Instituto Juan March de Estudios e Investigaciones

87

CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Novel Approaches to Study Plant Growth Factors

Organized by

J. Schell and A. F. Tiburcio

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D. Bartels
A. B. Bleecker
N-H. Chua
S. D. Clouse
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J. E. Mullet
M. Pagès
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C. A. Ryan
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T. Schmülling
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Introduction

Jeff Schell and Antonio F. Tiburcio

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Plant hormones are the biochemical messengers translating the genetic blueprint of plants into their fully developed, final form. Traditionally, they were grouped as: auxin, cytokinins, gibberellins, ethylene and abscisic acid. However, it is becoming increasingly clear that the classic phytohormones may not be the only molecules involved in the control of plant growth and development. Thus, it is now accepted that a variety of novel signal molecules, such as polyamines, brassinosteroids, jasmonates, salicylic acid, oligossacharides and most recently peptide growth factors, can play a crucial role as signals in the development either participating in the action of, or supplementing traditional phytohormones.

The mechanism(s) by which plant hormones and growth factors trigger physiological processes has been a very difficult question to approach. The advent of modern molecular biology and molecular genetics has revolutionised our ability to unravel the complexities of plant hormone signal action in general, as well as helped to define novel forms of biochemical signalling in plants. Different approaches are now available, each technique having its own strength and limitations. The researcher, therefore, must carefully select the technique(s) to be used according to the question(s) that she or he would like to answer.

Molecular cloning has identified a number of genes that respond to several plant hormones as well as genes involved in the biosynthesis and the metabolism of plant hormones. Promoter analysis of hormone regulated genes has lead to the identification of cis acting elements that control the expression of those genes. Progress has also been made in identifying some of the specific trans-acting factors that interact with such promoters. With the availability of some of the key genes involved in the biosynthetic pathways, it has become possible to manipulate hormone levels using antisense and sense transgenic approaches. This technology eliminates or greatly reduces problems associated with uptake, transport and metabolism of exogenous applied compounds. By using tissue specific or inducible promoters it is also possible to direct hormone alterations to specific tissues or to induce hormonal changes at specific developmental stages. Transgenic plants have proved valuable not only for the study of hormone biosynthesis and action, but also from an agronomic point of view. The work with delayed ripening tomatoes is one of the most advanced examples of a practical application of hormone manipulation in transgenic plants. However, this technology has its limitations since some unexpected results have also been obtained.

The genetic approach has also led to significant advances in several areas of plant hormone biology. The availability of hormone-insensitive mutant plant-gene libraries will likely encompass and allow us to identify hormone receptors, critical components of the signal transduction pathway, and possibly elements involved in uptake and/or metabolism of the hormone to an active form. The screening of mutant plant libraries created by radiation, chemical, transposon or

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T-DNA tagging techniques has led to the identification of a number of genes implicated in hormone signal transduction as well as to the cloning of some hormone receptors, like the ethylene ETR-1 receptor. The problem usually resides with the nature of the screen used to identify novel signalling genes. In this regard, the use of the green fluorescent protein (GFP) fused to hormone-regulated genes may allow the screening of large numbers of plants simply by looking for the presence or absence of fluorescence. Novel techniques such as differential display and amplified fragment length polymorphism (AFLP) in combination with GFP promoter fusion expression in mutant libraries could provide a powerful means of identifying signal transduction genes. However, the genetic approach also has limitations: some genes may act at more than one stage in development and growth, or may function in more than one cell or tissue type, making phenotypic analysis difficult. For example, the identification of a cyclic ADP-ribose in abscisic acid signalling through microinjection experiments might not have been possible with a traditional genetic screen.

A number of plant signalling genes have been cloned by mutant complementation in yeast. The real power of yeast is the abundance of mutant strains in which specific signalling pathways have been knocked out. Consequently, genes may be cloned in these mutants by their ability to restore function to the mutant. In addition to complementation, a technique known as the two-hybrid system can also be used. This system can be used to identify new proteins that bind to a protein that has previously been identified within a signal transduction pathway. Hormone signalling pathways in plants may thus start to be delineated by using these techniques.

This workshop brought together scientist actively engaged in plant hormone signalling using molecular and genetic approaches. The workshop was very fruitful and provided a unique opportunity for the participants to exchange and share information, to review and discuss new approaches, and to propose directions for future research.

J. Schell
A. F. Tiburcio

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Session 1: Cytokinins and Auxin

Chair: Jeff Schell

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NOVEL METHODS TO STUDY METABOLISM AND FUNCTION OF CYTOKININS

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Various research strategies are used to study the correlation between hormone metabolism and functions of hormones in plant development. In this paper some case studies of the use of transgenic plants and cell cultures will be discussed.
Transgenic plants carrying genes that interfere with auxin and cytokinin metabolism or mutants which are affected in hormone metabolism show aberrant morphological treats. Using powerful LC-MS/MS (Prinsen et al. 1995; Witters et al. 1998) and immunocytochemical techniques it was possible to correlate both endogenous levels and cellular localisation of hormones with the observed effects of the expression of these genes. As an example the role of cytokinins in the flowering process of normal and *ipt*-transgenic tobacco plants will be elaborated (Estruch et al. 1991; Dewitte et al. 1998).
Based on the abundant data on the endogenous cytokinins levels some species specific cytokinin metabolisms may be recognised, e.g. *ipt*-transgenic tobacco (SR1) accumulates mostly zeatin type cytokinins whereas *ipt*-transgenic potato's produce iP -type cytokinins. This phenomenon raises questions about the specificity of the mode of action of each cytokinin type.
This was illustrated by studying the role of cytokinins in the cell cycle progression of a synchronised tobacco BY-2 cell suspension culture. Results of these studies point to a stringent structure-specific effect (zeatin only) rather than an overall function-specific effect of cytokinins on G_2-M transition in tobacco BY-2 cells. More emphasis is thereby put on the need for an accurately regulated zeatin metabolism during G_2-M transition and the existence of a putative zeatin specific receptor Laureys et al. 1998).

ESTRUCH J., PRINSEN E., VAN ONCKELEN H., SCHELL J. AND SPENA A. Viviparous leaves produced by somatic activation of an inactive cytokinin- synthesying gene. SCIENCE 254,1364-1367 (1991)

PRINSEN E., REDIG P., STRNAD M., GALIS I., VAN DONGEN W.& VAN ONCKELEN H. Quantifying Phytohormones in Transformed Plants. In: Methods in Molecular Biology, Vol 44: Agrobacterium Protocols. Ed. K.M.A. Gartland & M.R. Davey. Humana Press Inc., Totowa, NJ pp 245-261, 1995

LAUREYS F., W. DEWITTE, E. WITTERS, M. VAN MONTAGU, D. INZE, H. VAN ONCKELEN Zeatin is indispensable for the G2-M transition in tobacco BY-2 cells. FEBS Lett. 426, 29-32, 1998.

WITTERS E., VANHOUTTE K., DEWITTE W., MACHÁCKOVÁ I., BENKOVÁ E., VAN DONGEN W., ESMANS E.L. EN VAN ONCKELEN H.A. **1998**. Analysis of cyclic nucleotides and cytokinins in minute plant samples using phase-system switching capillary electrospray-LC-MSMS. *Phytochemical Analysis*, In Press.

DEWITTE W., CHIAPPETTA A., AZMI A., WITTERS E., STRNAD M., REMBUR J., NOIN M., CHRIQUI D. AND VAN ONCKELEN H.A., **1998**, Dynamics of Cytokinins in Apical Shoot Meristems of a Day Neutral Tobacco during Floral Transition and Flower Formation. *Plant Physiology*, In Press.

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Cytokinins: Light at the end of the tunnel?

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Cytokinins have proven to be rather recalcitrant in attempts to unravel their mode of action. However, in recent years research into the biology of cytokinins is beginning to benefit from the development of new methods (Schmülling et al. 1997, 1998). Important steps have been made in the identification of molecular components of cytokinin action. One G-protein-coupled seven transmembrane receptor kinase homolog, GCR1, that possibly functions in cytokinin perception and signal transduction has been identified by a combination of database searches and analyses of transgenic plants (Plakidou-Dymock et al. 1998). Activation tagging has been used to identify genes whose overexpression mimicks cytokinin activity. These genes, *CKI1* and *CKI2*, code for proteins with homology to histidine kinases of the bacterial two-component signalling system (Kakimoto et al. 1996; Kakimoto, personal communication). We have recently identified a putatively membrane located cytokinin responsive Ser/Thr kinase, CRK1, which is currently being analyzed.

Of importance to future research is the recent identification of primary cytokinin response genes. These *ARR/IBC* genes encode homologs of bacterial response regulators (Sakakibara et al. 1998; Taniguchi et al. 1998; Brandstatter and Kieber 1998). The *ARR/IBC* gene products may function as a molecular switch in the His-Asp phosphotransfer system in *Arabidopsis* downstream of CKI1 or other pathways. Transcriptional activation of the response regulator would result in a feed-forward reaction that could amplify the cytokinin response. Rapid response genes will provide the basis to establish tests for fast cytokinin action and permit the application of new research strategies.

Another tool to study cytokinin metabolism and action are transgenic plants that overproduce cytokinins endogenously in a regulated fashion. This overcomes some of the problems associated with uptake, translocation and metabolism of exogenous cytokinins. The experimental approach makes use of a two-component system consisting of the bacterial *ipt* gene, which codes for an isopentenyl transferase catalysing the rate-limiting step of cytokinin biosynthesis and, of equal importance, the promoter that directs the expression of the gene in a spatially or temporally distinct manner. The approach has confirmed many of the known

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cytokinin effects and yielded new information that would have been difficult to obtain otherwise.

The analysis of sixteen different cytokinin metabolites in tobacco and *Arabidopsis* plants by LC-MS and HPLC/ELISA techniques has yielded similar results (Redig et al. 1996; Faiss et al. 1997; Rupp et al., unpublished results). In both species no increase in iP-type cytokinins was detectable. This could indicate that the likely catalytic product of ipt activity, iPRP, is rapidly metabolised in higher plants to form the zeatin-type cytokinins. Large increases in the concentration of zeatin riboside were found in both species with more moderate increases of zeatin and the dihydrozeatin cytokinins (Redig et al. 1996; Faiss et al. 1997). The latter compounds are particularly rare in *Arabidopsis*. *Arabidopsis* forms higher concentrations of N-glucosides and ribotides than tobacco, where O-glucosides are the prevalent conjugates that are detected following cytokinin overproduction (Rupp et al., unpublished results). The relevance of glucoside formation is under study. Interestingly, an investigation of the kinetics of accumulation of different cytokinin metabolites in tobacco has shown that zeatin, dihydrozeatin and glucosides accumulate later than zeatin riboside. Organ specific differences in the formation of cytokinin metabolites were described too, however, the biological relevance of this finding is unclear at present (Faiss et al. 1997). The activity of cytokinin oxidase, which inactivates cytokinins by side chain cleavage, increased up to 10-fold in different organs after increases in cytokinin concentration (Motyka et al. 1996). This apparently substrate-induced increase in cytokinin oxidase was accompanied by the accumulation of a glycosylated form of the enzyme. These results indicate an autoregulatory mechanism in a response to elevated cytokinin levels, possibly linked to differences in the subcellular localisation of the catabolic enzyme.

Large increases in endogenous cytokinin are not necessary to influence the plant phenotype. Two- to fourfold increases of endogenous cytokinin can be correlated with major phenotypic alterations in sensitive target tissues (Hewelt et al. 1994; our unpublished results). This marks an important difference from exogenous application, where much higher concentrations of exogenously applied cytokinins are needed to obtain phenotypic effects.

Deregulated cytokinin overproduction has a number of well known pleiotropic effects on plant growth. The general cytokinin syndrome consists of root growth inhibition, stunted shoots with shortened internodes, retarded leaf senescence, an increased stem diameter with reduced xylem content, and reduced apical dominance (Faiss et al. 1997 and references therein). Therefore, *ipt* gene expression needs to be targeted precisely to specific tissues or

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developmental periods to avoid undesired side effects, which are caused by very low levels of *ipt* gene transcription. Fine-tuned expression of the *ipt* gene permitted the study of specific aspects of enhanced cytokinin levels. One important result of the work with transgenic plants with a more specific *ipt* gene expression is the finding that localised production of cytokinins causes local alterations (Hewelt et al. 1994; Gan and Amasino 1995). This finding questions the textbook opinion that developmental effects are due to root-derived cytokinins and favours the possibility that cytokinins are produced locally at their site of action. Faiss et al. (1997) investigated this question further by analysing reciprocal grafts between facultative cytokinin overproducing tobacco plants and wild type plants. The growth stimulatory effect of cytokinin on dormant lateral buds and its inhibitory influence on leaf senescence remained restricted to the transgenic cytokinin overproducing graft partner and was not transmitted through the graft union. This was observed irrespective of the direction of grafting. This argues against a role for cytokinins as a long-range root-to-shoot signalling substance in the regulation of apical dominance and sequential leaf senescence in tobacco and lends support for a role for the hormone in localised, i.e. paracrine, signalling.

Additional findings indicate that an important site of cytokinin action is the shoot meristem where cytokinins are most likely involved in the regulation of cell division and sink formation. Recent results indicate that cytokinins could have an additional function. The steady state mRNA levels of meristem specifying genes were increased in cytokinin overproducing *Arabidopsis* (Rupp et al., unpublished results). This outcome could provide a molecular link between this hormone and developmental regulator genes.

References

Brandstatter I, Kieber JJ (1998) Plant Cell 10, 1009-1019.
Faiss M, Zalubílova J, Strnad M, Schmülling T (1997) Plant J. 12, 401-415.
Gan S, Amasino RM (1995) Science 270, 1986-1988.
Hewelt A, Prinsen E, Schell J, Van Onckelen H, Schmülling T (1994) Plant J. 6, 879-891.
Kakimoto T (1996) Science 274, 982-985.
Motyka V, Faiss M, Strnad M, Kamínek M, Schmülling T (1996) Plant Physiol. 112, 1035-10430.
Plakidou-Dymock S, Dymock D, Hooley R (1998) Curr. Biol. 8, 315-324.
Redig P, Schmülling T, Van Onckelen H (1996) Plant Physiol. 112, 141-148, 1996.
Schmülling T, Schäfer S, Romanov G (1997) Physiol. Plant. 100, 505-519, 1997.
Schmülling T, Rupp HM, Frank M, Schäfer S (1998) *In* Strnad M, Pech P, Beck E (eds) Advances in regulation of plant growth and development. In press.
Sakakibara H, Suzuki M, Takei K, A Deji A, Taniguchi M, Sugiyama T. (1998) Plant J. 14, 337-344.
Taniguchi M, Kiba T, Sakakibara H, Ueguchi C, Mizuno T, Sugiyama T (1998) FEBS Lett. 429, 259-262.

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Polar auxin transport - molecular genetic dissection of an elusive pathway

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Polar auxin transport has been recognized to play an essential role in many processes such as apical dominance, vascular development and tropisms. The molecular mechanisms of this transport have remained poorly understood, despite extensive research. We have started to systematically identify proteins and genes involved in this process using biochemical and genetic strategies. Of particular interest for our studies was the Arabidopsis pin1 mutant. Phenocopies of this mutant could be generated using inhibitors of polar auxin transport.

