SIGNAL TRANSDUCTION IN PLANTS

Organizers: Daniel F. Klessig and Winslow Briggs

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Plenary Sessions *Page*

March 30
Hormonal Signaling I 470
Hormonal Signaling II 471

March 31
Signaling in Plant-Pathogen Interactions I 471
Cell Cycle (Joint) 472

April 1
Signaling in Plant-Pathogen Interactions II 473
Signaling By Light I 474

April 2
Signaling By Light II 474
Cell-Cell Communication (Joint) 475

April 3
Stress Signaling 476
Second Messengers and Phosphorylation 477

Poster Sessions

March 30
Hormonal Signaling (J6-100-126) 478

March 31
Signaling in Plant-Pathogen Interactions (J6-200-223) 485
Cell Cycle (J6-224-231) 491

April 1
Signaling By Light (J6-300-324) 493

April 3
Stress Signaling (J6-400-417) 499
Second Messengers (J6-418-426) 504
Phosphorylation (J6-427-435) 506

Late Abstracts 510

Hormonal Signaling I

J6-001 SIGNAL TRANSDUCTION OF THE OXIDATIVE BURST, Philip S. Low[1], Peter F. Heinstein[2], Sreeganga Chandra[1], Shannon Dwyer[1], and Laurent Legendre[1], Dept. of Chemistry[1] and Dept. of Medicinal Chemistry and Pharmacognosy[2], Purdue University, W. Lafayette, IN 47907.

In response to elicitors, cultured soybean cells release $\sim 10^{-14}$ moles H_2O_2/min/cell, an amount comparable to activated human neutrophils. This oxidative burst is likely initiated by binding of an elicitor to a cell surface receptor. Elicitors known to induce the oxidative burst include polygalacturonic acid, harpin, homogenates of many pathogens, several kinase activators and phosphatase inhibitors. Binding of the elicitors to the plant cell surface can be visualized by fluoresence and epipolarization microscopy. Following binding, the elicitor is carried into the cell by receptor-mediated endocytosis. However, prior to endocytosis, the receptor-elicitor complex is believed to initiate a series of signaling events that may include activation of a trimeric GTP binding protein, induction of phosphatidylinositol-4,5-bisphosphate hydrolysis by phospholipase C, release of lysolipid and free fatty acid by phospholipase A, phosphorylation of several distinct proteins, and assembly of a protein complex that can generate O_2^- and H_2O_2. This latter oxidase complex appears to be immunologically and functionally related to the neutrophil oxidase complex. Importantly, the oxidase can be activated by at least three separate signaling pathways that distinguish themselves in their use of phospholipase C and phospholipase A as signaling intermediates. Different elicitors independently initiate these disparate pathways. The properties of these pathways will be described to the extent they are currently known, and the molecular identity of one protein kinase essential to the signal transduction pathway will be reported.

J6-002 ETHYLENE AND THE ETR1 TWO-COMPONENT REGULATOR FAMILY IN ARABIDOPSIS, Caren Chang, Jian Hua and Elliot M. Meyerowitz, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Little is known of the signal transduction pathways through which plant growth substances act to transmit signals from one part of a plant to another. It is not known if these pathways resemble known pathways from other kingdoms, or if they have features unique to plants. To find out, my laboratory has attempted to study key components in the ethylene signal transduction pathway in the flowering plant *Arabidopsis thaliana*. We started with the molecular cloning of the *ETR1* gene. *ETR1* was first identified by Bleecker et al. (1988) in a screen for *Arabidopsis* mutants that lacked the seedling triple response to ethylene. There are four known mutant alleles, all dominant, and all of which confer resistance to ethylene as assayed by any of the many known ethylene responses in *Arabidopsis*. Because of this, and because the mutation reduces ethylene binding to leaves, it seemed that the product of the *ETR1* gene could play a critical role in ethylene signal transduction, perhaps as a receptor or part of a receptor complex. We cloned the *ETR1* gene by map-based cloning (Chang et al., 1993). The sequence of the gene showed it to be a member of a family of genes previously known only in prokaryotes, involved in signal transduction across bacterial membranes. The family is that of the two-component regulators, which typically consist of a sensor component which includes an input portion and a histidine protein kinase, and a response regulator component that includes an aspartate phosphotransferase that takes the phosphate from the histidine of the transmitter and attaches it to an aspartate, and as a consequence activates an output domain. The output domain in turn activates some downstream function. The *ETR1*protein contains parts of both components, and shows homology with both the histidine protein kinases and the aspartate phosphotrasferases. In bacteria these two components may be found on a single protein, or may be coded by separate genes. The similarity of *ETR1* to bacterial two-component regulators has not only shown that this type of protein is found in eukaryotes (additional eukaryotic members are now known from yeast), but also indicates that *ETR1* is indeed a good candidate for an ethylene receptor, or at least a key component of the ethylene signal transduction machinery. One other known part of the ethylene signal transduction path in *Arabidopsis* is the product of the *CTR1* gene (Kieber et al., 1993). This is a homologue of a part of a MAP-kinase cascade. One open possibility is thus that ethylene signals are received by a signal transduction pathway that is a mixture of what used to be considered a prokaryotic input end, connected to a typically eukaryotic output end. One open question is why all known *ETR1*mutations are dominant. One possibility is that loss of function has no effect because of redundancy. To test this we have cloned two additional genes that code for two-component regulators from *Arabidopsis*, and have shown that mutations in at least one of them also give dominant insensitivity to ethylene.

Bleecker, A.B., Estelle, M.A., Somerville, C. and H. Kende (1988) Science **241**, 1086-1089.
Chang, C., Kwok, S.F., Bleecker, A.B. and E.M. Meyerowitz (1993) Science **262**, 539-544.
Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K. and J.R. Ecker (1993) Cell **72**, 427-441.

