

03 01

A possible role of phosphatidylinositols in transduction of temperature signal in winter cereals

T.P. BUKOLOVA, N.V. VOLOVIK and V.S. KRAVETS

Institute of Plant Physiology and Genetics, Academy of Sciences of Ukraine, Vasilkovskaya 31/17, 252022 Kiev 22, Ukraine

The recent discovery showed that inositol phospholipids play essential role in cellular perception and transduction of extracellular signals. It was presumed also that phosphatidylinositol system takes part in plant – environment interaction, but there are no data about the phosphatidylinositol cycle participation in the transduction of temperature signal in higher plants. Experiments were carried out on plants of winter wheat (*Triticum aestivum* L.) cv Mironovskaya 808 and winter barley (*Hordeum vulgare* L.) cv Cyclone. Plants were grown in soil at 22°C under cycles of 16 h light : 8 h dark. 7 day old plants were stand at 0°C during 1 h. The phosphatidylinositols were extracted from the leaves and analysed by TLC systems. It have been found that under these conditions changes of levels of phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate in winter cereals were significant. Phosphatidylinositols are supposed to participate in the cold stress response of plants.

03 02

Calcium and signal transduction in *Allium cepa* suspension cell culture

J.V.DYACHOK*, O.M.DYACHOK**

*Institute of Cell Biology and Genetic Engineering, 148 Zabolotnogo St., 252022 Kiev, Ukraine**
*Dept. of Biophysics, Kiev University, 64 Vladimirskaya St., 252017 Kiev, Ukraine***

We are studying the mechanisms of signal perception and transduction in plants in the process of elicitation of phytoalexins (PA) synthesis. Fast growing callus and suspension cell cultures of onion have been obtained. Conditions of PA synthesis in suspension culture has been investigated. PA accumulation in onion suspension culture could be induced by pretreatment with biotic elicitor derived from *Botrytis cinerea* culture filtrate. It has been shown that Ca^{2+} is likely to be involved in elicitation of PA synthesis. Ca^{2+} - binding by EGTA or blockade of plasmalemma Ca^{2+} - channels by inhibitor verapamil led to strong decrease of PA accumulation. However, PA synthesis significantly increased after treatment of onion cell culture both with biotic elicitor and agonist Ca^{2+} - ionophore A23187. Ionophore A23187 was able to induce PA accumulation in onion suspension culture. The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was measured in single onion cells from suspension culture using Ca^{2+} -sensitive fluorescent dye indo-1. Rapid transient increasing of $[\text{Ca}^{2+}]_i$ has been observed immediately after treatment of cell with biotic elicitor. It is suggested that Ca^{2+} takes part in the mechanism of inducible plant resistance, probably as a second messenger.

03 03

Zeatin-binding protein from barley leaves: isolation and properties

N.N. KARAVAIKO, Ya.V. ZEMLYACHENKO, S. Yu. SELIVANKINA, and O.N. KULAEVA
*Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35,
Moscow, 127276 Russia*

Zeatin-binding protein (ZBP) was isolated and purified from barley leaf cytosol (160 000 g) by consequent chromatography including Phenyl-Sepharose and Zeatin-Sepharose (*trans*-zeatin was immobilized on epoxy-activated Sepharose 6B). Non-denaturing PAGE revealed a single band (67 + 1 kD) in fraction eluted from Zeatin-Sepharose with 1 M NaCl. SDS-PAGE showed also the single polypeptide with the same molecular mass. This polypeptide was revealed by Western blot with anti-idiotypic antibodies against zeatin. Zeatin-binding properties of the protein were demonstrated by competition of the protein with antibodies against zeatin. Zeatin-binding properties of the protein were demonstrated by competition of the protein with antibodies against zeatin for the hormone in ELISA. ZBP activated rRNA synthesis *in vitro* in the presence of zeatin. The data obtained give a base to consider this protein as a putative zeatin receptor in barley leaves.

