ABSTRACTS
LECTURES

REGULATION OF PLANT GROWTH AND DEVELOPMENT BY CYTOKININ

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The recent years have witnessed dramatic progress in understanding metabolism and signalling of the plant hormone cytokinin. This is particularly true for *Arabidopsis* where the major genes of cytokinin biosynthesis, cytokinin breakdown and cytokinin signaling were discovered. These genes were used as tools to establish cytokinin loss-of-function mutants. This was achieved either by constitutive or tissue-specific overexpression of different cytokinin oxidase/dehydrogenase (*CKX*) genes or by isolating and combining genetically knockout alleles of the three receptor genes. This presentation will mainly address the question what we have learned from the resulting mutants - which have either a lowered cytokinin content or a reduced cytokinin signaling - about the functions of cytokinins in regulating plant development. A number of cytokinin-dependent processes during vegetative development of roots and shoots, as well as during generative development, were identified. Potential biotechnological applications will be discussed. In addition, some analytical data of the Arabidopsis *CKX* and *AHK* gene families and progress in finding links between the cytokinin signaling system and the rest of the *Arabidopsis* genome and proteome will be addressed.

PHOSPHOLIPASES AND PHOSPHATIDIC ACID IN HORMONAL SIGNALING AND STRESS RESPONSES

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Cell membranes are the initial and focal points of stimulus perception and signaling messenger production. Signal transduction, vesicular trafficking, and many other critical cellular functions are initiated by the assembly of cytosolic protein complexes to specific sites in cellular membranes. Binding to specific lipid ligands are required for the recruitment and/or regulation of the signaling and meatbolic complexes. In recent years, phosphatidic acid (PA), the simplest membrane phospholipid and also a central intermediate of glycerolipid metabolism, has emerged as a class of pivotal lipid mediators in various cellular processes. The effects of PA have been linked to signaling and production of phytohormones and to plant growth, development, and responses to abiotic and biotic stresses. The modes of PA action are multifaceted and include membrane tethering, direct modulation of enzymatic activity, and effects on membrane structures and metabolism. Signaling PA can be produced by multiple enzymes, and the activation of specific enzymes regulates the timing, location, and molecular species of PA. Phospholipase D (PLD) is one major family of enzymes that produce PA. All the PLDs characterized display distinguishable regulatory properties, and the functions of specific PLDs and PA have been linked to plant responses to various stresses, including water deficits, freezing, salinity, and nutrient deficiency. Recent results have provided insights into different mechanisms by which different PLDs and PA mediate hormonal and stress responses.

SENSING CHANGES IN AUXIN CONCENTRATION

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Auxin has been recognised as an important plant hormone for many decades. Its role in many diverse responses attracts an ever increasing number of scientists. As a community we now have detailed insights into how auxin signalling is initiated. The mechanisms of how auxin is recognised by receptors (how the plant measures its auxin concentration) will be described, drawing on the structural detail provided by crystallography of both TIR1 and ABP1. Questions of sensitivity will be discussed. In the second part of the presentation I will present the case for developing auxin biosensors which can be quantitative, real-time and accessible for living plant tissues. Neither of the receptor proteins has proved adaptable as a tool for reporting quantitative fluxes in auxin concentrations. I will review methods which have been used to assess auxin concentrations in plant tissues, genetic reporters, immunology and mass spectrometry to name a few. Only the last is reliably quantitative and this requires tissue homogenisation. There is a need for further development of auxin biosensor technologies and the prospects will be reviewed.

REGULATION OF CYTOKININ ACTION IN PLANTS: BIOSYNTHESIS AND ACTIVATION

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Cytokinin (CK) plays a crucial role in various phases of plant growth and development and the concentration is finely controlled by internal and external environmental factors such as phytohormones and nitrogen sources. CK in plants is first synthesized as isopentenyladenine (iP) nucleoside phosphate by adenosine phosphate-isopentenyltransferase (IPT) and then hydroxylated to trans-zeatin (tZ) nucleoside phosphate by a cytochrome P450 monooxygenase, CYP735A. The expression of a subset of IPT genes is tightly regulated by nitrogen sources, such as nitrate, and that of CYP735A is induced by cytokinin and repressed by auxin. Our recent study with a rice mutant, *log*, an activation step of CK is catalyzed by LOG, a cytokinin-specific phosphoribohydrolase, which converts the CK-nucleotide to the biologically active free-base form. Spatial expression patterns of the genes indicate that CK is locally synthesized and activated at various sites where it is needed. However, occurrence of CK in xylem and phloem, and our recent study on *cyp735a1/a2* double knock out mutant strongly support that tZ, which is translocated from root to shoot via xylem, plays an important role for shoot normal development. We will outline the recent progress of study on metabolic pathway of CK and discuss the regulatory system in plants.

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LIGAND-BINDING PROPERTIES AND ACTIVATION OF CYTOKININ RECEPTORS

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Cytokinins were discovered in the F. Skoog's laboratory (USA) more that 50 years ago, but only recently the important progress has been made in elucidation of the molecular mechanism of their action. At present, three histidine kinases are considered as cytokinin receptors in Arabidopsis: AHK2, AHK3 and CRE1/AHK4. In order to study the properties of cytokinin receptors in more detail, we have used the model system based on transgenic bacteria expressing individual cytokinin receptors (Suzuki et al., Plant Cell Physiol., 2001). It was shown previously, that the receptors CRE1/AHK4 and AHK3 expressed in bacteria were responsive to cytokinin, i.e. retained their functional properties (Yamada et al., Plant Cell Physiol., 2001; Spíchal et al., Plant Cell Physiol., 2004). We have developed a live-cell binding assay to characterize and compare the hormone-binding ability of the receptors (Romanov et al., An. Biochem., 2005; J. Exp. Bot., 2006). By using highly labeled ^{3}H-zeatin, it was shown that bacteria transformed with *CRE1/AHK4* or *AHK3* acquired the ability to bind specifically cytokinins but not related non-hormonal compounds or other phytohormones. Some important characteristics of cytokinin-receptor interaction (affinity constants, ligand specificity of binding, pH-dependence, etc.) will be presented in the lecture. We found a clear difference in binding specificity between two types of receptors, CRE1/AHK4 and AHK3, regarding their affinity towards the iP-type cytokinins: CRE1/AHK4 binds iP-cytokinins much more strongly than does AHK3. Both cytokinin receptors strongly bind *trans*-zeatin. Thus it appears that AHK3 is tuned to respond mainly to a long-distance signal representing root-derived *trans*-zeatin.

It now becomes clearer how the cytokinins activate the appropriate receptors and what happens thereafter. By analogy with the bacterial sensor histidine kinases, it was suggested and strongly confirmed (Kakimoto, Annu. Rev. Plant Biol., 2003; Hwang & Sakakibara, Physiol. Plant., 2006) that receptors are activated by autophosphorylation and then the signal is transduced via phosphotransfer proteins to a set of primary response genes. Our data show that other factors might be important for cytokinin signal transduction as well, in particular phospholipase(s) D. As regards cytokinin primary response genes, they were shown to consist of a small portion (less than 1%) of the plant genome and are enriched with genes participating in transcriptional regulation. But the secondary transcriptional effects which occur a bit later concern a rather large proportion of the genome (thousands of genes, Brenner et al., Plant J., 2005). To sum up, we can conclude that:

Cytokinin receptors differ in functional (ligand-binding) properties. This could ensure tight signal communication between aerial and underground parts of plant, thus maintaining the integrity of plant organism;

Cytokinin signal transduction probably involves not only elements of two-component system of bacterial type, but also some elements of eukaryotic signaling, particularly phospholipase D;

Cytokinins seem to change overall gene expression via transcriptional cascades, firstly activating a small number of primary response genes which encode regulatory proteins (transcription factors).Supported by grants RBFR (Russia) NN 07-04-91211-ЯФ and 07-04-00331

METABOLIC CONVERSION OF CYTOKININS

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Cytokinins are plant hormones that contribute to regulation of a variety of developmental processes including apical dominance, flower and fruit development, leaf senescence, and seed germination. Cytokinins bind to cell surface receptors and initiate a signal transduction cascade leading to activation of specific genes. Structural features including the nature of the side chain that is attached to the adenine moiety, conjugation with sugars and phosphorylation greatly affect the biological activity of cytokinins.
Cytokinins are selectively inactivated by oxidative cleavage of their side chain by cytokinin dehydrogenase (CKX, EC 1.5.99.12), a flavoprotein containing covalently bound redox cofactor FAD that is presumed to recycle during the catalytic reaction via quinones derived from oxidation of plant phenolics. Genome-wide studies revealed that in higher plants, CKX proteins are encoded by small gene families with a varying number of members. Study of the seven members of the *Arabidopsis thaliana* CKX family shows that secreted enzymes AtCKX2, AtCKX4, AtCKX5 and AtCKX6 regulate the level of cytokinins in apoplast, cleaving preferably free cytokinin bases. AtCKX1 that may recycle components of cytokinin molecules in vacuoles shows specificity to cytokinin ribosides and cytokinin N^9-glucosides. Similarly, AtCKX7, showing also high preference for cytokinin N^9-glucosides, may perform this function in cytoplasm. The physiological meaning for such a preference is unclear so far, because cytokinin N^9-glucosides were found inactive in many biotests. Another vacuolar enzyme, AtCKX3, degrades preferentially cytokinin nucleotides. Substrate specificity of CKX enzymes seems to be predetermined by the nature of a single amino acid residue positioned near the entrance to the substrate channel that interacts with N^9 atom of the cytokinin. Neither AtCKX enzyme exhibits specificity towards aromatic cytokinins that are very weak substrates. Further investigation on cytokinin degradation showed that yeast adenine deaminase (EC 3.5.4.2) from *Schizosaccharomyces pombe* hydrolyzes cytokinins at significant rates (including the aromatic ones), but this activity has not been detected yet with any plant enzyme.

