L1-1: Genetics of auxin regulation in *Arabidopsis*

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Indole-3-butyric acid (IBA) and amide-linked conjugates of indole-3-acetic acid (IAA) and can act as auxin storage forms. We have exploited the observation that IBA and certain IAA-amino acid conjugates mimic IAA in bioassays to isolate Arabidopsis mutants defective in IBA or conjugate responses. Developmental defects in the absence of exogenous sucrose suggest that some IBA response mutants are impaired in peroxisomal fatty acid chain shortening. Genes defective in IBA response mutants include those required for peroxisome biogenesis and functioning. These results imply that the conversion of IBA to IAA is disrupted in the mutants and that IBA acts, at least in part, through its conversion to IAA. Genes defective in the IAA-conjugate response mutants encode IAA-amino acid conjugate hydrolases, proteins implicated in metal homeostasis, and a transcription factor. Embryonic and seedling defects in IBA response mutants and hydrolase mutants suggest that IAA released from IBA and IAA-amino acids contributes to embryo and seedling development. (Supported by the NSF and the Robert A. Welch Foundation.)

L1-2: Cytokinin glycosyltransferases: genes, substrates, molecular models, and transgenics

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Cytokinins occur as free bases, nucleoside, nucleotides, N-glucosides, O-glucosides, and O-xylosides. Zeatin O-glycosylation genes and enzymes were isolated and characterized. The first genes were cloned from *Phaseolus* and subsequently, homologs were identified in other species including maize, rice, *Arabidopsis*. The enzymes from dicots and monocots obtained thus far prefer zeatin and cis-zeatin respectively as substrate. Interestingly, topolins are also substrates of these enzymes, with parallel preference for *m*-topolin and trans-zeatin, and for *o*-topolin and cis-zeatin. Based on enzyme/substrate interactions and receptor assays, similarities are evident between cytokinin-binding sites on the enzymes and receptors. Cytokinin O-glycosyltransferases display sugar donor preference, recognizing UDP-glucose, UDP-xylose, or both. Sequence comparisons, nucleotide exchanges, and enzyme modeling have pinpointed regions of importance to substrate binding. Transgenic experiments with tobacco and maize indicated that effects of increasing zeatin O-glucosylation are partly species-specific. The relevance of these findings to cytokinin biology will be discussed.

L1-3: Cytokinin biosynthesis pathway and regulation in *Arabidopsis*

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Most natural cytokinins (CKs) are adenine derivatives that carry an isoprene-derived side chain at the N^6-terminus. The first step of *de novo* synthesis of CKs is catalyzed by adenosine phosphate-isopentenyltransferase (IPT), which produces isopentenyladenine nucleotide with adenine nucleotide and dimethylallyldiphosphate (DMAPP). In higher plants, *trans*-zeatin (tZ), a major CK, is formed by subsequent hydroxylation, which is catalyzed by a cytochrome P450 monooxygenase, CYP735A1 or CYP735A2. Biochemical characterization of IPTs from various organisms revealed that the substrate specificities differ between *Agrobacterium* and higher plants. *Agrobacterium* IPTs have the ability to produce tZ-type species directly by use of hydroxymethylbutenyl diphosphate (HMBDP) as the side chain donor. Analyses of expression patterns of genes for CK metabolic enzymes suggest that CK biosynthesis and homeostasis are finely controlled by internal and external environmental factors such as phytohormones (auxin and ABA) and inorganic nitrogen sources. This regulatory system would be important in linking nutrient signals and morphogenetic responses. I will outline recent progress in CK biosynthesis and regulation, which is studied in *Arabidopsis.*

O1-1: Isolation, molecular characterization and functional analysis of a cytokinin biosynthesis gene (ZmIPT2) expressed in corn