03 04

cAMP-dependent protein kinase activity determination in etiolated pea seedlings

F.G. KARIMOVA, L.A. ZAKIROVA, N.U. MURSALIMOVA, Y.A. TARCHEVSKY
*Institute of Biology, Kazan Science Centre, Russian Academy of Sciences. P.O.Box 30, 420503
Kazan, Russia*

In plant tissue homogenates cAMP-dependent activity represented 20 - 70 % of the basal protein kinase activity. cAMP-dependent activity was determined by using high specific protein inhibitor of cAMP-dependent protein kinases from rabbit skeletal muscles. Charged agents changed the magnitude of cAMP-dependent protein kinase activities. The importance of susceptibility of protein-substrate phosphorylation sites to cAMP-dependent protein kinases was demonstrated on crude nuclei fraction from pea roots after its treatment with detergents SDS and Triton X-100. The evidence for the Ca²⁺-dependent protein phosphatase participation in regulation of cAMP-dependent protein phosphorylation has been obtained.

03 05

The effects of spermidine biosynthetic inhibitor methyl bis-(guanyldihydrazone) on spore germination, growth and ethylene production in *Alternaria consortiale*

E. KĘPCZYŃSKA and J. KĘPCZYŃSKI

Department of Plant Physiology, University of Szczecin, Felczaka 3a, 71-412 Szczecin, Poland

The spermidine synthesis inhibitor methyl bis-(guanyldihydrazone) (MGBG) was found to reduce spore germination, hyphal and mycelial growth in *Alternaria consortiale*. Simultaneously application of spermidine and its inhibitor MGBG caused complete reversing the inhibitory effect of this inhibitor. Spermidine or MGBG had not significant influence on ethylene production by mycelium.

The data suggest that spermidine may play a role in the development of *Alternaria consortiale*.

03 06

Effects of osmotic stress on jasmonates in barley leaf segments

R. KRAMELL, R. ATZORN, O. MIERSCH and B. PARTHIER

Institute of Plant Biochemistry, P.O.B. 250, D-06018 Halle, Germany

Osmotic stress (sorbitol, mannitol, PEG) induces in leaf segments of barley (*Hordeum vulgare* L.) different jasmonates, which are possible signal transducing substances between stress and gene expression. Strength and duration of stress influenced the quantity of produced substances. NaCl does not stimulate the jasmonate biosynthesis. Induced substances were isolated, purified and structural elucidated to be mainly (-)-jasmonic acid (JA) and (+)-7-iso-JA. Further minor compounds were cucurbitic acid and amino acid conjugates of isoleucine, leucine and valine. From the isolated jasmonates the major compounds and JA amino acid conjugates are able to induce JIPs (jasmonic acid proteins).

03 07

The effect of cytokinin and CaM on Ca^{2+} transport to plasma membrane vesicles isolated from zucchini cotyledons grown in different light conditions**D. KUBOWICZ***Department of Forest Botany, Warsaw Agricultural University, Rakowiecka 26/30, 02-528 Warsaw, Poland*

A plasma membrane preparation from zucchini (*Cucurbita pepo* L.) cotyledons, possesses an active ATP-dependent calcium (netto) transport. This transport can be increased by one-day pretreatment of 10 μM zeatin or (to less extend) by calmodulin (1 $\mu\text{g}/10 \mu\text{g}$ membrane protein) added into $^{45}\text{CaCl}_2$ solution. White, red and blue /UV irradiation (in the contrary to far red) increased these stimulatory effects. An increase by zeatin of ^{45}Ca accumulation by plasma membrane vesicles appears also without applied 1mM ATP and independently on light conditions. The results obtained suggest that in zucchini cotyledon cells active cytokinin-dependent Ca^{2+} transport (probably *via* calcium pumps) is regulated by light but passive Ca^{2+} transport (probably *via* calcium channels) can be light - independent.

