L3-1: Auxin recognition and sensing

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The structure of the auxin-binding protein ABP1 demonstrated in detail how auxins bind inside the binding pocket. Since publishing the data for bound 1-NAA, the details for a set of other bound molecules will be shown. Associated with each is a small change in the position of part of the protein, including the C-terminus and these data will be discussed in relation to the activity of ABP1 in signalling. In addition, a chemical structure-activity binding analysis has been completed on both maize and strawberry ABP1s using 5 families of auxin, many of which have been used as selective herbicides. Detailed comparisons of monocot and dicot binding have been preculded before due to the difficulties of purifying ABP1s, but recombinant strawberry ABP1 has been expressed in the baculovirus system and purified. The binding kinetics for many auxins are similar between the two species, although the affinity for IAA does differ being greater in the dicot. The data will be discussed in terms of auxin recognition and other developments in the field of auxin perception. We will also explain our strategy for developing auxin sensors.

L3-2: Characterization of the TIR1/AFB family of auxin receptors

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The plant hormone auxin functions in many aspects of plant growth and development. Auxin acts by promoting the degradation of transcriptional regulators called Aux/IAA proteins through the action of the ubiquitin protein ligase SCFTIR1. In our recent work we have shown that TIR1, the F-box protein subunit of SCFTIR1, functions as an auxin receptor. Auxin binds directly to TIR1 to promote the interaction between TIR1 and the Aux/IAA proteins. However, loss of TIR1 has a modest effect on auxin response indicating that additional auxin receptors must be present. Indeed genetic and biochemical studies indicate that three additional F-box proteins called AFB1,2 and 3 also regulate auxin response. Like TIR1, these proteins interact with the Aux/IAA proteins in an auxin dependent manner. The successive loss of TIR1 and the AFB proteins results in a progressively more severe phenotype. Plants that are deficient in all four proteins are auxin insensitive and exhibit a severe embryonic phenotype similar to the mp/arf5 and bdl/iaa12 mutants. Our results indicate that TIR1 and the AFB proteins define a new family of auxin receptors that collectively mediate auxin responses throughout plant development.

L3-3: Auxin response factors and aux/IAA repressors

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ARFs and Aux/IAA proteins function together in regulating a variety of early/primary auxin response genes. Arabidopsis contains more than twenty different ARFs, and five of these function as transcriptional activators , while others function as transcriptional repressors on auxin response genes in transfected protoplasts. On the other hand, more than twenty different Aux/IAA proteins that have been tested function as transcriptional repressors on auxin response genes in transfected protoplasts. ARFs contain a portable repression or activation domain, while Aux/IAA proteins contain a portable repression domain. ARFs are thought to bind to TGTCTC Auxin Response Elements (AuxREs) in promoters of auxin response genes, while Aux/IAA repressors are thought to be recruited to auxin response genes by associating with ARF activators bound to AuxREs. We initiated ChIP assays to monitor the association of ARFs on AuxREs in promoters of both integrated reporter genes and natural auxin response genes, using antibodies specific for individual ARF activators as well as epitope tag antibodies specific for epitope-tagged ARF activators. The ARF antibodies have also been used to monitor the abundance of ARF activators in response to hormones and environment.

L3-4: The role of two-component elements in cytokinin signaling and plant growth and development

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Cytokinin signal transduction occurs through a classic bacterial two-component signaling system. Using primarily a genetic approach, we have defined the role of the Arabidopsis two-component genes in cytokinin signaling. There is extensive functional redundancy in these gene families. Analysis of lines harboring multiple disruptions has indicated that most of these two-component like genes play a role in cytokinin signaling. Single loss-of-function mutations in the type-A genes have no detectable effect on cytokinin responsiveness. However, multiple type-A arr mutants are hypersensitive to cytokinin in various assays, and the severity of the hypersensitive phenotype generally correlates with the number of type-A ARRs disrupted. The multiple mutants also display various phenotypes associated with increased cytokinin function. Single and various double mutants in the 5 Arabidopsis AHP genes exhibit no differences in cytokinin responsiveness, but the *ahp1,2,3* mutant displays reduced sensitivity to exogenous cytokinin in root elongation assays. The *ahp1,2,3,4,5* mutant is severely impaired in the induction of cytokinin primary response genes and shows developmental defects. A model incorporating these and other results will be presented.