CYTOKININS: THEIR UNEXPECTED ROLE AS PCD INDUCERS

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High levels of cytokinins induce programmed cell death (PCD) both in animals and in plants. A high dosage of the cytokinin BA induces PCD in proliferating plant cell cultures as demonstrated by DNA laddering and chromatin condensation and the release of cytochrome *c* from mitochondria into the cytosol. Usually, the release of cytochrome *c* occurres prior to the cleavage of DNA, suggesting a temporal sequence of apoptotic steps.
Apparently, high levels of BA induce PCD by accelerating senescence. In fact, the process is relatively slow, taking place in 4-5 days, and cell death and DNA fragmentation is preceded in *Arabidopsis* by early expression of *SAG12* , a gene coding for a cysteine protease specifically associated with leaf and cell culture senescence. During cell death induced by cytokinin in *Medicago truncatula* cell culture, the reticular arrangement of mitochondria, characteristic of healthy growing cells, disintegrates rapidly. MtSAG (an ortologue of *Arabidopsis SAG12*) transcript levels increase and giant mitochondria, usually associated with high levels of cell death, are detected together with an increase of release of cytochrome *c*. Hence mitochondria appear to play a central role in this pathway of cell death.
In order to identify signalling intermediates of cytokinin-induced PCD, the role of nitric oxide (NO), a key signalling molecule, was analysed in *Arabidopsis* suspension cells. We observed that BA induces NO synthesis in a dose dependent manner. NO appears to be produced via a NOS enzyme since both its level and cellular effects were strongly reduced by pre-treatment with a NOS inhibitor and a NO scavenger. More interesting, in cells incubated with BA in the presence of NOS inhibitor, cell death was significantly reduced and cell growth inhibition was attenuated, suggesting for NO an early signalling role in this pathway of PCD induced by BA.
In BA-treated cells, mitochondrial functionality is altered via inhibition of respiration. It was not unexpected as we have previously showed that NO affects mitochondrial functionality in plant cells reducing total cell respiration by inhibiting the cytochrome pathway. But this inhibition can be prevented by addition of NO scavenger or NOS inhibitors implying that NO acts at the mitochondrial level.
Preliminary results on the physiological role of high cytochinin concentration in plants will also be discussed.

CONTROL OF *AHP6*, A CENTRAL PLAYER OF VASCULAR DEVELOPMENT IN THE *ARABIDOPSIS* ROOT

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During vascular development in the *Arabidopsis* root, cytokinins promote pluripotent cell as well as phloem identity and inhibit protoxylem cell identity. Protoxylem cell fate is dependent on the localised inhibition of cytokinin signalling by AHP6, a pseudo-phosphotransfer protein that acts to inhibit the phosphorelay associated with cytokinin signalling. *AHP6* is expressed specifically in both protoxylem cell files. Conversely, cytokinin signalling negatively regulates the spatial domain of *AHP6* expression. Consequently, a negative regulatory feed-back loop operates where cytokinin signalling counteracts expression of its inhibitor facilitating protoxylem formation. The identity of either the negative regulatory (cytokinin mediated) or promotive factors which converge on *AHP6* is unknown. To identify and characterize upstream factors controlling *AHP6*, a forward genetic screen was performed to identify altered expression patterns of *pAHP6::GFP* within an EMS mutagenized line. A set of novel mutants was identified and the phenotypic description of these genetically interacting loci will be presented. Further functional and molecular characterization of those loci can reveal the basic genetic mechanisms underlying vascular development.

CYTOKININ CONTENT IN SHOOTS IN RESPONSE TO ROOT TREATMENT. IS CYTOKININ TRANSPORT INVOLVED IN ROOT/SHOOT SIGNALING?

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Plants have to coordinate numerous processes taking place in their different parts. Exchanges of hormonal signals between shoots and roots are believed to be implicated in the control of their coordinated growth (Jackson, 1993). But it remains unclear if cytokinin signals always come from treated roots. In our research we applied different treatments resulting in activation of root growth: partial derooting of wheat plants (cv. Bezenchukskaya 139) and 10-fold dilution of their Hoagland-Arnon nutrient solution. Excision of 4 out 5 seminal roots decreased cytokinin content in xylem sap determined by means of immunoassay. Since transpiration did not change significantly delivery of cytokinins remained the same as in intact plants. Restoration of cytokinin flow to shoot was observed only one day after the treatment. Unexpectedly cytokinin content in shoots was higher in partially derooted plants than in intact plants already in 1 hour. Which mechanisms of cytokinin accumulation may be involved? It may be a reduced rate of cytokinin (CK) decay first of all. Activity of cytokinin oxidase (CKO) was determined as the rate of degradation of isopentenyladenine after addition of proteins extracted from leaves to incubation medium. Expression of the gene coding for CKO was estimated by means of RT-PCR. A decline in activity of the enzyme and CKO gene expression were observed. Accumulation of CK due to a reduced rate of their decay contributed to maintaining shoot growth of partially derooted plants at the level of intact plants both directly and through their effect on photosynthesis.

Deficit in mineral nutrition led to no changes in cytokinins content in xylem sap and their delivery from roots to shoots within one day after the treatment. However by this time cytokinin content in shoots decreased two times. Unlike experiments with partial derooting dilution of the nutrient solution resulted in accumulation of ABA in leaves as compared to control plants. The source of ABA accumulation may be the bound ABA transported from roots, which elevated level was registered in xylem sap of plants grown on diluted nutrient solution. According to literature data ABA may influence metabolism of cytokinins (Brugiere et al., 2003). We measured activity of CKO and expression of CKO gene in shoots and observed an increase in both enzyme activity and expression of its gene. Consequently cytokinin signal may be generated in shoot itself under the influence of ABA on the rate of cytokinin decay. It is obvious that a study of implication of hormones in shoot/root interaction demands analysis of not only the transport of hormones from roots to shoots, but also of the mechanisms generating hormonal signal in the target tissue.

Thus under different influences cytokinins alongside with ABA are involved in root signaling and play a great role in coordination of roots and shoot growth. Mechanisms of signaling may be different depending on environment and internal factors.

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THE MEKK1-MKK1/2-MPK4/6 MAPK PATHWAY COORDINATES STRESS RESPONSES WITH REACTIVE OXYGEN SPECIES AND HORMONE SIGNALING

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We have shown that the *Arabidopsis* MAPK kinase MKK2 is involved in mediating abiotic stress responses (Teige et al., 2004). In agreement with such a function, transcriptome analysis of constitutively active MKK2-EE plants showed altered expression of genes induced by abiotic and biotic stresses, including enhanced levels of genes encoding enzymes of ethylene (ET) and jasmonic acid (JA) synthesis. Our recent analysis showed that in contrast to wild type, MKK2-EE plants are comropmised in JA and SA accumulation upon *P. syringae* infection, indicating that MKK2 is involved in regulating hormone levels in response to pathogens (Brader et al., 2007). MKK2-EE plants were found to be more resistant to infection by *P. syringae* and *Erwinia carotivora*, but less resistant to the fungal necrotroph *Alternaria brassicicola*. Various experimental evidence suggests that MEKK1 is the upstream activator of the MKK2-MPK4/MPK6 module and its downstream targets. A genetic analysis of *MEKK1, MKK2, MPK4* and *MPK6* revealed that *MEKK1*- and *MPK4-,* but not *MKK2* or *MPK6*-deficient plants accumulate reactive oxygen species and exhibit a lethal phenotype that is correlated with misregulation of ROS biosynthesis and detoxification genes (Nakagami et al., 2006). These and other data suggest that the MAPK module MEKK1-MKK1/2-MPK4/6 is involved in mediating abiotic and biotic stress responses.

AUXIN RESPONSE NETWORKS IN EMBRYONIC ROOT FORMATION

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The root meristem is first initiated in the developing embryo, and requires specification of an extra-embryonic suspensor cell as hypophysis, the quiescent centre precursor cell. Specification of the hypophysis is controlled by the Auxin Response transcription Factor (ARF) MONOPTEROS (MP), and its inhibitor IAA12/BODENLOS. We have found previously that MP acts in a small group of cells adjacent to the future hypophysis, which implicates cell-cell communication through secondary signals in hypophysis specification. Auxin itself is such a signal, since MP controls PIN1-dependent auxin transport from the embryo to the upper suspensor cell, where it elicits a second auxin response. However, auxin accumulation alone seems insufficient for hypophysis specification. In order to identify additional signalling pathways, we have used microarrays to isolate a number of transcription factor genes that are immediate targets of MP. We will present functional analysis of these target genes in root initiation. Furthermore, we have used a reverse genetics approach to identify additional ARF and AUX/IAA proteins in hypophysis specification and found that in addition to hypophysis cell fate, many cell fates in the early embryo are controlled by ARF transcription factors. We will also present our progress on the systematic dissection of auxin responses in embryo development.

ROLE OF THE AUXIN-BINDING PROTEIN 1 IN THE CONTROL OF ROOT GROWTH

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Auxin Binding Protein 1, ABP1, was identified over 30 years ago by its capacity to bind auxin. It was rapidly established, via the accumulation of a large range of electrophysiological data, that ABP1 is involved in the control of very early auxin responses, including activation or deactivation of ion channels (K^+, anions) or transporters (proton pump ATPase) in an auxin dose dependent manner[1]. More recently, ABP1 was shown to be essential for early embryo development[2], reflecting a critical importance to the plant. To further investigate the role of ABP1, we have generated conditional knock-down for ABP1 using cellular immunisation. This approach is based on the *in vivo* expression of recombinant immunoglobulin fragments termed scFv (single chain Fragment variable) consisting of the heavy and light chain variable domains of an antibody linked by a flexible peptide. We have made use of well characterised monoclonal antibodies to construct such scFv fragments and target inactivation of ABP1. This approach was used first to conditionaly impair ABP1 function in tobacco BY2 cells[3], thus demonstrating that ABP1 is involved in the control of the cell cycle in these cells, most likely by mediating auxin action. This approach was then tranferred to the whole plant and conditional knock-down were generated in Arabidopsis. Inactivation of ABP1 provokes severe growth and developmental defects amongst which a strong inhibition of root growth.