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Cytokinins (CKs) are a class of plant hormones that play a central role in plant growth and development. The first plant cytokinin, zeatin, was isolated from immature corn seeds. It was first shown to induce cell division in the presence of auxins and to influence differentiation of plant cells in culture. Later, CKs were also shown to be involved in induction of stomata opening, delayed senescence, suppression of auxin-induced apical dominance, signaling of nitrogen availability, plastid differentiation and controlling sink strength. Using a bioinformatics approach, we identified several isopentenyl transferase (IPT) candidate genes. This allowed us to isolate and characterize the first CK biosynthetic gene from corn, which we have called *ZmIPT2*. The gene is specifically expressed in developing kernels and its expression overlays with CKs levels. The functionality of the corresponding protein was tested *in vivo* in Arabidopsis calli. We showed that organ regeneration from transgenic calli ectopically over-expressing ZmIPT2 on media containing auxin was consistent with CK overproduction. The role of *ZmIPT2* in cytokinin biosynthesis will be discussed with an emphasis on endosperm cell divisions, embryo development and kernel sink strength.

O1-2: Cytokinin oxidase/dehydrogenase overexpression in the moss *Physcomitrella patens*

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The generation of low cytokinin plants of *Physcomitrella patens* by overexpressing a heterologous cytokininoxidase/ dehydrogenase, demonstrated that cytokinins play a main role for the development of moos. The *AtCKX2* gene from *Arabidopsis* was cloned into an overexpression vector under the control of the actin1 promoter from rice. Enzymatic measurements of cytokinin oxidase/dehydrogenase activity in the transformants revealed an increase up to 190-fold in the medium and ca 30-fold in the tissue when compared to the wild type. In order to monitor effects on cytokinin metabolism we carried out *in vivo* labelling studies using radioactive cytokinins. During incubation with tritiated isopentenyladenine (iP) and isopentenyladenosine (iPR), we measured a strong reduction of iP and iPR, as well as a reduction of iPR-nucleotides in the transformants. HPLC-mass spectrometry measurements of endogenously produced cytokinins showed a 14fold reduction of iP and a 5-fold reduction of iPR in the culture medium. The effect of lowered cytokinin levels resulted in reduction of growth, retarded formation of buds and smaller leaflets and gametophors. These morphological changes show analogies to effets of cytokinin deficiency in higher plants.

O1-3: Functional analysis of the active site of the maize β-glucosidase Zm-p60.1

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β-glucosidases Zm-p60.1 (maize) and Bgl4:1 (*B. napus*) can release active cytokinins from their storage cytokinin-O-glucosides, although their active site amino acid residues differ dramatically. To analyze molecular evolution in the active sites, we generated single and multiple mutants of Zm-p60.1 reproducing the active site of Bgl4:1 as follows: F193A, F200K, W373K, F461L; F193A F200K W373K F461L; T189V F193A F200K D256S M258A W373K F461L A462D and Q188Q T189V F193A F200K D256S M258A R331F P372K W373K F461L A462D E466A. The multiple mutants could be produced in *E. coli* as soluble proteins. However, CD spectroscopy revealed significant alterations in their structure compared to Zm-p601. No enzyme activity was detected in the proteins. Kinetic analysis on the single mutants confirmed the essential role of F200 and W373 in aromatic aglycone binding. F461L exerted little effect on enzyme activity. Surprisingly, F193A showed a 16- and 1.3-fold decrease in K_m, and a 160- and 15-fold decrease in k_{cat} against PNPG and MUG respectively, compared to Zm-p60.1. Molecular modelling suggests energetically favourable non-productive binding of PNPG and MUG to the F193A active site as the main cause of the changed kinetic parameters. (Supported by GACR203/02/0865, MSM0021622415, AVOZ50040507.)