We cloned the AtPIN1 gene using the maize transposable element En/Spm. The AtPIN1 gene is localized on chromosome 1 on a 4 kb long stretch of DNA consisting of 5 exons. The protein deduced from the DNA sequence consists of about 10 putative transmembrane spanning domains interrupted by a stretch of hydrophilic amino acid residues. The predicted topology of the AtPIN1 protein, its polar localization in xylem parenchyma cells and the drastic reduction of polar auxin transport in "knockout" plants corresponds with the proposed role as auxin efflux carrier. The AtPIN1 gen is a member of a large gene family. Several members of this family have been isolated and are currently studied using transposon mediated gene disruptions as well as biochemical and biophysical methods. One of the genes analyzed contributes to the understanding of the molecular mechanisms underlying gravity perception. Roots of the AtPIN2 transposon null-mutant were agravitropic and showed altered auxin sensitivity, a phenotype characteristic of the agravitropic wav6-52 mutant.

The AtPIN2 gene was mapped to chromosome 5 (115.3 cM) corresponding to the WAV6 locus and subsequent genetic analysis indicated that wav6-52 and Atpin2::En701 were allelic. The AtPIN2 gene consists of 9 exons defining an open reading frame of 1944 bp that encodes a 69 kDa protein with 10 putative transmembrane domains interrupted by a central hydrophilic loop. The topology of AtPIN2p was found to be similar to members of the major facilitator superfamily of transport proteins.

We have shown that the AtPIN2 gene was expressed in root tips. The AtPIN2 protein was localized in membranes of root cortical and epidermal cells in the meristematic and elongation zones revealing a polar localistaion.

The results suggest that AtPIN2 plays an important role in control of gravitropism regulating the redistribution of auxin from the stele towards the elongation zone of roots.

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Session 2: Polyamines and Ethylene

Chair: Csaba Koncz

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GENETICS AND EVOLUTION OF ARGININE DECARBOXYLASE IN PLANTS

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Putrescine and polyamines are produced by two alternative pathways in plants. One pathway starts with the enzyme arginine decarboxylase; the other with ornithine decarboxylase. We have been interested in studying arginine decarboxylase (ADC) function and regulation using both mutational and evolutionary approaches. We developed an in vivo screening strategy to identify mutants with low levels of arginine decarboxylase activity. We used the method to screen EMS-mutagenized M2 seedlings for low levels of ADC activity and identified seven mutants that fall into two complementation groups. These mutants have from 20% to 50% of wild type enzyme activity.

We also identified a mutant that overexpresses ADC. Several different morphological alterations, including changes to root growth, are apparent in the mutants. At the molecular level, we have identified two expressed ADC structural genes in Arabidopsis. Some of the mutants we have isolated identify regulatory genes that control the activity of both ADC structural genes. Phylogenetic studies suggest the gene duplication that gave rise to the two ADC genes occurred early in the evolution of the Brassicaceae; several neighboring genes were included within the region that was duplicated. ADC is encoded by a conserved, low copy number nuclear gene, with no reported introns. We have used these features of ADC genes to study the evolution of angiosperms.

Russell L. Malmberg, Mark B. Watson, Gregory L. Galloway, Wei Yu. 1998. Molecular genetics of plant polyamine synthesis. Critical Reviews in Plant Sciences 17:199-224.
Mark B. Watson, Kim K. Emory, Ruth Marie Piatak, Russell L. Malmberg. 1998. Arginine decarboxylase (polyamine synthesis) mutants of Arabidopsis thaliana exhibit altered root growth. The Plant Journal 13:231-239.
Gregory L. Galloway, Russell L. Malmberg, Robert A. Price. 1998. Phylogenetic utility of the nuclear gene arginine decarboxylase: an example from Brassicaceae. Molecular Biology and Evolution, in press.

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Genetic engineering of the polyamine metabolic pathway

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Polyamines (PAs) are biologically active compounds present in all organisms. In plants, they are considered as important modulators of biological processes such as cell division, development and stress responses (Galston et al 1997; Tiburcio et al 1997), but the mechanisms underlying their effect in such various phenomena are not clear (Walden et al 1997; Kumar et al 1997; Malmberg et al 1998). For example, increase in endogenous PAs by several environmental stresses such as potassium deficiency, low temperature, low pH, osmotic stress, herbicide treatment, UV irradiation or salt stress has been reported (Evans and Malmberg 1989; Flores 1991), but the physiological significance of such PA accumulation is at present unkown. A number of protective functions for PAs have been proposed. These include a possible substitution for divalent cations in the regulation of nucleic acid function, maintenance of cellular pH, maintenance of membrane integrity, and a possible mechanism for detoxification of ammonia (Evans and Malmberg 1989; Flores 1991). However, several experiments involving exogenous PA application have been used to argue that stress-induced putrescine (Put) accumulation is a deleterious response. For example, Coleman and Richards (1956) fed Put through cut barley leaves and found necrotic areas characteristic of potassium deficiency. Similar effects have been reported in black currant and grapevine leaves (Flores 1991).

We have used the transgenic approach to study the phenotypic effects resulting from the cellular perturbation of endogenous PA levels. We generated transgenic tobacco plants containing the oat ADC gene (Bell and Malmberg 1990) under the control of a tetracycline-inducible promoter (Masgrau et al 1997). Most of the transgenic plants displayed abnormal phenotypes including short internodes, small stems and leaves, leaf-chlorosis and necrosis, and inhibited root growth (Masgrau et al 1997). A similar root growth inhibition phenotype has been observed in EMS mutagenised Arabidopsis mutants overexpressing ADC (Watson et al 1998). Leaf chlorosis, necrosis and inhibition of plant growth are common plant symptoms of potassium deficiency-stress, and this phenotype has been correlated with increased levels of both ADC activity and Put (Young and Galston 1984).

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Therefore, it is tempting to speculate that a common mechanism involving ADC and Put enhancement could mediate the abnormal phenotypes induced either by a stress reaction (i.e. potassium deficiency) or by genetic manipulation (i.e. over-expression of ADC). The possible mechanisms of this cytotoxicity will be discussed.We have also generated transgenic tobacco plants containing the homologous ADC, ornithine decarboxylase (ODC) and spermidine synthase genes (Cordeiro et al unpublished) in both sense and antisense orientations. In the case of ADC, the observed phenotypic alterations upon induction with tetracycline are in accordance with the results obtained previously with the heterologous oat ADC gene.

References

Galston AW, Kaur-Sawhney R, Altabella T, Tiburcio A-F (1997) Plant polyamines in reproductive activity and response to abiotic stress. Bot Acta 110: 197-207

Tiburcio A-F, Altabella T, Borrell A, Masgrau C (1997) Polyamine metabolism and its regulation. Physiol Plant 100: 664-674

Walden R, Cordeiro A, Tiburcio A-F (1997) Polyamines: small molecules triggering pathways in plant growth and development. Plant Physiol 113: 1009-1013

Kumar A, Altabella T, Taylor M-A, Tiburcio A-F (1997) Recent advances in polyamine research. Trends Plant Sci 2: 124-130

Malmberg R-L, Watson M-B, Galloway GL, Yu W (1998) Molecular genetic analysis of plant polyamines. Crit Rev Plant Sci 17: 199-224.

Evans P-T, Malmberg R-L (1989) Do polyamines have roles in plant development? Annu Rev Plant Physiol Plant Mol Biol 40: 235-269

Flores H-E (1991) Changes in polyamine metabolism in response to abiotic stress. In: Slocum RD, Flores HE (eds) Biochemistry and Physiology of Polyamines in Plants . CRC Press, Boca Raton, pp 213-228

Instituto Juan March (Madrid)

Coleman T-G, Richards F-J (1956) Physiological studies in plant nutrition. XVIII. Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. Ann Bot 20: 393-409

Bell E, Malmberg R-L (1990) Analysis of a cDNA encoding arginine decarboxylase from oat reveals similarity to the E. coli arginine decarboxylase and evidence of protein processing. Mol Gen Genet 224: 431-436

Masgrau C, Altabella T, Farrás R, Flores D, Thompson A-J, Besford R-T, Tiburcio A-F (1997) Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. Plant J 11: 465-473

Watson M-B, Emory K-K, Piatak R-M, Malmberg R-L (1998) Arginine decarboxylase (polyamine synthesis) mutants of Arabidopsis thaliana with altered root growth. Plant J 13: 231-239

Young N-D, Galston A-W (1984) Physiological control of arginine decarboxylase activity in K-deficient oat shoots. Plant Physiol 76: 331-335

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Use of hormone response mutants to study the ethylene mediated signal transduction in Arabidopsis Anthony B. Bleecker, Department of Botany, University of Wisconsin-Madison, Madison Wi. 53706, USA

Recent breakthroughs in understanding ethylene signal transduction have come from pursuing a genetic approach in *Arabidopsis thaliana*. A number of mutants affecting ethylene responses in *Arabidopsis* have been identified, and these define a common initial pathway for all ethylene-induced changes in the plant. The cloning of genes represented by these mutants has revealed much about the biochemical mechanism of ethylene signal transduction. The ethylene receptors are related to a superfamily of histidine kinases common in bacteria (1), while down-stream events appear to be mediated by a RAF-related kinase, CTR1 (2). Recent genetic and biochemical studies indicate that a family of ETR1-like receptors interact with CTR to repress ethylene response pathways and that ethylene binding inhibits this activity (3). According to this model, dominant mutations in any one receptor isoform produce ethylene insensitivity by locking the mutant receptor in this negatve signaling mode. These mutations are all located in the ethylene sensor domains of the receptors and some, but not all, mutations work by disrupting ethylene binding activity (4).

The ability to sense nanomolar concentrations of ethylene may be a unique feature of the Plant Kingdom. The stability of the ethylene/receptor complex allows for the measurement of binding sites in live plant tissues using ^{14}C-ethylene. An extensive survey of organisms representing all three domains of life indicates that detectable binding activity is restricted to land plants and a subset of Eubacteria–the Cyanobacteria (blue-green algae). The ethylene binding site in higher plants is represented by the hydrophobic domain of the ETR1 receptor from Arabidopsis (4). When this domain (a.a. 1-128) was exprcssed as a GST fusion in yeast, high-affinity binding activity was detected. Similar ethylene-binding activity was detected in the Cyanobacterium, Synechocystis. The genome of Synechocystis includes an open reading frame, designated slr 1212, that contains an encoded protein domain with similar topology and 25% amino acid identity to the hydrophobic domain of ETR1. The slr1212 protein does not contain the histidine kinase domain found in ETR1, but, like ETR1, does contain a GAF domain related to cGMP binding domains. The slr 1212 gene also contains PAS domains related to the domain thought to be essential for signal output by higher plant phytochromes. Knockout of slr1212 by homologous recombination eliminated ethylene binding activity, indicating that a homologous structure is responsible for ethylene binding in higher plants and Cyanobacteria, thus supporting a monophyletic origin for this functional domain.

It has been hypothesized that the ethylene binding site could contain a transition metal cofactor that would mediate the interaction of ethylene with the protein moiety. Addition of copper enhanced by 20-fold the binding activity of the recombinant ETR1 extracted from yeast. Furthermore, stoichiometric amounts of copper co-purified with the ETR1 binding activity. A structural model for the copper-based binding site has been developed that incorporates topological analysis, sequence conservation between ETR1 and slr1212, and extensive in vitro mutagenesis. According to this model, the Cu(I) cofactor is embedded in a hydrophobic, electron-rich pocket formed by 3 membrane-spanning a-helicies. Ligands for the metal are provided by a His and a Cys residue located in the second membrane-spanning helix. This chemical environment that allows ethylene to bind

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copper with exceptional affinity may be particularly unique as it apparently has arisen only once in evolution.

The observed stability of the receptor/ethylene complex ($T_{0.5}$ for dissociation = 11 hr.) must be reconciled with the relatively rapid recovery from ethylene responses once ethylene has been removed. For example, etiolated seedling growth in Arabidopsis returns to pretreatment rates within 45 minutes of removal of ethylene from the atmosphere. Evidence will be provided that the rapid recovery of seedling growth is not an intrinsic property of the system, but rather is due to biochemical changes to the system that are induced by ethylene pretreatment. A model that can account for the rapid recovery of this system that utilizes a slowly dissociating signal receptor complex will be presented.

1. Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) Arabidopsis ethylene-response gene ETR1, similarity of product to two-component regulators. Science 262: 539-544

2. Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell 72: 427-441

3. Hua J, Meyerowitz, EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell 94: 261-271

4. Schaller GE, Bleecker AB (1995) Ethylene binding sites generated in yeast expressing the Arabidopsis ETR1 gene. Science 270: 1809-1811

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Transgenic Plants Altered in Their Response to Ethylene

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Tomato is an excellent model for evaluating the control of ethylene responses during growth and development. While control of ethylene perception is known to occur in multiple tissues such as flowers, fruits and abscission zones, the mechanisms of regulation are not well defined. We have been characterizing the ethylene receptor gene family in order to determine whether there are specific developmental functions for the individual receptors. To date, we have isolated five receptor genes, each of which exhibits a distinct pattern of expression. Only one of the genes, *NR*, is positively regulated by ethylene. That ethylene inducibility is also developmentally regulated. In order to identify unique roles for each receptor, we have produced transgenic plants that are either over- or under-expressing each receptor gene. Phenotypes of the various transgenic lines are helping to sort out roles for each receptor. In separate and parallel experiments, we have used an ethylene insensitive mutant of tomato, *Never ripe*, to evaluate the roles for ethylene in various responses to the environment. We determined that ethylene has a very significant role in symptom formation during susceptible disease responses; elimination of ethylene responses causes a reduction in symptoms following infection by several bacterial and fungal pathogens. Finally, we have used the information gained from these experiments to genetically engineer plants that are resistant to ethylene. The consequences of constitutive ethylene insensitivity on plant growth and development will be discussed.

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A D-type cyclin is an essential target of cytokinin for *Arabidopsis* cell division

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Cytokinins are purine derivatives essential for cell division in most plant tissue cultures[1], with diverse additional roles in differentiation processes such as greening, shoot production and senescence[2]. Despite the significance of cytokinins as plant growth hormones, the lack of mutants in cytokinin biosynthesis or perception has hindered molecular analysis[3], although roles in cell cycle progression have been suggested[4-8]. Here we demonstrate that the *Arabidopsis* D-type cyclin CycD3 is a major and sufficient target of cytokinin for the induction and maintenance of cell division, since constitutive CycD3 expression in leaf explants bypasses normal cytokinin requirements for high frequency callus induction. CycD3-expressing calli green normally and remain cytokinin independent, and failure to induce shoots indicates that CycD3 can also disrupt morphogenesis. Expression of CycD3 is rapidly induced by cytokinins in tissue culture and plants without *de novo* protein synthesis and is elevated in the high cytokinin mutant *amp1*. Based on cell cycle expression analysis, we propose that cytokinin control of cell proliferation in *Arabidopsis* is mediated through a CycD3 requirement at the G1/S phase transition.

1. Miller, C.O., Skoog F.S., Okumura, M.H., Von Saltza, H. & Strong, F.M. Kinetin, a cell division factor from deoxyribonucleic acid. *J. Am. Chem. Soc.* **77**, 1392 (1955).

2. *Phytohormones and Related Compunds: A complete treatise Vol. II* (eds Letham, D.S., Goodwin, P.B., Higgins, T.J.V.) Elsevier/ North Holland (1978).

3. Gray, W.M. & Estelle, M. Biochemical genetics of plant growth. *Current Op. Biotechnol.* **9**, 196-201 (1998).

4. Cooke, R. & Meyer, Y. Hormonal control of tobacco protoplast nucleic acid metabolism during in vitro culture. *Planta* **198**, 9 (1981).

5. Zhang, K., Letham, D.S. & John P.C.L. Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of $p34^{cdc2}$-like histone kinase. *Planta* **200**, 2-12 (1996).