O3-1: Floret abscission in "Red Cestrum" cut flowers may involve members of the Aux/IAA gene family

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Floret abscission in `Red Cestrum` (*Cestrum elegans*) cut flowers was delayed by pulse treatment of 2,4-D but not by NAA. 2,4-D moved acropetally in a significant amount sufficient to reduce floret abscission, while NAA failed to do so. In florets and leaves, NAA was quickly conjugated while 2,4-D remained in the active form. Significant amount of ethylene was evolved from shoots pulsed with 2,4-D compared to those treated with NAA. We hypothesize that differences in the mode of transport and metabolism of the two auxins might result in differences in gene activation. Expression patterns of the early auxin responsive (*Aux/IAA*) genes were used as molecular markers. Northern hybridization results for two of the six different *Aux/IAA* cDNAs cloned from the floret AZ showed differences in both temporal and spatial expression levels of their encoding genes. Higher level of expression was induced by 2,4-D as compared to NAA. The level of expression of the two genes seems to be inversely related to floret abscission. Thus higher level of *Aux/IAA* gene expression may delay the abscission process. To further elucidate our hypothesis an expression analysis of the additional genes is underway.

O3-2: Auxin binding protein 1 - is it a growth limiting auxin receptor ?

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We investigated the possible growth-relevance of auxin binding protein 1 (ABP1) by several approaches: Transport inhibitor studies, analysis of ABP1 mutants in *Arabidopsis*, effects of anti ABP1 antibodies in a protoplast system and analyisis of signalling mutants. Anti-ABP1 antibodies inhibit auxin induced protoplast swelling. Antibodies against the putative auxin binding domain and peptides with the sequence of the docking protein interaction domain had auxin agonist activity in the same system. While these data suggest extracellular ABP1 being a significant auxin receptor, inhibitor studies and the analysis of auxin transport mutants suggest the involvement of an intracellular receptor in growth control. To check for the growth relevance of ABP1 we used the *diageotropica* (*dgt*) auxin signalling mutant of tomato. Auxin triggered neither growth nor protoplast swelling in *dgt*. Agonistic anti-ABP1 antibodies and C-terminal peptides failed to induce swelling in the mutant, showing that DGT is an element of an auxin signalling chain using extracellular ABP1 as a receptor. The data are compatible with the idea that ABP1 regulates growth, perhaps in concert with intracellular receptors.

O3-3: Expression of AHP genes in *Arabidopsis*: organ specificity and regulation by cytokinins

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Histidine-containing phosphotransmitters (AHPs) transfer the phosphoryl group from membrane receptors to effectors in the nucleus. Five *AHPs* have been identified in *Arabidopsis*. Analysis of expression pattern in the *AHP* gene family was conducted to initiate its functional characterization. Real-time RT-PCR was employed to quantify steady state levels of individual transcripts in *Arabidopsis* leaves, roots, stems, flowers and siliques. The transcripts of all five members of the gene family were detected in every organ examined, although at different levels. High organ specificity of gene expression was found in *AHP1*, *AHP2*, *AHP4* while expression of *AHP3* and *AHP5* appears more uniform. Effects of cytokinins (CKs) on expression of *AHP* genes were investigated in 8 day-old Arabidopsis seedlings. Wild-type and transgenic (pOp-ipt/LhGR) seedlings were treated with exogenous CKs and dexamethasone, respectively. Short-term (1 to 24h) treatment with exogenous CKs and dexamethasone-induced increase in endogenous CK levels resulted in a comparable pattern of expression induction in *AHP1-4* genes while *AHP5* gene expression remained unaltered. However, long-term effects of the two treatments differed dramatically. (Supported by LN00A081, MSM0021622415, AVOZ50040507 and IAA600380507.)