O1-4: Kinetic analysis and structural correlations of the reaction of cytokinin dehydrogenase (EC. 1.5.99.12)

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Cytokinin dehydrogenase (CKX) is a flavoprotein that cleaves cytokinins to adenine and corresponding side-chain aldehyde using a quinone-type electron acceptor. Reactions of maize CKX with five different substrates were studied using the Stopped-flow monitoring of the reductive half-reaction revealed formation of a binary ES complex between the formed cytokinin imine and the reduced enzyme. K_D values for cytokinin binding range from <2 μM for isopentenyladenine to 66 μM for kinetin and reasonably correspond to respective K_m values. Reduction rate was high for isoprenoid cytokinins that showed specific formation of a charge transfer complex with the enzyme, but very low for other cytokinins. The ES complex decays to form a free imine-product which accumulates in the reaction mixture and only slowly hydrolyzes to adenine and the corresponding aldehyde. The imine products were identified using Q-TOF MS/MS. Mixing of the substrate-reduced enzyme with Cu^{2+}/imidazole as an electron acceptor, was used to monitor the oxidative half reaction. Using this artificial electron acceptor a high turnover rate was observed with isopentenyladenine. Correlations of the kinetic data with the known crystal structure will be discussed.

O1-5: Auxin conjugate hydrolysis from an evolutinary perspective

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Both higher and lower plants regulate auxin balance through interactions among *de novo* synthesis, degradation, efflux, influx, and conjugate synthesis/hydrolysis. We are using molecular genetic approaches to isolate and characterize components involved in the regulation of auxin conjugate hydrolysis in a variety of plant species. Several genes encoding putative hydrolases were isolated from the dicots *Arabidopsis suecica*, a close relative to *Arabidopsis thaliana*, *Medicago truncatula*, a model legume, the monocot *Triticum aestivum* and the gymnosperm *Pinus taeda*. Different temporal and spatial expression patterns for the hydrolase genes from different species were found. Expression of the hydrolase genes in *E. coli* allowed the investigation of substrate specificity which also differed among the amidohydrolases. Correlation of the number of amidohydrolase sequences in databases and their ability to convert a variety of substrates suggests an inverse correlation between the number of genes in this gene family and their substrate spectrum. We will extend our study in the future to other plant species, including lower plants such as the moss *Physcomitrella patens*, to understand how auxin homeostasis has evolved.

P1-1: Auxins stimulated the axillary branching of *Atriplex nummularia* L. *in vitro*

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The effect of three types of auxins; indole-3-acetic acid (IAA), indole-butyric acid (IBA), α-naphtalene acetic acid (NAA), at various concentration (0.0, 0.5, 2.0, 3.0, 4.0 mg/L) on axillary branching of *Atriplex nummularia* L. was tested using Murashige and Skoog medium (MS). *In vitro* shoots exhibited different response to the auxins. The maximum shoot proliferation rate was obtained at 0.5 and 2.0 mg/L IBA. IAA levels higher than 0.5 mg and the lowest level of NAA gave slightly lower shoot numbers compared with 0.5 and 2.0 mg IBA. The control treatment and the highest levels of IBA and NAA produced the longest shoots. Largest callus diameter was observed at 4.0 mg/L IBA and NAA. The best rooting occurred on media containing IAA and lower levels of IBA. IAA at 4.0 mg/L yielded the highest rooting percentage. Rooted microshoots were successfully acclimatized in the greenhouse under the mist.

P1-2: Loss-of-function mutants of cytokinin dehydrogenase genes and suppressor-mutagenesis of cytokinin-deficient *Arabidopsis*

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The catabolic inactivation of cytokinins is catalyzed by cytokinin oxidases/dehydrogenases (CKX). To assign functions to each individual *AtCKX* gene of *Arabidopsis*, we isolated 10 homozygous insertional mutants of six of the seven genes. Knockouts of single *AtCKX* genes have minimal consequences for plant development. To overcome the problem of functional redundancy, insertion lines have been systematically crossed to produce double and multiple knockout mutants. In addition, transgenic *Arabidopsis* plants expressing RNAi-constructs of individual *AtCKX* genes as well as regions common to all genes have been generated. A phenotypic description of mutant phenotypes will be presented. We carried out supressor mutagenesis of *AtCKX1*-overexpressing transgenic *Arabidopsis* plants which display a strong cytokinin-deficient phenotype. By this approach we aim to identify genes which are necessary for the establishment of the cytokinin-deficiency syndrome, e.g. negative control elements of cytokinin signal transduction or of cytokinin synthesis. We identified several recessive and dominant second site mutations which are being characterised. Intragenic suppressor mutations in the *AtCKX1* transgene identified several amino acids essential for the activity of the CKX enzyme.

