**L4-1: Auxin and cytokinin represent key regulators of lateral root initiation and development in *Arabidopsis***

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Lateral roots provide a good developmental model to study the regulatory signals that influence post-embryonic organogenesis in higher plants. In *Arabidopsis*, lateral roots are derived from a small number of pericycle founder cells located adjacent to the xylem pole which undergo a defined program of oriented cell divisions to form a lateral root primordium (Casimiro *et al*, 2003). Auxin represents a key promotive signal. We will (1) describe how auxin transport influences the apical-basal patterning of lateral root primordia; and (2) discuss the developmental functions of 2 presumptive auxin influx carriers, AUX1 and LAX3. Cytokinins act as negative regulators of lateral root development (Werner et al., 2003). However, little is known about the mode of cytokinin action. To delimit which root cells were sensitive to the cytokinin signal, we targeted the expression of the *Agrobacterium* isopentenyltransferase (cytokinin biosynthesis enzyme) transgene in xylem-pole pericycle cells and young primordia, respectively. Our results indicate that xylem-pole pericycle cells are sensitive to cytokinin inhibition, whereas young lateral root primordia are not. Casimiro et al., 2003, Trends Plant *Sci.* 8, 165, Werner et al.,, 2003, Plant Cell 15, 2532)

**L4-2: Shoot branching**

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Balancing root and shoot growth, and primary and secondary shoot growth, necessarily requires long-distance signalling between the root and the shoot, and between primary and secondary shoot apical meristems. Classically both shoot-derived auxin and shoot and root-derived cytokinin have been implicated in these processes. More recently, additional components of the network of signals that regulate shoot branching have been identified. In Arabidopsis, mutants at 4 loci, called MAX1-MAX4, with increased shoot branching appear to define an additional branch-inhibiting pathway that has roles both downstream of, and independent of auxin. Grafting studies have demonstrated that three of these loci are involved in the production of a long-range graft transmissible signal that inhibits bud growth, and this is consistent with the molecular identities of these genes. Progress in identifying this new hormone and in unravelling its interactions with auxin and cytokinin will be presented.

**O4-1: Regulation of source-sink relations by cytokinins to mediate developmental and physiological responses**

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Soluble sugars are not only substrate to sustain heterotrophic metabolism but are also metabolic signals that regulate various aspects of growth and development of plants. Extracellular cleavage of the transport sugar sucrose into the hexose monomers by an invertase is essential for assimilate partitioning. A relation between cytokinins and sink activity was supported by the induction of an invertase by cytokinins. Using complementing functional approaches in tobacco plants it has been established that extracellular invertase is an essential component of cytokinin-mediated delay of senescence. The effect of cytokinins can be substituted by this metabolic enzyme through developmentally or chemically inducible expression. Cytokinins fail to delay senescence under conditions when invertase activity is inhibited, showing that nutrient mobilisation is an essential component of the underlying mechanism. It is suggested that also other cytokinin mediated processed such as the stimulation of cell division are mediated via the regulation of assimilate partitioning and of the sugar status, thus linking cytokinin responses to primary metabolism.

**O4-2: The RAMOSUS branching genes in pea regulate a network of novel signals, auxin and cytokinin**

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In *Pisum sativum* L., the *RAMOSUS* genes *RMS1*, *RMS2* and *RMS5* regulate shoot branching via long-distance signalling. Our work provides molecular, genetic and physiological evidence that *RMS1* plays a central role in a shoot-to-root-to-shoot feedback system that regulates shoot branching in pea. *RMS1* and *RMS5* control levels of an as yet unidentified mobile branching inhibitor required for auxin inhibition of branching. Indole-3-acetic acid (IAA) positively regulates *RMS1* transcript level, a potentially important mechanism for regulation of shoot branching by IAA. In addition, *RMS1* transcript levels are dramatically elevated in *rms3*, *rms4* and *rms5* plants, which do not contain elevated IAA levels. This degree of up-regulation of *RMS1* expression cannot be achieved in WT plants by exogenous IAA application. Nevertheless, grafting studies indicate a mobile feedback signal contributes to the elevated *RMS1* transcript levels. Grafting studies also show *RMS* genes regulate xylem sap cytokinin content via long-distance signalling, with some mutations in the shoot causing up to 40-fold depletions in zeatin riboside content in the root xylem sap. Therefore, branching control in pea involves IAA, cytokinin, the *RMS1* inhibitor and a potentially IAA-independent feedback signal.