O3-4: Cytokinin-mediated leaf longevity control by phosphorelay of AHK3 to ARR2 in *Arabidopsis*

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Cytokinins are plant hormones with profound roles in cell division, shoot formation, and longevity control. However, molecular mechanisms underlying cytokinin-mediated plant responses are largely unknown. Here, we show that, among the three *Arabidopsis* histidine kinase cytokinin receptors, AHK3 is the specific receptor in leaf longevity control. In mature leaf cells, AHK3 led phosphorylation specifically on a conserved Asp residue of ARR2, a response regulator, in a cytokinin-dependent manner, which rendered faster degradation of ARR2 through a proteasome-dependent pathway. Furthermore, wild type but not an unphosphorylatable mutant ARR2 led to increased leaf longevity in transgenic plants. Thus, the specific phosphorelay cascade from AHK3 to ARR2 positively controls cytokinin-mediated leaf longevity along with a proteasome-dependent desensitization mechanism.

O3-5: The *Arabidopsis* F-box TIR1 protein is an auxin receptor

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Despite 100 years of evidence showing a central role for auxin in plant development, the mechanism of auxin perception has remained elusive. The best-characterised auxin binding protein is the endoplasmic reticulum-localised Auxin Binding Protein 1 (ABP1). However, there is no evidence linking ABP1 with auxin-induced gene expression, a fundamental primary response underlying auxin's influence over diverse developmental processes. Auxin regulates transcription by enhancing the interaction between the Aux/IAA transcriptional repressor proteins and the ubiquitin-ligase complex SCF^{TIR1}, targeting them for proteolysis. Regulated SCF-mediated protein degradation is a widely occurring signal transduction mechanism. Specificity is conferred by the F-box protein subunit of the SCF (TIR1 in the case of Aux/IAAs) and there are multiple F-box protein genes in all eukaryotic genomes examined to date. Although SCF-target interaction is usually regulated by signal-induced modification of the target, we have previously shown that auxin signalling involves modification of SCF^{TIR1}. Here we will describe work showing that this modification involves the direct binding of auxin to TIR1, and thus that TIR1 is an auxin receptor that mediates transcriptional responses to auxin.

O3-6: Identification of common and specific cytokinin receptor functions by phenotyping single and multiple receptor mutants

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The cytokinin signal is perceived in *Arabidopsis* by three membrane-located receptor histidine kinases. Single, double and triple loss-of-function receptor mutants have been isolated and an extensive phenotypical analysis has been carried out. Changed morphological phenotypes and/or an altered cytokinin sensitivity has been observed in shoot growth, including hypocotyl elongation, leaf size, shoot height, plastochrone and leaf senescence, as well as seed size, germination and in roots. The experiments indicate that the three receptors have in part overlapping functions, but some functions seem to be specifically controlled by one receptor or distinct receptor combinations. The studies provide physiological evidence for the role of cytokinins in diverse processes, in particular growth control and reveal the complexity of cytokinin sensing and allow to assign specific functions to the three receptors.

O3-7: Is NO involved in cytokinin signalling?

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We have tested the hypothesis that nitric oxide (NO) plays an important role in cytokinin signalling [Tun et al., 2001, FEBS Letters 509, 174). Inhibitors of NO synthase (NOS), L-NMMA and L-NAME, inhibited the accumulation of GUS activity in transgenic Arabidopsis harbouring the *GUS* gene under control of the cytokinin-sensitive *ARR5* promoter. Not only did the active compounds L-NMMA and L-NAME have inhibitory activity but so did the usually inactive analogues D-NMMA and D-NAME. NO donors alone or together with cytokinin and L-NMMA did not induce GUS activity. Northern blot analysis of induction of the P_{ARR5}::*GUS* transgene and of the host *ARR5* gene revealed that transcript accumulation was not altered by NMMA-treatment indicating that NMMA acts post-transcriptionally. Similarly, kinetic analysis in Amaranthus showed that NMMA-inhibition of the cytokinin response acted later than the onset of transcriptional gene induction. Taken together, the data show that NOS inhibitors suppress the cytokinin effect after the signal transduction stage and suggest that NO has no direct role in eliciting the primary cytokinin response in plants. (Supported by grants RFBR.)