P1-3: Use of ror-1 mutant to understand the cytokinin N-glucosylation pathway in *Arabidopsis*

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N-glucosylation is considered to be a cytokinin detoxification or inactivation mechanism in plants. Evidence for an N-glucosyltransferase (N-GT) pathway in Arabidopsis includes the presence of N7 and N9-glucosyl conjugates and the recent report of two glucosyltransferase genes expressed in yeast that recognized cytokinin substrates. We report the identification of a third gene that may encode an N-GT enzyme involved in cytokinin metabolism. We have isolated an Arabidopsis mutant, *roscovitine-resistant* (*ror-1)*, using T-DNA tagged lines and a roscovitine-resistance screening protocol. The sequence for the over-expressed gene in *ror-1* was retrieved by plasmid rescue. Blast analysis showed that the insertion was located in the gene *At3g59660.1* and the protein contained a GRAM (Glycosyltransferases Rab-like GTPase activators and Myotubularins) domain typically associated with glucosyltransferase enzymes and the endomembrane system. Analysis of endogenous cytokinins showed a smaller decrease in N-glucosides levels in *ror-1* compared to wild type plants in the presence of roscovitine. Mutants also showed an increase in the *cis*-cytokinin compounds and a decrease in cytokinin oxidase activity compared to controls.

P1-4: Expression, purification and characterization of recombinant *Arabidopsis thaliana* cytokinin dehydrogenase AtCKX2

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Cytokinin dehydrogenase (CKX, EC 1.5.99.12) is a flavoprotein catalyzing irreversible oxidative degradation of plant hormones cytokinins. Plants contain small families of genes coding for different CKX proteins. In *Arabidopsis thaliana*, seven distinct genes have been identified (*AtCKX1-AtCKX7*) and six of them have been shown to code for functional proteins. The isolation and purification of individual proteins from plant material is hindered by their simultaneous occurrence and low concentration in plant. *AtCKX2* gene was cloned into pYES2 and pDR197 yeast expression plasmids or integrated into yeast genome using vectors YIplac211 and pYESX under constitutive promoter PMA1 and inducible promoter GAL1. The *Saccharomyces cerevisiae* strain 23344c (MATa ura3) was used. CKX activity was in all cases released into the growth medium. AtCKX2 was purified to homogeneity from concentrated cell-free medium of the pYESX::AtCKX2/*ApaI* yeast clone producing about 1.5 mg of CKX per liter of medium. The purification was accomplished by chromatography on Concanavalin A, followed by ion exchange, gel filtration and hydroxyapatite chromatographies. AtCKX2 shows reaction optimum at pH 7 to 8, reacts with both isoprenoid and aromatic cytokinins and uses quinones and DCPIP as electron acceptors.

P1-5: Changes of cytokinin oxidase/dehydrogenase levels during senescence in cereals

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Senescence is the sequence of biochemical and physiological events comprising the final phase in development, which culminates in the cellular breakdown and death. Senescence initiation is programmed new gene expression caused naturally or induced by space competition, light, environmental stress and nutrition factors. It was proven that endogenously enhanced cytokinin contents in leaves leads to the delayed senescence. In cereals, the velocity of senescence of the flag leaf determines subsequent power and quantity of assimilate transport to the ripening seeds. Hence, a triggered manipulation of cytokinin content via engineered regulation of *CKX* gene expression becomes a promising tool to alter yield attributes in cereals. High levels of cytokinins were found in chloroplasts extracted from green leaves. There is no evidence for any CKX isoforms to target to chloroplast up to present time. However, an enormous increase in the CKX level was detected in early stages of leaf senescence in many cereal species. CKX activities were also detected in subcellular fractions isolated from leaf cells focusing to chloroplast fractions. Changes in expressions of all-known *CKX* genes during senescence in maize and barley are characterized.