03 08

The effect of phytohormones on ATP-driven Ca^{2+} uptake into plasma membrane vesicles**E. LADYZHENSKAYA***Bakh Biochemistry Institute of Russian Academy of Science, Leninsky Prosp. 33, 117071 Moscow, Russia*

The influence of GA, ABA, IAA and kinetin on ATP-driven Ca^{2+} uptake into inside-out plant plasma membrane vesicles was studied. The Ca^{2+} uptake was inhibited by GA, ABA and IAA and was stimulated by kinetin. The stimulation of Ca^{2+} uptake by kinetin was abolished by both DCCD and Na_3VO_4 treatment. IAA-induced inhibition was increased by DCCD and was decreased by Na_3VO_4 . Both DCCD and Na_3VO_4 influenced the effect of GA and of ABA on Ca^{+2} uptake.

03 09

Stomatal movements in starchless *Arabidopsis thaliana* L.

G. LASCEVE*, M. BRESTIC** and A. VAVASSEUR*

Département de Physiologie Végétale et Ecosystèmes. Centre d'Etudes Nucléaires de Cadarache, F-13108 Saint Paul-lez-Durance, Cedex, France*

Department of Plant Physiology, University of Agriculture, 949 76, Nitra, Slovakia**

Starch breakdown is assumed to be the main source for organic acid anions which counter-balance the K^+ uptake during stomatal opening. In almost all guard cell chloroplasts so far studied, starch content is reduced after stomatal opening, specially under darkness and CO_2 -free air. The only plants lacking starch in their guard cells which have been studied were those in the *Allium* or *Paphiopedilum* genus. With *A. cepa*, Schnabl et al (1978) suggested that stomata could exchange organic compounds with the other cells and Darbyshire and Allaway (1981) showed that fructans could replace starch. With *P. leanum*, guard cell chloroplasts are also devoid of chlorophyll and stomatal movements are weak.

Despite of their small size and a growth in a "rosette" pattern, mutants of *Arabidopsis thaliana* are potentially useful in stomatal physiology. Guard cells of TC 75, a mutant lacking chloroplastic P-glucomutase activity (Caspar et al 1985) are devoid of starch. First results indicate that with starchless plants, light-induced stomatal opening, measured by gaseous exchange techniques, was comparable in amplitude but slower than with wild type plants. Interestingly, under darkness and CO_2 -free air, there was no difference in opening kinetics between deficient and wild type plants indicating that starch breakdown does not play an essential part in the response to a decrease in CO_2 concentration.

03 10

Fluorescence ratio microscopy in plant cell signaling

S.M. LINDBERG

Dept of Plant Physiology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

In signal transduction, external stress factors and plant hormones induce rapid changes in e.g. cytosolic ion concentrations which may lead to different metabolic events within the cell. The primary reactions, such as changes in cytosolic free calcium and pH, can be demonstrated by use of dual wavelength microscopy. Several fluorescent probes are now available for determination of intracellular concentrations of ions in living cells or protoplasts. These can be loaded with dyes, specific for different cations by e.g. microinjection, electroporation or by addition of the dye in an tetra (acetoxymethyl) ester form. By the latter technique, esterases of the cell cytoplasm will split the ester to an acid, ion-binding form.

Addition of $AlCl_3$ or synthetic auxin 1-naphthaleneacetic acid in active form, to protoplasts from wheat induce transient changes in cytosolic free calcium, potassium and pH. Aluminium causes oscillations of cytosolic calcium. Results will be presented from fluorescence measurements using the probe BCECF, bis-carboxyethyl-carboxyfluorescein, which has pH sensitive excitation spectra, the calcium specific indicator Fura-2, a stilbene chromophore, whose excitation spectra are shifted to shorter wavelengths as Ca^{2+} increases, and the new potassium binding fluorescent benzofuran isophthalate, PBFI. When the latter dye binds to free K^+ inside the cytoplasm, the fluorescence intensity ratio 340/380 nm increases in direct relation to the K^+ -concentration.