O3-8: X-ray crystallography and in silico studies of specificity of cytokinin-binding proteins.

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There is much evidence for interactions of cytokinins with plant lectins. In order to understand the molecular recognition mechanism between the plant hormones and their receptors, we have solved crystal structures of mistletoe lectin 1 from *Viscum album* in complex with kinetin and with *trans*-zeatin. The crystals are of good quality and give diffraction at 2.6 Å resolution. Cytokinins have been identified in the binding site previously assigned as the adenine binding active site. The cytokinins fit tightly to the shape of the binding site. The adenine residue interacts specifically though hydrogen bonds to N1 and N7 while the N6-substituents bind non-specifically in the depth of the hydrophobic binding pocket. We have also applied the Gene Relate Sequence Database (GRDB) to identify distant homologs of the CHASE domain (cyclases/histidine kinases associated sensory extracellular) and performed molecular modelling of the CRE1 cytokinin receptor with the MODELER program. We superimposed the model and related structures with their ligands. We docked the *trans*-zeatin and kinetin molecule in the pocket in the same manner as other ligands in their structures. The docked ligands were entirely buried. None of their atoms were accessible outside the protein. Molecular modeling showed the importance of Thr 278. It is located at the entry of the sensing pocket and is ~3 Å from the oxygen of kinetin or zeatin side chain. The role of this amino acid residue in the function loss of the protein may be due to the ligand interaction with the backbone oxygen group at the entrance to the binding pocket. Additional highly conserved amino acid residues in cytokinin receptor pockets are in contact with the ligands.

O3-9: Auxin influences erotein trafficking - a novel mode of hormone action

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Auxin regulates a variety of developmental processes in plants such as embryogenesis, organ initiation, directional growth or apical dominance. Auxin is distributed between cells by a polar transport system, which requires asymmetrically localized auxin transport facilitators of the PIN family. Using Brefeldine A, an inhibitor of subcellular vesicle trafficking it has been shown that PIN proteins are constitutively recycling between the plasma membrane and internal endosomes. Recent data suggests that auxin inhibits the endocytotic step of this recycling, leading to an accumulation of PIN proteins at the membrane and presumably increased auxin transport. This inhibition of cycling is specific to biologically active auxins and affects also other PM associated proteins, but does not interfere with other trafficking processes. The mechanism by which auxin influences protein recycling is not clear, but our data suggests that it is independent of any known auxin signalling pathway. We want to discuss some possibilities for the mechanism, especially the question whether the effect is genomic or non-genomic and a possible involvement of auxin binding protein 1 in this process.

O3-11: Rapid cytokinin-induced NO biosynthesis and potential function of NO as a cytokinin signaling intermediate

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Nitric oxide (NO) biosynthesis in Arabidopsis wild type and NR-deficient *nia1/nia2* seedlings, measured as release of NO into the incubation medium, was up-regulated after 2 min upon zeatin addition. Hence, NR is not the enzyme regulated by cytokinin. Release of NO was inhibited by amino-2-ethyl-thiourea and the NO scavenger PTIO. In *nia1/nia2* seedlings the tissue distribution of NO biosynthesis and the physiological response to cytokinin was changed with respect to wild type. Differences in NO-induced fluorescence were strongest in the cotyledons, mature root, and hypocotyls where also the differences in morphological changes in response to zeatin were observed. Moreover, the cytokinin receptor knockout mutant *cre1/ahk4* produced less NO in the main root base where this receptor is strongly expressed. The different regulation of NO biosynthesis in *nia1/nia2* and *cre1/ahk4* plants argues for a participation of NO as a mediator in the response to zeatin. A knockout line nos1 of the plant NO synthase showed a physiological response to cytokinin indistinguishable from wild type plants which argues against a role of this enzyme in cytokinin action. The zeatin-regulated NO-producing enzyme remains an open question.