6. Jacqmard, A., Houssa, C. & Bernier, G. in *Cytokinins. Chemistry, Activity and Function* (eds Mok, D.W.S. & Mok, M.C.) 197-215 (CRC Press, Boca Raton, 1994).

7. Nagata, T., Ishida, S., Hasezawa, S. & Takahashi, Y. Genes involved in the dedifferentiation of plant cells. *Int. J. Dev. Biol.* **38**, 321-327 (1994).

8. Bayliss, M.W. in *The Cell Division Cycle in Plants* (eds Bryant, J.A. & Francis, D.) 157-177 (Cambridge University Press, Cambridge, 1985).

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Session 3: Brassinosteroids, Gibberellins and Oligosacharides

Chair: Nam-Hai Chua

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Genetic analysis of brassinosteroid and glucose signaling in *Arabidopsis*

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Post-embryonic development of higher plants is governed by complex frame-works of signaling pathways controlling cell division and elongation. Molecular analysis of *Arabidopsis* mutants displaying hypocotyl elongation defects in both dark and light uncovered recently that steroids play an essential role as hormones in plants. Deficiencies in brassinosteroid biosynthesis or signaling allow photomorphogenic development and light-regulated gene expression in the dark, as well as result in severe dwarfism, male sterility and de-repression of stress-induced genes in the light. Remarkably, a cytochrome P450 steroid hydroxylase (CYP90), controlling a rate limiting step in brassinosteroid biosynthesis, is feed-back regulated by intermediates and end-products of brassinosteroid biosynthesis. CYP90 also functions as a signaling molecule which in the yeast two hybrid system binds to novel regulatory proteins with homology to known animal signaling factors implicated in oxysterol-dependent cell death pathways. Catabolic/glucose repression represents a second conserved signaling pathway controlling cell elongation and stress responses in plants. *Arabidopsis* homologs of the yeast SNF1 and animal AMP-activated protein kinases (AMPKs) play a chief role in the regulation of glucose repressible and inducible genes. The activity of *Arabidopsis* SNF1-like kinases, AKIN10 and AKIN11, is negatively controlled by PRL1, an ∀-importin-binding nuclear WD-protein which plays a central role as general repressor of glucose regulated genes in *Arabidopsis*. The PRL1 function is evolutionarily conserved, its absence abolishes cell polarity in fission yeast, whereas in plants results in inhibition of root elongation and de-repression of glucose and stress-regulated genes. The *prl1* mutation confers hypersensitivity to glucose, cold and several plant hormones, including cytokinin, ethylene, abscisic acid and auxin. Studies of PRL1 protein interactions in a yeast two hybrid system suggest that the SNF1-PRL1 interaction represents a central regulatory step in co-ordination of basic cellular responses to metabolic, hormonal, and environmental stress stimuli.

1: Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei GP, Nagy F, Schell J and Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* **85**, 171-182.

2: Kauschmann A, Jessop A, Koncz C, Szekeres M, Willmitzer L, and Altmann T (1996) Genetic evidence for an essential role of brassinosteroids in plant development. *Plant J.* **9**, 701-713.

3: Koncz C (1997) *BRI1* codes for a LRR receptor kinase: cross-talk between brassinosteroids and pathogenic signalling? *Trends Plant Sci.* **3**, 1-2.

4: Szekeres M and Koncz C (1997) Biochemical and genetic analysis of brassinosteroid metabolism and function in *Arabidopsis*. *Plant Phys. Biochem.* **36**, 145-155.

5: Mathur, J., Molnár, G., Fujioka, S., Takatsuto, S., Sakurai, A., Yokota, T., Adam, G., Voigt, B., Nagy, F., Maas, C., Schell, J., Koncz, C., and Szekeres, M. (1998) Transcription of the *Arabidopsis CPD* gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. *Plant J.* **14**: 593-602.

6: Salchert, K., Bhalerao, R., Koncz-Kálmán, Z. and Koncz, C. (1998) Control of cell elongation and stress responses by steroid hormones and carbon catabolic repression in plants. *Phil. Trans. R. Soc. Lond.* B **353**: (in press)

7: Németh, K., Salchert, K., Putnoky, P., Bhalerao, R., Koncz-Kálmán, Z., Stankovic-Stangeland, B., Bakó, L., Mathur, J., Ökrész, L., Stabel, S., Geigenberger, P., Stitt, M., Rédei, G.P., Schell, J. and Koncz, C. (1998) Control of glucose and hormone responses by PRL1, a nuclear WD-protein, in *Arabidopsis*. *Genes & Devel.* (in press)

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Molecular genetic analysis of brassinosteroid action

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Brassinosteroids (BRs) are growth-promoting natural products found at low levels in pollen, seeds and young vegetative tissues throughout the plant kingdom. Detailed studies of BR biosynthesis and metabolism, coupled with the recent identification and analysis of BR-insensitive and BR-deficient mutants, has implicated these plant steroids in a number of essential developmental programs including organ elongation, leaf development, photomorphogenesis, fertility, apical dominance and vascular differentiation (1). We identified a BR-insensitive mutant in *A. thaliana* (*bri*1) that confers pleiotropic phenotypic effects including severely dwarfed stature, reduced apical dominance, delayed flowering and senescence, male sterility and nearly complete insensitivity to BRs in a variety of assays (2). We have also recently identified a BR-insensitive mutant in tomato with similar phenotype and physiological properties as *bri*1. The *BRI*1 gene has been cloned by others (3) and shown to encode a putative receptor-like kinase. Studies are currently underway to investigate the properties of the BRI1 kinase domain, including possible intracellular substrates.

We are also investigating the molecular mechanisms underlying BR regulation of genes encoding xyloglucan endotransglycosylases (XETs) to further clarify the role of these wall modifying enzymes in BR-mediated cell elongation and differentiation. We have cloned a BR-regulated gene from soybean, called *BRU*1, and have shown that it encodes a xyloglucan endotransglycosylase whose transcript levels and enzyme activity are correlated with BR-promoted stem elongation (4,5). Recently, it been has determined that the *TCH*4 gene of *Arabidopsis thaliana* also encodes a xyloglucan endotransglycosylase that is regulated by BR in expanding tissue (6). To identify cis-acting sequences in the promoter of the Arabidopsis *TCH*4 gene that are responsible for mediating BR regulation of this gene, we have generated a complete set of deletions which cover the *TCH*4 promoter in 100 bp increments and have constructed a set of linker scanning mutants covering 10 bp increments. Preliminary analysis suggests that the BR response element(s) lies somewhere between -288 and -114. We are also attempting to clone and characterize genes encoding nuclear proteins that bind to the *TCH*4 promoter. One such *Arabidopsis* gene, *TCH4-BF1*, shares sequence similarity to PHD finger transcription factors that regulate the expression of homeotic genes during development in *Drosophila* and humans. Gel mobility shift assays show that TCH4-BF1 binds to a specific sequence in the –288 to -114 region of the *TCH*4 promoter. We are currently investigating whether this binding is critical for BR-regulated gene expression.

1. Clouse S, Sasse J (1998) Brassinosteroids: Essential Regulators of Plant Growth and Development. Annu Rev Plant Physiol Plant Mol Biol 49: 427-451
2. Clouse SD, Langford M, McMorris TC (1996) A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol 111: 671-678
3. Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinsteroid signal transduction. Cell 90: 929-938
4. Zurek DM, Clouse SD (1994) Molecular cloning and characterization of a brassinosteroid-regulated gene from elongating soybean(*Glycine max* L.) epicotyls. Plant Physiol 104: 161-170
5. Oh, MH, Romanow, WG, Smith, RC, Zamski, E, Sasse, J, Clouse, SD (1998) *BRU*1 encodes a xyloglucan endo-transglycosylase that is expressed in inner and outer tissues of elongating soybean epicotyls. Plant Cell Physiol 39: 124-130
6. Xu W., Purugganan M.M., Polisensky D.H., Antosiewicz D.M., Fry S.C. & Braam J (1995) Arabidopsis *TCH*4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell 7: 1555-1567

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Regulation and genetic manipulation of gibberellin biosynthesis

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The gibberellins (GAs) are a large group of diterpenoid carboxylic acids with currently 121 fully characterized structures known from higher plants and fungi. Certain GAs function as hormones, controlling development throughout the life cycle of higher plants (Hooley, 1994). Developmental processes influenced by GAs include seed germination, stem and root elongation, leaf expansion, flower intiation and development, and fruit growth. GAs also mediate the effects of environmental signals on development; notable examples are stimulation of seed germination by light, and induction of stem elongation in association with flowering in rosette plants by long-day photoperiods or exposure to low temperature (vernalization).

The biologically active GAs are biosynthesized in a complex pathway involving the action of diterpene cyclases, membrane-associated monooxygenases and soluble, 2-oxoglutarate-dependent dioxygenases (reviewed recently by Hedden and Kamiya 1997; Lange, 1998). The cyclases, which convert the common diterpene precursor geranylgeranyl diphosphate (GGPP) to the hydrocarbon, *ent*-kaurene, are localized in proplastids (Aach et al., 1997), whereas the oxidases that catalyze the subsequent reactions are located outside the plastid. Currently, cDNA or genomic clones for more than half of the enzymes catalysing the conversion of GGPP to the bioactive GAs have been obtained. The availability of these clones has led to rapid progress in our understanding of the regulation of GA-biosynthesis by endogenous and environmental factors.

The later reactions of the pathway are catalyzed by the 2-oxoglutarate-dependent dioxygenases GA 20-oxidase, 3β-hydroxylase and 2β-hydroxylase; the first two catalyze the final steps in the production of the active hormones, which are deactivated by 2β-hydroxylases. The GA dioxygenases are encoded by multigene families, with differential and tissue-specific expression of the different members. Furthermore, expression of these genes is regulated in certain tissues by the action of GA, so providing a mechanism to maintain GA homeostasis. Thus, the abundance of mRNA for GA 20-oxidase (Phillips et al., 1995) and 3β-hydroxylase (Cowling et al., 1998) in Arabidopsis is decreased and that of GA 2β-hydroxylase is increased by GA (S.G. Thomas, A.L. Phillips and P. Hedden, unpublished results). The effects of light on GA biosynthesis are due to specific changes in expression of dioxygenase genes. For example, bolting in spinach and Arabidopsis after exposure to long days is accompanied by increased expression of GA 20-oxidase genes (Wu et al., 1997; Xu et al., 1997) and the phytochrome-mediated induction of seed germination in lettuce and Arabidopsis by red light is associated with increased expression of specifc GA 3β-hydroxylase genes (Kamiya et al., 1998).

Work with recombinant enzymes obtained by heterologous expression of cDNAs in *E. coli* has revealed that several of the dioxygenases are multifunctional, catalyzing successive reactions in the pathway. For example, GA 20-oxidases convert C-20 from a methyl group to an aldehyde and then remove this atom to form the C_{19}-GA skeleton. Gibberellin 2β-hydroxylases catalyze successive oxidations at C-2, leading to the formation of 2β-hydroxy GAs and the GA catabolites, which have a ketone function at C-2.

Modification of the expression of GA biosynthesis genes in transgenic plants is providing a means of manipulating GA content and, hence, plant development. Such experiments are yielding information on the contribution of specific enzymes to the rate of GA production. They also provide a new technology with considerable potential for crop improvement. At IACR-Long Ashton Research Station, we have overexpressed GA 20-

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oxidase genes in Arabidopsis, resulting in enhanced seedling growth, accelerated bolting and a taller stem at maturity (Hedden et al., 1998). This result indicates that GA is limiting for these developmental processes in Arabidopsis and, moreover, that the activity of GA 20-oxidase plays a major part in determining the content of bioactive GAs. We are evaluating three strategies for reducing GA content by genetic manipulation, using several model species. In one approach, we have reduced expression of GA 20-oxidase genes by introducing antisense copies of these genes. We have also overexpressed a pumpkin GA 20-oxidase gene, the product of which forms biologically inactive by-products of GA biosynthesis and should divert precursors from the main pathway. Finally, we have overexpressed a GA 2β-hydroxylase gene and, thereby, increased turnover of the bioactive compounds. This last approach, in particular, offers considerable potential as a non-chemical method for controlling plant stature.

References

Aach H, Bode H, Robinson D.G., Graebe J.E. (1997) *ent*-Kaurene synthase is located in proplastids of meristematic shoot tissues. *Planta* **202,** 211-219.

Cowling, RJ, Kamiya Y, Seto H, Harberd NP (1998) Gibberellin dose-response regulation of *GA4* gene transcript levels in arabidopsis. Plant Physiol **117,** 1195-1203.

Hedden, P. and Kamiya, Y. (1997) Gibberellin biosynthesis: enzymes, genes and their regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48,** 431-460.

Hedden P, Coles J.P., Phillips A.L., Thomas S.G., Ward D.A., Curtis I.S., Power J.B., Lowe K.C., Davey M.R. (1998) Modification of plant morphology by genetic manipulation of gibberellin biosynthesis. In: Cockshull K.E., Gray D., Seymour G.B., Thomas B., eds. *Genetic and Environmental manipulation of horticultural crops,* pp. Wellingford: CAB International, in press.

Hooley R. (1994) Gibberellins: Perception, transduction and responses. *Plant Mol. Biol.* **26,** 1529-1555.

Kamiya,Y., Ait-Ali S., Frances S., Toyomasu T., Yamaguchi S., Kendrick R. (1998) Light regulation of gibberellin biosynthesis. *Abstracts of the 16th International Conference on Plant Growth Regulators* p. 48.

Lange T. (1998) Molecular biology of gibberellin biosynthesis. *Planta* **204,** 409-419.

Phillips A.L., Ward D.A., Uknes S., Appleford N.E.J., Lange T., Huttly A.K., Gaskin P., Graebe J.E., Hedden P. (1995) Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. *Plant Physiol.* **108,** 1049-1057.

Wu K., Gage D.A. Zeevaart J.A.D. (1996) Molecular cloning and photoperiod-regulated expression of gibberellin 20-oxidase from the long-day plant spinach. *Plant Physiol.* **110,** 547-554.

Xu Y.-L., Gage D.A., Zeevaart J.A.D. (1997) Gibberellins and stem growth in *Arabidopsis thaliana*. Effects of photoperiod on expression of the *GA4* and *GA5* loci. *Plant Physiol.* **114,** 1471-1476.

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OLIGOSACCHARIDE SIGNALS IN PLANTS. Peter Albersheim, Jocelyn K. C. Rose, Kyung-Sik Ham, Sheng-Cheng Wu, Carmen Catala, Ronald Clay, Brian Cook, Carl W. Bergmann, and Alan Darvill, Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, University of Georgia, 220 Riverbend Road, Athens, GA 30602-4712, U.S.A.

The primary walls of all plant cells that have been analyzed contain six well-characterized polysaccharides: cellulose; the two hemicelluloses, xyloglucan (XG) and arabinoxylan, which bind to and interconnect cellulose microfibrils; and the three interconnected pectic polysaccharides, homogalacturonan (HG), rhamnogalacturonan I, and rhamnogalacturonan II. The cell walls of some plant tissues contain a seventh polysaccharide; these are exemplified by the β-1,3/1,4-mixed-linked glucan of the coleoptile walls of Gramineae, the apiosylgalacturonan of aquatic plants, and the xylogalacturonans of reproductive tissues. At least two of the six ubiquitous polysaccharides, XG and HG, are the source of biologically active oligosaccharides (oligosaccharins) that regulate growth and development in some if not all plants (Darvill *et al.*, 1992, *Glycobiology* **2(3):** 181-198). The oligogalacturonide oligosaccharin isolated from HG not only inhibits the formation of roots and stimulates the formation of flowers in certain explants, it can also activate defense responses in some plants. Oligosaccharins have also been isolated from chitin and β-1,3/1,6-linked glucans, both fungal cell wall polysaccharides; these oligosaccharins also participate in activating plant defenses. All of the systems referred to in this talk focus on bioactive oligosaccharides generated by the action of *endo*glycanases on cell wall polysaccharides .