**O4-3: Auxin, PINs and flower phyllotaxy**

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Leaves, flowers and floral organs develop from plant meristems in exquisitely ordered phyllotactic patterns. Genetic or chemical disruption to polar auxin transport can severely disturb organ initiation and phyllotaxy, thus revealing that the active transport of auxin is an important signalling system in directing organ patterning. The auxin canalization model explains how spiral or decussate patterns occur. PIN proteins concentrate auxin levels and trigger organ initiation. Depletion of auxin around each new primordium creates a zone of organ inhibition, and overlapping depletion zones specify the phyllotactic pattern. While the initiation of leaves and flowers follows a spiral pattern in Arabidopsis, floral organs occur in consecutive whorls. It is not yet known how this pattern is established in relation to auxin transport. Using reporter gene expression, we have observed auxin distribution in flowers. Interestingly, the whorled pattern of sepal initiation does not match the auxin distribution pattern, which appears to be decussate. Thus, an apparent delay in sepal initiation, despite auxin build-up, may compel floral organs to follow a whorled pattern. In addition, while *PIN1* is the primary PIN gene required for flower and floral organ phyllotaxy, other PINs are also involved.

**O4-5: ABPHYL1, a cytokinin-inducible response regulator that regulates phyllotaxy in maize.**

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Phyllotaxy describes the pattern of leaf initiation at the shoot apical meristem (SAM). While auxin has long been implicated in control of phyllotaxy, our data also suggest an essential involvement of cytokinins. A maize mutant, abphyl1 (abph1) displays an altered phyllotaxy (opposite and decussate) and is defective in a two-component response regulator that may operate in the cytokinin signaling pathway. ABPH1 expression is normally confined to the SAM, and is cytokinin-inducible. When maize SAMs are treated with cytokinin, they become enlarged to a similar size as seen in abph1 mutants. This result suggests that cytokinin regulates phyllotaxy through ABPH1, a repressor of cytokinin-stimulated SAM growth. A failure in this signaling pathway in abph1 mutants results in more available space for leaf initiation. To ask if ABPH1 might function as a transcriptional repressor, we have made a yellow fluorescent protein fusion, and found that ABPH1 is nuclear localized. We are also testing the nature of the interaction between auxin and cytokinin in phyllotaxy regulation, by studying ABPH1 expression in plants grown on auxin polar transport inhibitors, and auxin polar transporter (PIN1) expression in abph1 mutants. Giulini, A., Wang, J. and Jackson, D. (2004). Nature, 430, 1031-4.

**O4-4: Auxin response factors mediate *Arabidopsis* organ polarity via modulation of KANADI activity.**

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Members of the KANADI gene family in Arabidopsis regulate abaxial identity and laminar growth in lateral organs. The auxin response protein ETTIN (ETT/ARF3) mediate KANADI activity as determined via rescue of growth restricting effects of ectopic KANADI1 in petals. While ett plants have normal leaves and mildly affected flowers, double mutants between ett and arf4, have many of the characteristics of kan1 kan2 mutant plants. ARF4 RNA expression is restricted to abaxial side of emerging leaf primordia while ETT is detected throughout. In floral organ primordia these patterns are reversed. Both gene transcripts are present in kan1 kan2 mutant plants, and ectopic ARF4 or ETT expression fails to rescue the KANADI mutant phenotype. We propose that ETT and ARF4 act in conjunction with KANADI or its downstream targets to facilitate lateral organ asymmetry. This function is common with other ARF proteins as well, implied by the negative function of a truncated ETT N-terminal region, which is highly conserved in most ARF proteins. As ARF proteins mediate between auxin levels and auxin response, we propose a model where establishment of lateral organ asymmetry utilizes localized auxin gradients to gradually partition emerging primordia along the abaxial-adaxial axis.