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BRASSINOSTEROIDS: A NEW TYPE OF SIGNALING MOLECULES AND BASIS FOR DEVELOPMENT OF ECOLOGICALLY FRIENDLY AGROCHEMICALS

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Recognition of brassinosteroids (BS) as a new class of plant hormones was an epoch-making discovery of XX century, because to the previously known role of steroids as hormones of humans, animals, insects and fungi their hormonal functioning in plants has been added [1,2]. This brought a new understanding of steroids as versatile bio-regulators characteristic for all living creatures. An important physiological effect of new plant hormones, when applied exogenously to growing plants, is their capability to stimulate plant growth and development. That is why their application in agriculture was considered to be promising from the beginning of BS studies and later found its realization [3].

After the discovery of brassinolide, the first member of the series, progress in brassinosteroid research has been extremely rapid, and less than twenty years were necessary to start agricultural use of BS. During this period, extensive fundamental and applied studies were carried out including elaboration of economically feasible approaches to BS synthesis, laboratory and field-scale biological trials with different crops, toxicological studies, solving the problems on industrial-scale production and official status of new agrochemicals, etc. For none of the other plant hormones, although studied for a much longer time, there has been similar development.

All the data on BS activities in plants, the discovery of genes that are specifically expressed by BS, and the identification of BS-receptor make sure that BS are real plant hormones. During recent years, the molecular genetic methodology and use of special mutants of *Arabidopsis* brought real break-through in the mechanistic studies of BS-action. The findings in disclosure of the mechanism stimulate further efforts directing to the localization of the effect of BS in the chain of signaling events in the plant cell. One of the indicators on the promising area is the data on the genetically-determined involvement of BS in the light-regulated plant development. Our study on the effect of BS on the hormonal balance in light-dependent development of wild type *Arabidopsis* and its mutants defective in genes encoding synthesis of some photoreceptors showed clear relationships between the responses mediated by these photoreceptors and action of BS. The results suggest an important function of BS in light and hormone signaling cross talk and give a new confirmation of their central role among other phytohormones.

Although a number of problems have been solved, there are still those playing a critical role for further development of the area and demanding deeper investigation. Among them: further development of synthetic and analytical methodology and search for structural species-specificity in natural distribution of BS, further mechanistic studies and localization of BS-effect in cell signaling, study on structural species-specificity of plant physiological response to exogenous BS in connection with the biosynthesis and metabolism of the endogenous BS. All these are very important for higher reliability and predictability of agricultural use of BS. Our recent results in the area will be discussed.

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REGULATION OF BRASSINOSTEROID SIGNALING

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Brassinosteroids are a unique class of plant polyhydroxysteroids critical for normal plant growth and development. Arabidopsis mutants defective in brassinosteroid biosynthesis or signaling exhibit a characteristic set of growth/developmental defects including a dwarf stature, reduced male fertility, delayed flowering, abnormal vascular differentiation, and aberrant skotomorphogenesis. Genetic and biochemical studies in the past decade have revealed a linear brassinosteroid signal transduction pathway that involves two cell surface receptor kinases, a GSK3-like kinase, and two GSK3 substrates that can directly bind DNA to regulate gene expression. I will summarize the current knowledge of this phosphorylation-mediated signaling pathway. In addition, I will discuss a potential regulatory mechanism for restricting the GSK3-like kinase activity in response to activation of two receptor kinases and potential roles of two families of helix-loop-helix proteins in brassinosteroid signaling.

ABSCISIC ACID AND BRASSINOSTEROIDS HAVE AN OPPOSITE EFFECT ON THE MODULATION OF THE PROTON PUMPING AND THE ANION CHANNEL ACTIVITY AT THE PLASMA MEMBRANE OF *ARABIDOPSIS THALIANA SUSPENSION C*ELLS: VACUOLAR CALCIUM DEPENDENCY ?

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The plant growth regulators abscisic acid (ABA) and 28-homobrassinolide (HBL) play key roles in the control of plant development and cell volume by regulating ion channel activities and water exchanges across the plasma membrane (PM).
In *Arabidopsis thaliana* suspension cells, our results clearly show that both ABA and HBL had opposite effect on the modulation of the proton pump and anion channel activity. These modulations were associated with the control of the PM electrical gradient magnitude involved in phytohormones signaling pathways.
Using experiments employing combined voltage clamping and continuous measurement of extracellular pH during PM phytohormone signaling on cells where physiological wall functions are maintained, we demonstrate that HBL induced both medium acidification (ΔpH $\approx$ 0.45 units in less than 10 min) and PM hyperpolarization (ΔEm $\approx$ -12 mV), whereas ABA simultaneously induced rapid alkalinization of the medium (ΔpH $\approx$ 0.06 units) and PM depolarization (ΔEm $\approx$ 6 mV). These data revealed that the PM H^+-ATPase is activated by HBL (Zhang et al. 2005), but inhibited by ABA (Brault et al. 2004) in *A. thaliana* suspension cells. Upon ABA treatment, we observed an increase in the anion current (anion efflux) in suspension cells (ΔI $\approx$ 62%). This increase is abolished by a subsequent addition of the anion channel inhibitor 9-AC (ΔI $\approx$ 17%) or strongly reduced when ABA was added in the presence of 9-AC. In opposite manner, we observed HBL treatment decrease anion current in suspension cells (ΔI $\approx$ 70%) during the PM hyperpolarization. Therefore, anion channels may also be good candidates, in addition to proton pumps for the controls of PM potential during the responses to phytohormones signaling (Zalejski et al. 2006). These effects could be prevent by the targeted interruption of the signaling pathway either at the level of the PM Ca^{2+} channels or (and) the tonoplast level of the cADPR, IP_3 and ryanodine-induced intracellular calcium increase.

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THE INFLUENCE OF *CYP11A1* GENE EXPRESSION ON THE REGULATORY SYSTEM AND PHENOTYPE OF TRANSGENIC TOBACCO PLANTS

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Close similarity of steroid biosynthesis in animal and plant cells, where cytochromes P450 take a considerable role, should be noted. The main difference between steroidogenic systems in plants and animals is the absence of cytochrome P450scc in plants. This enzyme catalyzes the conversion of cholesterol into pregnenolone – the precursor of all steroid hormones in animals. This process occurs only in animal mitochondria and proceeds with participation of the other two proteins of electron transfer chain – adrenodoxin and adrenodoxin reductase that homologes are also found in plant mitochondria. All above mentioned give us the possibility to suppose that cytochrome P450scc can display its catalytic activity in plant cells.

The presence of animal steroid hormones in a range of higher plants and their involving in growth and development regulation has been established recently. Moreover it has been shown that progesterone at low concentrations can promote plant growth and suppresses it at higher concentrations.

With the aim to study the synthesis of mammalian hormones and its possible influence on the plant growth and development the transgenic *Nicotiana tabacum sv. Petit Havana SR1* plants carrying cDNA of *CYP11A1* gene encoding this protein have been created. Transformation vector *pGBP450f* was constructed on a basis of *pGreen0229* binary plasmid where the cDNA of *CYP11A1* was under the *CAMV 35S* promoter. Southern blot and RT-PCR analyses confirmed the presence and the expression of *CYP11A1* in transgenic plants obtained. The pregnenolone in the steroid fraction has been identified by gas chromatography mass spectrometry method. Its further conversion into progesterone was verified using the enzyme immunoassay method.
Transgenic plants exhibit an enhanced growth and development rate as compared to the wild type plants. The earlier flowering of all transgenic lines has been observed as well.
The protein and carbohydrates contents in leaves and seeds of transgenic plants exceed noticeably those in control plants. At the same time the difference from line to line has also been observed.
The results obtained indicate that *CYP11A1* expression in transgenic tobacco plants makes considerable alterations in their regulatory system that is accompanied by changes in phenotype.

THE ROLE OF BRASSINOSTEROIDS IN TRANSDUCTION OF GREEN LIGHT SIGNALS

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It was assumed that brassinosteroids (BRs), possibly, participate in transduction of light signal (T. Takematsu, 1988). It is known that biosynthesis and mechanism of BRs inactivation as well as response reaction to BRs are under red light (phytochromes) and blue light receptors (cryptochromes) control. Despite numerous physiological studies of this question, it is little known about the participation of BRs in transduction of light signal. The interaction between green light and brassinosteroids have not been researched yet. The reception of green light signals has not been studied enough. In 1995 C. Lin presupposed that cryptochrome was one of probable green light photoreceptors.

We studied the role of brassinosteroids (brassinolide, epibrassinolide and homobrassinolide) in transduction of green light signal. The universal plant model to study regulatory function of BRs in plant morphogenesis at green light is *Arabidopsis thaliana*. Plants of *A. thaliana* ecotypes Landsberg *erecta* (L*er*) and Columbia (Col) as well as mutants *hy4* (mutated in cryptochrome) and *det2* (disturbed synthesis of brassinolide) were used.

The study of growth of etiolated seedlings of *A. thaliana* showed that the length of hypocotyls and areas of cotyledons of *hy4* were significantly less than those of wild type L*er*. The mutant *det2* differed from wild type Col having shorter hypocotyl and also bigger areas of cotyledons. The reaction of ecotypes to BRs in the darkness differed from mutants' one and manifested in inhibition of Col, L*er* and *hy4*'s length of hypocotyls and roots and also in stimulation of *det2* axes organs growth. The biggest bioactivity with respect to Arabidopsis seedlings was noted for brassinolide, than bioactivity of epibrassinolide for Col and *det2* and homobrassinolide for L*er* and *hy4* came.

Deetiolation of Arabidopsis seedlings for 15 minutes with green light (λ = 543 nm, 29 $\mu mol/m^2s$) induced the processes analogous to the action of exogenous BRs. The green light activated photomorphogenesis in Arabidopsis seedlings of wild type and of mutants with disturbed synthesis of brassinosteroids and cryptochrome. The inhibiting influence of green light on *det2* hypocotyl growth was displaced by exogenous BRs. The analogous effect was observed for wild type seedlings (Col) while using epibrassinolide and brassinolide in concentration 10^{-8} M. Simultaneous influence of brassinosteroids (brassinolide, epibrassinolide and homobrassinolide) and green light resulted in significant increase of Col and *det2* cotyledons areas comparing to their separated influence.