A nine-residue fragment of XG is one of the first oligosaccharides shown to be a signal molecule. This oligosaccharin is able to regulate the rate of auxin-induced growth of pea stems. A few years ago, a paper was published in *Science* identifying a mutant of *Arabidopsis* that had less than 2% of the fucose of the parent plant in the above-ground portion of the plant (Reiter *et al.*, 1993, *Science* **261:** 1032-1035). The mutant is slightly dwarfed and the flower stalk more brittle than the parent. But the fact that the mutant survived was taken as evidence against the oligosaccharin theory, since L-fucose was reported to be an essential component of the XG nonasaccharide that regulates growth (York *et al.*, 1984, *Plant Physiol.* **75:** 295-297; Augur *et al.*, 1993, *Plant J.* **3:** 415-426). The explanation of this apparent contradiction, which is described in this talk, provided additional support for the oligosaccharin theory (Zablackis *et al.*, 1996, *Science* **272:** 1808-1810).

The mechanism(s) by which oligosaccharins function have been hard to unravel, as have the mechanisms by which the products of plant resistance genes and fungal avirulence genes function. Harold Flor's discovery (Flor, 1971, *Annu. Rev. Phytopathol.* **9:** 275-296) of the gene-for-gene relationship between the avirulence genes of pathogens and the corresponding resistance genes of plants is, once again, a center of attention in plant science, with numerous pairs of these genes having been cloned. Many of us anticipated that the resistance gene products would be revealed as receptor proteins located in the plasma membrane and the avirulence gene products as proteins interacting directly with the receptors or as enzymes synthesizing ligands (elicitors?) to interact with the corresponding receptor. A dozen or so gene-for-gene pairs have now been cloned, and rather than the expected clarification, the biochemical mechanisms controlling these activities remain obscured.

Although the mechanism of action of oligosaccharins remains to be elucidated, quite a bit is known about the formation of oligosaccharins, especially those involved in host-pathogen interactions. These biochemically defined systems in which the products of

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gene pairs are highly suggestive of pathogenesis and resistance factors appear to meet the biochemical requirements of gene-for-gene interactions. But these molecules have not yet been established as the products of resistance and avirulence genes. One of these systems involves pathogen-secreted polygalacturonases, the polygalacturonase-inhibitor proteins of their hosts, and elicitor-active oligogalacturonide products resulting from the interactions of these proteins with HG (Toubart *et al.*, 1992, *Plant J.* **2:** 367-373; Desiderio *et al.*, 1997, *Mol. Plant-Microbe Interac.* **10:** 852-860). A somewhat inversely related but equally informative system involves the "pathogenesis-related" *endo*-β-1,3-glucanases of the host, glucanase-inhibitor proteins of the pathogen, and elicitor-active oligoglucoside products resulting from the interactions of these proteins with a wall polysaccharide of the pathogen (Ham *et al.*, 1997, *Plant J.* **11:** 169-179). A third system with great promise for providing important information vis-à-vis oligosaccharide signals involves the *endo*xylanases secreted by a fungal pathogen and the arabinoxylan of the host (Wu *et al.*, 1997, *Mol. Plant-Microbe Interac.* **10:** 700-708). This lecture describes these systems and speculates on the direction we think the study of oligosaccharins is headed.

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Auxin Response in *Arabidopsis* is Mediated by Conjugation of the Ubiquitin-like Protein RUB1.

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The phytohormone Auxin is implicated in almost every process of the plant growth and development. Genetic and physiological studies showed that the AXR1 protein is required for normal response to auxin in *Arabidopsis*. This protein showed homology with the N-terminal half of the Ubiquitin Activating Enzyme (E1), which catalyzes the first step in the Ubiquitin conjugation. We found that AXR1 functions together with the ECR1 protein (E1 C-terminal related) to form an heterodimer E1-like enzyme. This heterodimer is able to activate the RUB/NEDD8 family of Ubiquitin-related proteins. We identified the active Cysteine in the ECR1 protein, which is necessary to form a thiolester bond between ECR1 and the C-terminal of RUB1. We found that the *axr1-3* mutation, which replaced the Cysteine 154 with Alanine in AXR1 and causes reduced response to auxin, also prevents the thiolester formation between ECR1 and RUB1. In vitro assays using wt and *axr1-12* protein extracts show that seedlings lacking a functional AXR1 protein do not promote formation of the RUB1-ECR1 thiolester. However, this biochemical phenotype is restored in the axr1-12 background by adding recombinant AXR1 to the reaction, suggesting that, at least in seedlings, AXR1 is responsible for the majority of RUB1 activation. Using immunolocalization, AXR1 is localized primarily to the nucleus of dividing and elongating cells, suggesting that the targets of RUB1-modification are nuclear. The principal target of RUB1 in yeast is the cell cycle protein Cdc53p, which form part of the protein ligase complex of the Ubiquitin conjugation pathway. We have found that, *in vitro*, CDC53 from *Arabidopsis* is modified by RUB1, suggesting that postranslational modification of CDC53 could be necessary for normal auxin response. TIR1 gene was identified as a mutation that impaired the normal response to auxin. TIR1 is an F-box protein that also may form part of the protein ligase complex as well as Cdc53p. These results suggest that the auxin response is mediated through modification of a nuclear protein(s) by RUB1 and likely targeting repressors of the auxin response with Ubiquitin for their degradation. Finally, previous results show that seedling that overexpress AXR1 and/or ECR1 under control of an inducible promotor are more susceptible to auxin than wt and have several aspects of development altered. More data of these lines would be present.

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Session 4: Abscisic Acid

Chair: Clarence A. Ryan

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Molecular and Genetic Dissections of ABA Signal Transduction Pathways

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Abscisic acid (ABA) is the primary hormone that mediates plant responses to stresses such as cold, drought, and salinity. We have used two approaches in an attempt to elucidate the signal transduction pathway for activation of ABA-responsive genes.

(1) Microinjection approach:
We have developed a single-cell microinjection approach that allows dissection of signaling intermediates between ABA and its target genes. This approach led to the identification of cyclic ADP-ribose (cADPR) as a signaling molecule in the ABA response and cADPR was shown to exert its effects by way of calcium. We also demonstrated that an okadaic acid-sensitive phosphatase and a K252-sensitive kinase act upstream and downstream of the calcium pool, respectively (Wu et al., 1997). In collaboration with Dr. E. Grill's laboratory, we have shown that abi1 and abi2 inhibited ABA-induction of kin2 and rd29A. The inhibitory efffects of the mutant proteins can be reversed by higher concentrations of their respective wild type proteins. The site of action of abi1 and 2 was localized to downstream of the calcium pool.

(2) Genetic approach:
We have used a genetic approach to screen for Arabidopsis mutants that exhibit deregulated ABA-responsive gene expression. To monitor this ABA response, a line of Arabidopsis thaliana carrying a transgene composed of the ABA-responsive Arabidopsis kin2 promote fused to the coding sequence for the firefly luciferase gene, kin2::luc, was generated. Patterns of ABA-responsive luciferase activity were monitored by photon counting using a CCD camera. In contrast to wild-type plants that display a transient activation of kin2::luc, an ABA deregulated gene expression mutant (ade1) exhibits both sustained and enhanced levels of transgene activity. Genetic analyses indicate that the ade1 mutant is a monogenic recessive trait. Levels of kin2, cor47, and rab18 expression in ade1 plants are also enhanced and prolonged indicating that the molecular mechanism(s) altered in ade1 plants also affect other ABA responsive genes. Our results suggest that ADE1 encodes a negative regulator in ABA signaling.

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REGULATION OF THE ABA-INDUCIBLE EM GENE: THE ROLE OF VIVIPAROUS-1 AND 14-3-3 PROTEINS

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The Em gene is a member of a gene set which is expressed only in response to ABA during embryo maturation. This specific pattern of expression requires ABA and the product of the viviparous-1 (vp1) locus in maize and its homologue in Arabidopsis ABA insensitive-3 (abi 3) (1,2). Overexpression of the ABI3 protein in vegetative tissue of Arabidopsis treated with ABA results in the ectopic expression of Em, indicating that tissue-specific expression is regulated by both ABA and ABI3 (2). VP1 and ABI3 proteins are over 85% identical in three regions representing over 35% of the protein (2). Vp1 has a transcriptional activation domain in its N-terminal region, suggesting a role in transcription (1).

Using a protoplast transient assay (3), we have identified a 76 bp ABA-response element (ABRE) within a 650 bp promoter of the Em gene from wheat. Overexpression of VP1 in cereal protoplasts transactivates the same Em promoter without exogenous ABA (1), possibly by making the protoplasts more sensitive to endogenous ABA. In the presence of excess ABA and VP1, a synergistic response is observed (1). A tetramer composed of a 22 bp sequence within the ABRE, which includes a G-box (5'-CACGTG-3'), can support ABA-induced expression, Vp1 transactivation and the synergy between ABA and VP1(4). Deletion of 18 amino acids in a highly conserved region of VP1 (BR2) eliminates the ability of VP1 to transactivate the Em promoter (5).

A DNA binding protein (EmBP-1) of the basic-leucine zipper class (bZIP) can bind to the G-boxes in the ABRE(6). A 2 bp mutation in the G-box(es) reduces or eliminates the ability of the ABRE to bind EmBP-1(6), or to exhibit the responses to ABA and VP1 in the transient assay (4). EmBp-1 can simulate transcription from an Em template in HeLa cell-free transcriptional assay (7). A VP1 fusion protein enhances the DNA-binding activity of EmBP-1 to the G-box (5). Deletion of the BR2 domain in VP1 eliminates enhancement in vitro and transactivation in transient assays, while a 40 amino acid fragment containing BR2 can confer the ability to enhance EmBP-1 binding to lacZ (5). Although a weak interaction of BR2 with DNA can be demonstrated in vitro, no specificity of enhancement was observed; VP1 enhances the specific DNA-binding activity of several diverse transcription factors (5). However, VP1 can specifically stimulate transcription in HeLa nuclear extracts using a template from the Em promoter but not from the DHFR promoter (7).

Previous work demonstrated that a 30 kDa protein (GF-14) is part of the transcription complex that binds to a G-box in the ADH gene from Arabidopsis. GF-14 has homology with a class of proteins known as the 14-3-3 proteins which participate in many signaling pathways. Using Em1a as a probe, we recently showed (8) that three different antibodies to different regions of the GF14 protein can interact with nuclear protein-DNA complexes formed with nuclear extracts prepared from untreated, ABA- and NaCl-treated embryogenic rice suspension cultures. No interactions were observed when an AT-rich region of the Em promoter was used as a probe. GF-14 antibodies recognize a doublet protein (28-30 kDa) in both cytoplasmic and nuclear extracts. A bacterially-expressed maltose-binding GF14 fusion protein (MBP-GF14) from rice, when added to nuclear extracts, can form a G -box binding complex when assayed by gel retardation (8). MBP-GF14 cannot bind to the G-box element alone. VP1 does not appear to be required to form G-box complexes containing GF14, since nuclear extracts prepared from

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maize wild and vp1 mutant embryos show similar gel retardation patterns with and without GF14 antibodies(8). However, we showed that VP1 and GF-14 can interact using a yeast two-hybrid assay and by immunoprecipitation. These GF-14 proteins can also physically interact with EmBP1 (8).

The possible role(s) of these 14-3-3 and VP1 in the regulation of Em expression will be discussed (9).

REFERENCES

1). McCarty, D.R., et al. (1991). Cell 66:895-905.
2). Giraudat, J., et al. (1992). Plant Cell 4: 1251-1261.; Parcy, F., et al. (1994). Plant Cell 6:1567-1582.
3). Marcotte, W.R., Russell, S.H. and Quatrano, R.S. (1989). Plant Cell 1:969-976.
4). Vasil, V., Marcotte, W. R., Jr., et al. (1995). Plant Cell 7:1511-1518.
5). Hill, A.H. et al. (1996). J. Biol. Chem. 271:3366-3374.
6). Guiltinan, M.J., Marcotte, W.R. and Quatrano, R.S. (1990). Science 250:267-271.
7). Razik, M.A. and Quatrano, R.S. (1997). Plant Cell 9:1791-1803
8). Schultz, T.F., Medina, J., Hill, A.H. and Quatrano, R.S. (1998). Plant Cell 10: 837-848.
9). Quatrano, R.S., Bartels, D, Ho, T-H, and Pages, M. (1997). Plant Cell 9: 470-475.

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REGULATION OF ABSCISIC ACID INDUCED TRANSCRIPTION

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The ABA responsive genes are induced by ABA and water stress in embryo and vegetative tissues of maize (1). Recently, important advances have been made in understanding the pathways that induce ABA and how ABA is transduced into physiological and molecular responses. The study of gene expression in response to ABA and stress has lead to the detection of several intermediates of the ABA signal cascade and the dissection of ABA responsive promoters has given new insight into the integration of ABA into stress response and seed development.

By in vivo footprinting and transient transformation of maize embryonic and vegetative tissues with the maize rab promoters we defined the cis-elements involved in ABA- and stress-response in maize (2). The functional relevance of the cis-elements identified was assessed by gel retardation assays and transient and permanent transformation in maize and Arabidopsis of constructs containing intact or mutated versions of the promoters (3).We are using the "one-hybrid" strategy to screen for maize cDNAs encoding binding factors for the cis elements involved in ABA and stress gene regulation. Current status of these approaches will be presented.

The ABA response genes encode proteins that may serve different functions. Several proteins may be components of the ABA signal transduction cascade, other group can function as specific regulators (4) or in protecting the cells from water deficit. Moreover, phosphorylation and dephosphorylation are also involved in the ABA signal transduction pathway. Therefore, kinases and phosphatases and they respective phospho /dephospho-rylatable substrates (5) may have a role in the response of plants to desiccation.

For the regulatory proteins fusion of reporter genes with different protein domains are used to study targeting in transgenic systems. They are also used in the yeast two hybrid system to identify possible interacting molecules. From these approaches several genes have been isolated. The potential role during ABA mediated gene expression of regulatory proteins will be discussed.

References

1-Gomez et al. (1988) Nature 334 262-264

2-Busk P.K.et al. (1997). Plant Journal. 11,6 1285-1295

3- Busk, P.K. et al. (1997) Plant Cell 9 2261-2270

4-Jensen A. et al.(1998). Plant Journal 13(5) 691-697.

5-Alba, et al. (1998) Trends in Plant Sci.3(1),15-21.

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CONTROL OF ABA-REGULATED GENE EXPRESSION IN THE RESURRECTION PLANT C. PLANTAGINEUM

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The resurrection plant *Craterostigma plantagineum* (Fam. Scrophulariaceae) is being used as an experimental model system to understand pathways leading to desiccation tolerance. Analysis of gene expression in this plant has shown that desiccation tolerance involves many different pathways. Therefore we try to isolate regulatory genes which control complete pathways.

In order to analyse general regulatory mechanisms three promoters from different Lea type genes and four promoters from genes encoding inducible enzymes of the carbohydrate metabolism (sucrose synthase I, II, sucrose-phosphate-synthase and GAPDH) have been analyzed for their responsiveness to drought and ABA in transient assays and transgenic plants. Dot matrix sequence comparisons, promoter deletions and electrophoretic mobility shift assays did not reveal general motifs triggering drought or ABA-induced gene expression, but the analysis showed different factors for each gene.