03 11

Calcium control and polar transport of auxin in the cambial region of *Acer pseudoplatanus* and *Fraxinus excelsior* stem cuttings

K.D. MARCISZEWSKA

Department of Forest Botany, Warsaw Agricultural University, Rakowiecka 26/30, 02-528 Warsaw, Poland

Effects of different substances affecting concentration of cytosolic calcium (D_{600} , DES, W7) on polar transport of natural and synthetic auxin in the cambial region of *Acer pseudoplatanus* and *Fraxinus excelsior* stem cuttings were investigated. W7, the calcium-calmodulin complex formation inhibitor, significantly decreased basipetal as well as acropetal efflux of both endogenous and synthetic auxins. The effects of calcium antagonist (D_{600}) and inhibitor of plasmalemma Ca^{2+} -ATPase (DES) appeared less distinct. The observations seem to support the hypothesis that the system controlling cytosolic calcium concentration can be involved in auxin signal transduction.

03 12

Characterization of polyphosphoinositide phospholipase C in *Chenopodium rubrum* leaf plasma membranes

J. MARTINEC and I. MACHÁČKOVÁ

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Ke dvoru 16, 166 30 Praha 6, Czech Republic

The polyphosphoinositide phospholipase C has been identified in *Chenopodium rubrum* leaf plasma membrane purified by aqueous dextran - polyethyleneglycol two phase partitioning. The enzyme hydrolyzed phosphatidylinositol-4,5-bisphosphate into mixture of inositolbisphosphate and inositoltrisphosphate. It was Ca^{2+} dependent ($[Ca^{2+}]_{opt} = 1 \times 10^{-5} M$) and deoxycholate (0.05 - 0.1%) further increased its activity. Optimum pH was 7.5. We have also observed *in vitro* sensitivity of phospholipase C activity to the red light. This enzyme may participate in signal transduction of light across plant plasma membrane.

03 13

Response coupling to hypertonic salt shock

L. NAVEH, D. BENO-MUALEM and B. JACOBY

Agricultural Botany Department, The Hebrew University of Jerusalem, P.O.Box 12, Rehovot 76100, Israel

Aged red beet (*Beta vulgaris* L.) tissue-slices were exposed to a hypertonic, non plasmolyzing, DASW (1:5 diluted artificial sea water) shock. Coupling of adaptive metabolic responses to perception of changes in environmental water potential and salinity was investigated. Enhanced PM (plasma membrane) ATPase activity was the adaptive response of reference for salt shock. Within 1.0 min of DASW application, Ins(1,4,5)P₃ (inositol 1,4,5-trisphosphate) content of the tissue increased 2-fold, plasma membrane ATPase activity increased 45 % and a small, but definite, dephosphorylation of PM proteins was observed. Two minutes after exposure to the DASW shock, an intense phosphorylation of some plasma membrane proteins occurred. DASW-dependent dephosphorylation, enhancement of PM ATPase-activity and Ins(1,4,5)P₃ formation were all prevented by microcystin - a phosphatase inhibitor. Manoolide and neomycin, inhibitors of phosphoinositide-metabolism, prevented the DASW-dependent increase in Ins(1,4,5)P₃ and PM ATPase activity.

03 14

Senescence-dependent changes in cytosolic Ca²⁺ of leaf plasma membranes

S. PHILOSOPH-HADAS, R. SABATO, E. BAUDOUIN and S. MEIR

Dept. of Postharvest Science of Fresh Produce, The Volcani Center, Bet Dagan 50250, Israel

