTOKININ IN THE LIGHT STRESS RESPONSE

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Although light is indispensable for photosynthesis, it is a serious stress factor at the same time. Light-induced decrease in photosynthetic capacity (photoinhibition) is a general phenomenon in all oxygenic photosynthetic organisms. Plants with a reduced cytokinin status, i.e. overexpressors of a *CKX* gene and mutants of cytokinin receptor genes, show an increased sensitivity to light stress. A consequence of the increased sensitivity is the formation of leaf chlorosis after a shift from low light to high light or after a shift from short day conditions to long day conditions. In order to analyze this phenomenon in more detail we exposed detached leaves from cytokinin receptor double mutant (*ahk2 ahk3*) and cytokinin-deficient *Arabidopsis* plants to high irradiance. Pulse-amplitude-modulated chlorophyll fluorescence measurements revealed a stronger decline in maximum quantum efficiency of photosystem II photochemistry (Fv/Fm) in cytokinin-deficient plants compared to wild-type plants, while it was similar under moderate light conditions. This effect was accompanied by a delayed emergence of protective anthocyanins in plants with a reduced cytokinin status. The possible connection between cytokinin and light stress, including sensing mechanisms and acclimation responses, will be addressed.

P6-9 OXIDATIVE STRESS IN NICOTIANA TABACUM WITH ELEVATED LEVEL OF CYTOKININS

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Cytokinins (CKs) can, among others, positively regulate shoot development and delay onset of senescence. However, recently opposite effects of CK action, namely promotion of programmed cell death, and cytotoxic effects of over-expression of the CK-biosynthetic gene *ipt* in tobacco, were recognized. Here we investigated the cytotoxic effects of *ipt* expression in tobacco in detail. We show that lesion formation in expanded tobacco leaves proceeds shortly after *ipt* induction – the first lesions being observed in app. 60 hours after induction of *ipt* expression, and lesions can spread over the entire leaf area within 5 days after induction. Formation of visible lesions was preceded by increase in reactive oxygen species (ROS) as indicated by DAB and 2´7´-dichlorofluorescein diacetate staining despite increase in a key ROS scavenging enzyme – APX. Further, we demonstrate that lesion formation is a light-dependent process as it is prevented by shading. Concomitantly with increase in ROS levels, transcript levels of genes involved in light phase of photosynthesis (*FNR1* and *CAB*), and xanthophyll metabolism (*VDE*) were markedly down regulated as revealed by RT-qPCR analysis.

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P6-10 MUTATION OF THE MEMBRANE-ASSOCIATED M1 PROTEASE APM1 RESULTS IN DISTINCT EMBRYONIC AND SEEDLING DEVELOPMENTAL DEFECTS

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Aminopeptidase M1 (*APM1*), a single copy gene in Arabidopsis, encodes a peripheral membrane metallopeptidase originally identified via its affinity for and hydrolysis of the auxin transport inhibitor 1-naphthylphthalamic acid (NPA). Loss-of-function mutants are haploinsufficient & show irregular & uncoordinated cell divisions throughout embryogenesis often resulting in embryo abortion and seedlings die at 5 dag. Quiescent center & cell cycle markers show no signals in *apm1-1*, & the ground tissue specifiers *SHR* & *SCR* are misexpressed or mislocalized. *apm1* alleles show defects in gravitropism & auxin transport. Gravistimulation decreases *APM1* expression in auxin accumulating (lower) root epidermal cells. Auxin treatment increases *APM1* expression in the vascular cylinder. On sucrose gradients APM1 is observed in unique light membrane fractions not characterized by other subcellular markers. Electron microscopy shows APM1 signal at the margins of Golgi cisternae, plasma membrane, in select multivesicular bodies, at the tonoplast, in dense intravacuolar bodies, & in maturing metaxylem cells. Visualization by immunolocalization and N-terminal YFP fusions associates APM1 with BFA-sensitive endomembrane structures and the plasma membrane in cortical and epidermal cells. NPA treatment abolishes protein localization & dimerization. The auxin-related phenotypes & mislocalization of auxin efflux proteins observed in the *apm1* are consistent with APM1 biochemical interaction with NPA.

P6-11 THE ROLE OF PLANT GROWTH REGULATORS (AUXIN AND CYTOKININ) ON THE GROWTH OF FOREST TREE SPECIES GROWN UNDER STRESS CONDITIONS.