**O4-6: sow1 suppresses the vascular phenotype and the reduced cytokinin response of wol**

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The *Arabidopsis wooden leg* (*wol*) mutant root has a reduced number of provascular cell files and all cell files differentiate as xylem during vascular morphogenesis resulting in determinate root growth (Scheres et al. 1995). Additionally, *wol* shows reduced sensitivity to cytokinin. *WOL* is allelic with *CRE1*/*AHK4* and encodes a cytokinin receptor (Mähönen et al. 2000, Inoue et al 2001, Suzuki et al 2001). In order to identify molecules acting downstream of CRE1, we performed a suppressor screen for the determinate growth habit of *wol.* In the *wol* background, the *suppressor of wol1* (*sow1*) mutant shows an increased number of vascular cell files in the root-hypocotyl junction with undifferentiated files present, as opposed to the exclusively protoxylem cell files present in *wol.* Moreover, the impaired cytokinin response of *wol* can partially be suppressed by *sow1*. Alone, *sow1* shows defects in maintaining the balance between cell division and differentiation during root vascular morphogenesis.

**P4-1: Diversity of cellular and organogenetic responses of *Arabidopsis* pericycle in response to auxins and cytokinins**

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Arabidopsis pericycle is able to regenerate both root (RAM) and shoot (SAM) apical meristems when grown *in vitro* according to the hormones added in the medium. In order to determine the respective roles of auxins and cytokinins in these opposite morphogenetic events, the effects of a range of 2,4-D, IAA, NAA and kinetin concentrations on the xylem and phloem pericycle cells have been examined on explants expressing GUS or GFP fusions with promoters of cell cycle-, RAM- or SAM-specific genes. The number of xylem pericycle cells involved in RAM formation and the subsequent RAM size vary according to the auxin, and a range of true RAMs to RAM-like structures more or less fasciated is obtained in presence of 2,4-D. These structures have a decreased RAM identity and can switch to a SAM identity upon cytokinin treatment. Combination of 2,4-D with kinetin stimulates the phloem pericycle to divide periclinally and gives rise to pericycle derivatives which are able to regenerate SAMs following transfer on high cytokinin-containing medium. These data reveals the bipotentiality of the pericycle and the versatility of the RAM identity, this identity being possibly controlled by the auxin to cytokinin ratio.

**P4-2: Establishing of polar auxin transport in pea axillary buds released from apical dominance**

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Apical dominance is a dominance of the shoot apex over the axillary buds. It is well-known that auxin accumulated in the apex is transported towards the roots by polar transport. The mechanism, whereby polar auxin transport in the shoot regulates outgrowth of axillary buds, is unknown. One of the possibilities is that shoot auxin transport inhibits polarization of the cells in axillary buds, disabling the export of auxin from the inhibited buds. We studied the expression patterns of genes involved in auxin transport and bud dormancy (*AUX 1, PIN 1* and *PsDRM1*) and localization of the putative PsPIN1 protein using anti-AtPIN1 antibodies in axillary buds during release from apical dominance. Semi-quantitative RT-PCR revealed a decrease of*PsDRM1* expression after decapitationor BA application to the axillary buds of intact plants. Expression of *PsPIN1* and *PsAUX1* increases already 2 hours after decapitation with a maximum of about 150% at 6 hours and also the application of BA dramatically increases expression of both genes. Immunolocalization of the putative PsPIN1 protein proved polarization of the protein in provascular strands of axillary buds 6 hours after decapitation and also after BA application.

**P4-3: Growth control of the *Arabidopsis* root meristem by cytokinins**

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Two plant hormones, auxins and cytokinins, have long been recognized as essential signaling molecules in diverse processes of plant growth and development. They are believed to act synergistically or antagonistically to control fundamental developmental process such as cell division and cell differentiation which ultimately lead to shoot and root organogenesis. Although, molecular, genetic and biochemical studies have already provided evidence for a role of auxin in controlling cell specification, cell division and cell polarity, the role of cytokinin at the cellular and tissue level during plant development has remained elusive. Recently a number of molecular and genetic tools have been developed to study cytokinin signal transduction pathway in *Arabidopsis*. We are taking advantage of these tools to shed light on the role of cytokinin *in planta* by focusing on the well-characterized *Arabidopsis* root meristem.