P1-7: *Plasmodiophora brassicae* and the role of the *GH3*-gene family

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Plasmodiophora brassicae is the causal agent of clubroot disease which is one of the most plasmapus among Brassicacae. This disease was analysed among others with a microarray experiment (Affymetrix 22k) using the model plant *Arabidopsis thaliana*. One interesting result were changes in auxin metabolism and homeostasis during the infection. So the *GH3*-gene family, of which some genes adenylate auxins to form aminoacid conjugates (Staswick et al., 2005), is upregulated by *Plasmodiophora brassicae* during colonization of host roots. To repress the expression we took the two most upregulated *GH3*-genes and made antisense constructs with a root specific promoter from *Arabidopsis thaliana*. The testing of transformants is in progress.

P1-6: The forms and metabolism of cytokinins in pea (*Pisum sativum* L.) leaves

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A wide range of endogenous cytokinins (CKs), both isoprenoid and aromatic, was identified and quantified by HPLC-MS in pea (*Pisum sativum* L. cv Gotik) leaves indicating diverse metabolic conversions of primary products of CK biosynthesis. The occurrence of some CK forms such as N^6-benzyladenine, N^6-benzyladenosine and *cis*-zeatin derivatives was reported for the first time in pea. In order to obtain more information about CK interconversions, the metabolic fate of [2-^{3}H]*trans*-zeatin ([2-^{3}H]Z) was monitored *in vivo* after uptake by de-rooted pea plants. Radiolabelled *trans*-zeatin riboside, its ribotide and CK degradation products adenine and adenosine were detected as predominant [2-^{3}H]Z metabolites during 2-24 h incubation. Increasing formation of adenine and adenosine indicated extensive degradation of [2-^{3}H]Z by cytokinin oxidase/dehydrogenase (CKX). High CKX activity was confirmed in crude protein preparations from pea leaves by *in vitro* assays. Inhibition of CKX by dithiothreitol (15 mM) revealed relatively high activity of zeatin reductase (ZRED) catalysing *in vitro* conversion of [2-^{3}H]Z to [^{3}H]dihydrozeatin. Potential role of CKX and ZRED activities in establishment and maintenance of CK homeostasis in legumes will be discussed. (Supported by GAČR grant 206/03/0313.)

P1-8: Differential expression of maize cytokinin oxidase/dehydrogenase genes

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Cytokinin oxidases/dehydrogenases (CKO/CKX) play a major role in the regulation of cytokinin levels in plants. Significant enzymatic activity has been reported for *Zea mays*, especially in kernels. We cloned *ZmCKO1, ZmCKO2, ZmCKO3* cDNAs from immature kernels and the respective genes were mapped. Expression of these 3 genes and two additional ones, *ZmCKO4* and *ZmCKO5* (sequences retrieved from databases), was analyzed in different maize organs and during kernel development by using semi-quantitative RT-PCR. *ZmCKO1* was mainly expressed in the kernel while *ZmCKO2* to *ZmCKO5* genes were mainly expressed in vegetative tissues, with unique expression patterns. *ZmCKO1* (expressed in embryo) and *ZmCKO2* to *4* (expressed in pedicel) showed bi-phasic pattern of expression. *ZmCKO1* and *2* were expressed only after pollination contrarily to *ZmCKO5* (expressed in pericarp), which is probably not involved in kernel development. Enzymatic activity was confirmed for ZmCKO1 and ZmCKO3 produced in the yeast *Yarrowia lipolytica.* ZmCKO1 to ZmCKO5 share from 45 to 53 % identity with the exception of ZmCKO2 and 3 (93%) that are encoded by duplicated genomic regions. A duplication event could also account for two other genomic sequence pairs recently found in databases.