When Lea gene promoters were analysed in heterologous systems, they differed in their response to ABA. It was shown for two genes that the presence of the Arabidopsis ABI-3 gene was necessary to regain ABA inducibility. This suggests the presence of a repressor or a factor missing in the heterologous system.

Two putative transcription factors have been isolated: one (HDZIP-1) is structurally closely related to the family of the homeodomain leucine zipper gene family and the other one (HSF-1) to the family of the heat shock transcription factors. Characteristic for both genes is that they are members of gene families, of which only one gene is responsive to drought and the corresponding transcripts are expressed before putative target genes. The yeast two-hybrid system revealed three types of interaction: HDZIP forms homodimers, heterodimers and interacts with a non-HDZIP protein. Both HDZIP transcripts are inducible by dehydration in leaves and roots, but steady state transcript levels vary in response to exogenously applied ABA. The HDZIP1 transcript is not inducible by ABA but the HDZIP2 transcript level increases during ABA treatment. This differential expression suggests that they act in different branches of the dehydration-induced signalling network.

References:

Frank W, Phillips J, Salamini F, Bartels D (1998) Two dehydration-inducible transcripts from the resurrection plant *Craterostigma plantagineum* encode interacting homeodomain-leucine zipper proteins. The Plant Journal 15, 413-421

Furini A, Parcy F, Salamini F, Bartels D (1996) Differential regulation of two ABA-inducible genes from *Craterostigma plantagineum* in transgenic *Arabidopsis* plants. Plant Molecular Biology 30, 343-349

Velasco R, Salamini F, Bartels D (1998) Gene structure and expression analysis of the drought- and abscisic acid-reponsive CDeT11-24 gene family from the resurrection plant *Craterostigma plantagineum* Hochst. Planta 204, 459-471

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frs1, A RECESIVE MUTATION AT THE *ABA3* LOCUS, AFFECTS FREEZING AND DROUGHT TOLERANCE IN ARABIDOPSIS

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The abscisic acid (ABA) is a phytohormone that plays an important role in many physiological processes, including the response of plants to environmental stresses (1, 2). During the last years, the involvement of ABA in the process of cold acclimation, the proces whereby many plants increase their freezing tolerance in response to low nonfreezing temperatures, has been a matter of some controversy. While gene expression analyses showed that ABA can induce the expression of some low temperature regulated genes (3, 4), experiments with arabidopsis mutants deficient or insensitive to ABA originated contradictory data on the implication of ABA in the cold acclimation process (5, 6).

We have employed a genetic strategy to identify genes of arabidopsis involved in cold acclimation and freezing tolerance. This strategy consisted in using EMS mutagenized populations of arabidopsis to select mutants showing an altered response to cold acclimation. We isolated a *freezing sensitive mutant* (*frs1*) that showed a reduced freezing tolerance in acclimated plants. Genetical characterization revealed that *frs1* is produced by a single recesive mutation. The *frs1* mutation also causes a decrease in the freezing tolerance of nonacclimated plants, as well as in the drought tolerance. Phenotypically, *frs1* mutant plants are smaller and darker than the L*er* wild type and show a wilty phenotype. Seeds from the *frs1* mutant can not germinate in a medium containing ABA, indicating that *frs1* is not an ABA insensitive mutant. Furthermore, exogenous treatments with ABA rescue almost completely the wild phenotype of the mutant, and its capability to tolerate freezing temperatures and dehydration. Endogenous ABA measurements revealed that *frs1* is an ABA deficient mutant, and mapping experiments disclosed that the *frs1* mutation maps on chromosome 1 very close to the *aba3* mutation. Complementation tests indicated that *frs1* is, in fact, a new allele of *aba3* and, therefore, should be affected in the conversion of ABA aldehyde to ABA, which is the last step in the ABA byosynthetic pathway. The fact that *frs1* is an ABA deficient mutant is a confirmation that ABA mediates the process of cold acclimation, and that ABA controlled processes are required for development of cold acclimation and acquisition of full freezing and drought tolerance in plants. Results obtained from gene expression analyses in *frs1* suggest that ABA mediates cold acclimation, and therefore freezing and drought tolerance, by regulating gene expression.

References

1. Bray, E. A. (1993). Plant Physiol. 103: 1035-1040.

2. Jensen, A. B. et al. (1996). Plant Growth Regulation 20: 105-110.

3. Thomashow, M. F. (1994). In: Arabidopsis. Cold Spring Harbor Laboratory Press. Cold Spring Harbor. pp. 807-834.

4. Hughes, M. A. and Dunn, M. A. (1996). J. Exp. Bot. 47: 291-305.

5. Gilmour, S. J. and Thomashow, M. F. (1991). Plant Mol. Biol. 17: 1233-1240.

6. Mantyla, E. Et al. (1995). Plant Physiol. 107:141-148.

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Session 5: Peptide Growth Factors, Jasmonates and Salicylic Acid

Chair: Ralph S. Quatrano

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Polypeptide Signaling for Plant Defensive Genes

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Systemin, an 18 amino acid polypeptide, regulates the systemin acivation of defensive genes in plants in response to attacking herbivores and pathogens. Systemin is released in response to wounding by attacking herbivores and is transported throughout the plant where it activates a signaling pathway that regulates the transcription of nearly twenty systemic wound response genes. Systemin is similar to most animal and yeast polypeptide hormones in that it is processed from a larger prohormone protein by proteolytic cleavages. The polypeptide activates a signal lipid-based transduction pathway in which the 18:3 fatty acid linolenic acid, released from plant membranes, is converted to the oxylipin signaling molecules phytodienoic acid and jasmonic acid, analogous to the conversion of the 20:4 fatty acid arachidonic acid to prostaglandins in cells of higher animals. Our recent studies of the intracellular events leading to linolenic acid release has found that levels of a MAP Kinase activity and phospholipase activity, and a calmodulin mRNA and protein, rapidly increase in resoponse to wounding and systemin in both wild-type tomato plants, as well as in a mutant line defecient in the octadecanoid pathway. These components appear to have roles early in the defense signaling pathway leading to the the intracellullar release of linolenic acid from membranes in response to wounding and systemin. (Supported in part by Washington State University College of Agriculture and Home Economics, and grants from the National Science Foundation , The Department of Energy, and the National Institutes of Health).

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PEPTIDE SIGNALLING IN ROOT NODULE FORMATION

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Legume root nodule formation is the result of a compatible interaction between the plant and soil-borne bacteria belonging to the genera Rhizobium, Bradyrhizobium and Azorhizobium (collectively called rhizobia). In these nodules the bacteria are able to reduce atmospheric nitrogen into ammonia.
This process involves the following steps; Upon inoculation root hairs deform and the bacteria invade the plant using a tubular invagination called the infection thread, which develops within the root hair and the cortical cells. Simultaneously, cortical cells are mitotically activated, giving rise to the nodule primordium. The position of the primordium is either in the outer (e.g. soybean) or in the inner cortex (e.g. pea, clover). In the latter case, in the outer cortex, cells also enter the cell cycle but become arrested in G2, leading to a radial alignment of cytoplasmic bridges, named preinfection threads. Via these bridges the infection threads grow towards the primordium and upon arrival the transported bacteria are released into the cytoplasm of the host cells. Subsequently, the nodule primordium develops into a nodule.
To redirect the fate of root cells, the Rhizobium bacteria secrete lipo chitin-oligosaccharides (LCOs) based signal molecules. Purified LCOs are able to induce several morphological changes similar to those observed after inoculation with bacteria.
Each stage of bacterial penetration and nodule development is accompanied by the expression of specific host genes, the so-called nodulin genes. One of the first plant genes that is activated by rhizobia is ENOD40. It is induced long before the onset of cell divisions and it encodes a 12-13 amino acid peptide. Homologs of ENOD40 have been isolated from nonlegumes, implying that the peptide may have a function in plant developmental steps other then nodule development.
The following aspects of ENOD40 will be discussed; 1.How this peptide might be involved in root nodule development as well as its putative role in nonlegumes. 2. approaches to dissect the molecular mechanism underlying ENOD40 action.

Geurts, R and Franssen H. (1996) Plant Physiol. 112, 447-453.
Van de Sande et al (1996) Science 273, 370-373.
Charon et al (1997) PNAS 94, 8901-8906.

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Jasmonate - Linking Defense, Development and Metabolism

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Jasmonates (JAs) mediate plant responses to wounding and activate plant defenses. Similar inflammatory responses are mediated by the structurally related compounds prostaglandins and eicosanoids in animals. Jasmonates and eicosanoids are derived from fatty acids via the action of lipoxygenase. In plants, jasmonate levels increase rapidly in response to wounding. The increases in JA in turn induces the expression of numerous genes known to be involved in plant defense, especially insect defense.

Hence, it is not surprising that JA deficient A. thaliana plants are very susceptible to fungal gnats and treatment of these plants with JA confers resistance. Recent analysis of JA insensitive mutants in combination with elucidation of downstream cis and trans-factors is gradually filling in the JA signal transduction pathway. Interestingly, JA response elements in the soybean vegetative storage protein phosphatase act in conjunction with a positively acting carbon response element and a negative phosphate responsive cis-element. The complexity of regulation revealed by analysis of this promoter may provide insight into the strategic use of JA in plants for defense and the counter balance between assimilation and growth vs, allocation of nutrients to defense.

High levels of JA in young leaves, stems, and apices, as well as young buds and flowers, may reflect the plant's developmentally regulated production of JA to provide preemptive defense of these important tissues. The mechanism for regulating JA levels during development will be examined.

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SALICYLIC ACID-MEDIATED SIGNAL TRANSUCTION IN DISEASE RESISTANCE RESPONSES

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Exogenous salicylic acid (SA) is known to affect many processes in plants. Particularly noteworthy are the studies of Cleland and Raskin which suggested roles for endogenous SA in flowering and thermogenesis, respectively. However, the major focus of SA research during the past decade has been on its role in disease resistance. During the past several years, we have used a variety of biochemical, molecular, and genetic approaches to identify components of the host signal transduction pathway(s) activated during plant defense responses to pathogen attack, particularly those involved in SA-mediated signaling.

To help elucidate the mechanisms of SA action, several tobacco proteins which interact with SA have been identified including catalase and ascorbate peroxidase (1,2). SA inhibits these two major H_2O_2-scavenging enzymes by serving as an one-electron donating substrate (3). In so doing, it is converted to a phenolic radical, which may be responsible for SA-induced lipid peroxidation (4). Lipid peroxides induce defense genes such as the well characterized pathogenesis-related (*PR*)-1 genes. Thus, the signals that activate defense gene expression may be generated by SA's interaction with these enzymes. SABP2 is another SA binding protein (5). The affinity for SA of this low MW, soluble protein ($K_d \cong 90nM$) is 150 fold higher than that of catalase. SABP2 has even higher affinity for the commercial plant defense activator benzothiadiazole, which is more effective at inducing *PR* gene expression than SA. Benzothiadiazole is also a very effective inhibitor of catalase and ascorbate peroxidase (6).

Protein phosphorylation and/or dephosphorylation have been implicated in plant defense responses. We identified a 48-kD salicylic acid-induced protein kinase (SIPK) in tobacco suspension cells. This kinase was purified to homogeneity and its encoding gene cloned based on peptide sequences (7). *SIPK* is a member of the tobacco MAP kinase family and is distinct from *WIPK* (wounding-induced protein kinase), another tobacco MAP kinase family member whose mRNA is induced by wounding and was thought to encode the wounding-activated protein kinase (8). During the past year we, with the aid of several collaborators, have analyzed the effects of pathogen-derived elicitors, pathogen infection, and wounding on the activity of SIPK and also WIPK (9-11). The results are summarized in the table below.

Stimuli	SIPK			WIPK		
	mRNA⇧	protein⇧	activity⇧	mRNA⇧	protein⇧	activity⇧
SA	-	-	+ +	-	-	-
CWD elicitor	-	-	+ +	+ +	+ +	- /+
elicitins	-	-	+ +	+ +	+ +	+ +
harpin	-	-	+ +	NT	NT	NT
TMV	-	-	+ +	+ +	+ +	+ +
Avr9	NT	-	+ +	+ +	-	+ +/+
wounding	-	-	+ +	+	- /+	- /+

SIPK was activated by SA, a cell wall-derived (CWD) carbohydrate elicitor and two elicitins from *Phytophthora* spp, bacterial harpin, TMV, and Avr9 from *Cladosporium fulvum*. In addition to these pathogen-associated stimuli, wounding also activated SIPK, suggesting that this enzyme is

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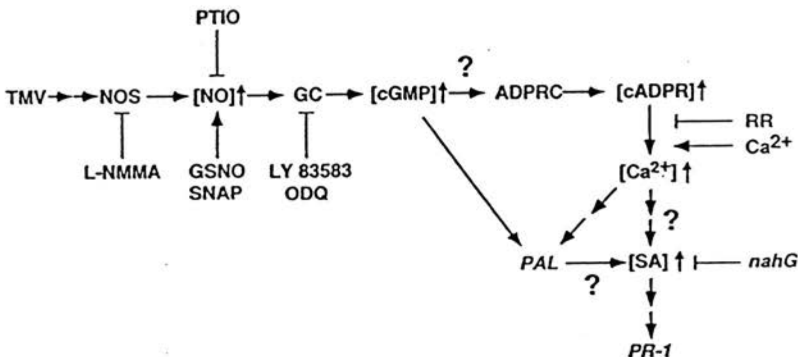
involved in multiple signal transduction pathways. In all cases tested, SIPK activation was exclusively posttranslational via tyrosine and threonine/serine phosphorylation. WIPK was activated by only a subset of these stimuli including infection by TMV and treatment with the CWD elicitor, elicitins or Avr9. In contrast to SIPK, WIPK was activated at multiple levels. Low level activation (e.g. by the CWD elicitor) appeared to be primarily posttranslational whereas dramatic increases in kinase activity (e.g. by TMV or elicitins) required not only posttranslational phosphorylation, but also preceding rises in mRNA levels and *de novo* synthesis of WIPK protein. Activation of SIPK and WIPK at the enzyme level by TMV and the Avr9 peptide of *C. fulvum* follows the gene-for-gene paradigm. That is, activation by these two stimuli require the presence in the host of the corresponding disease resistance genes *N* and *Cf-9*, respectively.

Reactive oxygen species are believed to perform multiple roles during plant defense responses to microbial attack, acting in the initial defense and possibly as cellular signaling molecules. In animals, nitric oxide (NO) is an important redox-active signaling molecule. We found that infection of resistant, but not susceptible, tobacco with TMV resulted in enhanced NO synthase (NOS) activity (12, see figure below). In addition, TMV induction of *PR-1* gene expression was blocked by the NOS inhibitor, L-NMMA. Furthermore, administration of NO donors or recombinant mammalian NOS to tobacco plants or tobacco suspension cells triggered expression of the *PR-1* and phenylalanine ammonia lyase (*PAL*) genes. These genes were also induced by cyclic GMP (cGMP) and cyclic ADP ribose, two molecules that can serve as second messengers for NO signaling in mammals. Consistent with cGMP acting as a second messenger in tobacco, NO treatment induced dramatic and transient increases in endogenous cGMP levels. Moreover, NO-induced activation of *PAL* was blocked by LY 83583 and ODQ, two inhibitors of guanylate cyclase. *PR-1* gene activation by NOS or cyclic ADP ribose was SA dependent, because this induction was blocked in NahG transgenic tobacco. In contrast, *PAL* induction was independent of SA.