J6-003 REGULATION OF GENE EXPRESSION BY ABSCISIC ACID (ABA), Ralph Quatrano, Alison Hill, Andre Nantel, Chris Rock, Joaquim Medina, Leigh Brian, and Janice Davis, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

The Em gene is a member of a gene set which is expressed only in response to ABA in developing embryos. This specific pattern of expression requires several known genetic loci, including *viviparous-1* (*vp1*) in maize and *ABA insensitive-3* (*abi 3*) in *Arabidopsis* (1). Mutations at these loci result in pleiotropic effects, one of which is an insensitivity to ABA when assayed for inhibition of seed germination. 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(3). Using a protoplast transient assay(4), 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 was shown to transactivate the same Em promoter without exogenous ABA(3), possibly by making the protoplasts more sensitive to endogenous ABA. In the presence of excess ABA and VP1, a synergistic response is observed(3). A tetramer composed of a 20 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. Deletion of an 18 amino acid in a highly conserved region of VP1 (BR2) eliminates its ability to transactivate the Em promoter. A DNA binding protein (EmBP-1) of the basic-leucine zipper class (bZIP) was shown to bind to the G-boxes in the ABRE(5). A 2 bp mutation in the G-box(es) reduces or eliminates the ability of the ABRE to bind EmBP-1(4,5), or to exhibit the responses to ABA and VP1 in the transient assay. EmBP-1 and a partial VP1 without its activation domain were purified from *E. coli* as fusions with the maltose-binding protein. We demonstrate that VP1 can greatly enhance the DNA-binding activity of EmBP-1 to the ABRE in a gel retardation assay. The enhancement by VP1 is most pronounced at low concentration of the bZIP factor or when using a low-affinity DNA target. Deletion of the BR2 domain eliminates the enhancement while a 40 amino acid fragment containing BR2 can substitute for VP1. Attempts at demonstrating a physical interaction between VP1 and EmBP-1 have not yielded convincing results, but a weak interaction of BR2 with DNA can be demonstrated by UV-crosslinking. The capacity of VP1 to enhance DNA-binding activity has also been observed on transcription factors as diverse as opaque-2, max, NF-kB and Sp1. This lack of specificity might explain why a mutation in *vp1* has pleiotropic effects. Implications of these results for ABA-regulated gene expression will be discussed. Supported by research grants from the National Institutes of Health to R.Q. (GM 44288), A.H. (GM 13588) and C.R. (GM 14752).

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Hormonal Signaling II

J6-004 GENETIC STUDIES OF AUXIN ACTION AND AUXIN TRANSPORT IN ARABIDOPSIS, Alex Cernac, Elizabeth Dewey, Lawrence Hobbie, Doug Lammer, Jason Pitts, Max Ruegger, Serena Smith, Candace Timpte, Jocelyn Turner, Loni Walker, and Mark Estelle. Dept. of Biology, Indiana University, Bloomington IN 47405

By screening for auxin-resistant mutants of Arabidopsis, we have identified a number of genes that function in auxin response. Mutations in at least three of these genes, *aux1, axr1,* and *axr2* result in a reduction in the auxin-regulated *SAUR-AC1* gene suggesting that each gene functions at an early step in auxin action. Genetic studies indicate that *AXR1* and *AUX1* function in distinct auxin response pathways. We have cloned the *AXR1* gene and shown that it encodes a protein related to ubiquitin activating enzyme E1, the first enzyme in the ubiquitin conjugation pathway. Although the biochemical function of the AXR1 protein remains uncertain, our studies suggest that AXR1 has an important role in cell growth. *In situ* hybridization studies indicate that *AXR1* gene expression is restricted to the meristem and early organ primordia. Overexpression of AXR1 in transgenic plants results in a number of dramatic changes in cell growth. We are presently pursuing several approaches to understanding the biochemical function of AXR1 in cell growth.

We are also using a genetic approach to studying auxin transport. By screening for resistance to inhibitors of auxin efflux, we have identified three genes that may play a role in auxin transport (*tir1, tir2, tir3*). Mutations in each of these genes affect a variety of auxin regulated growth processes, including gravitropism and lateral root initiation. In addition, the *tir* mutations enhance the effects of the *axr1* mutations on plant morphology, suggesting that the *TIR* genes play an important role in auxin regulated growth. Our current working hypothesis is that each of the *TIR* genes is important for the regulation of intracellular auxin levels. The *TIR1* gene has been cloned and encodes a novel protein. The results of *in situ* hybridization studies, as well as additional physiological studies of the *tir* mutants will be presented.

Signaling in Plant-Pathogen Interactions I

J6-005 ELICITOR-BINDING PROTEINS AND SIGNAL TRANSDUCTION IN THE ACTIVATION OF PLANT DEFENCE, Jürgen Ebel, Markus Feger, Ulrich Kissel, Axel Mithöfer, and Tom Waldmüller, Botanisches Institut der Universität, D-80638 München, Germany.

Soybean (*Glycine max* L.) tissues respond to infection with the oomycete *Phytophthora megasperma* f. sp. *glycinea* and to treatment with a ß-glucan elicitor isolated from it by the rapid activation of a phytoalexin defence response at the level of transcription. The ß-glucan elicitor derived from the cell walls of *P. megasperma* was successfully used for studies on signal perception, signal transduction, and defence gene regulation in soybean. Elicitor-active ß-glucans were shown to be released early during germination of *P. megasperma* zoospores. Soybean membranes were shown to possess ß-glucan-specific, saturable binding sites that displayed highest affinity (K_d = 3 nM) of all ligands tested for a branched hepta-ß-glucoside exhibiting a structural motif of the mycelial wall glucans. The affinity of the binding sites for several glucan fractions correlated well with the ability of the ligands to stimulate phytoalexin production in a bioassay utilizing soybean cotyledons. A major component of the ß-glucan-binding sites is a protein with an apparent M_r of 70000 that was highly enriched after affinity purification and was identified by photoaffinity labeling using a photoreactive, radiolabeled conjugate of the hepta-ß-glucoside. The results provided evidence that the glucan-binding proteins are elicitor receptors. The signal transduction pathway leading to the transcriptional activation of the genes encoding enzymes of phytoalexin biosynthesis appears to involve rapid and transient changes in the permeability of the plasma membrane to Ca^{2+} and H^+ and in the level of phosphorylation of specific proteins. Chloride channel antagonists were more effective than calcium channel antagonists in inhibiting both the stimulation of the defence response and the inducible ion fluxes. (Supported by the Deutsche Forschungsgemeinschaft, SFB 369, and the Bundesministerium für Forschung und Technologie).

J6-006 A MECHANISM OF ACTION OF SALICYLIC ACID IN PLANT DISEASE RESISTANCE, Daniel Klessig, Zhixiang Chen, Uwe Conrath, Paloma Sánchez-Casas, Joseph Ricigliano, and Herman Silva, Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, N. J. 08855, U.S.A.