Simultaneous influence of brassinosteroids and green light on L*er* and *hy4* Arabidopsis morphogenesis displayed addition of effects in the growth of hypocotyl and cotyledon areas. Thus, it may be suggested that BRs can be involved in transduction of green light signals in Arabidopsis seedlings morphogenesis as alternative messengers.

EFFECTS OF PLANT GROWTH SUBSTANCES ON MEMBRANE PHOSPHOLIPID METABOLISM

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We have studied the changes in phospholipid metabolism that are caused when pea shoot elongation is altered by growth substances. Both gibberellic acid (GA_3) and indole acetic acid (IAA) were used but the latter gave larger, and more reproducible, effects. Altered stem growth was characterised by several changes in lipid metabolism, of which altered phosphatidylcholine synthesis was noticeable.
Phosphatidylcholine is the main non-chloroplast membrane lipid in plants and is synthesised mainly by the CDP-base pathway. Of the enzymes involved in this pathway, the first (choline kinase) and the middle (cholinephosphate cytidylyltransferase: CPCT) have been claimed to exert regulation. We have purified both enzymes and isolated cDNAs for them. Studies on the regulation of phosphatidylcholine formation in response to IAA showed that CPCT was the most important enzyme for control of flux. Mechanisms for its regulation have been examined and will be discussed.

ROLE OF PHOSPHOLIPASES C AND D IN THE PHYTOHORMONES ACTION AND STRESS RESPONSE OF THE PLANT MERISTEM AND MATURE CELLS METABOLISM. *IN VIVO* INVESTIGATION

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Plant hormones plays important roles in many cellular processes including seed development, dormancy, germination, growth, and environmental stresses, such as drought, cold and salinity, but the molecular mechanism of their action is far from being understood. Individual plant cells can both directly sense and respond to the extra cellular condition. It is well known that phospholipids are key regulators of a plant cells metabolism. Many extra cellular signals are perceived by plasma membrane receptors and converted into intracellular responses via phospholipases C and D (PI-PLC and PLD). The effect of different stress and phytohormones on the *in vivo* activity PI-PLC and PLD of the mature plant tissues as well as plant meristems were studied. Detached leaves and roots of corn were placed in flasks containing [^{33}P]orthophosphate for 16 h at 25°C. It was shown that in maize leaves ^{33}P incorporated into phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid (PtdOH) and polyphosphoinositides . For the analyses PI-PLC activity the level phosphatidyl inositol 4,5-bisphosphate (PIP2), phosphatidyl inositol 4-phosphate (PI(4)P),as well as inositol (1,4,5) - trisphosphate was investigated. PLD activity was determined as the level incorporation ^{33}P in the phosphatidylbutanol (PBut) *in vivo.* Our results strongly suggest that stress conditions and Phytohormones can activate PLC and PLD of the mature plant tissues as well as plant meristems. Theses phospholipases closely cooperates in the signaling network and involved other lipid-signaling enzymes, first of all phosphatidylinositol kinases and phosphatases.

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PARTICIPATION PA IN TRANSPORT OF IONS AND BAP SIGNALING

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Phosphatidic acid (PA) takes special place among signal lipids because it is a key link in the membrane lipid metabolism, on the one hand, and it performs functions of lipid signal molecule, on the other hand. Amount of PA in plant cells elevates transiently under the influence of pathogens, ROS, elicitors, ABA and ethylene. Changes in PA level influence physical properties of membranes and their ability to form vesicles. Some data confirms the ability of PA to transport Ca^{2+} ions through membranes of muscular and nervous cells.

We investigated action of PA with different composition (dioleoyl, dipalmitoyl, from egg yolk) on membrane transport of Ca^{2+}, Mg^{2+} and H^+ using plasma and endomembranes vesicles from maize (Zea mays L.) roots and coleoptiles. Also, of this investigation was to study the influence of BAP on the level of PA.
The 4-day-old etiolated maize seedlings were used. The vesicle preparations were obtained by differential centrifugation and subsequent separation in the PEG/DEX aqueous polymer two-phase system (for isolation of plasma membranes) or in the sucrose density gradient (for isolation of endomembrane fraction). The transport of ions were assayed using Ca-sensitive fluorescent probe Indo-1 (loaded into the membrane vesicles) and Ca^{2+}(Mg^{2+})-sensitive probe chlortetracycline (added to the incubation medium). The proton gradient on the tonoplast vesicles was created by activation of vacuolar H^+-ATPase. The reaction was initiated by the addition of ATP. The increasing of H^+ concentration inside vesicles was registered by the potential density probe acridine orange. The preparation of total pure lipids extracts was isolated on method Bligh and Dyer. The fraction of phospholipids was divided TLC a method Vaskovsky V.E.
Three types of PA were used in experiments; PA was allocated from an egg yolk; PA containing two residues of palmitic acid and PA containing two residues of oleic acid. The greatest effect was observed with PA consisted of two residues of oleic fatty acid. PA including in structure two residues of palmitic fatty acid had the least ionophore properties. The ability of PAs to transport calcium ions across membranes is higher at alkaline conditions than at acidic conditions. We had showed that the ionophore properties of PA are not specific. They are capable to function as membranous transmitting agent not only for Ca^{2+}, but also for Mg^{2+}.
The movement of protons along pH gradient was facilitated by PA as well as FCCP, a well-known protonophore. The highest rate of proton membrane gradient dissipation of tonoplast vesicles caused PA, which consists of two residues of oleic acid. PA with two residues of palmitic acid had the least ionophore activities.
Than we analyzed the influence BAP on change level of PA in cells. We show that the action of BAP on coleoptiles or roots induced the increase of quantity PA and change of fatty-acid composition PA. The received results testify that is probable the action BAP activated PLD. We assume, that PA produced by PLD in plant cells.

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THE EFFECT OF PHOSPHATIDIC ACID ON POTATO TUBERS 9-LIPOXYGENASE ACTIVITY

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In plants the activity of lipoxygenases on polyunsaturated fatty acids generates hydroperoxide products, which are known as oxylipines. These compounds take part in plant response on biotic and abiotic stresses. 9-lipoxygenase(9-LO) is membrane associated enzyme. Adsorption stage of enzyme on biologic membrane is the first step in 9-LO activity regulation. Anionogenic lipids, such as phosphatidylserine, cardiolipin, phosphatidylinositol and phosphatidic acid have an effect on 9-LOX.

It was studied as phospholipid phosphatidic acid (PA) influence on oxidation of linoleic acid by 9-LO from *Solanum tuberosum*. Reaction mixture was consisted of 9-LO and mixed micelles of linoleic acid (LA), Lubrol PX and different quality of enzyme effector – PA. It was established that 9-LO had two $pH_{opt.}$ 5.0 and 6.9 in presence of 50mkM phosphatidic acid. In concentration of 50mkM PA had ability to activate 9-LO (13- 15) fold by pH 5.0. In such condition reaction maximum velocity (V_{max}) concurred with lipoxygenase reaction V_{max} without effector by pH 6.9. It was displayed, that 30mkM phospholipid in reaction mixture decreased concentration of half saturation of substrate on 43-67%. Enzyme demonstrated positive cooperation of substrate, reaction curve circumscribed by Hill equation. Hill coefficient value (h) of substrate was 3,34±0.277(pH 6,9) and 5,607±0.889(pH 5.0), that is substrate molecules number increased with change pH to acidic region and they possible to interact with enzyme molecule from 4 to 6. In case of substrate insufficiency 50 and 100mkM LA enzyme demonstrated positive cooperation of PA, it bonded from 4 to 3 effectors' molecules by pH 5,0. Comparative analyze influence of 4-hydroxy-TEMPO displayed, that nonenzymatic process level lower on 20-80% by unphisiological pH in presence of 30-80mkM PA in comparison 9-LO product level without PA in such condition.

According to our data phospholipid phosphatidic acid may direct interact with molecule of 9-LO and increase enzyme activity in condition of substrate limitation and unphysiological pH cellular matrix. PA supports primary product level of 9-LO linoleic acid oxidating via allosteric displacement substrate molecules in enzyme allosteric center, rising enzyme congeniality to substrate and decrease nonenzymatic product level of linoleic acid oxidating. For phospholipase D has been demonstrated analogue ability of PA to allosteric interact with this enzyme. Thus PA can function not only as second messenger in the cell, but this phosphilipid can direct to regulate membrane associated enzymes.

JASMONATE SIGNALLING NETWORK IN *ARABIDOPSIS THALIANA*: CRUCIAL REGULATORY NODES AND NEW PHYSIOLOGICAL SCENARIOS

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Plant development and stress responses are regulated by complex signalling networks that mediate specific and dynamic plant responses upon activation by various types of exogenous and endogenous signals. Jasmonates mediate responses to stress and act like growth inhibitors. The latest work on jasmonates (JAs) signalling has identified new regulatory nodes in the transcriptional network that regulates a number of diverse plant responses to developmental and environmental cues. Therefore the key elements mediating cross-talk between JAs with other signalling pathways that are activated during stress and defence response will be discussed. Most of the work on JAs has been traditionally done in the context of stress; however, new findings implicating JAs in regulating senescence and plant responses to pathogens suggest a common mechanism of JAs action via distinct groups of transcription factors. Moreover, analysis of JA mutants has mostly focused on altered JA-inducible gene expression and defence responses, whereas a detailed analysis of the causes underlying the stunted growth that characterizes some of them has been seldom performed.In my laboratory, we are interested in discovering the cellular components linking plant stress responses to growth processes with the aim to improve seed production, yield and adaptation of plants to their environment. JAs blocks cell cycle progression by inhibiting G1/S and G2/M transitions in tobacco cells. While the molecular mechanisms and downstream responses have not been clarified yet, we are excited by the likelihood that jasmonate is a distress signal, a physiological role of which is to block cell cycle, slowing vegetative growth during defense responses. A summary of the results obtained so far will be presented. In addition, we will report on new physiological scenarios for JAs signalling such as anti-cancer therapy.