In animal systems, another major target of NO besides guanylate cyclase is aconitase. We have found that tobacco aconitase, like its mammalian counterpart, is inhibited by NO. In summary, it appears that critical players in animal NO signaling are also operative during plant defense responses to microbial pathogens.

References
1. Chen Z, Silva H, Klessig DF, 1993. Science 262, 1883-1886.
2. Durner J, Klessig DF, 1995. Proc. Natl. Acad. Sci. USA 92, 11312-11316.

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3. Durner J, Klessig DF, 1996. J. Biol. Chem. 271, 28492-28501.
4. Anderson M, Chen Z, Klessig DF, 1998. Phytochemistry 47, 555-566.
5. Du H, Klessig DF, 1997. Plant Physiol. 113, 1319-1327.
6. Wendehenne D, Durner J, Chen Z, Klessig DF, 1998. Phytochemistry 47, 651-657.
7. Zhang S, Klessig DF, 1997. Plant Cell 9, 809-824.
8. Seo S, Okamoto M, Seto H, Ishizuka K, Sano H, Ohashi Y, 1995. Science 270, 1988-1992.
9. Zhang S, Du H, Klessig DF, 1998. Plant Cell 10, 435-449.
10. Zhang S, Klessig DF, 1998. Proc. Natl. Acad. Sci. USA 95, 7225-7230.
11. Zhang S, Klessig DF, 1998. Proc. Natl. Acad. Sci. USA 95, 7433-7438.
12. Durner J, Wendehenne D, Klessig DF, 1998. Proc. Natl. Acad. Sci. USA 95, 10328-10333.

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REGULATION OF CKII ACTIVITY IN BY2 CELLS IN RELATION TO CELL DIVISION CYCLE.

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ABSTRACT

The synchronizable BY2 tobacco cell line is being used to characterize CKII activity and CKII gene expression in relation to cell division cycle. By treatment of the cells with aphidicolin (arrest in phase S) and with propyzamide (arrest in prophase) we have accomplished synchronization rates of 80%. The CKII activity and mRNA and protein levels for both α and β CKII subunits have been measured in those conditions. Our data show important changes in CKII activity along the cycle, and depletion of CKII-activity by using a specific inhibitor support those data by blocking cells at stated points of the cycle. The regulation of CKII activity seems to be more at a post-transductional than at a transcriptional level, since no significant changes in the amount of the mRNA or protein are detected.

We have been able to correlate the peaks of CKII activity with peaks of polyamine's concentration (spermine, spermidine and putrescine) in BY2 cycling cells, meaning that interaction of polyamines might be the main form of regulation of CKII activity during the cell cycle.

However, gene transcription of CKII α and β subunits is significantly higher in dividing than in non-dividing cells, suggesting that in the transition G0/G1 transcriptional regulation also contributes substantially to regulate the CKII activity.

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POSTERS

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INDUCIBLE OVEREXPRESSION ARGININE DECARBOXYLASE IN TRANSGENIC TOBACCO PLANTS. EFFECTS OF PUTRESCINE ACCUMULATION IN PLANT GROWTH.

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Since all the steps in the polyamine (PA) biosynthetic pathway are known, this route is an excellent candidate for studying metabolic modulation through gene manipulation and, in this way, to determine regulatory functions of PAs in plants.
In most studies, the PA biosynthetic genes have been overexpressed using constitutive promoters. However, this could not be the most suitable way to determine the role of PAs in specific plant growth responses (Kumar et al., 1997). To overcome this problem, we have generated transgenic tobacco plants containing the oat arginine deacarboxylase (ADC) gene under the control of an inducible promoter, the tetraciclyne (Tet)-repressor system described by Gatz et al. (1992) (Masgrau et al., 1997). Inducible overexpression of oat ADC in transgenic tobacco led to the accumulation of ADC mRNA, increased ADC activity and changes in polyamine levels. Transgenic lines, induced during vegetative stage, displayed different degrees of a toxic phenotype, the severity of which was correlated with putrescine content. Now we are investigating if the toxicity comes directly from putrescine, or indirectly from its catabolytes.
The availability of plants from the F2 generation coming from line 52 (a low expressor) (Masgrau et al., 1997), has allowed us to analyze the differences between homozygous (high expression) and heterozygous (low expression) individuals. The obtained results confirm that the toxic effect observed in the transgenic plants is dependent of putrescine levels.
DiTomaso et al. (1989) have suggested that the basis for putrescine toxicity is the presence of a diamine oxidase (DAO) leading to the formation of oxidation products (i.e. hydrogen peroxide and free radicals), which most probably causes damage to the plasma membrane. To prove this hypothesis, we are determining DAO activities in F2 transgenic plants from line 52, homozygous (toxic phenotype) and heterozygous (non-toxic phenotype observed) for the transgene. On the other hand we are feeding the homozygous plants (toxic phenotype) with aminoguanidine, a DAO inhibitor, in order to determine if it is possible to revert the toxic phenotype.
Another alternative hypothesis to explain detrimental effects of ADC overexpression could be that excessive decarboxylation of arginine may lead to decreased arginine availability for protein synthesis (Slocum and Flores, 1991). To verify this hypothesis we are feeding transgenic plants overexpressing oat ADC (homozygous line 52, toxic phenotype) with 1μM arginine.
On the other hand, we have used the Tet-repressor system to transform tobacco plants with the homologous ADC, ODC (ornitine decarboxylase) and Spdsyn (spermidine synthase) genes (Cordeiro et al., unpublished) in sense and antisense orientations. In the case of ADC, the overexpression of the transgene and the resulting alterations in polyamine levels correlate with the observed phenotypic alterations upon induction with tetracycline, and are in accordance with the results obtained previously with the heterologous oat ADC gene.
The results obtained with these experiments could help to clarify the mechanism of action of polyamine as plant growth regulators.

References

- DiTomaso, J.M., Shaff, J.E. and Kochian, L.V. (1989). Plant Physiol., **90**: 988-995.
- Gatz, C., Frohberg, C. and Wendeenburg, R. (1992). Plant J., **2**: 397-404.
- Kumar, A., Altabella, T., Taylor, M.A. and Tiburcio, A.F. (1997). Trends Plant Sci., **2**: 124-130.
- Masgrau, C., Altabella, T., Farras, R., Flores, D., Thompson, A.J., Besford, R.T. and Tiburcio, A.F. (1997). Plant J., **11**: 465-473.
- Slocum, R.D. and Flores, H.E. (1991). Biochemistry and Physiology of Polyamines in Plants. Boca Raton, FL: CRC Press.

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EXPRESSION AND SUBCELLULAR LOCALIZATION OF THE PROTEIN AtRAB27 in *ARABIDOPSIS*

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Several studies have demonstrated that most plant species accumulate a set of proteins in the seed embryo during the desiccation period which coincides with the maturation of the seed (1). These proteins have been grouped in several classes and are named LEA (Late Embryogenesis Abundant). Since they are regulated by abscisic acid (ABA), are also called Rab (Responsive to ABA). However, there is no clear and definitive role for the LEA proteins.

The maize abscisic acid responsive gene *rab28*, has been shown to be ABA-inducible in embryos and vegetative tissues. The *rab28* gene product accumulates during late embryogenesis. In vegetative tissues, dehydration stress induced *rab28* gene expression. Using immunoelectron microscopy the Rab28 protein has been located in the nucleolus of different cell types (2). Two cDNA clones with homology to the well-characterised maize *rab28* have been identified in *Arabidopsis*: *atrab26* and *atrab27*.

The purpose of the present work is to characterize the Atrab27 protein, comparing its pattern of expression and subcellular localization with its homologous protein Rab28 in maize.

Generation of antibodies against Atrab27 has allowed us to study the protein pattern in *Arabidopsis*. The protein is only detected in dry seeds. The antibody recognizes two bands, which correspond to the two proteins, Atrab26 and Atrab27. The 2D-analysis show that these proteins are acidic. Following *in vitro* translation and immunoprecipitation with Atrab27, the pattern is the same that the one for *in vivo* protein, indicating that the protein is not modified post-translationally.

The results obtained by immunocytochemistry at the E.M. level and subcellular fractionation show that the protein is mainly located in the nucleolus, as the homologous maize protein Rab28.

To detect possible consensus of targeting to the nucleolus, deletions of the cDNA were analysed by transient expression and by stable transformation. Particle bombardment of onion cells has shown different localizations of the protein. There is some diffusion of the GFP alone to the nucleus, so now we are improving this method studying the localisation of Atrab27 in fusions which have both GUS and GFP (4).

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REFERENCES:

(1) Niogret, M.F. et al. Expression and cellular localization of *rab28* mRNA and Rab28 protein during maize embryogenesis. *The Plant Journal*: 9(4) 549-557 (1996).

(2) Pla, M. et al. Regulation of the abscisic acid-responsive gene *rab28* in maize *viviparous* mutants. *Mol Gen Genet* 230: 394-400 (1991).

(3) Goday, A. et al. Nuclei isolation from dry embryos of maize. In *Plant Responses to Cellular Dehydration During Environmental Stress*, Timothy J. Close and Elizabeth A. Bray, eds. p. 177-184 (1993).

(4) Grebenok, R.J. et al. Green-fluorescent protein fusions for efficient characterization of nuclear targeting. *The Plant Journal*: 11(3) 573-586 (1997).

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FUSION GENETIC STUDIES OF THE GIBBERELLIN SIGNALING PATHWAY

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The aim of this work is to identify signalling components mediating transcription of gibberellin (GA)-regulated genes. This involves screening mutagenized *Arabidopsis* plants carrying reporter fusions of GUS and LUC under control of promoters of GA-regulated genes as generally described by Susek et al. (1993). One such gene, ga5, encodes a GA20-oxidase, involved in the last steps leading to synthesis of biologically active GAs, which is negatively feedback-regulated at the transcriptional level by GAs (Xu et al., 1995; Phillips et al., 1995). We therefore isolated 0,9 kb of the ga5 gene promoter by means of Long Range-PCR (Mundy et al., 1995). Transcriptional fusions with either the LUC or the GUS reporter genes were constructed and transgenic lines generated via *Agrobacterium*-mediated transformation. Homozygous lines were selected and analyzed for the expression pattern and the regulation of the transgenes following treatment with exogenous GA or ancymidol (an inhibitor of GA biosynthesis). Seeds were mutagenized by gamma irradiation. M1 seeds were sawn in 20 pools of 1250 plants each. LUC activity of the M2 progeny (5-day-old seedlings grown on 50 uM GA3) was monitored by a liquid nitrogen-cooled CCD camera after spraying the plate with 5 mM luciferin. Approximately 30 putative mutants, showing LUC deregulation, were selected and classified upon their LUC activity levels, associated or not with an altered phenotype, into 3 different categories: late-flowering mutants, GA-insensitive mutants and high LUC expressor without visible phenotype.

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Polyamine biosynthesis during early fruit development and ovary senescence in tomato and pea.

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The last step of ovary development is a senescence process. However, this pattern of development can be changed by pollination or application of some growth regulators to unpollinated ovaries that induce fruit development. Polyamine levels and some of their biosynthetic enzymes in ovaries and young fruits of pea (Pisum sativum L. cv Alaska) and tomato (Lycopersicon esculentum Mill. cv Rutgers) shows some interesting changes associated to ovary senescence or early fruit development. In both pea and tomato, a relative high level of spermine is associated with ovary senescence, while a marked decrease is observed after induction of fruit development. Putrescine and spermidine do not show any difference between senescent ovaries and developing fruits. In tomato ovaries the levels of polyamine conjugates are drastically reduced after treatment, while no conjugates are found in pea ovaries. In tomato ovaries both ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) activity are present, as well as arginase, that catalyzes the conversion of arginine in ornithine. However, only ADC is present in pea ovaries. In tomato and pea, early fruit development is characterized by increases in the activity of putrescine biosynthetic enzymes. In tomato ovaries a marked reduction of arginase is also observed. A cDNA coding for ADC and two cDNAs coding for spermidine synthases have been isolated from a cDNA library from pea young fruits, and a cDNA coding for ODC from tomato ovaries has also been isolated. Expression of the cDNAs in Saccharomyces cerevisiae show that actually encoded ADC, ODC and spermidine synthase activities. Analysis of expression of those genes shows interesting correlations with development of different tissues.

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Salt and drought stress and ABA treatment induce phosphoenolpyruvate carboxylase (PEPC) expression in roots of wheat seedlings

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Phosphoenolpyruvate carboxylase (PEPC) catalizes the β-carboxylation of PEP in a reaction that yields Pi and oxaloacetate, which is converted to malate by the action of malate dehydrogenase (Chollet et al., 1996; Vidal and Chollet, 1997). This enzyme plays an important role in C_4 and CAM photosynthetic C metabolism. In C_3 plants this enzyme is found in most organs and, among other roles, it plays an anaplerotic function which consists of the replenishment of oxaloacetate in the tricarboxylic acid cycle whenever the demand of C skeletons for amino acid biosyntheis is high (Hupe and Turpin, 1994). In a previous work, we have analyzed the expression and localization of PEPC in cereal grains (González et al., 1998). In this study we showed a high expression of PEPC in tissues with a high rate of protein synthesis as the developing endosperm. In addition, a high expression of PEPC was found in the vascular tissue of developing grains. This finding prompted us to analyze further the presence of PEPC in vascular tissue from other plant organs.

In roots of 3-4 days-old wheat seedlings two PEPC polypeptides (with 108 and 103 kDa, respectively) were detected by western blot analysis. *In situ* hybridization and immunolocalization experiments show a high expression of PEPC in the meristematic tissue at the root tip while in the rest of the root expression was localized to the vascular bundle and epidermal cells. When grain imbibition was carried out under salt stress conditions (170 mM NaCl or 50 mM LiCl), PEPC activity in root extracts was duplicated. Western and northern blot analysis showed a high accumulation of PEPC polypeptides and mRNA, respectively. A similar effect on PEPC induction was also observed when wheat seedlings were subjected to drought stress. The induction of PEPC in response to salt or drought stress is specific of roots since no effect was observed in shoots. Immunolocalization experiments on root sections from stressed plants, showed that the accumulation of PEPC polypeptides in response to stress was localized in most root tissues. When seedlings were treated with ABA, this hormone induced PEPC mRNA accumulation. We have cloned and sequenced a 7 kb *SalI* fragment containing a wheat PEPC gene. Preliminar analysis of its promoter region shows the presence of an ABA-responsive element (ABRE) and other elements found in genes which respond to drought or salt stress (Busk and Pages, 1998). These results suggest that the induction of PEPC in roots of wheat seedlings in response to salt or drought stress is mediated by ABA. Our working hypothesis is that PEPC activity promotes the accumulation of malate in the root, which would play a role as osmolite in response to salt stress or water deficit.