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Abiotic and biotic stresses elicit changes in normal physiology of trees. Plant growth regulators (PGR) are involved in the stress response and appear to have two roles: 1) to minimize the impact of the stress on the tree and; 2) to trigger stress resistance mechanisms. In the latter case the PGR-induced changes appear to enhance resistance to subsequent stress. This cross-adaptation to stress is important in trees. The role of PGRs in the physiological response to the abiotic stresses of water deficit, water excess, temperature, nutrition and mechanical perturbation is discussed along with cross-adaptation in the interactions of these stresses. Disease response and defense, and plant-plant communications involve PGRs and are topics covered with respect to biotic stress. Stress leads to early senescence and abscission in trees. These processes are controlled by PGRs and are briefly discussed.

O6-12 PHYSIOLOGICAL DISSECTION OF CYTOKININ INDUCED DROUGHT AVOIDANCE IN PLANTS.

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Cytokinins (CK´s) are known to induce cell division, seed germination and delay senescence, whereas constitutive exposure of CK´s has server effects on plant growth and development such as release of apical dominance and inhibited root growth.

To control the endogenous levels of CK´s in Arabidopsis we used a chimeric promoter composed of a stress inducible promoter (salt, drought and ABA) and a leader intron (for stabilization of mRNA) to express a homologues version of the cytokinin-biosynthesis gene *ipt*, the rate-limiting step in the synthesis of CK´s.

We have observed that the transformed plants display an increased drought tolerance. We are exploiting the molecular and physiological background for the observed drought avoidance in the plants. Data related to water potential, photosynthesis and stomatal conductance will be discussed in relation to the level and composition of cytokinins. Furthermore we have tested CK´s inhibitory effect on senescence.

P6-13 ROOT-SPECIFIC REDUCTION OF CYTOKININ STATUS CAUSES ENHANCED ROOT BIOMASS PRODUCTION AND LEAF MINERAL ENRICHMENT

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The root system is an important plant organ and optimized root system architecture is relevant for access to water and nutrients. Classical breeding approaches for optimizing root systems are difficult because the trait is governed by many genes and difficult to score. We have chosen a metabolic engineering approach to generate transgenic *Arabidopsis* and tobacco plants with enhanced root-specific degradation of cytokinin, which is a negative regulator of root growth. Compared to wild type plants, these transgenic plants form a larger root system, whereas growth of the shoot is similar to the wild type. Elongation of the primary root, root branching and root biomass formation of transgenic lines was increased by up to 80%. Thus, it was demonstrated that a single dominant gene can be used to regulate a complex trait, root growth. Root enhancement caused a significant increase in the content of several elements in the aerial plant parts. This indicates that our approach could be used for biofortification of crop plants or for phytoremediation of metal-contaminated soils.

O7-1 QUANTITATIVE APPROACHES TO PLANT DEVELOPMENT

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In the plant shoot apical meristem, gradients of auxin are set up not by diffusion from a localized source or by a reaction-diffusion mechanism, but through a feedback loop between auxin and its transporter, the PIN1 protein. Two distinct molecular mechanisms for the subcellular polarization of PIN1 have been proposed. For leaf positioning (phyllotaxis), an “up-the-gradient” PIN1 polarization mechanism is proposed to direct auxin towards cells with higher auxin concentration, yielding localized auxin maxima that determine the positions of initiating leaves. In contrast, the canalization hypothesis proposes a “with-the-flux” PIN1 polarization that reinforces the direction of auxin flow, leading to the formation of vascular strands. During the initiation of the midvein, these two patterning mechanisms intersect, and thus the question arises as to how two different PIN1 polarization mechanisms may work together. Our detailed analysis of PIN1 polarization during midvein initiation in combination with computer simulations suggests that both mechanisms for PIN1 polarization are operating simultaneously, and that some cells in both the epidermis and in internal meristem tissue appear to switch from one polarization strategy to another.

O7-2 A COMPUTATIONAL MODEL OF PHYLLOTAXIS IN COSTUS

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The phyllotaxis of Costaceae is unusual in two respects. First, divergence angles may be as low as 30 degrees. This violates Hofmeister's rule, according to which primordia at the apex should appear as far as possible from each other. Second, divergence angles between consecutive primordia in Costaceae change in a continuous fashion. In contrast, common phyllotactic patterns show preference for specific values, such as the golden angle of 137.5 degrees. In spite of almost 150 years of studies of costoid phyllotaxis, its mechanism has remained a mystery. I will present a computational model which supports Hirmer's (1922) conjecture that costoid phyllotaxis is a derivative of the distichous phyllotaxis observed in the related ginger family. The model is consistent with the conceptual model of phyllotaxis introduced by Reinhardt *et al.* (2003), and supported by computational models by Smith *et al.* (2006) and Jönsson *et al.* (2006). According to the proposed Costus model, the distinctive features of costoid phyllotaxis result from different dynamics of auxin induction and propagation, compared to that observed in most plants. Analysis of the Costus model leads to more general observations regarding the role of finite signal propagation rates in plant morphogenesis.