Salicylic acid (SA) is an endogenous inducer of several plant defense responses. We have purified and characterized a soluble SA-binding protein (SABP) from tobacco, whose binding specificity and affinity are consistent with a receptor function. SABP is a catalase whose ability to convert H_2O_2 to H_2O and O_2 is inhibited by SA binding. The resulting rise in H_2O_2 levels then appears to act as a signal to activate defense-related genes such as pathogenesis-related (PR)-1 genes. In support of this model, increasing H_2O_2 levels by treatment of tobacco leaves with SA or with the known catalase inhibitor 3-amino triazole induces PR-1 gene expression. Similarly, several different prooxidants induce PR-1 expression while antioxidants prevent PR-1 activation by SA. PR-1 genes are also induced in transgenic tobacco plants expressing an antisense copy of the catalase cDNA thereby inhibiting synthesis of endogenous catalases. In addition, INA, a synthetic inducer of PR gene expression and enhanced disease resistance, also binds to and inhibits catalase activity.

J6-007 SYSTEMIC ACQUIRED RESISTANCE SIGNALING IN PLANTS. John Ryals[1], Terrance Delaney[1], Urs Neuenschwander[1], Leslie Friedrich[1], Kris Weymann[1], Kay Lawton[1], Helmut Kessmann2. [1]Ciba Agricultural Biotechnology Research Unit, Research Triangle Park, NC. [2]Ciba Crop Protection Division, Basel , Switzerland.

Many plants respond to a pathogen infection by inducing a broad-spectrum, systemic resistant state that is effective against many pathogens for several weeks to months. This systemic acquired resistance (SAR) is an important defense response in plants and it could serve as the basis for future crop protection strategies by providing either engineered plants with increased pathogen tolerance or novel mode-of-action chemicals that stimulate the plants inherent defense mechanisms. SAR is also a facile, reliable system to study signal transduction in plants. We have identified a number of genes (SAR genes) whose expression correlates well with the onset of SAR. Plants expressing these cDNA's provide significant levels of pathogen tolerance. The expression of these genes increases in healthy plants from very low levels to levels approaching 1% of the total mRNA. Thus, the system provides for a dynamic range of gene expression.

We have previously shown that salicylic acid (SA) is required for SAR signaling. The role of SA appears to be in the signal transduction process, not as the translocated signal. Plants that are engineered to express a bacterial enzyme that degrades SA are not only incapable of inducing SAR but also are severely compromised in general health. This data suggests that the SAR pathway is central to disease susceptibility, resistance and SAR. Recently, it has been shown that salicylic acid can bind and inhibit a particular isozyme of catalase. In this case, high-levels of SA would lead to a build-up of active oxygen species. This mode of action for SA is apparently an important aspect of its role in plant pathogen interactions, however, it does not appear to be the mode of action involved in signaling SAR gene expression.

We have developed several chemicals that induce SAR when applied to plants. There is potential for some of these chemicals to be used as crop protection compounds. one of these compounds is the synthetic chemical 2,6-dichloroisonicotinic acid (INA). INA works by triggering a signal transduction pathway that is indistinguishable from the bona-fide SAR pathway. INA does not induce the accumulation of SA and it is an active inducer in plants that are unable to accumulate SA. However, it's action is blocked in Arabdiopsis by a pathway mutation that also blocks SAR. Thus INA appears to mimic the action of SA at a step downstream of SA accumulation.

We have studied the SAR signaling pathway using Arabidopsis genetics. Several mutants have been isolated that constitutively express SAR. Also, one mutant has been isolated that can no longer be induced by INA, SA or other chemical induces. The progress of understanding these mutants will be discussed.

J6-008 PEPTIDE ELICITOR RECOGNITION AND SIGNAL TRANSDUCTION IN PLANT DEFENSE, Dierk Scheel[1], Klaus Hahlbrock[2], Thorsten Jabs[2], Dirk Nennstiel[1], Thorsten Nürnberger[1], and Wendy R. Sacks[2], [1]Institut für Pflanzenbiochemie, D-06120 Halle/Saale, Germany, [2]Max-Planck-Institut für Züchtungsforschung, D-50829 Köln, Germany.

Cultured parsley (*Petroselinum crispum*) cells or protoplasts respond to treatment with a crude cell wall preparation from *Phytophthora megasperma* f.sp. *glycinea* with the transcriptional activation of the same set of defense-related genes as are activated in parsley leaves upon infection with fungal spores. Proteinaceous components of this preparation were found to be the elicitor-active substances. A 42-kDa glycoprotein elicitor was purified from fungal culture filtrate that was also present in hyphal cell walls. Its elicitor activity was found to reside exclusively in the protein moiety. Endoprotease Glu-C treatment of this glycoprotein released an oligopeptide consisting of 13 amino acids (Pep-13) that was found to stimulate the same responses as the intact elicitor, namely defense gene activation, formation of phytoalexins, an oxidative burst, Ca^{2+} and H^+ influx as well as an efflux of K^+ and Cl^- ions. Inhibition of these ion fluxes, which were the earliest reactions of parsley cells to elicitor treatment, prevented the activation of all other responses. Stimulation of the ion fluxes with the polyene antibiotic, amphotericin B, in the absence of elicitor initiated defense gene activation, phytoalexin production and an oxidative burst.

A fungal cDNA encoding the elicitor protein has been isolated and characterized. The full-length cDNA, as well as clones in which the region corresponding to the 13 amino acids (Pep-13) was replaced by nucleotides encoding either 2 or 6 unrelated amino acids, were overexpressed in *Escherichia coli*. While the product synthesized from the intact cDNA showed full elicitor activity in parsley cells, the proteins lacking the original Pep-13 sequence were completely inactive, indicating that this region is necessary and sufficient for activity of the intact elicitor.