**P4-4: *Arabidopsis* shoot apical meristem development after ipt transcriptional activation in pOp/LhG4 system**

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A transcription activation system pOp/LhG4 developed for regulated gene expression (Moore et al., 1998, PNAS USA 95, 376) was used in *Arabidopsis.* Expression of *ipt* is achieved by crossing a reporter plant (pOp-*ipt*) with an activator plant expressing the transcription factor 35S-*LhG4*. We attempted to quantify the impact of *ipt* expression on the structure of seedlings in comparison with reporter, activator and WT (*A. thaliana* Col0) controls. Aseptically grown seedlings of all lines were collected at three intervals. Fixed samples were treated by the classical histological method and stained with Safranin and Fast green. An Olympus BX-61 equipped with CCD camera DP 50 and analySIS® software were used for section evaluation. Phenotypic signs of cytokinin overproduction were observed in crosses of reporter and activator lines. Distinct differences were observed between lines after 7 days. Plantlets with activated *ipt* developed larger shoot apical meristem. Meristems of 15-day-old *Arabidopsis* plants were changed to generative ones to various degrees. The shift to latter developmental stages (Smyth D.R. et al., 1990, Plant Cell 2, 755) was traced in *ipt*-activated lines. (Supported by grants nos. MSM143100008, MSM0021622415, IAA600380507 and AVOZ50040507.)

**P4-5: Tuber formation, cytokinin content and carbohydrate metabolism in potato plants carrying *rolC* and invertase genes**

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Effect of *Agrobacterium rolC* gene, gene of yeast invertase, and their combination on the morphogenesis, cytokinin content and carbohydrate metabolism was studied on tubers of transgenic potato (*Solanum tuberosum* L. cv. Désirée) plants. Nontransformed plants ($Cont_{nt}$) and transgenic plants harbouring *rolB* or GUS gene, were used as control lines. Single node cuttings were cultivated *in vitro* in the dark at 20°C on MS medium with 8 % sucrose. Expression of *rolC* or *rolC + inv* genes resulted in specific changes of microtuber shape. Content of cytokinins (IPA, ZR, and IP9G) was higher in *rolC* tubers. High activity of sucrose synthase was found in tubers of all transgenic and control lines. The activity of acid invertase (soluble and cell-wall bound forms) was low in tubers of $Cont_{nt}$, GUS, and *rolB* variants and high in *inv* tubers. In *rolC* tubers, the activity of soluble and cell-wall bound invertase was significally enhanced comparing to control plants. The content of sucrose was 4-5-fold higher in *rolC* tubers and 3.5-4-fold lower in *inv* tubers than in $Cont_{nt}$. The level of fructose and glucose varied between lines to a less extent as compared to sucrose content. The data indicate the possible involvement of cytokinins in tuber shape determination. (Supported by RFBR 04-04-49117.)

**P4-7: Overexpression of the bacterial ipt gene in developing *Arabidopsis* seedlins carrying auxin reporters**

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Constitutive (pOp/LhG4) and/or inducible (pOp/LhGR) CaMV S35-directed transactivation of the expression of the cytokinin biosynthetic gene *ipt* from *A. tumefaciens* was carried out in *Arabidopsis* first to compare its phenotypic effects if applied at distinct stages of early seedlings' development, second to show whether it can lead to altered auxin distribution. The response of the root system and its ability to regenerate depends on a particular developmental stage at which the *ipt* overexpression is turned on. Long-term activation, triggered immediately at germination, allowed us to visualize DR5 expression patterns in seedlings displaying *ipt* phenotypes of different strength. Intermediate phenotypes lack lateral roots and have reduced number of lateral root primordia which still preserve local DR5 gradients. Highly activated seedlings feature severe morphological changes accompanied by alteration in the DR5 expression. We hypothesize there is a threshold level of endogenous cytokinin which, when exceeded, leads to developmental changes that may be irreversible. Whether they precede or are a result of the impaired auxin homeostasis and transport still needs to be studied. (Supported by MSM0021622415, AVOZ50040507 and IAA600380507.)