O7-3 AGENT BASED MODELLING OF AUXIN TRANSPORT CANALISATION

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Auxin transport canalisation describes how auxin organises and promotes its own transport through cells. It is therefore an interesting target for computer simulation as it is a selforganising process where auxin in cells promotes its own transport between cells. Auxin is able to enter cells passively or activity , but can only leave cells by being actively pumped out by proteins including PIN. During vascular tissue formation a narrow transport path, or canal, forms from an area where auxin is accumulating, to a sink elsewhere in the tissue. A missing link in this process is the regulation of the positioning of the PIN proteins on the cell membrane. During canalisation, PIN proteins are observed to be polarised in the direction of auxin flux. This could be by targeted removal or targeted insertion of PIN proteins causing them ultimately to become polarly localised on the cell membrane in the correct location to pump auxin towards the sink. To investigate canalisation, in the hope that we can direct future wet laboratory work, we have developed a flexible agent base modelling framework to allow for testing of different hypotheses about how PIN localisation might be regulated. The models are built by putting agents representing auxin, and proteins including PIN, into a tissue consisting of a number of cells. We then look for the global behaviour of canalisation as an emergent property of the simple interactions between the agents and their environment.

O7-4 TOWARDS A MODEL OF AUXIN RESPONSE IN ROOT EPIDERMIS

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The basic mechanism of auxin perception and signal transduction to gene expression via the proteolysis of Aux/IAA repressor proteins is deceptively simple. In contrast, the sheer diversity of responses to auxin illustrates the complexity of downstream signalling and has also hampered the analysis of specific auxin-regulated developmental phenomena. To address this problem we are investigating auxin response in defined developmental contexts, initially the hair and non-hair cell lineages of the root epidermis. Quantitative genomic, biochemical and metabolomic data obtained using fluorescence activated cell sorting of GFP-marked cell-types are being combined with kinematic data to parameterise, build, and refine mathematical models capturing the essential properties of the auxin signalling system. Simple preliminary models also highlight the interesting potential differences in capacity of this system to interpret gradients of auxin in the root. This work illustrates how auxin can regulate different developmental outcomes in neighbouring cell types of the root and has implications for understanding auxin action throughout development.

O7-5 MODELLING OF POSITIVE-FEEDBACK MECHANISM FOR AUXIN CARRIER POLARIZATION DURING AUXIN-DEPENDENT PLANT DEVELOPMENT

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The key aspect of hormone action in developmental biology relates to a rather complex issue of how individual cells translate hormone signals into polarity cues in the whole macroscopic ontext. The directional transport of plant hormone auxin has been shown to play a pivotal role in defining plant tissue polarity. Interestingly, spatio-temporal auxin gradients trigger the whole variety of developmental cues. The dynamic subcellular localization of PIN-FORMED (PIN) auxin efflux carriers largely determinate the direction and rate of auxin flux. Hence, the mechanism underlying dynamics of PIN proteins and involved auxin signaling is a key to understand plant development. Here we present the unified and plausible mechanism underlying early PIN polarization events for guiding auxindependent plant development. Using available cell biological data and computer modeling, we study the positive-feedback loop between apoplastic auxin concentrations and PIN abundance at the cell surface that leads to the polarization at the cellular and tissue levels. We found that the novel mechanism can account for competitive canalization, venation guidance, tissue regeneration, phyllotaxis, and auxin gradient formation and maintenance in organs, such as roots. These observations were confirmed experimentally, suggesting that non-genomic spatial inhibitory effect of auxin on PIN internalization might be central to auxin-guided plant development.

O7-6 MODELLING OF AUXIN TRANSPORT PROCESSES ON A SINGLE CELL LEVEL

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Phytohormone auxin is transported through plant body typically by specialized polar transport machinery. This machinery consists of a balanced system of passive diffusion combined with activities of the auxin influx and efflux carriers. These carriers possess the rate-limiting function in capacity of auxin flow. Besides, there are processes affecting auxin availability for carriers such as metabolism and compartmentalization. Detailed kinetic characterization of selected auxin transport components was performed using accumulation assays on suspension-grown tobacco BY-2 cells (*Nicotiana tabacum* L., cv. BY-2). During cellular auxin transport measurement, metabolism of particular auxin was also determined. Using synthetic auxin naphthalene-1-acetic acid (NAA) that enters cells almost exclusively by diffusion, we propose a mathematical model of NAA flow, taking into account the contribution of diffusion, compartmentalization and metabolism.