A single class binding site with high affinity (K_d = 2.4 nM) for Pep-13 was detected in binding assays with parsley microsomes and protoplasts using [^{125}I]Pep-13 as a radioligand. The binding was found to be specific, saturable and reversible. [^{125}I]Pep-13 was covalently cross-linked with a 91-kDa microsomal protein from parsley. The labeling of this band was specific in that it was prevented by excess of unlabeled Pep-13. Functional relevance of the binding site was demonstrated by comparing competitor and elicitor activities of a series of Pep-13 derivatives in which single amino acids had been replaced by alanine. In all cases, the ability of these peptides to compete for binding of [^{125}I]Pep-13 to parsley microsomes correlated perfectly with their activity to induce ion fluxes, oxidative burst and phytoalexin production in parsley cells. Therefore, binding of the oligopeptide elicitor, Pep-13, to its receptor on the parsley plasma membrane appears to initiate signal transduction processes that involve plasma membrane-located ion channels and result in transient activation of plant defense genes. COS cell expression cloning of the gene encoding this receptor is currently in progress.

Cell Cycle (Joint)

J6-009 NOVEL CYCLINS ARE INVOLVED IN EARLY EVENTS IN THE CELLCYCLE, Laszlo Bögre, Irute Meskiene, Marlis Dahl, Dang Cam Ha, Manfred Pirck, Karin Zwerger, Erwin Heberle-Bors and Heribert Hirt, Institute of Microbiology and Genetics, Biocenter Vienna, Dr. Bohrgasse 9, 1030 Vienna, Austria.

In all eukaryotes, transition through the cell cycle is regulated by the activation of protein kinase complexes composed of catalytic cdk (cyclin-dependent kinase) and regulatory cyclin subunits. Protein kinase activity of these complexes is controlled at different checkpoints through the action of kinases and phosphatases and association with other regulatory proteins. In alfalfa, two cyclins, cyc1 and cyc2, were isolated that show homology to animal A and B type cyclins but transiently accumulate only at G2/M. Two novel types of cyclins were identified by either complementation of yeast G1 cyclins or suppression of pheromone-induced cell cycle arrest. Whereas cyc3 shows highest similarity to animal A and B type cyclins, cyc4 has highest homology to animal D type cyclins. Both cyclins show fluctuating transcript patterns in synchronously dividing cells. Whereas cyc3 is peaking at G1/S, cyc4 reaches maximal levels in S phase. Upon stimulation of quiescent cells, cyc3 mRNA levels rise severalfold within minutes. In agreement with other data, reentry into the cell cycle also stimulates cyc3 transcript levels but maximal levels are observed only after entry into S phase. Cdk kinase complexes have been identified that are active at the G1/S, S and G2/M transitions of alfalfa cells. Presently, antibodies against the different cdks and cyclins are used to study the composition and regulation of the complexes during the cell cycle. Dominant negative cyclin constructs have been transferred into alfalfa cells and into transgenic plants. Expression under an inducible promoter should reveal the effect of these genes in their natural context at different developmental stages and environmental conditions.

J6-010 HORMONAL INTERACTIONS WITH PLANT CELL PROLIFERATION CONTROLS, Peter C.L. John, Kerong Zhang, Ludger Diederich, Chongmei Dong, Plant Cell Biology, R.S.B.S., Australian National University, PO 475, Canberra City, ACT 2601, Australia.

Changes in $p34^{cdc2}$ level and activity are consistent with participation in control of the cessation[1] and resumption[2] of division under phytohormonal control, which allows cells to abstain from division and differentiate, or conversely to resume division in formation of lateral primordia, secondary thickening, or wound response. An indication that low $p34^{cdc2}$ relative to other proteins can enforce exit from the cell cycle was first seen in seedling wheat leaf where a fifteen fold decline in $p34^{cdc2}$ correlated with cell enlargement without division prior to differentiation into photosynthetic cells[1]. Auxin-induced division could only be elicited in wheat tissue that contained active $p34^{cdc2}$ histone H1 kinase[3,4]. In dicotyledonous tissues prior $p34^{cdc2}$ accumulation was necessary for resumption of cell division in phytohormone-stimulated cells of leaf[2], root[3] and stem pith[4]. In excised carrot cotyledon tissue fifteen fold elevation of $p34^{cdc2}$ levels, restoring the level to that in meristematic cells, preceded the resumption of proliferation in large photosynthetic cells. These changes are consistent with low $p34^{cdc2}$ being used by plants as an economical restraint of division in cells that are sufficiently large and active to divide but must switch to differentiation to allow organogenesis. In excised tobacco stem pith[5] auxin was capable of inducing $p34^{cdc2}$ -like protein but not division. In pith the additional presence of cytokinin was necessary for induction of catalytic activation of the $p34^{cdc2}$ kinase and cell proliferation. Cytokinin was specifically required for initiation of mitosis in suspension cultured *N. plumbaginifolia* cells, which in medium with 2,4-D but without cytokinin arrested in G2 phase and on supplementation with kinetin rapidly activated $p34^{cdc2}$ H1 histone kinase and entered nuclear division. We are using enzymes that can change $p34^{cdc2}$ phosphorylation *in vitro* and *in vivo* to investigate the mechanism of cytokinin activation. When division is hormonally terminated an additional control that involves removal of $p34^{cdc2}$ can operate. In pea root tissue stimulated by IAA to form lateral primordia[6] the addition of zeatin riboside caused rapid removal[3] of $p34^{cdc2}$ and sensitivity of the process to phenylmethylsulphonylfluoride indicates that the mechanism of $p34^{cdc2}$ decline involves proteolysis. Phytohormones may therefore exert effects on cell division during plant development by influencing $p34^{cdc2}$ accumulation, activation or breakdown.