**P4-6: Effect of different type growth regulators on the early phases of *Drosera rotundifolia* L. somatic embryogenesis**

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Conversion of differentiated on dedifferentiated plant cells is accompanied with large amount of processes closely connected with the genes, which transcription depends on the concentration gradients of growth regulators (GR). GR - $1.10^{-8}$ M NAA and $1x10^{-8}$ M BAP in MS cultivation media induced dedifferentiation in some mesophyl and epidermal cells of sundew giving base for somatic embryo globular structure. Ultra-thin section indicated that dedifferentiated cells had large nuclei, decondensed chromatin, big nucleolus with "transparent nucleolar vacuole" characteristic with active transcription. SEM showed well-developed fibrilar network of extracellular matrix. Analyses of protein patterns showed quantitative and qualitative changes for protein belonging to the group of auxin-binding protein. Content of *de novo* synthetized low-molecular proteins decreased after $24^{th}$ days of cultivation. Opposite effect was found for high molecular part of protein patterns. Slot-blot indicated that amount of some nonspecific chitinases is under control of GRs. During somatic embryogenesis their content decreased. SE so reflect complicated molecular and biochemical pathways induced with GR by dedifferentiation and redifferentiation.

**P4-8: Cytokinins in potato tuber initiation, growth and dormancy**

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Changes in cytokinin (CK) contents were determined during the periods of tuber initiation, growth and dormancy in 2 varieties: early Karin and late Granola. These were grown at stations Valečov and Velhartice at two levels of nitrogen fertilization (40 and 120 kg $N.ha^{-1}$). Cytokinin content was determined (by LC/MS) in leaves, stems, stolons and tubers, eventually in buds from tuber apical parts. Karin had in all organs much higher CK level than Granola during the whole growth season. Nitrogen fertilization had only slight effect on CK levels and also the differences between the stations were rather small. Most important developmental differences were found during tuber initiation and than during dormancy. At the beginning of tuber initiation there was a higher level of CKs in stolons and when the tuber started to grow, then CK level dropped in stolons and increased in tubers. At harvest the level in tuber buds was rather low, but during storage at low temperature it dramatically increased - more at $8^{o}$ C than at $4^{o}$ C. Dessication resulted in decreased CK levels in buds of stored potato tubers. Comparison of more cultivars with different earliness did not show any correlation between this factor and CK content in buds. (Supported by the GA AV grant S5038352.)

**P4-9: Influence of decreased level of endogenous cytokinins on antioxidative mechanisms in tobacco leaves during ageing**

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Cytokinins are known to inhibit plant senescence. Their content in plant is effectively regulated by irreversible degradation catalysed by cytokinin oxidase/dehydrogenase (CKX). Plant ageing and senescence as its final phase are associated with increased levels of reactive oxygen species (ROS) causing oxidative stress and damage of cell components. Control of ROS levels may occur through an antioxidative enzyme system. We investigated the antioxidative enzyme protection in tobacco (*Nicotiana tabacum* L. cv. Samsun NN) leaves of different age having reduced levels of endogenous cytokinins resulting from overexpression of the *Arabidopsis CKX* gene (AtCKX2). Transgenic plants showed significantly enhanced CKX activity. Control and AtCKX2 plants differed substantially in their phenotypes. Leaf developmental stage and onset of senescence was characterized by changes in chlorophyll fluorescence parameters and chlorophyll and soluble protein contents. Initiation of leaf senescence was not hastened in transgenic plants. Activities of antioxidant enzymes were higher in AtCKX2 plants suggesting their better equipment with antioxidant protection mechanism(s) and markedly decreased with age in both control and transgenic plants. (Supported by GAČR 522/03/0312 and 206/03/0313).