The idea that the capability to maintain low cytosolic Ca²⁺ levels ([Ca²⁺]_{cyt}) at homeostatic state is impaired during leaf senescence, was examined. Accordingly, the senescence-dependent changes occurring in the Ca²⁺-transport and in the Ca²⁺-dependent protein phosphorylation of sealed plasma membranes (PM), purified from detached leaves of parsley (*Petroselinum crispum*) and chrysanthemum (*Chrysanthemum morifolium*), were studied. A 2-3 fold increase in activity of the PM Ca²⁺-ATPase and a reduction in the Ca²⁺-dependent protein phosphorylation were obtained during the initial 24 h of leaf senescence. Subsequently, at advanced senescence stages (4 days), this PM Ca²⁺ transport activity was significantly reduced, but was not affected by ethylene. Application of benzyl adenine to chrysanthemum leaves prevented the senescence-dependent decrease of their Ca²⁺-transport activity. Results suggest that the increase in Ca²⁺-transport obtained at initial senescence stages, may represent an effort of the leaf cells to cope with higher-than-normal [Ca²⁺]_{cyt}. The loss of the ability to extrude Ca²⁺ out of the cell, observed later on, may be ascribed to the senescence-associated decrease of Ca²⁺-ATPase activity. This is likely to result in elevated [Ca²⁺]_{cyt}, which may be the signal that triggers turnover of membrane phospholipids, leading irrevocably to cell death.

03 15

Zeaxanthin: a putative blue light photoreceptor in corn coleoptiles

M. A. QUIÑONES and E. ZEIGER

Department of Biology, University of California, Los Angeles, CA 90024, USA

The identity of the photoreceptor mediating blue light-induced phototropism in plants remains unknown. Reported action spectra may imply a flavin or a carotenoid as the chromophore involved. Corn coleoptile chloroplasts contain all the main carotenoids found in leaves, including the components of the xanthophyll cycle: violaxanthin, antheraxanthin and zeaxanthin. Dark-grown coleoptiles lack zeaxanthin but have a large violaxanthin content. These zeaxanthin-less coleoptiles show no phototropic response to a blue light pulse. In the light, coleoptiles operate the xanthophyll cycle and accumulate zeaxanthin. Titration of the zeaxanthin content by exposure of the coleoptiles to red light, red light followed by darkness or incubation with dithiothreitol (an inhibitor of zeaxanthin formation), results in blue light-induced phototropic curvatures that are proportional to the zeaxanthin content. These results suggest that the xanthophyll, zeaxanthin, may be a blue light photoreceptor mediating the blue light-dependent phototropic response of corn coleoptiles.

03 16

Cytokinin binding proteins as possible targets for physiologically active phenylurea derivatives

G.A.ROMANOV

Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35, 127276 Moscow, Russia

Different type of cytokinins including adenine derivatives such as zeatin (Z), dihydrozeatin (DhZ) and 6-benzyladenine (BA) and phenylurea derivatives such as 3-thidiazuron (TD), 4PU-30 and diphenylurea were tested in parallel for their effect on retardation of senescence of detached barley leaves. At concentrations close to 10^{-5} M the most active PU-cytokinins (TD and 4PU-30) were able to restore approx. as much chlorophyll as "classical" Ade-cytokinins. Meanwhile, the amount of endogenous cytokinins (Z and Z-riboside) appeared not to increase but, on the contrary, to be significantly reduced after prolonged incubation on solutions of TD or 4PU-30 as compared to initial control level. Highly labelled ^3H -TD was assayed for its ability to interact with cytokinin binding protein (CBP) isolated from the same plant (i.e. barley leaves) by means of its specific binding of Ade-cytokinins. In both direct and competitive binding assays CBP was shown to bind PU-cytokinins specifically with reasonably high affinity (K_d approx. 10^{-6} M). Nevertheless, it was demonstrated that the binding site for PU-cytokinins on CBP is probably different than that for Ade-cytokinins, the former being able to inhibit the activity of the latter, but not *vice versa*. It was concluded that PU-cytokinins can transfer the hormonal signal in similar way as Ade-cytokinins without affecting the metabolism of the latter, although the mode of interaction with CBP is probably different for the two kinds of cytokinins.