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Signaling in Plant-Pathogen Interactions II

J6-011 CLONING AND CHARACTERIZATION OF THE *ARABIDOPSIS RPS2* DISEASE RESISTANCE GENE, Frederick M. Ausubel, Fumiaki Katagiri, Michael Mindrinos, Lynne Reuber, and Guo-Liang Yu, Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

We used a map-based cloning strategy to identify the *Arabidopsis thaliana RPS2* gene which corresponds to the *Pseudomonas syringae avrRpt2* gene. *RPS2* and *avrRpt2* display a typical "gene-for-gene" relationship. Fine-structure genetic mapping delimited the chromosome walk on the bottom of chromosome IV to a 35 kilobase region containing at least six transcribed genes. *RPS2* was identified by sequencing four independent *rps2* mutant alleles and by complementation of the *rps2* mutant phenotype using a transient assay for *RPS2* function that involves biolistic bombardment of *rps2* mutant leaves with *35S-RPS2* cDNA. The identification of *RPS2* was confirmed by complementation of the *rps2* phenotype in stably transformed plants. DNA sequence analysis of *RPS2* suggests that it encodes a 909 amino acid polypeptide with a relative molecular weight of 105 kilodaltons and a pI of 6.51. The C-terminal region of RPS2 protein contains 14 imperfect leucine-rich repeats (LRR). LRR motifs are present in many proteins and are thought to be involved in protein-protein interactions. The N-terminal portion of the RPS2 LRR is most closely related to the LRR of yeast adenylate cyclase CYR1. The N-terminal region of RPS2 contains a leucine zipper and domains predicted to constitute a nucleotide binding site (NBS). DNA blot analysis shows that *Arabidopsis* contains several DNA sequences that hybridize to *RPS2*, suggesting that there are several related genes in the *Arabidopsis* genome. RPS2 protein does not share similarity with the protein kinase encoded by the tomato *PTO* gene, another disease resistance gene that conforms to the gene-for-gene model. Instead, the overall structure of RPS2 protein, including the NBS and LRR motifs, is similar to that of the tobacco *N* gene product which confers resistance to tobacco mosaic virus (TMV). The overall similarity between the *Arabidopsis* RPS2 and tobacco N proteins suggests that there is a common or similar signal transduction pathway leading to the resistance response to both bacterial and viral pathogens. This is consistent with a model that postulates an array of related receptors that have evolved for the recognition of a wide variety of pathogens and is supported by our data which indicate the presence of a family of *RPS2*-related genes in *Arabidopsis*. We have used the mRNA differential display technique to identify genes that are specifically regulated by the signal transduction pathway containing *RPS2* by comparing RNA isolated from wild-type and *rps2* mutant plants following infection with *P. syringae* carrying *avrRpt2*. This analysis led to the identification of at least two previously unknown genes that are expressed in wild-type but not in *rps2* mutants. Interestingly, these two gene are not expressed in wild-type *Arabidopsis* plants following infection with *P. syringae* carrying the *avrRpm1* avirulence gene.

J6-012 *Cf*-DEPENDENT DEFENSE RESPONSES IN TOMATO INDUCED BY RACE-SPECIFIC ELICITORS OF THE TOMATO PATHOGEN *CLADOSPORIUM FULVUM*, **Pierre J.G.M. De Wit, Matthieu H.A.J. Joosten, Paul J.M.J. Vossen, Ton J. Cozijnsen, Guy Honée, Miriam Kooman-Gersmann & Ralph Vogelsang, Department of Phytopathology, Wageningen Agricultural University, Wageningen, The Netherlands.**

Host genotype specificity in the interaction between the biotrophic fungal pathogen *Cladosporium fulvum* and tomato complies with the gene-for-gene model. Success or failure of infection is determined by absence or presence of complementary genes, avirulence and resistance genes, in the pathogen and the host plant, respectively. Resistance, expressed by the induction of a hypersensitive response (HR) followed by other defence responses in the host, is envisaged to be based on recognition of the pathogen, mediated through (in)direct interaction between products of avirulence genes of the pathogen (the so-called race-specific elicitors) and receptors in the host plant, the putative products of resistance genes. Here we report on isolation, characterization and exploitation of the race-specific elicitors AVR4 and AVR9 of *C. fulvum*, respectively. The AVR9 elicitor is a 28 amino acid peptide which induces HR in tomato plants carrying the complementary resistance gene *Cf9*. The mature AVR4 elicitor is a peptide of 105 amino acids which induces HR in Cf4 genotypes of tomato. Plants transgenic for the *Avr9* gene are able to produce biologically active elicitor molecules. Based on this result the research group of Jonathan Jones, Norwich, has developed a strategy to successfully isolate the complementary resistance gene *Cf9* by transposon tagging. We and Mogen International, Leiden, are developing a strategy to obtain broad spectrum HR-based resistance in tomato plants which contain both the avirulence gene *Avr9* and the complementary resistance gene *Cf9* of which the first is under control of a pathogen-inducible promoter. Since the resistance gene *Cf9* has been cloned, now the so-called two-component system could also be tested in other crop plants. I^{125} labeled AVR9 peptide is presently used to isolate its receptor in tomato leaves. I^{125} -AVR9 binds preferentially to membranes indicating that its receptor resides in the (plasma)membrane. Surprisingly, binding occurs to the same extent to membranes of plants with or without the resistance gene *Cf9*. Also membranes of *Cf9*⁻ EMS-mutants which map at the *Cf9*-locus still show significant I^{125}-AVR9 binding. This might indicate that the cloned *Cf9*-resistance gene does not code for the AVR9 receptor. Possibly the AVR9 receptor, which occurs universally in tomato, interacts after elicitor binding with the product of the *Cf9* gene which is unique and required to activate the cascade of events leading to HR and HR-based resistance. In Cf9 genotypes the AVR9 peptide induces a number of responses including induction of active oxigen species, lipid peroxidation, ethylene, PR-proteins and opening of stomata. Some of these responses are also induced in Cf4 tomato genotypes treated with AVR4 peptide. Avoiding *Cf*-dependent resistance in tomato by *C. fulvum* is based on not producing an elicitor peptide (*Avr9*) or producing an altered version of a peptide elicitor (*Avr4*).

Signaling By Light I

J6-013 THE SIGNAL TRANSDUCTION PATHWAY FOR PHOTOTROPISM, Winslow R. Briggs[1], Emmanuel Liscum III[1], Paul W. Oeller[1], and Julie M. Palmer[2], [1]Department of Plant Biology, Carnegie Institution of Washington, 290 Panama St., Stanford, CA 94305, and [2]Department of Botany, University of Texas, Austin, TX 78713.