SIGNALING SPECIFICITY AND COMPLEXITY IN MAPK CASCADE NETWORKS

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Mitogen-Activated Protein Kinase (MAPK) cascades are pivotal and evolutionarily conserved regulatory modules controlling diverse signal transduction pathways in eukaryotic cells from yeast to human and plants. Plant genomes encode the largest number of putative MAPK cascade genes (e.g., Arabidopsis: more than 100; yeast: 14; human: 34) in all sequenced eukaryotes. Very limited information on MAPK cascade functionality and regulatory mechanisms has surfaced from classical genetic screens in plants. Biochemical, molecular, reverse genetic and transgenic studies have indicated that plant MAPK cascades are important for controlling broad and essential plant processes, including hormone, stress and innate immune signaling in diverse plant species. However, how MAPKs/MPKs and their immediate upstream regulators, MAPKKs/MKKs and MAPKKKs/MTKs, are integrated into the plant signaling networks connecting upstream signals and downstream transcription factors and target genes remains a major challenge in plant biology.
There are many unresolved puzzles regarding the precise physiological roles of MPKs, MKKs and MTKs in plant hormonal, stress, and defense signaling. To circumvent the limitations in classical and reserve genetic analyses, and to examine dynamic and complex actions in MAPK cascade signaling, we have developed MAPK cascade genomic resources and performed extensive cell-autonomous and systematic screens for *Arabidopsis* MAPKs acting downstream of MKKs and MTKs and various signals that activate putative endogenous MPKs in the cell-based transient expression systems. Arabidopsis mesophyll protoplasts have been used to show the conservation of MAPK cascade signaling and similar physiological responses in isolated cells and in intact plants. In the functional genomic analysis of Arabidopsis MAPK cascade signaling, 20 MPK, 10 MKK and 68 putative MTK genes have been analyzed in mesophyll protoplasts to establish a blueprint for potential MAPK cascade functions and connections. The information will serve as the foundation to launch new genome-wide studies linking dynamic and overlapping signal transduction pathways. The MAPK functional genomic project combines global gene expression profiling and bioinformatics tools to dissect distinct and overlapping MAPK cascades with gain-of-function and loss-of-function mutant analyses in response to hormonal, stress, and elicitor signals. The aims are to integrate broad resources and information on plant hormone, stress, and defense signaling and gene regulation to facilitate comprehensive and molecular understanding of the signaling specificity and complexity of the evolutionarily conserved MAPK cascade signaling networks in Arabidopsis.

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http://genetics.mgh.harvard.edu/sheenweb/mapk_cascades_nsf.html

DIVERSE STRESS SIGNALS ACTIVATE THE C1 SUBGROUP MAP KINASES

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Mitogen-activated protein kinase (MAPK) cascades play a key role in plant growth and development as well as in biotic and abiotic stress responses. They are classified according to their sequence homology into four major groups (A-D). A large amount of information about MAPKs in groups A and B is available but few data of the C group have been reported. In *Arabidopsis*, C1 subgroup is constituted by to MAPK genes: *AtMPK1* and *AtMPK2*. Gene expression data deposited in public microarray repertories indicate that these genes have very low expression with no relevant changes in their mRNA levels after hormone treatment and stress conditions. Moreover, both genes show very similar patterns of expression. We have studied the activation of AtMPK1/AtMPK2 in response to mechanical injury. In this study, we have used specific antibodies raised against the unique C-termini of AtMPK1 and AtMPK2 to measure the kinase activity by immuno-complex kinase assays. An increase in AtMPK1/2 kinase activity was detected in response to wounding that was blocked by cycloheximide. Jasmonic acid (JA) activated AtMPK1/AtMPK2 in the absence of wounding. Wound and JA-induction of AtMPK1/2 kinase activity was not prevented in the JA-insensitive *coi1* mutant. Other stress signals, such as abscisic acid (ABA) and hydrogen peroxide (H_20_2), activated AtMPK1/2. In addition, we report the isolation of a full-length cDNA for *PsMAPK2*, a C1 subgroup MAP kinase from *Pisum sativum*. The regulation of PsMAPK2 kinase activity in response to diverse stress signals was studied. The results obtained suggest that C1 subgroup MAPKs may have the same functions across species.

ENVIRONMENTAL STRESSES: POLYAMINES AND PLANT RESPONSES

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Plant development and productivity are negatively affected by environmental stresses. The abiotic stresses together represent the primary cause of crop loss worldwide. Plants respond and adapt to the continuous environmental fluctuation with appropriate changes to cope with these stress conditions. There is biological evidence suggesting that plants use general and conserved response mechanism to deal with abiotic stress. Polyamines are low molecular organic cations that are found in a wide range of organisms from bacteria to plants and animals. In plants, polyamines are involved in various physiological events and considered as plant growth regulator. It has been observed that plants significantly accumulate polyamines under biotic and abiotic stresses. However, the physiological significance and the role of polyamines are still under studies. During the last decade, many genes involved in polyamine metabolism have been identified from several species and their expression profiles in relation to the developmental stages have been analysed.
The *Mesembryanthemum crystallinum* L. (common name: ice plant), in native habitat, germinates and establishes in short, cool and moist winters, followed by dry summer coupled with increasing drought and salinity. The plant shows high adaptability to natural stress condition. *M. crystallinum* is used to compare the effect of salt stress and UV-B light. Plants, in hydroponical system, were subjected to 400 mM NaCl or/and to exposition to the range of 3 to 9 kJ m^{-2} d^{-1} UV-B_{BE} irradiation. The following day leaves and roots were used for analyses of polyamines and other metabolites changes.
Salt stress induced a general increase of polyamines either in leaves and roots, with also modification of composition. In particular the induction of cadaverine could have a role in resistance and adaptation. After UV-B irradiation the total polyamines showed a general decrease in root. In leaves increase of putrescine and spermidine seemed affected by the UV-B dose applied. Interesting is the absence of cadaverine after UV-B. The application of UV-B on plant under salt stress condition can block and modify the polyamine patter induced by NaCl. The data provide evidence that, at least on this plant, the UV-B stress caused polyamine responses divergent from that of salt stress. On the contrary, for many other cases the same metabolic responses under different stresses had been reported.
More then 25,000 ESTs sequences are available in *M. crystallinum* genome database. Only 20% of the most abundant transcripts in unstressed plants are also found in salt stressed. Several thousand transcripts in salt stressed state are under represented in EST collections from unstressed plants. Furthermore, are some of transcript related to different polyamine path presented by salt or UV-B stressed plants?

TRANSPORT OF GROWTH REGULATORS FROM ROOTS IN DRYING SOIL DURING PARTIAL ROOTZONE DRYING: THE MECHANICS OF A NEW DEFICIT IRRIGATION TECHNIQUE

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Decreased availability of water resources has increased pressure on crop managers to deliver improved crop water use efficiency into agriculture. To realise this aim, one strategy (termed "deficit irrigation") deliberately withholds water from plants. A recent form of deficit irrigation known as partial rootzone drying (PRD) aims to manipulate plant root-to-shoot signalling mechanisms to decrease crop water use. During PRD, water is distributed unevenly to the root system such that part is irrigated while the remainder is allowed to dry the soil. Theoretically, irrigated roots supply sufficient water to the shoots to prevent water deficits while the remainder sense drying soil and produce chemical signals that partially close the stomata and limit vegetative growth. However, the proportion of total sap flow (J) derived from drying roots (J_{dry}) may decrease as soil water potential (Ψ_{soil}) decreases, limiting transport of growth regulators to the shoots. The contribution of different parts of the root system to J and xylem ABA concentration ([X-ABA]) was investigated by grafting shoots onto two root systems of plants grown in two separate pots. The graft union resembled an inverted 'Y'. In PRD tomatoes, $[X\text{-}ABA]_{root}$ from the irrigated side underestimated $[X\text{-}ABA]_{leaf}$, while $[X\text{-}ABA]_{root}$ from the dry side overestimated $[X\text{-}ABA]_{leaf}$. The arithmetic mean of $[X\text{-}ABA]_{root}$ best explained variation in $[X\text{-}ABA]_{leaf}$, implying continued sap flow from the dry part of the root system at a Ψ_{soil} (-0.1 MPa) at which J_{dry} had ceased in a previous study. This was directly confirmed by sap flow measurements in grafted PRD sunflowers: J_{dry} began to decline when Ψ_{soil} = -0.18 MPa, and ceased when Ψ_{soil} = -0.55 MPa. Evaluating the relationship between J_{dry} and Ψ_{soil} may assist in maintaining export of ABA (and other growth regulators) from the drying part of the root system, to achieve desirable horticultural outcomes during PRD.

PLANT HORMONES IN CROPS UNDER STRESS: ENDOGENOUS RESPONSES AS THE BASIS TO DESIGN PALLIATIVE TREATMENTS

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The study of hormonal regulation of crop responses to abiotic stresses is crucial for modern agriculture. In this work, using citrus as a model crop, we studied the different patterns of hormone accumulation in response to water, salt and flooding stresses. Data revealed that ABA plays a central role as modulator of protective responses of citrus to abiotic stress whereas ethylene appears to act as an effector of the response. Jasmonates and polyamines also seem to be involved in the regulation of different events in woody plants under adverse situations. The understanding of the endogenous hormone regulation provided useful information for the development of palliative treatments with different hormone analogues on intact citrus plants under stress. In a first set of experiments, leaf abscission, chloride accumulation, ethylene production and net photosynthetic rate were the parameters used to characterize the performance of plants. Data indicate that exogenous applications of either ABA or a synthetic analog of this hormone were effective in delaying the deleterious effects of high salinity on citrus plants. The effect of agronomical treatments such as substrate amendment were also evaluated by examining endogenous hormonal changes. Current research in our laboratory follows a metabolomics approach to investigate the effects of stress on total metabolite profiles. High performance liquid chromatography coupled to mass spectrometry was used for metabolite profiling to assess physiological changes in response to environmental factors and/or endogenous signals with no previous information on the analyzed mass signals. In a parallel project, we are focusing in the annotation of mass signals and the search for markers linked to specific responses to environmental stresses.