P1-9: Two cytochrome P450s involved in cytokinin homeostasis

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Cytochrome P450s are involved in the metabolism of many secondary plant products as well as most phytohormones. However, the function of most P450s remains unknown. P450s may have some functional overlap due to sequence redundancy among other members of the same subfamily. In this study, we generated a double mutant for two P450 genes showing similar expression pattern. The double mutant showed pleiotropic phenotypes including short stature, sterility, multiple inflorescences, short hypocotyl, abnormal skotomorphogenesis, and abnormal vascular patterning. Microscopic analysis showed that SAM of the double mutant is bigger than wild type and single mutants. The morphological phenotypes of the double mutant mimic those of amp1 mutants, which contain high cytokinin levels. Further, transgenic plants overexpressing either P450 genes showed strong apical dominance and defects in floral development. amp1 mutation and NPA application suppressed the strong apical dominance observed in the overexpression lines. Callus induction assay also revealed that cytokinins are accumulated in the double mutant. These results suggest that these two P450 genes play a role in cytokinin homeostasis in Arabidopsis. (AVOZ50040507, the GA AS CR No. IAA600380507 and NSF (INT-9600462).

P1-10: Retargetting a maize β-glucosidase: evidence from intact plants that zeatin-O-glucoside is stored in the vacuole

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Subcellular compartmentation is expected to play a significant role in modulating active cytokinin levels. Overexpressing Zm-p60.1, a maize β-glucosidase, disrupts zeatin metabolism in transgenic tobacco. We constructed tobacco overexpressing a recombinant *Zm-p60.1* retargetted to the vacuole. On medium containing zeatin, the transgenic seedlings between 21 and 28 days after sowing are significantly heavier than wild-type and show ectopic outgrowths at the base of the hypocotyl. In several cases these outgrowths resemble leaf-like structures. β-glucosidase is found preferentially in these structures. Differences in both spatial distribution and staining intensity were observed in the retargetted version. The ectopic presence of the enzyme leads to disruption in zeatin metabolism. These plants however are completely unable to accumulate zeatin-O-glucoside, the substrate of Zm-p60.1, thus showing, for the first time in intact plants, that the vacuole is the storage organelle for ZOG. Detailed histological analyses are in progress and conclusions derived from them will be presented. (Supported by grants from the Ministry of Education of the Czech Republic Nos. MSM 143100008 and LN00A081, the AS CR)

P1-11: High-level expression, characterization and crystal structure of *Zea mays* cytokinin oxidase/dehydrogenase CKO1/CKX1

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Cytokinin oxidase/dehydrogenase (CKO/CKX) is a flavoenzyme, which irreversibly degrades cytokinins. *Zea mays* CKO1 was expressed in the yeast *Yarrowia lipolytica*. The secreted recombinant enzyme was purified using two chromatographic steps and obtained as a homogeneous protein (12 mg/l culture). ZmCKO1 exhibited a molecular mass of 70 kD, pI of 6.3 and high thermostability (T_{50} = 55 ºC for 30 min incubation). Neutral sugar content of the molecule was 22%. Absorption and fluorescence spectra were in accordance with the presence of FAD as a cofactor. MALDI-TOF peptide mass fingerprinting correctly assigned the enzyme in MSDB protein database. Stoichiometry of aerobic CKO reaction was assessed using spectrofluorimetry. In the presence of oxygen, ZmCKO1 produces one molecule of H_2O_2 per one molecule of substrate. Oxidase-mode K_m values of cytokinin substrates ranged from 0.5 to 1.9 μM with pH optima varying between 7.3 and 8.0. The enzyme was crystallized in the presence of different inhibitors and the respective structures were solved up to 1.9 Å resolution. An allenic-type inactivator was found to be covalently linked to FAD cofactor. Five of eight predicted glycosylation sites were found to be occupied in the crystal structure of recombinant ZmCKO1.

P1-12: Cytokinin mononucleotides: harmless cytokinin species or potential killers of plant cells?