**P4-11: Cytokinin control of KNOTTED1-like homeobox genes expression during *Arabidopsis* shoot development**

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Formation of plant body can be modulated by hormones including cytokinin (CK). Identification of molecular mechanisms of CK action in plant development is a matter of intense research. Recently, cross-talk between CKs and homeobox genes of the *KNOTTED1*-like class I (*KNOX I*) has been proposed. We characterized alterations in expression patterns of *KNOX I* genes following CK application and regulated increase in endogenous CK levels. Histochemical analysis of pKNAT1:GUS plants and mRNA *in situ* hybridization revealed that *KNAT1* expression is localized to the base of shoot apical meristem, leaves and stipules and along hypocotyl vasculature regardless of CK treatment. Real time RT PCR proved that quantitative shifts in *KNAT1* expression correlate with morphological changes following CK action but they do not precede them. It was demonstrated that all *KNOX I* genes follow similar trend in apices and hypocotyls. On the contrary, the members of *KNOX I* that are commonly expressed in leaves (*KNAT2, KNAT6*) seem to be positively upregulated by CKs only in cotyledons and true leaves. (Supported by grants from the Ministry of Education of the Czech Republic Nos. LN00A081 and MSM0021622415, the AS CR AVOZ50040507 and the GA AS CR (No. IAA600380507).

**P4-10: Cytokinin levels, cytokininoxidase and beta-glucosidase activity in tobacco leaf segments during *in vitro* organogenesis**

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Shoot organogenesis on the tobacco leaf segments *in vitro* is controlled by the levels of endogenous cytokinins. Relationship between regeneration and cytokinin activity was studied by using hydroxylated cytokinins (zeatin, dihydrozeatin or meta-topolin). Higher regeneration on transgenic (cv. Petit Havana SR1 transformed with cytokinin specific beta-glucosidase Zmp60.1) tobacco leaf segments than wild type (cv. Petit Havana SR1) was observed. The most effective cytokinin for the shoot organogenesis was zeatin, followed by meta-topolin and the least effective was dihydrozeatin. Taken up cytokinin bases were converted into ribosides, ribotides and glucosids during the cultivation period. The higher activity of cytokininoxidase in tobacco leaf segments (wild type) and β-glucosidase activity in transgenic tobacco leaf segments were detected. Regenerating segments had lower β-glucosidase activity than non-regenerating segments. (This research was supported by the project of Grant Agency of MUAF IGA 4/4.)

**P4-12: A genomic approach to identify epigenetic modifications associated with 2,4-D use during oil palm somatic embryogenesis**

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The inclusion of plant growth regulators in *in vitro* cell culture media may provoke somaclonal variation that gives rise to abnormal developmental phenotypes. Such phenomena may be caused by epigenetic changes that perturb gene expression. We have begun to investigate the epigenetic effects of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) used during oil palm somatic embryogenesis (SE). 2,4-D induces a global genome hypomethylation while remethylation occurs after SE is initiated by the removal of 2,4-D. In addition, higher doses of 2,4-D result in reduced remethylation during SE. Expression profiles of selected candidate genes change during the initiation of SE after the removal of 2,4-D. To investigate global genome expression changes during this transition, we produced suppression subtractive hybridization (SSH) libraries enriched in cDNAs derived from cells cultured in the presence of 2,4-D and cells undergoing the initiation of SE after the removal of 2,4-D. SSH derived cDNAs have been sequenced and an analysis of their putative functions during the initiation of SE will be discussed. The development of oil palm molecular resources will allow an analysis of genome expression during SE in relation to epigenetic changes induced by *in vitro* culture conditions.

**P4-13: Cytokinin pool dynamic changes and distribution of CKX activity in peas in relation to developmental senescence**

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Variable distribution of cytokinin oxidase/dehydrogenase activity (CKX: EC 1.5.99.12) in leaves of young pea seedlings (cv. Manuela) during the period needed for full leaf bud expansion was observed. Lower iPR/iPRP and *cis*ZR/*cis*ZRP contents were detected at stages characterised with higher CKX activity. *Cis*Z and its derivatives were found to be the dominant form of Z-type cytokinins at this particular stage of development in pea. Respective bases were poorly represented in the leaf tissue in this model system during the whole experimental period. CKX activity correlated with the changes in cytokinin pool, which may have triggered developmental events in leaves during bud expansion. These changes followed a regular pattern characterised with two peaks of enzymatic activity: the first one at stages corresponding to active organ development (with subsequent decrease of CKX in fully expanded leaves), and the second one related to beginning of senescence processes in the oldest leaves. The data suggest that CKX – mediated dynamic changes in cytokinin pool participate in internal control mechanisms of developmental senescence.