O3-10: The role of ABP1 in determination of plant cell sensitivity to auxin within cell elongation

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The mechanisms of plant cell sensitivity to auxin, which is strongly correlated with extension growth intensity, are still under elucidation. Maize coleoptile and tobacco cell cultureVBI-0 cells were investigated for sensitivity within cell elongation. In both models the youngest cells at the very beginning of the extension process were characterized by the highest intensity of auxin-induced elevation of H^+ and Ca^{2+} concentration in the cytosol. Both were measured by using fluorescent microscopy. With the advance of elongation the decrease in Ca^{2+} and H^+ shift was estimated. The observed alterations might coincide with changes in the initial steps of auxin perception and signal transduction, which could take place in maturation. Addition of ABP1 caused restoration of the amplitude of auxin-induced reactions, while antibodies to ABP1 had the opposite effect. We discuss the likelihood that the loss of plant cell sensitivity to auxin within the elongation process might depend on decrease in number of ABP1 and its supposed role as an external plasma membrane auxin receptor.

P3-1: Functional characterisation of *LeIAA3*, an *Aux/IAA* gene in the tomato

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Auxin responses have been shown to be mediated by transcriptional regulators from the Aux/IAA gene family. To identify Aux/IAA genes involved in auxin signalling during fruit development in tomato, we carried out a differential display-based screening that led to the isolation of LeIAA3 which showed ethylene and auxin-regulated expression suggesting a cross talk between these two hormones. Antisense LeIAA3 lines displayed auxin-related developmental defects including reduced apical dominance and altered phyllotaxy. The expression of a GUS reporter gene fused to the LeIAA3 promoter was mainly targeted to regions where auxin is supposed to play a major role, such as root tips, leaf vascular tissue and shoot apex. The LeIAA3 promoter activity is rapidly and strongly induced by auxin in tomato seedlings. Moreover, genetic crosses between the LeIAA3 promoter::GUS expressing lines and auxin-hypersensitive plants resulted in increase of the reporter gene expression, thus confirming the auxin responsiveness of the LeIAA3 gene. The LeIAA3 promoter also displayed an ethylene-regulated expression in the inner side of the apical hook region of dark-grown seedlings. These data support the hypothesis that LeIAA3 may represent a molecular link between auxin and ethylene signalling.

P3-2: Charecterisation of cytokinin responses in *Arabidopsis* cell suspension culture

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Cytokinins are well known plant growth regulators involved in various processes such as cell division. In Arabidopsis, cytokinin signalling is achieved by a cascade of protein phosphorelay leading to the activation of CK primary response genes. The aim of this project in two-fold: to find out if current model of CK signalling is active in cell suspension cultures and what effect CK has on the growth of these cultures. Use of cell suspension cultures allows the investigation of various biochemical processes in the absence of whole plant developmental processes, thereby making it simpler to understand various biological mechanisms. Menges and Murray (2002) have established a synchronisable Arabidopsis cell line, MM2d. Using this cell line, we demonstrated that MM2d responds to application of exogenous CK via the current model of signalling pathway (Hwang and Sheen, 2001). It was also found that signalling can be down regulated by the removal of exogenous CK and subsequent addition is able to restore it. This is the first report that CK signalling can be 'switched off'. However, it was found that MM2d does not require CK for its growth; nevertheless removal of CK has a slight inhibitory effect on G1/S transition thereby delaying S-phase progression.

P3-3: Does ABP4 integrate the auxin and light signaling pathways in corn seedlings?