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Recent studies indicated that cytokinin ribosides (Mlejnek and Procházka 2002, Mlejnek et al. 2003), and also cytokinin bases (Carimi et al. 2003, Mlejnek et al. 2005) could induce apoptosis in micromolar concentrations in plant cells. Although the cytokinin concentrations that induced apoptosis were somewhat higher than those used routinely for growth media, they are still in the range of concentrations applied for induction of organogenesis or for studies of gene expression. We demonstrated that externally added cytokinin bases and ribosides were very rapidly phosphorylated within BY-2 cells. While cytokinin ribosides seemed to be phosphorylated by adenosine kinase, cytokinin bases are likely converted into mononucleotides *via* phosphoribosyl transpherase pathway. Intracellular accumulation of cytokinin monophosphates was accompanied by massive production of endogenous reactive oxygen species (ROS), intracellular ATP depletion, and these events were followed by the loss of cell viability. Inhibition of intracellular phosphorylation of cytokinin bases by adenosine or cytokinin ribosides by adenosin kinase inhibitor diminished ROS production, abrogated ATP depletion, and consequently prevented cells from death. Our results represent the first evidence of direct relationship between intracellular conversion of cytokinin bases and ribosides into corresponding mononucleotides and apoptosis induction in BY-2 cells. (This work was supported by the National Research Centre: Signalling in Plants, Czech Republic; grant No. LN00A081.)

P1-13: Two cytochrome P450s involved in fruit and lateral root development in *Arabidopsis*

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Cytochrome P450s are involved in the metabolism of many secondary plant products as well as most phytohormones. However, the function of most P450s remains to be uncovered. In this study, we generated a double mutant for two cytochrome P450 genes, which showed high sequence homology and expressed in all tissues investigated. The silique length of the double mutant reduced 40% compared to wild type and single loss-of-function mutants, but the seed number per silique was not changed in the double mutant. The transgenic plants overexpressing either of the P450 genes showed defects in fruit development, stem growth, and lateral root development. GUS activity driven by the gene promoter was strongly detected in fast growing tissues such as young leaf primordial, developing siliques, axillary buds, and floral buds. We will discuss the functional roles of the cytochrome P450 genes in plant growth and development.

P1-14: Cellular resolution of the distribution of IAA, IAA precursors and conjugates in *Arabidopsis thaliana* root tips

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Indole-3-acetic acid (IAA) in the root tip induces among other things primary and lateral root growth. Auxin is present in a gradient in the tip of the root with an increasing concentration towards the apex. It is however not known which cell types that are responsible for the synthesis and catabolism of IAA. We are currently mapping the distribution of auxin, its precursors and conjugates at a cellular level, in order to resolve this. For this purpose we are using several *Arabidopsis thaliana (A.t)* GAL4-GFP lines, where GFP is constitutively expressed in specific celltypes, this in order to cover the entire root. Protoplasts are prepared from 8 days old *A.t* seedling roots and GFP expressing cells are selected by Fluorescence Activated Cell Sorting (FACS). Currently 50 000 protoplasts are enough to quantify the auxin content by GC-MS, which is equivalent to approximately 2 mg material. Preliminary results indicate that IAA levels are significantly higher in the stele compared to more peripheral tissues such as the endodermis and the lateral root cap. We will additionally quantify the levels of oxIAA, IAA-asp, IAA-glu and IAN to further elucidate the IAA metabolism in the *A.t.* root at a cell specific level.

P1-15: Transgenic maize with increased zeatin O-glucosylation

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O-Glycosylation plays an important role in regulating the levels of cytokinins. O-glucosides are likely to be important in cytokinin storage and transport since they are resistant to dehydrogenases (oxidases) and can be converted back to free bases. Maize transformants harboring the bean zeatin O-glucosyltransferase (*ZOG1*) gene driven by the ubiquitin promoter were characterized. Modifications were observed in both vegetative and reproductive development. Transgenic plants were shorter, with higher leaf chlorophyll contents and delayed senescence. Tassels were smaller with many empty florets, lacking anthers. Ears emerged sooner than expected, about two days after tassel emergence instead of the usual seven days. These changes resulted in dramatically decreased kernel production in the field but not in the greenhouse with artificial pollination. RT-PCR, Western blotting, and cytokinin analyses quantified transgene transcription, translation, and effects on cytokinin composition. Transformants had significantly higher levels of the O-glucosides of zeatin and *cis*-zeatin, and also higher levels of the respective aglycones. These results suggest that increased O-glycosylation of zeatin leads to complex changes in plant development and cytokinin composition.