There is strong physiological and genetic evidence that a plasma membrane-associated protein that becomes phosphorylated in response to blue light plays an important role in phototropism in higher plants. Progress in purifying and characterizing this protein and the gene encoding the protein will be discussed. We currently have three classes of mutants in the phototropic pathway for *Arabidopsis thaliana*. The first class is defined by four independent alleles at a single locus, *nph1* (*n*on-*p*hototropic *h*ypocotyl). This class is deficient in or lacks phototropic responses, and is deficient in or lacks the phosphoprotein. The second class is defined by two separate loci, *nph2* and *nph3*, with a single *nph2* allele and three *nph3* alleles. This class has a normal amount of the phosphoprotein, wild-type levels of blue light-dependent phosphorylation, and shows normal gravitropism, but is deficient in or lacks normal phototropic responses. The third class of mutants is defined by two alleles at a single locus, *nph4*. This class of mutants shows normal levels of the phosphoprotein and normal blue light-dependent phosphorylation but are deficient in both phototropic and gravitropic responses. Current progress in characterizing these mutants will be described. A recent report suggests that a carotenoid, zeaxanthin, may be the photoreceptor for phototropism. However, evidence both from mutant and norflurazon-treated wild-type maize seedlings indicate that a carotenoid can not be the photoreceptor. Both blue light-dependent phosphorylation and phototropic responsivity are normal in the absence of detectable carotenoids. Evidence implicating other potential chromophores will be discussed.

J6-014 SIGNAL TRANSDUCTION PATHWAYS CONTROLLING LIGHT-REGULATED DEVELOPMENT IN ARABIDOPSIS, Joanne Chory, Kim Cook, Richard Dixon[1], Tedd Elich, Enrique Lopez, Hsou-min Li[1], Nobuyoshi Mochizuki, Punita Nagpal, Alan Pepper, Daniel Poole, and Jason Reed. Plant Biology Laboratory, The Salk Institute, La Jolla, CA 92037 and [1]The Samuel Roberts Noble Foundation, Ardmore, OK 73402.

We are taking a combined genetic and molecular biological approach to identify components of the light signal transduction pathways. Our studies have identified a class of Arabidopsis mutants that show many characteristics of light-grown plants even when grown in complete darkness (*de-etiolated*). Because these mutations are both pleiotropic and recessive, we have hypothesized that *DET* genes play a negative regulatory role in photoregulated gene expression and leaf and chloroplast development in Arabidopsis. To determine the molecular mechanisms of light signal transduction, we are cloning the various *DET* genes by the method of chromosome walking. *DET1* encodes a nuclear-localized protein that appears to be ubiquitously expressed during Arabidopsis development. We are testing the idea that DET1 acts to repress the expression of light-regulated promoters by interactions with other nuclear proteins. In a separate set of experiments, we have identified mutations in two of the 5 Arabidopsis phytochrome apoprotein genes. Our results suggest that phytochrome B plays a general role in cell elongation in response to light and also controls flowering time and chlorophyll accumulation. In contrast, phytochrome A appears to play a highly specialized role in Arabidopsis development. The phenotype of *phyA phyB* doubly null mutants, however, indicates that for some processes PHYA and PHYB appear to have complementary functions. We are continuing our studies on phytochrome signaling by identifying and characterizing suppressors of *phyB* mutations. Lastly, we have utilized a genetic approach that focuses on one particular downstram light-regulated response, the transcription of the *CAB3* promoter. Using transgenic lines, we have identified mutations that define at least 5 complementation groups in which the *CAB3* promoter is expressed at aberrant high levels in the dark or low levels in the light. Some of the mutations also affect the levels of *RBCS* mRNAs, while others affect only *CAB*. We used the same transgenic lines to identify mutations in 3 genes defining an intracellular signal transduction pathway from the chloroplast to the nucleus. Presumably, this pathway functions to coordinate the expression of nuclear and plastid-encoded genes involved in photosynthesis. The mutations also impede the early steps in the transition from etioplast to chloroplast. The results from epistasis studies suggest a hierarchical regulatory network among the various genes in the control of light-regulated responses.

Signaling By Light II

J6-015 PHYTOCHROME PHOTOTRANSDUCTION PATHWAYS: BIOCHEMICAL AND GENETIC DISSECTIONS, Chris Bowler, Hiroshi Yamagata, Gunther Neuhaus*, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399; *Institute for Pflanzenwissenschaften, ETH-Zurich, Universitatstrasse 2, CH-8092 Zurich, Switzerland.

Three signal transduction pathways, dependent upon cGMP and/or calcium, are utilized by phytochrome to control the expression of genes required for chloroplast development and anthocyanin biosynthesis in plant cells. For example, *chs* is controlled by a cGMP-dependent pathway, *cab* is controlled by a calcium-dependent pathway, and *fnr* is regulated by a pathway that requires both cGMP and calcium. Using a soybean photomixotrophic cell culture and microinjection into the cells of a phytochrome-deficient tomato mutant, we have studied the regulatory mechanisms acting within and between these three signalling pathways. We provide evidence that changes in cGMP levels mediate the observed induction and desensitization of *chs* gene expression in response to light and demonstrate that high cGMP concentrations cause negative regulation of both the calcium- and the calcium/cGMP-dependent pathways. Conversely, high activity of the calcium-dependent pathway can negatively regulate the cGMP-dependent pathway. We have termed these opposing regulatory mechanisms "reciprocal control". In all cases, the molecules that are involved appear to be downstream components of the signal transduction pathways, rather than calcium and cGMP themselves. Furthermore, we have found that the calcium/cGMP-dependent pathway has a lower requirement for cGMP than does the cGMP-dependent pathway. The role of these phenomena in the regulation of plant photoresponses will be discussed.

J6-016 THE SIGNALING MECHANISM MEDIATING LIGHT CONTROL OF ARABIDOPSIS SEEDLING MORPHOGENESIS, **Xing-Wang Deng**, Lay-hong Ang, Daniel A. Chamovitz, Shing Kwok, Timothy W. McNellis, Ning Wei, and Albrecht G. von Arnim, Department of Biology, Yale University, New Haven, CT06520.

A combinatorial approach has been taken to dissect the largely unknown signaling process responsible for the light control of Arabidopsis seedling development. Although a great deal is known about the photoreceptors that perceive the light signals and the developmental processes which are regulated by light, little is known about how the light signals are transduced and integrated to mediate plant growth and development. Started with a systematic genetic screen of mutants which exhibit constitutive photomorphogenic (*cop*) seedling phenotypes in darkness, fifteen genetic loci (*COP1* to *COP15*) have been identified and characterized in detail. Our genetic studies have led to a working model in which light signals perceived by multiple photoreceptors converged to a common signaling cascade to control the primary commitment for the two alternative programs of seedling development: photomorphogenesis or skotomorphogenesis. The gene products encoded by several of our *COP* genes, including *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, *COP12*, *COP13*, *COP14*, and *COP15* are likely involved in this common signaling cascade. We are pursuing several additional genetic approaches in an effort to identify additional components in the common cascade as well as to elucidate the regulatory relationships of those genes.