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We have tested the possibility that the physiological consequences of the selection of modern corn varieties capable of maintaining productivity in dense planting involve changes in responsiveness to light and auxin. Etiolated seedlings of a modern corn hybrid 3394 elongated significantly less than seedlings of an older hybrid 307. The level of endogenous auxin and activity of PAT were similar in both genotypes. 3394 shows resistance to auxin- and light-induced responses at the seedling, cell, and molecular levels. 3394 cells were insensitive to auxin- and light-induced hyperpolarization of the PM. Expression of *ABP4* was much less in 3394 than in 307, and in contrast to 307, it was not upregulated by NAA, R or FR. Our results confirm the understanding that auxin interacts with light in regulation of growth and development of young corn seedlings and suggest that ABPs may be involved in development of leaf angle. We hypothesize that *ABP4* may integrate the auxin and light signaling pathways in corn seedlings. We further hypothesize that during the selection of the modern corn hybrid 3394, *ABP4* has been "mutated," which could affect the interaction between *ABP4* and *ABP1*. It may result in the observed 3394 phenotypes, including upright leaves and seed productivity in dense planting.

P3-4: Elucidating the protein-protein interactions within the cytokinin signaling pathway in *Arabidopsis thaliana*

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In *Arabidopsis* the cytokinin signal is transduced via a phospho-relay through a complex two-component system (TCS). More than 30 proteins divided into four major protein families, *i.e.* sensor histidine kinases, phospho-transmitter, A- and B-type response regulator, are thought to be involved in this process. The succession of the signal along the different modules seems to be clear, but there is limited knowledge about the abilities of the TCS proteins to interact with each other and how much these contribute to signal specification. To test the specificity of interactions of the TCS proteins, we cloned most of the genes involved into bait and prey vectors of the yeast two-hybrid system. TCS protein interactions were tested in a matrix fashion. The putative new interactions were verified by using an *in vitro* affinity co-precipitation method. 26 previously known interactions were verified by this approach and more than 40 new interactions were detected. Of those only four could not be confirmed in the biochemical interaction assay. As expected, most interactions were found between members of the phospho-transmitter protein family and proteins of the other three TCS protein families. However, also some new and unexpected interactions were identified.

P3-5: Changes of cytoplasmic calcium signalling in *Zinnia* xylogenic cells after auxin, cytokinin or GGMOs treatment

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Calcium is considered the most versatile intracellular messenger able to couple a wide range of cellular signals to specific responses. Parenchyma cells differentiation into tracheary elements (TEs) is a useful model system for studies of cytodifferentiation in higher plants. Histochemical analysis revealed in cells differentiating to TEs larger amounts of Ca^{2+} than in non-differentiating ones. Our aim was to analyse the effect of auxin, cytokinin, and putative signalling molecules - galactoglucomannan oligosaccharides (GGMOs) on cytoplasmic Ca^{2+} signalization. NAA, BAP or GGMOs were applied directly on the mechanically isolated cells incubated in the medium without hormones, loaded with Fluo3 and fixed in 0.75% agarose. Signalling of exclusively cytoplasmic Ca^{2+} has been observed in confocal microscope. Temporary increase of Ca^{2+} signalling after addition of auxin, cytokinin or GGMOs showed different kinetics and confirmed their different biological activity in *Zinnia* culture. Hormones caused constant secretion of Ca^{2+} to the cytoplasm which supported cell differentiation into TEs. GGMOs had a protective effect on cells. (This work was supported by SIGNAL – Marie Curie Felowship QLK3-CT-2001-60067, VEGA No. 2/4146/04, and APVT No. 51-013304 projects.)

P3-6: Role of phospholipases C and D in the mechanism of cytokinin action

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Cytokinins are plant hormones that play central role in regulation of plant development, but the molecular mechanism of their action is far from being understood. To investigate the role of PLD and phosphatidylinositol specific PLC (PI-PLC) in cytokinin signaling, we analyzed changes of PtdBut (as a marker of PLD activity), Ins(1,4,5)P_3, PI(4)P and PI(4,5)P_2 content in response to benzyladenin treatment of 3-days old *Amaranthus* seedlings. Within 5 min of hormone action, level of Ins(1,4,5)P_3 increased more than twice over control seedlings. Changes in Ins(1,4,5)P_3 were accompanied by decrease of PI(4)P and PI(4,5)P_2 levels. Our results indicate that PI-PLC can be activated at early stage of the hormone action. Benzyladenin activated PLD as well, as revealed by the formation of PtdBut. The addition of chemical reagents modifying intracellular level of calcium and PI(4,5)P_2 inhibited the formation of PtdBut, showing that the cytokinin-induced activation of PLD was dependent on PI(4,5)P_2 and calcium availability. The role of lipid-signalling enzymes PLD and PI-PLC in cytokinin signal cascade is discussed. This work was supported by the INTAS grant N 01-0602.