P1-16: Impact of ipt gene expression in PSAG 12-ipt transgenic wheat on cytokinin metabolism and nitrogen uptake and allocation

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Leaf senescence is a programmed process, which is under control of internal mechanisms including hormonal regulation. Cytokinins are known to inhibit leaf senescence. We report here how expression cytokinin biosynthetic gene (*ipt*) driven by promoter of a senescence-associated gene (P_{SAG12}) affects leaf senescence, cytokinin levels and nitrogen uptake and allocation in wheat plants (*Triticum aestivum* L. cv. Scamp). Plants grown in a nutrient solution containing low level of nitrate (385 μM) exhibited leaf senescence symptoms beginning 10 d after anthesis. Transgenic plants showed delay of senescence, reduced growth and more compact appearance. Delay of leaf senescence was accompanied with higher levels of endogenous cytokinins and increased activity of cytokinin oxidase/dehydrogenase in the flag- and 2nd leaf of the main stem. Lowering of the rate of nitrate uptake after termination of vegetative growth was slowed-down and up taken [^{15}N] from [^{15}N]O_3^- was preferentially allocated in slowly senescing leaves. The differences in nitrogen allocation were decreasing during the subsequent grain maturation. As compared to controls number of grains per ear, grain weight and grain nitrogen accumulation were slightly deceased. Possible practical applications will be discussed.

P1-17: Spatio-temporal study on gene expression of CYP735A2, a cytokinin hydroxylase, in *Arabidopsis*

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In Arabidopsis, CYP735A1 and CYP735A2 have been identified as genes for cytokinin hydroxylase catalyzing trans-zeatin biosynthesis, but physiological roles of the genes in plant development are still unclear. To understand them, we generated transgenic lines of Arabidopsis carrying CYP735A-promoter:GUS chimeric gene and analyzed their expression patterns in various tissue and stages of plant development. In analysis with quantitative RT-PCR (qRT-PCR), accumulation level of CYP735A1 mRNA was quite low and we could not detect any GUS activity in CYP735A1:GUS plants. On the other hand, expression of CYP735A2:GUS gene was found in various tissues such as root vascular system, pollen grain, base of primary and lateral inflorescence, abscission zone of floral organ and base of silique. The qRT-PCR analysis revealed that CYP735A2 expression at basal part of main inflorescence was up-regulated coordinately with the bolting process. These results suggest that trans-zeatin biosynthesis by CYP735A2 is involved in this developmental aspect.

P1-18: Bacterial auxins and cytokinins synthesis: phylogeny versus ecology

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Auxins and cytokinins are not unique for plants. Microbial phytohormone synthesis is reported for plant pathogens, endosymbiots as well as soil and plant-associated bacteria. The bacterial phytohormone production is complex: depending on the microbial species, growth phase and even plant host species. Several different auxin and cytokinin biosynthetic pathways are used by prokaryotes. Here we present a screening of the phytohormone production of a wide range of bacterial types including rhizosphere bacteria, endosymbionts and pathogens. The strains selected cover also all major bacterial classes. Soil bacteria and clinical isolates were analysed as control strains. Auxins and cytokinins were determined in bacterial growth media using LC-(ES+)-MS/MS. The pathogenic strain *Erwinia chrysanthemi* A470 and plant surface coloniser strain *Azospirillum brasiliense* Sp245 produce significant higher amounts of indole-3-acetic acid (IAA), whereas the highest cytokinin producers are found among the plant-associated bacteria. The phytohormone biosynthesis capacity and the pathways involved are discussed according to the ecological niche as well as the phylogeny of the bacteria.