**P4-15: Manipulation of Meristem Function by Local Manipulation of Cell Cycle Parameters**

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The main structure involved in plant vegetative development is the shoot apical meristem (SAM). It produces leaf primordia in a spiral phyllotaxy which requires highly coordinated patterns of gene expression linked with specific gradients of auxin. These patterns are superimposed on the highly organized and conserved tunica–corpus structure. However, whether this cellular organization has any functional significance is open to debate. To investigate this problem we changed locally parameters such as cell division orientation or frequency within the meristem. To do that we generated transgenic tobacco, a model plant in our study, containing either phragmoplastin or cyclinA under transcriptional regulation of a tetracycline inducible promoter. Overexpression of phragmoplastin led to disruption of the tunica structure while overexpression of cyclinA promoted cell proliferation. Neither manipulation overtly influenced meristem function. More recently we have turned our attention to a gene involved in the cellular decision to differentiate or proliferate - a retinoblastoma-related protein (RBR). After local increase of RBR gene expression in the meristem we observed a drastic inhibition of growth, supporting the hypothesis that RBR functions as a growth repressor.

**P4-14: Functional characterization of cytokinin oxidases/dehydrogenases in *Arabidopsis thaliana***

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The metabolic degradation of cytokinins is catalyzed by cytokinin oxidases/dehydrogenases (CKX), which are encoded by small gene families in different plant species. We have analyzed the expression of *CKX* genes in *Arabidopis*, the subcellular localization of their gene products and the developmental consequences of reduced cytokinin concentrations in transgenic plants. Retarded shoot development and an enlarged root system in these plants suggested that the hormone regulates shoot and root meristematic activity and morphogenesis in opposite modes. We present results on *AtCKX7*, which is expressed in specific domains of floral organs and in the vascular tissue. The protein shows a different cellular compartmentation than other family members. We have further investigated whether localized changes of the cytokinin content has local or systemic consequences. Enhanced CKX-expression in specific tissues revealed that the consequences of cytokinin deficiency are to a large extent tissue-autonomous, indicating a paracrine mode of cytokinin action, and opening the possibility to modulate organ growth in a targeted fashion.

**P4-16: *Arabidopsis* class 1 homeobox (KNOXI) genes act through activation of cytokinin biosynthesis**

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Continuous organ production is enabled through the action of plant meristems. *Class I homeobox* (*KNOXI*) genes are expressed in the shoot apical meristem (SAM), and play central roles in SAM function. Mutations in the Arabidopsis *KNOXI* gene *SHOOT MERISTEMLESS* (*STM*) result in compromised meristem function. KNOXI proteins and the plant growth regulator cytokinin are involved in overlapping pathways, such as meristem maintenance and repression of senescence. To investigate whether cytokinin acts downstream of KNOXI proteins, we applied cytokinin to Arabidopsis (*Arabidopsis thaliana*) *shoot-meristemless* (*stm)* mutants, and found that cytokinin application partially rescued the *stm* mutant phenotype. Specific meristem expression of a cytokinin biosynthesis gene through the *STM* promoter in *stm* mutants partially complemented *STM* function. STM activation in *35S:STM-GR* transgenic plants resulted in elevated cytokinin levels, and in increased expression of the cytokinin response gene *ARR5*. STM activation induced a fast and dramatic increase in the mRNA levels the Arabidopsis *isopentenyl transferase* 7 (At*IPT7*) gene, which encodes a cytokinin biosynthesis enzyme. Additional KNOXI proteins also induced expression of At*IPT7* and *ARR5*. These results indicate that KNOXI proteins act in part through activation of cytokinin biosynthesis.