Multiple pathways in calcium signalling

D. SANDERS, J.M.BROSNAN, S.R.MUIR, E. JOHANNES and G. ALLEN
Biology Department, University of York, York YO1 5DD, UK

The role of the plant vacuole in storage of inorganic and organic nutrients, waste products and defence compounds has long been appreciated. In the last few years, however, an additional key vacuolar function has emerged: this organelle - and in particular its bounding membrane - plays a key role in transduction of intracellular signals. This role stems from the considerable capacity for Ca^{2+} accumulation in the vacuolar lumen (mM levels of Ca^{2+} in this, the principal component of the intracellular volume), and from the fact that transient elevations of cytosolic free Ca^{2+} in the nM range function in stimulus-response coupling. In higher plants, especially, imaging studies have demonstrated that a large fraction of this Ca^{2+} signal results from intracellular Ca^{2+} mobilization from the vacuolar pool. Recent studies on the properties of two vacuolar Ca^{2+} release pathways will be described. The first pathway is gated open by inositol 1,4,5-trisphosphate (InsP_3) and, with respect to Ca^{2+} release, InsP_3 binding and protein chemistry, bears many similarities to the InsP_3 -gated Ca^{2+} release pathway across the ER of animal cells. The second pathway comprises a voltage-gated Ca^{2+} channel the opening of which is controlled by luminal pH and Ca^{2+} . Both channel types reside in beet and in guard cells. The presence of various classes of Ca^{2+} release channel at vacuolar membranes implies that stimulus-specificity in Ca^{2+} signalling might be achieved through flexibility in the duration and amplitude of cytosolic Ca^{2+} transients via differential operation of discrete channel types.

Inositol-1,4,5-trisphosphate receptor from *Chenopodium rubrum* is located on the tonoplast

C.H. SCANLON, P.J. LUMSDEN, J. MARTINEC*, I. MACHÁČKOVÁ* and C.E. ROLPH
Department of Applied Biology, University of Central Lancashire, Preston, PR1 2TQ, UK
*Department of Growth and Development, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Ke dvoru 15, 166 30 Prague 6, Czech Republic**

Inositol-1,4,5-trisphosphate (InsP_3) has been implicated in the coupling of signal perception and physiological response in a number of systems operating in plants. In animals, InsP_3 binds to a receptor on the endoplasmic reticulum causing an efflux of Ca^{2+} release, however, there is little data concerning the occurrence and properties of InsP_3 receptors in plant cells. Recently, high affinity InsP_3 binding sites were identified and characterised in a detergent-solubilised microsomal preparation from the storage root of red beet. We have detected similar high affinity binding sites in intact membrane vesicle preparations obtained from *C. rubrum* leaf tissue. When crude membrane preparations were subjected to sucrose density gradient centrifugation the InsP_3 binding sites co-equilibrated with tonoplast derived membrane vesicles. The parameters of InsP_3 binding to the receptor within this tonoplast-enriched preparation have been determined. The specificity of the receptor for InsP_3 has been established by a series of displacement assays employing potentially competing ligands. The existence of high affinity InsP_3 receptors in plant cells serves as further confirmation of the functioning of inositol based signal transduction pathways in plants. The localization of these receptors on the tonoplast complements existing evidence that the vacuole is the major storage site for InsP_3 mobilized Ca^{2+} .

03 19

Zeatin-dependent regulation of transcription in barley leaves

S. Yu. SELIVANKINA, N.N. KARAVAIKO, S.V. SHIPILOVA, Ya. V. ZEMLYACHENKO, and O.N. KULAEVA

Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35, Moscow, 127276 Russia

We present evidence that zeatin-binding protein (ZBP) with $M_r = 67 + 1$ kD isolated from barley leaf cytosol is involved in cytokinin activation of rRNA synthesis *in vitro*. The activation was observed in transcription elongation system contained chromatin-bound RNA polymerase I from barley leaves. ZBP in the presence of zeatin affected also rRNA synthesis in the system of isolated nuclei. Our results showed that the ZBP could be proposed as a putative zeatin receptor participating in hormonal regulation of RNA synthesis in barley leaves.