IN VIVO TRAFFICKING AND LOCALIZATION OF p24 PROTEINS IN PLANT CELLS

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Normal plant growth and development are dependent on specialized tissue and subcellular-specific components of the plant membrane trafficking machinery. The plant secretory pathway, which is critical for biosynthetic and endocytic trafficking to the plasma membrane and vacuole, is comprised of the endoplasmic reticulum (ER), Golgi apparatus and intermediate organelles such as the prevacuolar compartment. Secretory membrane trafficking mechanisms have been shown to be involved in a variety of plant-specific processes, including abcisic acid and auxin signalling, plant development, tropic responses, and pathogen defense. We have focused in proteins of the p24 family, which constitute a family of putative cargo receptors which traffic in the early secretory pathway, although their precise function has not yet been established. Interestingly, these proteins have specific properties in plants, and may thus play plant specific roles. The p24 family can be divided into 4 sub-families (p23, p24, p25 and p26) by sequence homology. While mammals and yeast contain p24 proteins belonging to all 4 sub-families, all plant p24 proteins are of the p25 subfamily, characterized by the presence of a dilysine motif in the -3,-4 position and a pair of bulky hydrophobic residues in the -7,-8 position (with respect to the cytosolic C-terminus). We have previously shown that the cytosolic tail of Arabidopsis p24 proteins has the ability to interact with COPI (through the dilysine motif) and with COPII subunits (through the diaromatic motif). However, plant p24 proteins seem to have a higher affinity for COPI than for COPII. The aim of this work was to establish the localization and trafficking properties of a protein of the p24 family in plant cells and to investigate the contribution of the sorting motifs in its cytosolic tail to its *in vivo* trafficking and localization. Using a fusion protein between an Arabidopsis p24 protein and RFP (*At*p24-RFP), we have found that *At*p24 localizes exclusively to the ER, as its mammalian counterpart p25. Using mutant versions lacking either the diaromatic motif, the dilysine motif or both, we have found that the dilysine motif is necessary and sufficient for ER localization. In contrast, *At*p24 mutants lacking the dilysine motif are transported to the prevacuolar compartment and the vacuole, probably uncovering a default pathway for membrane proteins in the secretory pathway. Finally, our data suggest that while ER export of *At*p24 is COPII dependent, its ER localization requires COPI function, suggesting a highly efficient Golgi to ER recycling.

CROP WATER USE EFFICIENCY, CAN WE IMPROVE IT FURTHER BY MANIPULATING SOIL DRYING SIGNAL?

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Water use efficiency (WUE), if defined as the carbon assimilated over the water transpired (the physiological sense), is known as a conservative parameter (e.g. the difference between C_3, and C_4 plants) and largely a function of stomatal opening that determines the CO_2 concentration gradient from inside leaf to the outside atmosphere. Soil drying may lead to partially closed stomata (a better WUE) but also a reduced biomass accumulation as a trade-off. We found that irrigated plants tend to open their stomata fully and some narrowing of stomatal aperture from full may reduce water loss without much effect on photosynthesis. This is possible when part of the plant root system is irrigated while the rest part is left drying. A root 'drying' signal is then transported to the shoots where shoot physiology would be regulated. In our research over the last ten years, we have confirmed that root drying signal can be generated in the field for long term if a partial rootzone irrigation (PRI) is applied and plant water consumption can be improved as a result.

If we define the WUE as the yield over water irrigated (the agronomic sense), it should be a function of biomass accumulation, harvest index and the total amount of irrigation. Our field experiments presented a case that WUE may be enhanced through an improved harvest index. Harvest index has been shown as a variable factor in cases where whole plant senescence of rice and wheat is unfavourably delayed. Such delayed senescence can delay the remobilisation of pre-stored carbon reserves in the straw and results in lower harvest index. A controlled soil drying at grain filling time can enhance whole plant senescence and therefore improve the remobilisation of pre-stored carbon reserve. The gains from the improved harvest index may outweigh any possible biomass loss due to shortened photosynthetic period.

Related reviews:

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SYNTHETIC PREPARATION METHYURE PROTECTS PLANTS UNDER SALT STRESS CONDITIONS

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Salinity is one of the hardest ecologic factors for plants represented a serious threat for agriculture in many countries including Ukraine. Global climate changes accrlerate its expansion especially on irrigated fields. Therefore increasing of plant salt tolerance becomes an actual practical problem which radical radical resolution consists in creation of salt tolerant transgenic forms of the main crop cultures. Besides there is a possibility of particular salt tolerance increasing with help of preparations capable to amlplificate adaptation processes in plant organisms. For this aim we have examinated some preparations and chose among them Methyure as a practically nontoxic and cheap synthetic compound tested before as a plant growth stimulator. As was found in our experiments its adaptogenic capacity is displayed at concentrations which are lower on some orders than ones provided a growth stimulation effect.

Protective mechanism of Mehyure has been investigated in comparison to synthetic preparation Ivine one on corn seedlings grown on Hoagland solution and exposed in NaCl presence. It was shown that seed soaking in 10^{-7}M water solutions diminished growth reduction and stress reaction indexes in NaCl-exposed sedlings. Protective effect of Methyure was stronger and realized mainly in roots whereas Ivine predominantly influenced shoots. Adaptation mechanism of these preparations consists in cell osmotic homeostasis supporting by soluble sugars and free aminoacids accumulstson accompanied by normalization ion homeostase due means of Na^+ transport influencing. Besides they can prevent stress peroxidation burst by activation of cell antioxidant system and by this way protect membrane structure and function.

Both preparations possess an antioxidant activity whereas Methyure has an additional antiradical property. However their efficacy in too low concentrations supposes that they don't act directly as antioxidants but their mechanisms are mediated on a genetic level.

Defence effect of these preparations during plant development has been studied in vegetation experiments on corn plants grown in vessels with soil contained 0.1M and 0,05M NaCl which are high concentrations for this culture. Salinity coused a death of plants at two month age but seed treating by Ivine couldn't prevent it. On the contrary Methyure provided surviving of the most plants during whole vegetation and permitted them to form cernels with seeds. It was found on one month plants that seed pretreating by Mrthyure protected their root system formation and nornalyzed ion balance in roots and leaves under salinity conditions.

It is known that plant organisms during their development have two periods of high sensitivity to negative factors what was confirmed by us under salinity conditions.We tried to support corn plants during their transition to generative period by their sprimkling by Methyure solution and this procedure showed perfect results.

Besides we carried out field experiments on slightly salinized soils and obtained results showed that under low salinity conditions. Not only Methyure but Ivine using too by two-time treating can provide a significant corn crop increasing.

Possibility of mass using of Methyure as antidepresant in agriculture on salinized soils and under other stress conditions has been discussed.

PRODUCTION OF PLANT HORMONES AND GROWTH REGULATORS BY ENDOPHYTIC MICROBES

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Endophytic microbes, which frequently emerge in plant tissue cultures, affect plant growth. For example, *Bacillus circulans* mediates somatic embryogenesis in *Pelargonium*, and many members of the genus *Methylobacterium* stimulate seed germination and promote plant growth and development *in vitro*. Production of phytohormones is a function typical for many plant-associated fungi and bacteria. The three types of growth-promoting plant hormones, auxins, cytokinins and gibberellins, are produced by mycorrhiza. Also fungal endophytes, such as *Colletotrichum*, can produce phytohormones. Production of plant hormones is also typical for many *Rhizobium* species and for the endophytic bacteria *Methylobacterium* spp., *Azospirillum* sp., *Acetobacter diazotrophicus*, and *Herbaspirillum seropedicae*. Endophytic microbes may act solely to increase the size of their habitat, but other, unknown mutualistic interactions may also exist. In animals, microbes are known to affect morphology, offer protection, and prepare a developing tissue for encounters with pathogens, but so far, very little is known of the interactions between the developing plant tissue and microbes.

GROWTH AND CYTOKININ CONTENT IN WHEAT PLANTS INOCULATED WITH CYTOKININ PRODUCING BACTERIA DURING RECOVERY AFTER DROUGHT

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In some experiments water shortage decreased cytokinins content in some plants (Hare et al, 1997, Kudoyarova et al., 2007) and this allowed us to hypothesise that plants may suffer from a deficit in cytokinins limiting their growth when they return to sufficient water supply. In order to test this prediction it was first necessary to follow the effect of drought on cytokinin content of wheat plants we worked with. We did find that water shortage decreased cytokinin content in the plants the effect being greater in roots than in shoots. The results suggested a decline in cytokinin synthesis by dried roots. Root/shoot cytokinin ratio was decreased by drought, which looked as if cytokinins were distributed in favour of shoots maintaining hormone content in the latter. The greatest drop in root cytokinins was observed in case of its transport form, which are ribosides (Mok and Mok, 2001) suggesting that the decline in root cytokinins in droughted plants may be due to export of hormones from roots to shoots. It is still possible that part of shoot cytokinins was synthesized in shoots of wheat plants themselves and there were indications that drought was likely to decrease production of cytokinins in shoots. Thus the content of phosphosylated cytokinins, in which form cytokinins are synthesised in plants (Mok and Mok, 2001), decreased more than that of other cytokinins in shoots of droughted plants.

The decline in cytokinin content in droughted plants was accompanied by and seemingly responsible for inhibition of shoot growth. The increase in water supply restored cytokinin content in wheat plants very quickly paralleled by an increase in relative leaf growth up to the level of continuously well watered plants. The only sign of cytokinin deficit was revealed one week after the increase in watering and manifested in lower content of phosphorylated cytokinins in shoots of previously droughted plants. It is possible that a longer and more severe drying of root zone may decrease ability of roots to supply shoot with cytokinins on re-watering, but in present experiments plants were able to increase cytokinin content up to the level of continuously watered plants. Nevertheless since droughted plants already had smaller leaves when water supply increased, they remained smaller one week later although during this week their relative growth rate was as fast as in continuously well watered plants. Thus after drought it was not sufficient to restore the level of cytokinins and growth rate. It was necessary to grow faster so that to catch up continuously well watered plants.