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O7-7 DEVELOPING A REAL-TIME, QUANTITATIVE BIOSENSOR FOR AUXIN AND ABA

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If we are to understand the timing, direction and extent of responses to hormonal stimuli we need to capture quantitative information from living, responding tissues.

Quantitative biosensors are widely used in several areas of biology and medicine and, although no one system will suit all applications, their continued development and application to plant physiology is overdue.

A novel configuration and application of a surface plasmon resonance instrument (Biacore 2000) is described such that it becomes a biosensor platform capable of measuring in real-time continuous changes in concentration of aqueous analytes from living tissue. The examples presented are for the plant hormones auxin and ABA. The biosensor gives time-resolved dataflow on a sensor with a dynamic range of approx four orders of magnitude. We will show how samples from live plant tissues are collected to flow continuously over the Biacore chip.

Previously, quantitation of plant hormones has tended to be either post-event, time fractionated assays of plant homogenates or assays have been qualitative using, principally, promoter-reporter constructs. Much detailed physiology has been determined from interpretations of these reporters. We discuss how much more information could be available from the application of suitable, time-resolved biosensors.

O7-9 HIGHLY SENSITIVE AND HIGH-THROUGHPUT ANALYSIS OF PLANT HORMONES USING MS-PROBE MODIFICATION AND UPLC-ESI-QMS/MS: AN APPLICATION FOR HORMONE PROFILING IN ORYZA SATIVA

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We have developed a highly sensitive and high-throughput method for the simultaneous analysis of 43 molecular species of cytokinins, auxins, ABA, and GAs. This method consists of an automatic liquid handling system for solid phase extraction and UPLC coupled with a tandem quadrupole mass spectrometer equipped with an electrospray interface (UPLC-ESI-qMS/MS). In order to improve the detection limit of negatively charged compounds, such as GAs, we chemically derivatized fractions containing auxin, ABA, and GAs with bromocholine that has a quaternary ammonium functional group. This modification, that we call “MS-probe”, makes these hormone-derivatives have a positive ion charge and permits all compounds to be measured in the positive ion mode with UPLC-ESI-qMS/MS in a single run. Consequently, quantification limits of GAs increased up to 50-fold. Our current method needs less than 100 mg fresh weight of plant tissues to determine phytohormone profiles and enables us to analyze simultaneously more than 180 plant samples. Application of this method to plant hormone profiling enabled us to draw organ-distribution maps of hormone species in rice and also to identify interactions among the 4 major hormones in the rice GA-signaling mutants. Combining the results of hormone profiling data with transcriptome data in the GA signaling mutants allows us to analyze relationships between changes in gene expression and hormone metabolism.

O7-10 NEW PURIFICATION AND MASS SPECTROMETRIC APPROACH FOR CYTOKININ ANALYSIS

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The major problem associated with plant hormone analysis is that their amount present endogenously in plant tissues is very low, usually in the range fmol-pmol/g F.W. Development of simple purification of real samples by batch immunoextraction (Hauserova et al.,2005) and application of new analytical approaches based on UPLC separation (Novak et al.,2008) makes possible a new direction in plant hormone research. A fast chromatography technique, the ultra performance liquid chromatography (Acquity UPLC,Waters) was coupled to triple quadrupole mass spectrometer (Xevo TQ MS,Waters) equipped with an electrospray interface (ESI) and the unique performance of collision cell - ScanWave. Small amount (1 mg) samples of 10-day-old *A. thaliana* plants were purified by SPE follow by an immunoaffinity step and process was completed by UPLC-MS/MS analysis of naturally occurring cytokinins (bases, ribosides, O- and N-glucosides, and nucleotides) in 5 minutes. In MRM mode, the detection limit was close to 50 amol and linear range was at least five orders of magnitude. The method provides substantial improvements in terms of robustness, sensitivity, selectivity, through-put and cost-effectiveness over previous methods published.