03 20

Investigation of auxin action on cation transport through plasmalemma

M.F. SHISHOVA, V.V. POLEVOI, N.I. INGE-VECHTOMOVA and K.A. VUIKHVALOV

Biological Institute of St. Petersburg University, Lab. Membrane Functional Activity, Oranienbaumskoye Sh. 2, St. Peterhof, 198904 St. Petersburg, Russia

The IAA action on different mono- and divalent cation transport was investigated on 4-days-old etiolated maize seedling coleoptile segments. The decrease of the primary auxin-induced negativation of the bioelectric tissue potential of coleoptile segments in presence of nifedipine and verapamil makes possible to support that Ca^{2+} -channels take part in initial phytohormone action on plant cells. On model systems (plasmalemma vesicles of maize seedling coleoptile segment cells) was shown that IAA influence on plasmalemma permeability is expressed by generation of K^{+} -diffusion potential and protonophore-similar action.

03 21

Effects of low temperature on inositol trisphosphate release, water potential changes and ABA content in winter rape leaves

G. SMOLEŃSKA and A. KACPERSKA

Laboratory of Plant Resistance Physiology, Institute of Plant Experimental Biology,

University of Warsaw, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland

Earlier experiments showed that short and rapid exposure to low temperature induced a breakdown of polyphosphoinositides to inositol phosphates in the leaves of winter rape (*Brassica napus* L. var. *oleifera* L. cv. Jantar). Using inositol - 1,4,5-trisphosphate [³H] assay system and ELISA for abscisic acid (ABA) determinations we found that the release of inositol trisphosphate in the low temperature-affected cells preceded an accumulation of ABA but was parallel to decrease of water potential in a tissue. Our work suggests that in the initial responses to the low temperature treatments both formation of inositol trisphosphate and decrease of water potential are involved. ABA accumulation seems to occur later on.

03 22

Localization and receptor function of maize auxin binding protein

M.A. VENIS and R.M. NAPIER

Horticulture Research International, East Malling, West Malling
Kent ME19 6BJ, UK

The auxin-binding protein (ABP) of maize microsomal membranes has been characterized thoroughly at gene and protein levels. Antibodies that were raised against the conserved auxin-binding domain show auxin agonist activity in several different electrophysiological assays, confirming a receptor function for ABP. These assays also imply that a functional pool of ABP is accessible at the outer face of the plasma membrane. However, cell fractionation studies show that the predominant location of ABP is in the endoplasmic reticulum, consistent with its C-terminal KDEL retention sequence and this is confirmed by immunogold and immunofluorescence localization (J. Henderson, C. Hawes, Oxford). We have shown that auxin induces a conformational change involving the C-terminus, which may allow some ABP to escape the KDEL receptor, but secretion of ABP is difficult to establish biochemically. However, recent silver enhanced immunogold studies on maize protoplasts (D. Robinson, Göttingen) have succeeded in visualizing ABP at the cell surface, as well as auxin-specific clustering of the signal induced within 30 minutes. The significance of these observations will be discussed.

03 23

Flavin type action spectra of the NO_3^- and Cl^- uptake in *Monoraphidium braunii*

F. G. WITT and P. J. APARICIO

Centro de Investigaciones Biológicas, CSIC, Velázquez 144, E-28006 Madrid, Spain

In the green algae *Monoraphidium braunii*, the uptake of monovalent anions, NO_3^- , NO_2^- and Cl^- , is triggered by blue light of relatively low photon fluence rate when the cells are illuminated with strong red light. The uptake of these anions is carried out with protons as counter ions and is independent of most common metal cations present in the solution. The action spectra of the uptake of NO_3^- and Cl^- resulted almost identical with a prominent band in the UV region, peaking at 267 nm and two other smaller bands, one in the UVA with a maximum at 360 and the other in the blue region, with two peaks at 450 and 480 nm. Radiations above 500 nm did not elicit the uptake of these anions, including those of 730 and 750 nm in the near infrared. These action spectra suggest that flavins may act as sensitizers of the process. Other results indicate that these two anions compete for the entrance in the cells, suggesting that the same transport system would operate in their uptake.