In our effort to understand the molecular and cellular mechanism of light signaling, we have succeeded in molecular cloning of two key genes, *COP1* and *COP9*, and several of their putative interactive partner genes. While *COP9* encodes a completely novel protein, *COP1* encodes a protein with putative DNA-binding Zn-finger motif, a coiled-coil domain, and a domain homologous to the beta-subunit of the trimeric G-proteins. With the molecular and biochemical tools available, we have been able to investigate the molecular and cellular features of light signaling, such as the specific post-translational mechanisms responsible for light modulation of COP1 or COP9, how COP1 and COP9 modulate their immediately downstream steps, and how light signals perceived in the cytoplasm are used to turn on genes in the nucleus. For example, we recently have demonstrated that Arabidopsis COP1 acts in the nucleus as a autonomous, light-inactivable repressor of photomorphogenic development and light inactivation of COP1 involves a cell-type specific regulation of its nucleocytoplasmic trafficking. In addition, we have shown that COP9, together with COP8 and COP11, are likely components of a novel signaling complex mediated light control of plant development. The possible involvement of this novel signaling complex in the light control of COP1 nucleocytoplasmic trafficking is currently under investigation.

J6-017 PHOTOREGULATION OF CHALCONE SYNTHASE: A MODEL SYSTEM TO ANALYSE SIGNAL TRANSDUCTION IN PHOTOMORPHOGENESIS, Eberhard Schäfer, Biological Institute II, University of Freiburg, Schänzlestrasse 1, D-79211 Freiburg, Germany

Anthocyanin as a visible marker has been used extensively to study signal transduction and developmental regulation in photomorphogenesis. We have, therefore, selected chalcone synthase - a key enzyme of flavonoid biosynthesis - as a tool to investigate the temporal and spatial pattern of gene expression as well as the light-mediated signal transduction.
The promotor of mustard chs 1 gene was fused to the bacterial uid A gene and transferred via Agrobacterium to *Arabidopsis*, tobacco and mustard. The observed photoregulation and tissue-specific expressions are different in the tree species and the transgene always followed the expression pattern of the homologous gene of the acceptor plant.
A promotor analysis yielded that an element conserved in chs genes (called unit 1) is necessary and sufficient to confer the correct photoregulation and tissue-specific expression pattern.
Gel retardation assays revealed that the unit 1 binds both b-zip proteins and myb proteins. Using a parsley cell suspension culture we could demonstrate that myb proteins are alost exclusively localized in the nucleus whereas b-zip proteins of the GBF, CPRF class are localized both in the cytoplasm and in the nucleus. The DNA binding activity of b-zip proteins is modified by phosphorylation. The phosphorylation/dephosphorylation can occur in the cytoplasm and is modified by light treatments. A newly developed in vitro cytoplasm nucleus transport system allowed to demonstrate that GBFs are transported from the cytoplasm to the nucleus in a light-dependent manner. Research is in progress to analyse localization and light-dependent transport of individual members of the b-zip protein family. Using a yeast expression system has, in addition, the advantage of analyzing the localization and transactivation of individual members of these factors.

Cell-Cell Communication (Joint)

J6-018 HOST CELL RESPONSES TO INVASION BY *RHIZOBIUM* DURING PEA NODULE DEVELOPMENT

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Rhizobium is a soil microbe that metamorphoses into a nitrogen-fixing endosymbiont. This transformation is achieved as a result of a network of interactive signals and responses between bacteria and host plant cells. There are three phases of interaction: pre-infection signalling; the establishment of *Rhizobium* as an intercellular endophyte; and an intracytoplasmic phase where the capacity for biological nitrogen fixation is progressively developed. At each stage, both positive and negative signalling elements are recognizable. The nature and specificity of cell-signalling is related to topological position. At the pre-infection stage, nodule induction is the consequence of the secretion of specific flavonoid molecules which can act as inducers (or anti-inducers) of the lipochito-oligosaccharide (LCO) biosynthetic pathway encoded by *Rhizobium nod* genes. In the appropriate host legume, LCO's specifically stimulate root cortical cell division and organogenesis, apparently by modifying cell-cycle control and the relative concentrations of plant growth regulators. Further development of nodule primordia is regulated by ethylene and other systemically acting feedback control systems. Tissue invasion by *Rhizobium* depends on reorientation of plant cell wall growth, forming an infection thread under the control of the cytoskeleton. Bacteria are embedded in a plant intercellular matrix that may serve as a potential antimicrobial system, being capable of conversion from a fluid to a solid phase following oxidative crosslinking of glycoproteins. This cross-linking could occur if non-host rhizobia elicit host defence responses that probably induce an oxidative burst - the sudden and local release of hydrogen peroxide. In order to avoid eliciting this host defence response, the bacteria apparently need to have appropriate cell wall components, e.g., acidic extracellular polysaccharide which complexes Ca^{++} ions, and lipopolysaccharide which may have both a surface-masking effect based on the O-antigen component and a host-elicitor function based on the core polysaccharide component. Following endocytosis into the host cytoplasm, the bacteroids take on organelle-like status, being individually enclosed by a plant-derived peribacteroid membrane. Differentiation of bacteroids is then controlled by host plant cell physiology, e.g. the supply of oxygen in a microaerobic niche, and by regulation of metabolic exchanges across the peribacteroid membrane. During differentiation of the "symbiosome" compartment, the composition of the peribacteroid membrane gradually changes from that of plasma membrane to a hybrid of this with tonoplast membrane. Genetic, immunological and biochemical analysis of peribacteroid membrane differentiation and vesicle targeting is currently being investigated. Components of the peribacteroid fluid, for example a nodule-specific lectin and a nodule-specific thiolprotease may be involved in the control of bacteroid senescence.

J6-019 PLASMODESMATA, MACROMOLECULAR TRAFFICKING AND THE SUPRACELLULAR NATURE OF PLANTS, William J. Lucas, Section of Plant Biology, Div. of Biol. Sci., University of California, CA 95616.