The results of inoculation of plants with cytokinin producing bacteria confirm that after normalization of water status plants needed more cytokinins. Inoculation of droughted plants prior to the increase in the level of watering led to an increase in the their cytokinin content obviously due to bacterially sourced hormones and in accordance with this they had higher growth rate and managed almost to reach the level of biomass accumulation of continuously well watered plants. It is of interest that cytokinins accumulated mostly in shoots of inoculated plants and not in roots, although they presumably arrived from the root zone. The results show that plants need additional cytokinins when they return from stressful to optimal environment and these cytokinins may be supplied by rhizosphere microorganisms.

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PHOTOTROPHIC PURPLE BACTERIA AS MODEL SYSTEMS IN STUDIES OF CYTOKININS

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Free living phototrophic purple bacteria are no traditional object in cytokinin research in contrast to symbiotic and parasitic bacteria which exhibit phytohormon activity on plants.
Zeatin riboside and non-purine substance 4-OH- phenethyl alcohol with high cytokinin activity have been isolated from phototrophic purple bacteria *Rhodospirillum rubrum* several years ago by us. However, following studies of cytokinins in other purple bacteria showed absence of purine cytokinins even in that relating to the same α-phylogenetic group as *Rs. rubrum* and soil bacteria. It was supposed that the reason of this may be absence of the cytokinin biosynthesis genes or their expression. To check this point, we studied presence and functioning of ipt genes and transcription regulators of their expression in phototrophic purple bacteria.
Computer analysis of genomes on the level of amino acid sequences in phototrophic purple bacteria has shown the possible presence of plant type isopentenyltransferases carrying DMAPP to ADP/ATF. However, isopentenyltransferases of agrobacterial type performing transport of isoprenyl group to AMP has not been found. High resemblance of tRNA-isopentenyltransferase of *Agrobacterium tumefacience* and the same in purple bacteria mentioned above was observed. More highest likeness of tRNA-isopentenyltransferase of *Salmonella typhi* and purple bacteria *Rv. gelatinosus*, which have also similar structures of cell wall lipid A determining the toxicity of the pathogenic enterobacteria was revealed. The conditionally pathogenous *Rv. gelatinosus* might be a good object in monitoring the connection of bacterial toxicity and the structure of tRNA cytokinins.
High homology (up to 58%) of agrobacterial negative transriptional regulator, Ros, which controls ipt gene expression and the same in phototropic bacteria *Rps. palustris* have been revealed *in silico*. At present prokaryotic Zn-finger Ros proteins attract interest by their specific structure against eukaryotic one. In this connection the studies of structure and functioning of Ros-like protein in *Rps. palustris* will be very interesting and informative.
One more transcriptional regulator like of agrobacterial Vir A-Vir G ipt gene expression was investigated in phototrophic purple bacteria. It is known that VirA-VirG processing is initiated by acetosyringone (AS). This signaling molecule causes in laboratory agrobacteria cultures the excretion into culture medium the cytokinins in large quantity. Cytokinin-like substance 4-OH- phenethyl alcohol and hypoxanthine secretion, as the product of purine cytokinin degradation by cytokinin oxidase in *Rs. rubrum*, under AS treatment was observed. The possibility of cytokinin oxidase action has been shown by us early in transformed by ipt gene purple bacteria *Rps. palustris*. So, future studies of cytokinin oxidase structure and studies of ipt gene expression regulation by two-component transcriptional systems with using of wild type and transformed phototrophic purple bacteria are pioneering and resulting.

EFFECTS OF SERINE/TREONINE AND TYROSINE KINASE AND PHOSPHOTASE. INHIBITORS ON CORTICAL MICROTUBULE ORGANIZATION IN *ARABIDOPSIS* ROOT CELLS

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Microtubules (MTs), polymers of α- and β-tubulins, is one of the cytoskeletal components which involved in control of various processes of plant morphogenesis and development. As it was revealed for higher plants, α- and β-tubulins can be intensively regulated by post-translational modifications, including phosphorylation (Blume et al., 1997; Blume et al, *in press*). Plant tubulin phosphorylation is a reversible modification which could undergo as on serine/threonine (Ser/Thr) as well as on tyrosine (Tyr) residues; whereas till now its distinct role in plant cells was not clearly elucidated. It is known that level of tubulin phosphorylation in plant cells is determined by the balanced activity of protein kinases (PKs) and protein phosphatases (PPs). Therefore, to investigate the functional role of plant tubulin phosphorylation the effects of different types of inhibitors of Ser/Thr (H7, olomoucine (OM), staurosporine, W7) and Tyr (genistein, herbimycin A, tyrphostin AG 18) PKs, and inhibitors of Ser/Thr (okadaic acid (OA)) and Tyr (sodium orthovanadate (SO)) PPs on morphology of *Arabidopsis thaliana* primary roots and MTs organization have been studied. Effects of inhibitors were examined *in vivo* on *A. thaliana* line expressing GFP-MBD using confocal laser scanning microscopy (LSM 510 META, Carl Zeiss, Germany).

It was found that treatment of *A. thaliana* seedlings with effective concentrations of Ser/Thr PKs inhibitors reduced elongation of primary roots and caused alteration in root morphology. Also significant effects of all tested Ser/Thr PKs inhibitors on MTs organization in *Arabidopsis* root cells were observed. Treatments with W7, H7 and OM resulted in changes of cortical MTs orientation from transverse to longitudinal in epidermis and cortex cells of elongation and differentiation zones of roots. However, treatment with OA (an activation of protein phosphorylation process) resulted in MTs stabilization, changes of native MTs orientation or even in MTs disorganization in epidermis and cortex cells of elongation and differentiation zones. Also, OA affected root hair morphology; namely, root hairs swelling and branching as a result of abnormal MTs orientation were observed.

Inhibitors of Tyr PKs caused disorientation and disruption of MTs in epidermis and cortex cells of elongation and differentiation root zones, led to alteration of normal root hairs growth and development in comparison with untreated roots. Changes in MTs orientation from transverse to longitudinal in epidermis and cortex cells of elongation and differentiation zones, and intensive root hair development and growth were observed after treatment with SO (Tyr PPs inhibitor).

Thus, it was established that tested inhibitors of Ser/Thr as well as Tyr PKs and PPs cause significant effects on *A. thaliana* primary root morphology, root elongation and on MTs organization in different root cells. We suppose also that processes of tubulin phosphorylation/dephosphorylation can be involved in the dynamic and MTs organization in different types of living plant cells.

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SENSITIVITY TO PARAQUAT IN CYANOBACTERIA LACKING ANTIOXIDANT GENES

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The effects of herbicide paraquat, named also methyl viologen (MV), on the mutant of cyanobacterium *Synechococcus* sp. PCC 7942 lacking *sodB* gene and on the mutant of cyanobacterium *Synechocystis* sp. PCC 6803 lacking *katG* gene were examined. Cultures of the mutants and their wild type strains were grown in liquid BG11 medium enriched by 10 mM $NaHCO_3$ under 30 μmol photons m^{-2} s^{-1}, 28 ºC, and aeration by the air with 3% CO_2. Growth of the cultures was monitored by light scattering at 750 nm. In the linear stage of growth, cultures were centrifuged, resuspended in fresh medium to an A_{750} of 0.5, and maintained under the same conditions without (control) of with the presence of 0.5 μM MV. Damages of the photosynthetic apparatus were evaluated by measuring of CO_2-depended O_2 gas exchange in cell suspensions and by the delayed light emission (DLE) of chlorophyll *a*.
The in concentration of 0.5 μM was found to be a strong stressor for the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 but had no significant negative effect on growth and photosynthesis in its wild type strain as well as in the wild type strain and the *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803. Exposure to 0.5 μM MV inhibited growth, light-saturated O_2 evolution, and PS II activity (tested by DLE) in the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 within 8 hr of stress treatment. In contrast, the wild type strain of *Synechococcus* sp. PCC 7942 remained nearly unaffected for 48 hr of 0.5 μM MV treatment. The oxidative damage to photosynthesis of the *sodB*$^-$ mutant of *Synechococcus* sp. PCC 7942 was not accompanied by essential changes in chlorophyll content and carotene/chlorophyll ratio but was accompanied by greater catalase activity [1]. Earlier [2], it was demonstrated in this mutant that PS II activity, PS I cyclic activity, and the P700 reaction center are all targets of O_2^- formed at PS I and that the cytosolic superoxide dismutase (Fe-SOD) protects these targets from oxidative damage. The earliest of these targets to be damaged by MV in the absence of *sodB* gene is PS I cyclic electron transport. This observation is consistent with the vulnerability of Fe_4S_4 clusters of PS I to disruption by superoxide.
Present work suggests that lack of catalase activity in the *katG*$^-$ mutant of *Synechocystis* sp. PCC 6803 does not sensitize to MV because Fe-SOD is active. However, if the Fe-SOD is absent, as in the *Synechococcus* sp. PCC 7942 *sodB*$^-$ mutant, activation of catalase appears to be an adaptive response to MV stress that may allow survival, if not continued growth.
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Kozyrovskaya N.
Abstract not received