References

Buck PK, Pages M (1998) Regulation of abscisic acid-induced transcription. Plant Mol. Biol. 37: 425-435
Chollet R, Vidal J, Oleary MH (1996) Phospho*enol*pyruvate carboxylase: A ubiquitous, highly regulated enzyme in plants. Ann Rev Plant Physiol Plant Mol Biol 47: 273–298
González MC, Osuna L, Echevarría C, Vidal J, Cejudo FJ (1998) Expression and localization of phosphoenolpyruvate carboxylase in developing and germinating wheat grains. Plant Physiol.116:1249-1258
Huppe HC, Turpin DH (1994) Integration of carbon and nitrogen metabolism in plant and algal cells. Annu Rev Plant Physiol Plant Mol Biol. 45:577–607
Vidal J, Chollet R (1997) Regulatory phosphorylation of C_4 PEPcarboxylase. Trends Plant Sci 2:203–241

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GENETIC MANIPULATION OF POLYAMINE LEVELS IN TRANSGENIC PLANTS

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Polyamines are ubiquitous cellular components that play an essential role in growth and development. In animals they have been shown to be involved in DNA, RNA and protein synthesis, and also to act as mediators of growth factor and hormone action, thus being essential for cell growth and proliferation (Tabor and Tabor, 1984). In plants, polyamines appear important in many aspects of development but their exact role needs to be established (Walden *et al.*, 1997; Kumar *et al.*, 1997; Tiburcio *et al.*, 1997). Intracellular levels of polyamines are related to many developmental events such as cell division, flower formation, membrane stability, pathogen and stress protection, and senescence (Galston and Tiburcio, 1991). However, the evidence that polyamines play an important role in plant development is largely correlative in nature (Evans and Malmberg, 1989). A molecular biological approach may allow us to definitively determine the basis of polyamine action in plants.

For this the tobacco polyamine biosynthetic enzymes ornitine decarboxylase (ODC), arginine decarboxylase (ADC), S-adenosylmethionine decarboxylase and synthase (SAMDC and SAMsyn) and spermidine synthase (SPDsyn) were cloned and characterised (Cordeiro, 1997). Using these cDNAs as probes, higher levels of the corresponding transcripts were detected in actively growing and/or dividing tissues, in accordance with the proposed roles of polyamine biosynthesis in growth and development.

The tetracycline-repressor system (Gatz *et al.*, 1992) was chosen to produce transgenic tobacco plants with controled expression of polyamine biosynthetic enzymes. For this the tobacco cDNAs for ODC, ADC and SPDsyn in both the sense and the antisense orientation were used. In each case plants were regenerated and submitted to PCR screening to confirm the presence of the transgene. For each construct 10 to 20 transgenic lines were obtained. After this first selection step, induction assays have been performed treating transgenic plants with tetracycline, initially in hydroponic culture and later transferring them to solid substrate. The mRNA levels corresponding to the transgene, as well as the enzyme activities and polyamine content were determined comparing induced plants with noninduced ones. The overexpresion of the transgenes and the resulting alterations in polyamine levels correlate with the observed phenotypic alterations upon induction with tetracycline. Basing on these results and on previous studies involving transgenic tobacco plants overexpressing the oat arginine decarboxylase gene (Masgrau *et al.*, 1997), we aim to dissect the molecular basis of polyamine action in plants and to study the possibilities for manipulating polyamine levels *in planta*.

References

Cordeiro, A. (1997) Molecular and biochemical analysis of methylglyoxal-bis(guanylhydrazone) resistance and of polyamine metabolism in relation to cell division in *Nicotiana tabacum*", PhD thesis, Univ. Cologne.

Evans, P.T. and Malmberg, R. (1989) Ann. Rev. Physiol. Plant Mol. Biol., **40**: 235 - 269.

Galston, A.W. and Tiburcio, A.F. (1991) Lecture course on Polyamines as Modulators of Plant Development. Fundación Juan March, Serie Universitaria, Madrid. Vol 257.

Gatz, C., Frohberg, C. and Wendenburg, R. (1992) Plant J., **2** : 397-404.

Kumar, A., Altabella, T., Taylor, M.A. and Tiburcio, A.F. (1997) Trends Plant Sci., **2**: 124-130.

Masgrau, C., Altabella, T., Farras, R., Flores, D., Thompson, A.J., Besford, R.T. and Tiburcio, A.F. (1997) Plant J., **11**: 465-473.

Tabor, C.W. and Tabor, H. (1984). Annu. Rev. Biochem., 53: 749-790.

Tiburcio, A.F., Altabella, T., Borrell, A. and Masgrau, C. (1997) Physiol. Plant., **100**: 664-674.

Walden, R., Cordeiro, A. and Tiburcio, A. F. (1997) Plant Physiol., 113: 1009-1013.

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SKP1p is a partner for PRL1-required protein kinases controlling glucose signalling in *Arabidopsis*

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We isolated a T-DNA tagged *Arabidopsis* mutant that displayed glucose hypersensitivity associated with pleiotropic alterations affecting cell elongation and hormone responses. The gene designated as pleiotropic regulatory locus PRL1 was found to encode a novel WD-protein carrying 7 C-terminal beta-transducin-like repeats conserved in eukaryotes.

Due to the fact that WD-40 repeat proteins are known to mediate their regulatory function through protein-protein interactions, yeast two-hybrid experiments have been performed to identify PRL1 interacting factors. The results of the yeast two-hybrid screen showed that PRL1 specifically interacts with two serine/threonine protein kinases, AKIN10 and AKIN11. By in vitro protein binding and kinase assays, it was found that PRL1 binds both *Arabidopsis* AKIN10 and AKIN11 and inhibits their activity both in vitro and in vivo. The prl1 mutation results in a cell elongation defect associated with numerous pleiotropic alterations resulting in altered leaf morphology and increased sensitivity to glucose and plant hormones, such auxin. The prl1 mutation also results in the activation of AKIN10 and AKIN11 kinases under glucose-repressive conditions in *Arabidopsis*. This leads to the derepression of glucose and stress controlled genes.

These alterations establish a connection between AKIN10 and AKIN11 controlled stress responses and tissue specific control of plant hormone action.

To search for further elements of the PRL1 signalling pathway, a yeast two-hybrid screen has been performed to identify AKIN10 and AKIN11 interacting partners. Both kinases were found to interact with yeast and human homologs of SKP1p. This interaction was also confirmed by in vitro protein binding assays .

The yeast SKP1 gene and its human homologue encode a kinetochoreprotein required for cell cycle progression during both DNA synthesis in S-phase and mitosis. In yeast, cell cycle progression and expression of genes encoding glucose transporters is strickly linked to nutrient availability. Nutrient sensing requires both SKP1p with Grr1p. The presence of glucose appears to enhance the association of Skp1p and Grr1p, and it has been suggested that this could be a mechanism of glucose sensing. Yeast two-hybrid experiments are currently performed to identify SKP1p interacting protein, with the prediction that yeast homolog involved in cell cycle progression and glucose signalling will be isolated from *Arabidopsis*.

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Cytokinin hypersensitivity and altered steady state mRNA levels of homeobox genes in tsd mutants of *Arabidopsis.*

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Plant cell division and differentiation need to be precisely controlled during development to ensure coordinated growth of tissues. A loss of this control could lead to cell divisions at ectopic positions and in the most severe cases to the formation of a plant tumor.
We have isolated recessive *tsd* (*tumorous shoot development*) mutants of *Arabidopsis* that show at different stages of development an altered hormone-independent differentiation *in vitro*. The members of one mutant class exhibit an early dedifferentiation and a hormone-autonomous callus growth. A second class of mutants shows several abnormalities reminiscent of cytokinin effects, including stunted growth, a hypertrophy of the green parts, a hormone-independent differentation of shoots, and a reduction of root growth. Analysis of the endogenous auxin and cytokinin contents showed no significant differences in comparison to wild type. Dose-response assays with exogenous hormones indicate a hypersensitivity to cytokinin. We detected in the mutants compared to wild type elevated steady state mRNA levels of the shoot-inducing *CKI1* and the shoot apical meristem specific homeobox genes *STM* and *KNAT1*. It is possible that mutations in negative regulatory elements of these genes are causally linked to the *tsd* phenotype.

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KNAT1 OVEREXPRESSION IN LETTUCE INDUCES CYTOKININ OVERPRODUCTION AND RE-ACTIVATION OF MORPHOGENIC PROGRAMS AT THE LEAF MARGINS.

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The products of homeobox genes control the expression of specific target genes and are thought to trigger important differentiation processes in a variety of organisms. The Knat1 gene from Arabidopsis belongs to the knotted-like class of homeobox genes that is thought to be involved in shoot meristem fate. The first homeobox gene knotted1 (kn1) was isolated by transposon tagging as a dominant leaf mutant in maize. In Kn1 mutants the ectopic expression of the *kn1* gene product in developing leaves has been correlated with the mutant phenotype. Overexpression of kn1 in a number of species has resulted in the modification of both leaf shape and plant architecture. The Arabidopsis Knat1 gene is expressed in the shoot apical meristem and downregulated before leaf initiation. Overexpression of Knat1 in *Arabidopsis thaliana* induces lobed leaves with ectopic meristems initiating in the sinuses of the lobed leaves in close vicinity to the veins.

Transgenic lettuce plants overexpressing Knat1 driven by the pea-plastocyanin promoter were obtained. Transformants showed a significant morphological alteration in leaf shape and were characterized by a drastic reduction in midvein elongation, decreased blade expansion and the formation of leaf-like structures at the margin. The immunolocalization by N^6-Δ^2-isopentenyl adenine (2iP) and N^6-Δ^2-isopentenyl adenosine (2iPA) showed a high increase of these cytokinins in the vascular leaf tissue. These data suggest that kn1-like genes may play a role in leaf morphogenesis and differentiation by affecting the cytokinin metabolic pathways.

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The cDNA-AFLP approach to study the post Imbibition abscisic acid synthesis mechanisms controlling seed domancy expression in Nicotiana plumbaginifolia.

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The physiological characteristics of seed dormancy in the Nicotiana plumbaginifolia wild-type line Pb H1D are described. Seed dormancy level is defined by the delay in seed germination (i.e the time allowed for germination), under favourable environmental conditions. This wild type line shows a clear primary dormancy which is suppressed by afterripening, whereas the abscisic acid-deficient mutant I217 shows a non-dormant phenotype. We investigated the role of ABA and GA in the control of dormancy maintenance or breakage during imbibition in suitable conditions. Fluridone, an ABA biosynthesis inhibitor, is almost as efficient as gibberellic acid (GA3), for breaking dormancy during imbibition. During early imbibition there is a transient accumulation of ABA in dormant seeds but not in afterripened seeds. In addition, fluridone and exogenous GA3 inhibit the accumulation of ABA in dormant seeds. This reveals an important role of ABA synthesis on dormancy maintenance in imbibed seeds and may lead to a better understanding of how GA and ABA interact to control seed-dormancy. Molecular studies of ABA and GA controlled processes in dormancy and germination are essential to elucidate the mechanisms by which these hormones operate. We are currently using a cDNA-AFLP approach with dry or early imbibed dormant and afterripened seeds to identify genes specifically involved in dormancy and its control. First results indicate that this approach is very efficient to identify genes differently expressed in dormant and non dormant seeds. Further, these identified function should be useful as possible biochemical markers for seed dormancy and germination.

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Interactions of cell cycle and growth regulators with geminivirus proteins

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Plant cells control their proliferation, growth and differentiation in response to both external and internal factors. Distinct molecular interactions, in particular at the regulatory level, have evolved in plants as a result of their unique growth properties, plasticity, body organization and response to pathogen invasion. We are interested in understanding how plant cells regulate the passage through the G1 and S phases of the cell cycle and the exit/entry from quiescence to an active cell proliferation and growth in response to growth factors. Our studies are also relevant to understand why tumor development is an extremely rare event in plants and how plant cells reactivate a proliferation program from fully differentiated cells. We are using geminiviruses as a model system since the replicative cycle relies absolutely on cellular functions. In fact, cell cycle activation or, at least, activation of some cell cycle functions as well as interference with other growth regulatory pathways are a likely response of plant cells to geminivirus infection. Thus, as a way to understand these processes in molecular terms, we have searched for cellular proteins which interact with geminivirus proteins.

Recently, we identified in a wheat dwarf geminivirus (WDV) RepA protein an amino acid motif (LXCXE) which mediates binding to human retinoblastoma (Rb) tumor suppressor protein (Xie et al., 1995, EMBO J. 14, 4073) and cloned a maize cDNA encoding a protein (ZmRb1; Xie et al., 1996, EMBO J. 15, 4900) with homology to human Rb family members (Rb, p107 and p130). Using plant Rb as a bait in a yeast two-hybrid screening, we have isolated several cDNAs encoding Rb-interacting proteins (RBIPs). We have also identified and cloned a family of proteins (GRAB) by their interaction with WDV RepA protein. GRAB proteins share a conserved domain with other plant proteins involved in development and senescence in plants. We will report the molecular characterization of two of these GRAB proteins. By deletion analysis, we have mapped the residues in the viral protein required for binding as well as identified a domain in GRAB proteins which is necessary and sufficient for their interaction with the viral protein.

The availability of these novel cDNA clones encoding key cell cycle and growth regulators should help in future studies to understand the cellular targets of plant growth factors as well as their mechanisms of action.

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Kinetics of jasmonate metabolism for defense signaling in the tobacco-TMV system

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The hypersensitive response (HR) is initiated after specific perception by plants of one or a limited number of pathogen components. Early cellular signaling events are followed by the synthesis of several defense response modulators, which act positively on the expression of downstream genes encoding a wide array of antimicrobial proteins. The best characterized defense gene activator is salicylic acid (SA), which plays a major role in the control of systemic PR gene expression but also in local defense reactions. Evidence is also emerging for the involvement of lipid-derived molecules ("the oxylipins") in the signaling of a subset of HR-induced defense responses.

We initiated a set of experiments to investigate the contribution of the jasmonate branch of oxylipin metabolism to the activation of pathogen-induced defense responses. Major steps in jasmonic acid (JA) biosynthesis include the release of insaturated membrane fatty acids, their oxygenation, cyclisation and ß-oxidation. Kinetic studies of activation of jasmonate metabolism were performed with plants undergoing HR.

Soluble phospholipase activity was found to increase dramatically in tobacco leaves between 2 and 4 days following TMV inoculation, markedly earlier than PR protein accumulation measured in the same extracts. A low abundance lipase was isolated and its N-terminal sequence determined. The cloning of this enzyme is in progress and should help in investigating its possible function in the release of fatty acid precursors of octadecanoid defense signals.

GC-MS measurements of phytodienoic acid (PDA), the biosynthetic precursor of JA, showed that this compound accumulated rapidly after inoculation, with a delay of 12-24 hours in comparison with the lipase activity, thus indicating a possible role of PDA in the regulation of downstream defense responses. JA levels, determined by ELISA, increased at later stages, and at lower levels than PDA.

As exogenous salicylic acid (SA) was described as an inhibitor of jasmonate synthesis and action during the wound response, we addressed the question to know if the high SA levels naturally occuring during the HR affected the levels and kinetics of octadecanoid signals. Quantitation of SA in the same material showed that both types of defense signals are produced in the HR, with SA accumulation occuring later than PDA. An inhibitory effect of SA on the octadecanoid pathway could not be visualized so far when SA is produced by the plant itself.

The relationships between the SA and JA pathways will be further examined in transgenic plants with altered metabolism of either one signaling compounds.