P3-8: Ligand specificity of cytokinin receptors from *Arabidopsis*

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The three cytokinin receptors (AHK2, AHK3, CRE1/AHK4) of *Arabidopsis* are sensor histidine kinases and located most probably in membranes. We used *E.coli* strains expressing individually AHK3 or CRE1/AHK4 (Suzuki et al., 2001, Plant Cell Physiol.,) to study the ability of intact bacteria expressing these receptors to bind ^{3}H-labeled *trans*-zeatin. The results showed that both receptors bound labeled zeatin with high affinity (Kd 2-5 nM). The binding was easily reversible and correlated well with the physiological activity of cytokinins. Furthermore, we performed competition assays using various cytokinin compounds and demonstrated differences in ligand recognition between AHK3 and CRE1/AHK4. The influence of environmental conditions (pH, mono- and divalent cations) on cytokinin-receptor interaction was studied and revealed differences between AHK3 and CRE1/AHK4. The physiological significance of these results is discussed. (Supported by grants RFBR.)

P3-7: Role of Ca^{2+} ions in transduction of cytokinin signal

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Calcium ions have unique properties and universal ability to conduct different signals that cause primary influence on the cell. The aim of our investigation was to analyse the role of Ca ions in transduction of cytokinin signal in plant cells. We investigated the influence of different types of calcium antagonists on the seedlings of *Arabidopsis thaliana* harboring a fusion of the cytokinin-responsive *ARR5* gene promoter and the *GUS* reporter gene. The standard *Amaranthus* bioassay was used too. Our results show that calcium agonists and antagonists can modify the cytokinin-induced *GUS*-activity in *Arabidopsis* and the cytokinin-induced amaranthin synthesis in *Amaranthus*. It was supposed that Ca-channels, Ca-ATPases and calmodulin can be involved in early stages of cytokinin signal transduction The special investigation of the role of phosphatidic acid (product of phospholipase D) was carried out. By using calcium fluorescent probes we show that the phosphatidic acid can induce Ca^{2+} and H^+ permeability of plasma membrane and tonoplast vesicles from maize roots and coleoptiles. The work was supported by a grants of INTAS 01-0602, RFBR 05-04-49610, UR.07.01.329.

P3-9: Cytokinin activity of differently substituted 6-benzylaminopurines

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Recent identification of cytokinin receptors opened new possibilities to study the cytokinin function. It was previously found that whereas aromatic cytokinins have very high biological activity in various cytokinin bioassays, they are recognised by the cytokinin receptors only partially. We used the receptor bacterial assay employing *ΔrcsC* and *cps::lacZ E. coli* mutant background expressing cytokinin receptors AHK3 and CRE1/AHK4 to study the structure-activity relationships of aromatic cytokinins. Thirty seven 6-benzylaminopurine (BAP) derivatives were compared in various *in vitro* assays. The majority of synthesised derivatives exhibited high activity in all three of the cytokinin bioassays employed (tobacco callus, wheat senescence and *Amaranthus* bioassay). The best results were obtained in the senescence bioassay. For some compounds tested, significant differences of activity were found in the different bioassays, indicating that specific recognition systems may operate and suggesting that it may be possible to modulate specific cytokinin-dependent processes with certain 6-benzylaminopurines. In contrast to their high activity in bioassays, the BAP derivatives were recognised with much lower sensitivity than *trans*-zeatin in both *A. thaliana* AHK3 and AHK4 receptor assays.