P7-1 THERMOLUMINESCENCE AND CHLOROPHYLL FLUORESCENCE AS TOOLS FOR EVALUATION OF THE HEAT STRESS TOLERANCE IN TOBACCO PLANTS WITH MODULATED CYTOKININ CONTENT

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The evaluation of plant stress tolerance requires relatively fast, but reliable techniques. The physiological relevance of the determination of photosynthetic characteristics by thermoluminescence and chlorophyll fluorescence was tested during the heat stress response. Tobacco plants differing in their endogenous cytokinin content were incubated at elevated temperatures (36°C - 45°C) for up to 6 h. Chlorophyll fluorescence was measured with a FluorCam instrument (PSI, CR) and thermoluminescence using a home-made thermoluminometer. The thermoluminescence curves revealed the strongest stress response after 2 h. Partial adaptation was observed after 6 h of heat stress. The evaluation of photosynthetic parameters (F0, FM, Fv, NPQ) indicated that the positive effect of elevated cytokinin content was highest at 42.5°C. Thermoluminescence and chlorophyll fluorescence proved to be suitable techniques for determining the appropriate experimental heat stress conditions, including temperatures and incubation periods.

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P7-2 USE OF GFP FOR LASER CAPTURE MICRODISSECTION IN ARABIDOPSIS

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Laser capture microdissection (LCM) is a powerful tool for isolating specific tissues or cell types. Cells isolated by LCM can be a source of cell-specific DNA, RNA, protein or metabolites. Where the morphology of the cells is not helpful in distinguishing particular cell populations in a tissue, the target cells, or non-target cells, can be identified by reporter gene expression or immunolocalization.

We are studying auxin response in the shoot apical meristem (SAM). Functionally the SAM can be divided into a central zone (CZ), which contains the stem cells, and a surrounding ring region, the peripheral zone (PZ), where primordia can form in response to auxin. To understand differences in auxin responsiveness across the SAM we are using LCM to sample CZ, PZ, and initiating primordia to define the topology and targets of the auxin response mechanism. The identification of these zones is hampered by a lack of distinct morphology but the different cell populations can be easily distinguished if a reporter line is used.

We describe a protocol adapted to use GFP as a marker to guide LCM of the meristem zones. For LCM, both the sample preparation must be adjusted to ensure that target cells can be identified and that the DNA, RNA, proteins, or metabolites are preserved and extractable.

P7-3 NEW CYTOKININS FOR TREE BIOTECHNOLOGY

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For long-term sustainability of tree genetic resources, micropropagation technologies are proven to be useful. However, some difficulties during standardized micropropagation procedures still remain to be solved. The investigations of mechanisms of action of cytokinins and their different derivatives could substantially contribute to rationalization of micropropagation. In the micropropagation industry, 6-benzylaminopurine (BAP) is widely used as one of the most effective and affortable cytokinins. Nevertheless, it has disadvantages such as heterogenity of growth and inhibition of rooting during acclimatization of some plant species. Thus, the development of alternative phytohormone would be still desirable Our LC-MS based search for naturally occurring aromatic cytokinins in plants led recently to the discovery of several new plant hormone substances. Surprisingly, these compounds exhibit a wide range of an interesting biological activities, not only on plant cells and tissues but also in various animal and human models. Subsequently, a large group of their synthetic analogues has been prepared and characterized. Based on these results, the best compounds were selected and tested further for their use in different micropropagation systems. Here, the influence of three different aromatic cytokinin derivatives on in-vitro multiplication, rhizogenesis and early senescence inhibition of the selected tree species (Sorbus torminalis and Ulmus glabra) were compared. Special attention has been paid to endogenous concentrations of cytokinins, auxins and ethylene, produced by the explants, grown on different cytokinins. Their importance as well as different roles in the process will be discussed.

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P7-4 MEASURING OXIAA, THE MAJOR AUXIN CATABOLITE, WITH A CELLULAR RESOLUTION. A WAY TO FURTHER UNDERSTAND AUXIN HOMEOSTASIS IN THE ROOT

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Indole-3-acetic acid (IAA) homeostasis is controlled by balancing the activities of IAA biosynthesis, metabolism and transport. Among catabolic processes, oxidation of IAA into 2-oxoindole-3-acetic acid (oxIAA) has been observed in a root apex.

For the tissue specific oxIAA/IAA quantitative analysis GFP fusion lines expressing GFP in specific root cells and tissues were used. Protoplasts were isolated from these lines and sorted by Fluorescent Activated Cell Sorting (FACS). This provided dissecting out certain tissues for auxins levels determination.

In order to construct an *Arabidopsis thaliana* root IAA/oxIAA concentration and distribution map with a cellular resolution, effective and sensitive analytical method is needed. Therefore, we developed efficient off-line coupling of the solid phase extraction (SPE) method with the highly sensitive technique - high performance liquid chromatography- electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), which allowed rapid and predictive screening of IAA and oxIAA in *Arabidopsis thaliana* root cells.