03 24

Cytokinins and cyclic nucleotide metabolismE. WITTERS, L. ROEF, H. VAN ONCKELEN, *Universitaire Instelling Antwerpen, Dept. Biology, Universiteitsplein 1, b-2610 Wilrijk, Belgium*

In order to investigate the influence of both isoprenoid and aromatic cytokinins (CK) on the *in vitro* adenylyl cyclase (AC) activity in chloroplasts from *Nicotiana tabacum* cv. Petit Havana SR1, we administered the phytohormones (zeatin, zeatinriboside, benzylaminopurin and benzylaminopurinriboside) in a micromolar to picomolar concentration range. At very low concentrations (1 to 10 pM) all of the assayed CK's had an inhibitory effect upon the AC activity. At a hundred fold higher concentration (1 to 10 nM) those CK's had a stimulative effect upon the AC activity. In this region (pM to nM) there seemed to be no significant distinction between the different CK types.

The observation that CK's influenced this AC activity in a regulatory way could be indicative for a link between cAMP metabolism and the known role played by CK's in chloroplast physiology and development. Those results will be discussed in relation with putative G-protein interaction and part of the data will be extrapolated in respect with 3',5'-cGMP and guanylyl cyclase activity.

E.W. is a grant student at the I.W.O.N.L., L.R. is Research Assistant and H.V.O. is Research Director at the N.F.W.O. (Belgium).

03 25

Auxin apical control of the auxin polar transport in stem cambium of *Pinus silvestris* L.

T.J. WODZICKI and A.B. WODZICKI

Department of Forest Botany, Warsaw Agricultural University, Rakowiecka 26/30, 02-528 Warsaw, Poland

Application of IAA (0.1 ppm) in 1.0% agar gel for 30, 45 or 60 min stimulates the natural auxin basipetal efflux from 6 mm high cambial region sections of the pine stem. Simultaneous application of an inhibitor of Ca-calmodulin complex formation (W7), [N-(6-aminohexyl)-5-chloro-1-naphtalene sulfamide]] does not prevent this additional auxin efflux although it reduces the total amount of growth stimulation produced by the auxin collected in basal receivers and measured by the oat coleoptile curvature bioassay for auxin. One of the possible explanations, is that the IAA signal (or increase of its concentration in apoplast) is transduced to a mechanism inducing the natural IAA release from a cellular pool of its conjugates, which seems to be not directly dependent upon the Ca-calmodulin mediated processes.

03 26

Photoperiodic induction of flowering in *Chenopodium rubrum* L. is controlled by an oscillatory mechanism

B. ZIVANOVIC, Z. VUCINIC

Center for multidisciplinary studies, University of Belgrade, 29. novembra 142, 11060 Belgrade, Yugoslavia

Photoperiodic induction of flowering takes place in the leaf in response to photoinductive light/dark cycles, and is mediated by phytochrome in all photoperiodic plants. According to the *Florigen hypothesis* a floral stimulus in the form of a hypothetical flower hormone or some other essential factor for flowering is transported from the induced leaves to the apex of the plant, initiating the subsequent developmental changes. However, nobody succeeded in isolating a flower-inducing substance. We performed long-term recordings of bioelectric potential difference across intact *Chenopodium* plants by subjecting them to non-inductive and inductive conditions for flowering for periods up to 10 days. Self-sustained oscillations of the trans-plant electrical potential difference were observed, the spikes appearing predominantly in the light-on period. As the age of the plant increases the frequency of the oscillations increased to more than 4 spikes per hour for induced plants on the tenth day of registration. In the case of induced plants the oscillations were more ordered and of higher frequency. The results obtained point to the possibility that instead of the florigen explanation of flowering, one should postulate the signal carrier hypothesis, where a frequency controlled oscillatory bioelectric mechanism is the basis for flowering control.