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THE ROLE OF LIPOXYGENASES IN THE REGULATION OF WOUND-MEDIATED GENE ACTIVATION, RESISTANCE AGAINST PESTS AND TUBERISATION IN POTATO

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De novo synthesis of the plant hormone jasmonic acid (JA) is required for the wound-induced expression of proteinase inhibitor II, cathepsin D inhibitor, and other defensive genes in potato. The first step in JA biosynthesis involves lipoxygenase (LOX) introducing molecular oxygen at the C-13 position of linolenic acid. We previously have shown that in potato at least two gene families code for 13-LOX proteins. Our approach to elucidate the regulatory role of 13-LOX in those processes consists in the generation of transgenic plants that either carry the LOX H1 or H3 cDNAs under the control of the 35S cauliflower mosaic virus promoter either in sense or antisense orientation. We have produced transgenic potato plants devoid of one specific 13-LOX isoform (LOX-H3) through the antisense-mediated depletion of its mRNA. LOX-H3 depletion largely abolishes accumulation of proteinase inhibitors upon wounding, indicating that this specific LOX plays an instrumental role in the regulation of wound-induced gene expression. As a consecuence, weight gain of Colorado potato beetles fed on antisense plants is significantly larger than on wild type plants. The poorer performance of LOX-H3 deficient plants towards herbivory is more evident with a polyphagous insect. Indeed, larvae of beet armyworm reared on the antisense lines have up to 57 % higher weight than those fed on non transformed plants. LOX-H3 thus appears to regulate gene activation in response to pest attack, and this inducible response is likely to be a major determinant for reducing performance of non specialised herbivores. However, the regulatory role of LOX-H3 is not due to its involvement in the wound-induced increase of JA, as wild type and LOX-H3 deficient plants have similar jasmonate levels after wounding. LOX-H3 deficient plants have higher tuber yields. The apparent effect of suppressing the inducible defensive response on plant vigour suggests that it may pose a penalty in plant fitness under non stress situations.
None of the more than 60 lines generated with the sense constructs resulted in significant overexpression of either H1 or H3 upon wounding. However, three lines harboring the H1 sense construct and other three with H3 sense construct were depleted of H1 and H3, respectively, as a result of cosupression effect. The selected transgenic lines have been characterized in terms of activation of marker genes upon wounding, levels of jasmonates, tuber production and flower setting.

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MOLECULAR GENETIC STUDIES OF THE ABA SIGNAL TRANSDUCTION PATHWAY.

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We have undertaken a genetic screen in order to identify molecular components of the abscisic acid (ABA) signal transduction pathway in Arabidopsis. The screen developed combines a classic physiological screen with a novel molecular screen. First, T-DNA or transposon-tagged plants able to grow in inhibiting concentrations of ABA (3uM) are selected. Second, the induction kinetics of ABA responsive genes are measured in single leaves of the selected plants. Using this two-staged screen, we have been able to identify at least five mutants that are insensitive to ABA at the germination and the molecular levels and one mutant that has normal responses at the molecular level but is insensitive to ABA in the germination test. Molecular and genetic analyses of these mutants will be presented.

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IDENTIFICATION OF PROTEINS MEDIATING ABA SIGNALING IN MAIZE

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The phytohormone abscisic acid (ABA) regulates key developmental and physiological processes in plants by activating specific target genes such as the *rab* (*responsive to ABA*) genes. However, little is known about how ABA signaling modulates the activity of target genes and how these genes elicit in turn specific responses in the plant. We are addresing these questions by identifying novel proteins involved in the process of ABA signaling at different levels. In one approach, we are carrying out yeast two-hybrid screenings to isolate proteins that interact with the product of the ABA responsive gene *rab* 28. This protein has been shown to localize normally within the nucleolus (1) but its function remains unknown. We will present the identification of several specific *rab* 28-interacting proteins and our progress in their molecular characterization.

In the second approach, we extent previous studies (2) on the role of the ABREs in the *rab* 28 promoter in induction by ABA using transient transformation and in vivo footprinting experiments. The analysis of transgenic *Arabidopsis* plants with the *rab* 28 promoter show that in embryos of these plants the ABREs of this promoter are necessary for expression during embryogenesis.

Finally, we are now identifying maize proteins homologous to the EmBP-1 transcription factor, which was initially identified in wheat, and is known to bind specific regulatory sites within the promoters of ABA responsive genes and mediate their activation in response to ABA. We find that in maize there is a family of EmBP-1 genes, and we are currently characterizing them with the aim of studying their role in different processes regulated by ABA.

References:

1.- Niogret, M.F., Alba, M.M., Culiañez-Macià, F.A., Goday, A. and Pagès, M. (1996). Expression and cellular localization of rab 28 mRNA and Rab 28 protein during maize embryogenesis. *The Plant Journal* 9:549-557.

2.- Busk P. K., and Pages, M. (1997). The seed-specific activator VP1 enhances transcription through a preformed complex on the abscisic acid responsive element. *The Plant Cell* 9: 2261-2270.

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TOWARDS THE CLONING OF NOD FACTOR RECEPTORS

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Rhizobial bacteria associate with plants belonging almost exclusively to the family *leguminosae*, to form a symbiosis which leads to the development of a new plant organ, the root nodule, in which gaseous nitrogen is reduced to ammonium that can be assimilated by the plant. It has been shown that a precise molecular dialogue takes place during the establishment of this symbiosis. The plant excretes flavonoids or other secondary metabolites which induce the bacterial genes essential for nodulation (the *nod* genes). The proteins encoded by these genes are responsible for the biosynthesis of lipo-chitooligosaccharidic signals (the Nod factors) which mediate the specific recognition between the two symbiotic partners (for reviews see Denarié *et al.*, 1996, Van de Sande *et al.*, 1997, Cohn *et al.*, 1998). In addition to their specificity Nod factors are active at extremely low concentrations suggesting a perception mechanism via high affinity receptors in the roots of their host plants. Biochemical studies revealed the presence of NFBS2, a high affinity binding site for NodRm-IV(Ac, S, C16 :2), the major Nod factor of *Rhizobium meliloti* , in the microsomal fraction of *Medicago varia* cell culture extracts (Niebel *et al.*, 1997). NFBS2 has characteristics compatible with a role in Nod factor perception during nodulation and could at least belong to a family of structurally related LCO binding proteins comprising the symbiotic Nod factor receptor(s) (Niebel *et al.*, 1997, and Gressent *et al.*, in preparation). We thus decided try to clone genes coding for Nod factor binding proteins such as NFBS2 using an expression cloning approach in mammalian COS cells. We constructed an expression library, starting from the cell cultures in which NFBS2 had been identified, of about 250000 clones which have been organised in 356 pools of about 700 clones. DNA minipreps have been prepared for each of these pools and we are currently transfecting them into COS cells. These cells are then incubated with radiolabelled Nod factor, washed and subjected to scintillation counting. Potential positive clones will subsequently be purified by subdividing the positive pool(s) and subjecting them to several cycles of transfection in COS cells.

References :

Cohn, J., Day, B., and Stacey, G. (1998): Legume Nodule Organogenesis. *Trends Plant Science*, 3:105-110.

Denarie, J., Debelle, F., and Prome, J.C. (1996): *Rhizobium* lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annu.Rev.Biochem.*, 65:503-535.

Niebel A, Bono, J.J., Ranjeva, R., and Cullimore, J.V. (1997): Identification of a high affinity binding site for lipo-oligosaccharidic NodRm factors in the microsomal fraction of *Medicago* cell suspension cultures. *Mol.Plant Microbe Interact.*, 10:132-134.

Van de Sande, K. and Bisseling, T. (1997): Signalling in symbiotic root nodule formation. *Essays.Biochem.*, 32:127-142.

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CALCIUM A CENTRAL SIGNAL IN PLANTS.

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Calcium fluxes control many biological processes and are essential steps in many signaling pathways in plants and animals. Although there is much knowledge about calcium changes due to different biotic and abiotic stimuli, information on the molecular level of calcium channels, channel regulators or associated proteins is still missing. Here we describe different approaches in order to isolate genes involved in the regulation of calcium signaling.
The first approach is based on T-DNA insertional mutagenesis in tobacco protoplasts, and subsequent selection during protoplast regeneration under non-permissive calcium concentration in the medium. Wild type tobacco protoplasts are unable to grow and develop to microcalli in culture medium without addition of calcium. We have mutagenized (through Agrobacterium T-DNA transfer) 57,000 protoplasts, and obtained 18 clones that were able to regenerate in selection medium. Currently we are characterizing the phenotype of these mutants, which carry the calcium indicator protein *aequorin.* Segregation analysis, and measurements on calcium changes of the putative clones in response to different stimuli will be discuss on the poster.
In a second approach, we are using yeast complementation with an Arabidopsis cDNA library to isolate genes involved in the regulation of calcium fluctuations in plants. Two mutant strains which are affected in the control of calcium homeostasis in yeast have been described: the calcium-sensitive growth mutants *csg1* and *csg2* (provided by Dr. Dunn). These mutants are affected by an increase in a calcium pool distinct from the vacuolar calcium pool, where most of the calcium in wild type cells resides. These strains are unable to grow on yeast media containing 100mM calcium at 37°C.From this screening we obtained four novel *Arabidopsis thaliana* genes which show no homology to known proteins. At the moment we are investigating the regulation and function of these genes in the plant.

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CROSS-TALK BETWEEN ALTERNATIVE SIGNALLING PATHWAYS DICTATES THE DISTRIBUTION OF WOUND-INDUCED GENE ACTIVATION IN *ARABIDOPSIS* THALIANA

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Plants have acquired sensor and signal transduction systems to react to environmental stresses affecting their development. Phytohormones have been shown to participate in the signalling cascades activating defensive responses against some of these stresses. Jasmonic acid (JA), cell wall oligosaccharides and ethylene forward the transmission of wound signals in tomato plants to induce, in damaged and systemic tissues, the expression of proteinase inhibitors and other defense-related genes. Interestingly, in arabidopsis these same signalling components interact in novel ways to activate distinct responses in injured (local response) and systemic tissues (systemic response) of the plant. The concentration gradient of cell wall oligosaccharides, released at the wound site, may determine the boundaries where the local or systemic wound responses are functional, by inducing in damaged tissues the expression of a specific set of wound responsive genes while repressing JA responsive (JR) genes, that are activated in systemic tissues not reached by oligosaccharides. Ethylene may be involved as a downstream component of the oligosaccharide regulated responses, mediating the repression of the JA-dependent pathway.

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Biochemical evidences of ADC activity regulation

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The synthesis of putrescine and polyamines in higher plants from arginine and ornithine is catalysed by arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) respectively. ADC is active in elongating cells, embryonic cells and in cells under various stress conditions. ODC activity is associated with rapid cell division, in a fashion similar to mammalian systems. In plants, the effects of various stresses on putrescine content have been studied. This increase is due to a specific enhancement of ADC activity since it is inhibited by alpha-difluoro-methylarginine but not by alpha-difluoromethylornithine (1).
One theme that has appeared in studies about ADC has been the existence of post-transcriptional and posttranslational means of regulating. In this way, a number of studies of regulation of plant ADC have shown that changes in enzyme activity occur without commensurate changes in mRNA levels. At present, is accepted that posttranscripcional regulatory mechanisms for polyamine synthetic enzymes is an aspect that cuts across kingdoms.
With ADC, we need to know if there are multiple forms of the enzymes, either derived from multiple genes or from various modifications of the product of a single gene.
Plant ADC has been characterized from a number of species, beginning with the work of Smith (1979) who suggested that oat ADC had native sizes of 118 KDa and 195 KDa (2). In 1990, Bell and Malmberg (3) isolated the cDNA clone encoding ADC. Subsequent studies (4) have shown the ocurrence of posttranslational proteolytic processing of oat ADC from 67,000 Da precursor into 42,000 and 24,000 Da products with an associated gain of enzyme activity.
In our laboratory, an aminoacid sequence near the C-terminus deduced from the nucleotide sequence of the oat ADC gene was selected for generation of polyclonal antibodies (5,6). Also we generated transgenic tobacco plants containing the oat ADC gene under the control of a tetracycline-inducible promoter (7).
In this work, by partial purification, molecular filtration, ELISA, Western blot and the use of DFMA, we have demostrated the existence of different enzymatic active and inmunoreactive molecular forms of ADC in oat with 195, 115, 66, 38 and 22.5 KDa molecular mass. Also, we demostrated that overexpression of oat ADC in tobacco increased the last one inmunoreactive band. These studies agree with previous papers but this is the first report to show all molecular forms simultaneously and is a preliminary contribution to resolve the question about the distribution of multiple forms of ADC and their importance in the correlation between the enzymatic activity and mRNA levels.

1) Tiburcio et al. (1997).Physiologia Plantarum, 100: 664-674.
2) Smith T.A. (1979). Phytochemistry 18: 1447-1452.
3) Bell E. and Malmberg R.L. (1990). Molecular and general genetics. 224: 431-436.
4) Malmberg R. L. and Cellino M.L. (1994). Journal of Biological Chemistry. 269:2703-2076.
5) Borrell et al (1995). Plant Physiology 109: 771-776.
6) Borrell et al (1996) Physiologia Plantarum 98: 105-110.
7) Masgrau C. et al. (1997). Plant journal 11: 465-473

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Abstract: Defense signaling in tomato plants involves the modulation of plasma membrane H^+-ATPase activity

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Tomato plants respond to local injury with the accumulation of systemic wound response proteins (SWRPs). The 18-amino-acid peptide systemin is a powerful inducer of SWRP-gene expression and is a candidate molecule for the systemically transmittable wound signal. In cultured cells of *Lycopersicon peruvianum*, systemin elicits a rapid alkalinization of the growth medium. A correlation has been observed between the extent of medium alkalinization and the SWRP-inducing activity of systemin analogues, which is indicative of a causal link between the two phenomena. The plasma membrane H^+-ATPase is responsible for building up and for maintaining the proton gradient across the plasma membrane. Inhibitors (VO_4^{3-}, erythrosin B, and diethyl stilbestrol) as well as an activator (fusicoccin) of the proton pump were employed to investigate this enzyme as a possible cause of systemin-induced pH-changes and SWRP induction.

In cultured cells of *L. peruvianum*, fusicoccin was found to cause a rapid acidification of the growth medium. Pretreatment of cells with fusicoccin abolished the response to subsequently added systemin. In whole tomato plants, fusicoccin inhibited the wound- and the systemin-induced accumulation of SWRP-mRNAs. Inhibitors of the proton pump, on the other hand, caused medium alkalinization in the cell culture as well as the induction of SWRP-mRNA levels in the whole plant assay. These data suggest that the changes in gene expression triggered by wounding and by systemin treatment are mediated by an inhibition of H^+-ATPase activity.

Inhibition of the proton pump seems to be preceded by an influx of $Ca^{2+}$and is dependent on the activity of a protein kinase. A model is proposed according to which the perception of the wound signal systemin results in elevated cytosolic Ca^{2+} concentrations. A calcium-activated protein kinase then mediates the inactivation of the plasma membrane H^+-ATPase and ultimately the induction of SWRP genes.

In contrast, the activation of the H^+-ATPase by fusicoccin treatment of tomato plants lead to the accumulation of mRNAs for pathogenesis-related proteins. Apparently, the signaling pathways triggered by wounding and by pathogen infection are differentially regulated at the level of the plasma membrane H^+-ATPase.

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ENOD40 homologues are present in the non legumes *Nicotiana tabacum* and *Lycopersicon esculentum*

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The *ENOD40* gene was first isolated from legumes as a gene induced during root nodule formation. Sequence comparisons showed the presence of two highly conserved regions, which were named region 1 and region 2. Region 1 encodes an unusually small peptide varying in length from 12-13 amino acids. Region 2 does not contain an ORF but is even higher conserved than region 1. *ENOD40* expression was found in the root pericycle prior to the first cell divisions in the cortical cells that later form the nodule primordium. This observation led to the hypothesis that *ENOD40* might be involved in establishing the onset of cell division and that it could do so by influencing the local auxin/cytokinin balance. Expression studies showed that *ENOD40* expression can be found in most plant organs albeit in much lower levels than during nodule development. This suggested a function not only in nodule development but also in plant development in general. To investigate the role of *ENOD40* in plant development tobacco and tomato *ENOD40* homologues have been isolated. Both homologues share the highly conserved regions 1 and 2 with the legume *ENOD40* genes and region 1 encodes a peptide of 10 amino acids. Experiments are being done to elucidate the role of *ENOD40* in plant development.

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