CHOLINE-CONTAINING GROWTH RETARDANTS INCREASE HEAT AND UV-B RESISTANCE OF PHOTOSYNTHETIC APPARATUS

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It is known that the use of growth retardants (GR) can enhance the plant resistance to stress-inducing factors such as drought, cold, salinity, ozone and УФ-В. However, it is little known about role of pre-treatment by GR in stress resistance of the photosynthetic apparatus (PA). Our objective was to study the influence of GR – choline-containing compounds, such as 2-chloroethyl-trimethylammonium chloride (CCC) and its analogue 2-ethyl-trimethylammonium chloride (CC) on stress resistance of the photosystem II (PS II). Photochemical activity of PS II was assessed by measurements of chlorophyll *a* variable and delayed fluorescence using phosphoroscope. The activities of antioxidant enzymes (catalase, ascorbate peroxidase, total peroxidase and glutathion reductase) and contents of pigments (carotenoids and flavoinoids) were determined spectrophotometrically by corresponding methods. The levels of abscisic acid (ABA) and cytokinins were assessed by enzyme–linked immunosorbent assay. We have showed that the application of CCC and CC both to bean seeds and seedlings diminished UV-B and heat induced inhibition of PS II activity (1). Membrane thylakoids isolated from the primary leaves of seedlings treated with choline-containing compounds were also more resistant to UV-B. The higher resistance of the PS II in beans pre-treated with choline-containing compounds assessed by the ratio (F_v/F_m) and maximal intensity delayed fluorescence of Chl *a* correlated with increased activities of antioxidant enzymes and higher amount of low molecular antioxidants such as carotenoids and flavonoids detected by us in primary bean leaves. Total peroxidase activity in thylakoid membranes isolated from leaves of pre-treated seedlings increased twice. Pretreatment with choline compounds increased contents of cytokinins (2) and ABA in leaves as well. We suggest that enhanced stress resistance of the PS II in plants treated by GR might be due to increased activities of the antioxidant enzymes as well as an increase in the content of low-molecular antioxidants (carotenoids, flavonoids) and hormones (ABA, cytokinins). The one of the reasons of high antioxidant activity in leaves and membrane thylakoids can be weak stress that develops as a result of choline pretreatment. Such weak stress was indicated in cells of green alga *Chlamydomonas* (3) treated with cholines.

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CYTOKININ SECONDARY HORMONE AND 14-3-3 PROTEINS ACTIVATES THE CA^{2+} ATP-ASE PUMP AND THE CYTOSOLIC CA^{2+} SWITCH ON THE ACTIVITY OF AMMONIA ASSIMILATION NADP -GLUTAMATE DEHYDROGENASE OF SPHEROSOME IN THE FILLING WHEAT GRAINS

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Our works is devoted to investigation of signal transduction of cytokinin. Earlier it was shown by us that cytokinin induces the formation of strong NADP-dependent GDh electrophoretic forms in whole wheat grain. In the same time the cytokinin inducing effect absent when cytokinine acts on unembryonated wheat seeds. In this reason it was assumed by us that cytokinine induces in the seeds embryos the formation of cytokinin`s secondary hormone (CSH). The CSH was purified from germinating wheat seeds by hydrophobic chromatography on column with octylsepharose 4B and by reverse phase chromatography on column type RP-18. It was shown that CSH is very close by its properties to fusicoccin.

So, the purified CSH strong inhibited the binding of tritium-labeled fusicoccin with fusicoccin receptors from roots of Zea maize seedlings**.** It was shown that CSH and 14-3-3 proteins activates Ca^{2+}- dependent ATP-ase of plasmatic membrane from aleuron layer of wheat seeds and increases the level of cytosolic Ca^{2+}. It was established that cytosolic Ca^{2+} activates NADP-GDH of spherosome in aleuron layer of wheat seeds. Using Ca^{2+} ionophore A_{23187} it was shown that artificial increasing of the level of cytosolic Ca^{2+} induces the formation of NADP-GDh of spherosome without effect of CSH whereas the artificial decreasing of the level of cytosolic Ca^{2+} canceled the effect of CSH. Thus level of cytosolic Ca^{2+} plays determining role in the activation of NADP-GDh of spherosome.

Thus we discover that the intracellular target for action of cytosolic Ca^{2+} ions is subcellular organelle - spherosome.

It was established that NADP-GDh of spherosome from filling wheat grains has the very high affinity to ammonia. Km to ammonia of NADP-GDh is equal 1,3 mkM. Dephosphorylation of this NADP-GDh let to decreasing the affinity of this enzyme to ammonia. Its necessary to know that the NADP-GDh activity shows only spherosomes as whole structure, but we don't be able to find this activity in soluble state. Thus it was shown that CSH and 14-3-3 proteins activates the Ca^{2+} ATP-ase pump and the cytosolic Ca^{2+} switch on the activity of ammonia assimilation NADP - GDh of spherosome in the filling wheat grains.

Our experimental data allow us to suggest the next scheme of signal transduction of cytokinin. Cytokinin induces the formation of CSH, then CSH activates the formation of 14-3-3 proteins. After translocation CSH and 14-3-3 proteins are bonded with receptors of Ca^{2+} - ATP-ase of plasmatic membrane. Then this gives the increasing of level of cytosolic Ca^{2+}. Ca^{2+} is bonded with GDh of spherosome and activates the NADP-GDh and its phosphorylation.

UKRANIAN PLANT GROWTH REGULATORS: FROM IDEA TO REALITY

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During 20 years since foundation of Bioorganic Chemistry & Petrochemistry Institute of NAS of Ukraine, we have developed the whole series of high efficiency plant growth regulators of new generation.

On the basis of the fundamental research results the authors offered the hypothesis concerning universal and narrow specific action of exogenous regulators on the plant growth processes. Regulators act through changing the synthesis of phytohormones which are accounted for the recognition and regulation of gene activity. These processes promote the development and growth of plants.

Hypothesis also explains the possible mechanism of the universal action of exogenous and endogenous plant growth regulators.

New plant growth regulators activate the basic of vital functional of plants, quality of products is improved, plant stability to the diseases and damages by insects are promoted content, content of nitrates, ions of heavy metals and radionuclide in products are lowered, mutagenic action of herbicides and other anthropogenic factors is descended in two times.

Developed regulators are ecologically safe. They positively influence on development of soil micro flora, growth of the root system, leaf surface and photosynthesis are strengthened, and stability to the stress factors (frost, drought, soil salinity) is promoted.

New plant growth regulators on the efficiency correspond with the best world standards, and on the technological index and the cost they are surpassed considerably. Cost of growth regulators application per 1 hectare almost to 3-7 $ USA.

The ISTC "Agrobiotech" was created in 2000 on the basis of Bioorganic Chemistry and Petrochemistry Institute for production and creation new plant growth regulators and now produce 30 items regulators for agrarian complex.

THIAZOLIUM ION: RING OPENING AND ACTIVITY IN MODEL SYSTEMS

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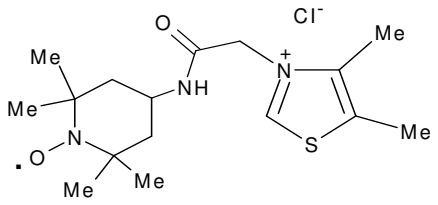
Much of the attention directed to mechanistic aspects of biological transport of thiamine has focused on mechanisms of reversible hydrolysis of thiazolium ion. We have investigated the kinetics of thiazolium ring opening of thiamine and its structural analogues in the presence of 5,5′-dithiobis(2-nitrobenzoic acid). It was found that values of the reaction rate constants catalyzed by general base correlate with inductive effect of substituents at position 3 of thiazolium cycle of 3-R-4-methyl-5-(2-hydroxyethyl)thiazolium ion, including 4-amino-2-methylpyrimidinyl-5-methyl substituent of thiamine.

Because of biochemical importance of thiazolium ion hydrolysis the properties of N-[(1-oxyl-2,2,6,6-tetramethylpiperidinyl-4)aminocarbonylmethyl] substituted thiazolium salts I, II in aqueous micellar solutions (physiological pH range) in the presence of sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) have been studied by EPR spectroscopy. Binding of paramagnetic thiazolium salts to the SDS and CTAB micelles resulting by increase of apparent rotational correlation time (τ_c) depends on the nature of the micelle, hydrophobisity of substituent in position 5 of thiazolium cycle, temperature and pH of solution. Dependences of free radical $\ln\tau_c$ on 1/T at pH 6 and pH 7,6 were analysed. The increase of τ_c at interaction of ion II with CTAB micelles at pH 7,6 in comparison with pH 6 is caused by thiazolium ring opening and formation of thiol form of nitroxide. Substantial increase of τ_c and relative parameter of hydrophobisity in the presence of 5,5′-dithiobis(2-nitrobenzoic acid) may be due to accumulation in CTAB micelle of mixed disulfide.



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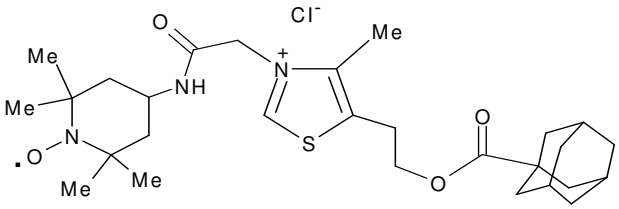

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We have examined also the binding of 3-[(1-oxyl-2,2,6,6-tetramethylpiperidinyl-4)aminocarbonylmethyl]-4-methyl-5-[2-(1-adamantoyloxy)ethyl] thiazolium chloride to the thylakoids of pea chloroplasts. Binding of spin probe to the membrane structures is revealed by significant increase of τ_c in a range of 20-60^{o}C.

It was established that cyclic photophosphorylation with phenazine methosulfate in isolated pea chloroplasts is inhibited by 3-benzyl-4-methyl-5-(2-acyloxyethyl) thiazolium salts containing at position 5 the acyl fragment of norbornane-2-carboxylic, adamantane-1-carboxylic, adamantane-2-carboxylic, adamantyl-2-acetic, 5-methyladamantyl-1-acetic and diphenylacetic acids. The degree of inhibition of ATP formation was increasing at combined action of the thiazolium salts and cationic detergent - cetyltrimethylammonium bromide - in concentrations which did not provide considerable effect of these compounds separately. Dependence of chloroplasts activity inhibition on the nature of substituents at positions 3 and 5 thiazolium ion was analysed. It was concluded that thiazolium cation localization on the membrane of thylakoids followed by thiazolium ring opening and interaction with ATP synthase causes inhibition of photophosphorylation.