P7-5 DEVELOPING A CYTOKININ BIOSENSOR

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If we are to understand the timing, direction and extent of responses to hormonal stimuli we need to capture quantitative information from living, responding tissues.

Previously, quantitation of plant hormones has tended to be either post-event, time fractionated assays of plant homogenates (mass spectrometry, HPLC, GC, ELISA etc.) or assays have been qualitative using, principally, promoter-reporter constructs. Much detailed physiology has been determined from interpretations of these reporters, but they remain largely qualitative and post-event with little or no temporal resolution. Quantitative biosensors are widely used in several areas of biology and medicine and, although no single system will suit all applications, their continued development and application to plant physiology is overdue.

We describe initial experiments using the enzyme cytokinin oxidase/dehydrogenase (CKX) incorporated into microelectrodes in order to develop an electrode sensitive to and specific for active cytokinins. Two approaches have been tested, the incorporation of CKX into a silicate sol with a quinone as an electron-carrying mediator, and the direct attachment of CKX to gold electrodes by use of a poly-His tag. Initial data on responsiveness will be shown and the potential of these electrodes as cytokinin biosensors will be discussed.

P7-6 FAST MONITORING OF ADVENTITIOUS SHOOT MERISTEM REGENERATION

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In vitro regeneration of plant tissue largely depends on the species and type of tissue studied. An important condition to obtain regeneration, is the development of a suitable shoot induction medium. To find a good composition of such medium, visual inspection of shoots has to be done. The slow growth of most shoot causes the analyses to be lengthy and in addition depends on the evaluation of the investigator. To rationalize the search for a medium composition with the optimal regeneration capacity, a system is being developed based on detection of molecular markers for shoot regeneration. Here we present a fast monitoring system for shoots as an alternative to visual inspection of shoot regeneration. We used the meristematic marker *SHOOT MERISTEMLESS* (*STM*), that is involved in the shoot apical meristem (SAM) formation in Arabidopsis thaliana. Arabidopsis root explants readily regenerate shoots on shoot inducing medium. By following the expression of *STM*, the emergence of shoot formation was predicted. The expression of STM was monitored using GFP as an *in vivo* live reporter. The use the STM reporter allowed us to evaluate the effect of various compounds on shoot regeneration. Drugs applied at different time points and the effect of different media was analysed. A major advantage of the STM marker was its predictive quality: shoot regeneration was observed before it was macroscopically visible. The expression of STM-GFP resulted in a robust fluorescence signal that correlated well with the emergence of newly developing shoots.

P7-7 ANALYSIS OF IAA AND ITS METABOLITES BY IMMUNOAFFINITY EXTRACTION AND HPLC-MS/MS

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IAA and its metabolites occur in a very complex matrix of plant samples at diverse concentrations, ranging from tens of nmol to hundreds of fmol per g of fresh weight. To isolate and quantify the metabolites, we developed an analytical protocol combining a highly-specific anti-IAA immunoaffinity extraction with a sensitive and selective LC-MS/MS analysis. By using internal standards for each of the analyzed compounds, we can study IAA and a wide range of its metabolites including amino acid conjugates. The protocol enables to quantify the metabolites in amounts of fresh material as low as 20 mg.

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P7-8 CYTOKININS: RECENT PROGRESS AND DEVELOPMENTS IN ANALYTICAL METHODS

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Chemical analysis of plant hormones is an integral part of the studies on plant development. Plant tissue represents a complex multi-component mixture that contains cytokinins (CKs) in trace amounts along with many other compounds with similar chemical structure and/or physico-chemical properties. This is very challenging for an analytical chemist especially due to the fact that majority of the experiments in plant biology are conducted with very subtle model plant Arabidopsis thaliana. Moreover, in order to intercept biological variability to obtain statistically significant sets of data, numerous biological replicates are often required, besides the technical ones. Therefore, the analysis demands high-throughput, sensitive and sufficiently selective analytical tools that should be applicable to the compounds of varying polarity, solubility and functional groups. The primary focus of this presentation is on novel analytical methods designed to meet the requirements for analysis of classic CKs, the group of CK nucleotides and 2-methyltio-derivatives with special emphasis on high-performance liquid chromatography (HPLC), ultra high-performance liquid chromatography (UHPLC) and capillary electrophoresis (CE) coupled with mass spectrometer.

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