Plasmodesmata form an important symplasmic pathway for cell-to-cell communication in higher plants (1, 2). The formation and complex structure of primary and secondary plasmodesmata (3, 4) will be discussed in terms of the creation of specialized cytoplasmic microchannels that permit the coordination of physiological and developmental processes at the tissue (2), organ and whole-plant level (4). These microchannels permit the cell-to-cell exchange of small molecules, such as metabolic intermediates and hormones, as well as various ions; this process is driven by diffusion (1). In addition, we now know that plasmodesmata have the capacity to engage in macromolecular trafficking of proteins as well as nucleic acids (see 5); this transport function appears to be highly effective, in terms of the macromolecules that can move from cell-to-cell, and the overall process appears to involve an active, energy-dependent, step. This new aspect of plasmodesmal biology will be discussed in terms of our molecular and cellular studies on viral and endogenous plasmodesmal movement (transport) proteins (6, 7, 8). These studies provide the experimental foundation for the hypothesis that, in higher plants, plasmodesmal trafficking of macromolecules plays a central role in coordinating plant growth and development (4). In our model, nuclear pore transport and plasmodesmal transport function in concert, to establish a unique system that allows plants to regulate their developmental and physiological functions at a supracellular rather than a multicellular level (2, 5).

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Stress Signaling

J6-020 CELL SIGNALLING IN THE WOUND RESPONSE OF TOMATO PLANTS, Dianna Bowles, Caroline Calvert, Philip O'Donnell, The Plant Laboratory, Department of Biology, University of York, YO1 5DD, UK.

Injury to a leaf of a tomato plant leads to changes in gene expression within the lamina of the wounded leaf and elsewhere throughout the plant. Living cells in the vicinity of the damage will respond to local signals released by the dead and dying tissue, whereas cells in distant leaves will respond to systemic, long-range signals that communicate the wound-induced events to the rest of the plant. There is evidence to indicate the local and systemic signals can be distinguished from one another and that events within the lamina of the wounded leaf are different from those in the distant unwounded leaf.

Gene markers to study these two responses include those encoding proteinase inhibitors, and those encoding enzymes involved in the biosynthesis of "wound" ethylene. We have been using the markers, together with gene products, such as the annexins, implicated in calcium- and phospholipid-mediated transduction, to study the sequence of events that link the wound stimulus to gene activation at the local and systemic sites. Recent results will be described.

J6-021 THE *IN VITRO* AND *IN VIVO* PHOSPHORYLATION OF THE PLASMA MEMBRANE PROTEIN PP34 IN RESPONSE TO OLIGOGALACTURONIDE DEFENSE SIGNALS. Edward E. Farmer, Kalanathee Paul, Suzanne Grünberger, Béatrice Kunz and Philippe Reymond : Institut de biologie et de physiologie végétales, Université de Lausanne, 1015 Lausanne, Switzerland.

Several classes of regulatory molecules exist in the angiosperm cell wall. Amongst these are the oligogalacturonides, 1,4-linked polymers of α-D-galacturonic acid. These polyanions trigger numerous defense responses in plants. We recently suggested that only a small spectrum of biologically active oligogalacturonides (OGA) are known due to the presence of the plant cell wall which may act as a barrier to exclude exogenous molecules of higher molecular weight. In contrast, naked plasma membranes can interact with a large range of higher pectic oligomers which stimulate the phosphorylation of a ~ 34 kDa protein (pp 34). The OGA stimulated-phosphorylation of pp34 is found throughout the vegetative tissues of tomato and in other distantly related dicotyledons. *In vivo*, pp34 exists as a phosphoprotein. Experiments to study the effect of exogenous OGAs and of pectic elicitors generated *in muro* on the *in vivo* phosphorylation of pp34 are underway. Additionally we are subfractionating a polygalacturonate-rich fraction of tomato leaf cell walls. Certain fractions contain molecules capable of stimulating pp34 thiophosphorylation *in vitro* more efficiently than purified oligogalacturonides. We wish to investigate whether a modified oligogalacturonide is responsible for this enhanced pp34 thiophosphorylation. The potential correlation of pp34 phosphorylation and defense gene expression is under investigation as are efforts to clone a pp34 cDNA.

Second Messengers and Phosphorylation

J6-022 CONTROL OF PLANT ENZYME ACTIVITY BY REVERSIBLE PROTEIN PHOSPHORYLATION, Joan L. Huber[1], Robert W. McMichael, Jr.[2], Markus Bachmann[2], and Steven C. Huber[2], [1]Department of Horticultural Science, and [2]USDA/ARS and Department of Crop Science, NC State University, Raleigh, NC 27695-7631.

Light is an important external "signal" influencing the phosphorylation status of leaf phosphoproteins in several compartments of the cell. The activities of several cytosolic enzymes, including phosphoenolpyruvate carboxylase, NADH:nitrate reductase (NR) and sucrose-phosphate synthase (SPS), are controlled via phosphorylation in response to light/dark signals such that the enzymes are activated in the light. The complex mechanisms for regulation of SPS and NR activity have poised these enzymes for pivotal roles in the control of photosynthetic carbon flow into sucrose and the assimilation of nitrogen, respectively. While both SPS and NR are activated (dephosphorylated) in the light and inactivated (phosphorylated) in the dark, it is clear that the phosphorylation process is mediated by different interconverting enzymes, the protein kinases and phosphatases, which are subject to distinct control mechanisms. The occurrence of spinach SPS phosphorylation in the dark on ser158 (ser162 in maize) has been confirmed by mutational analysis and is mediated by a ser/thr specific protein kinase (PK) predicted to be a homodimer with a native molecular weight of about 150 kDA. SPS-PK activity is sensitive to inhibition by hexose-P, primarily G-6-P, and is little affected by other metabolic intermediates of carbon and nitrogen metabolism. The regulatory seryl residue on the spinach NR protein has not yet been identified. The current model for reversible phosphorylation of NR includes several novel features which distinguish it from SPS protein phosphorylation. The NR-specific ser/thr protein kinase activity appears to be sensitive to a wide variety of glycolytic intermediates. Importantly, phosphorylation alone of NR via protein kinase activity is not sufficient to inactivate NR. An additional factor called the "inhibitor protein" (native molecular weight of about 70 kDA) is required for the inactivation of phospho-NR and is thought to bind to NR based on the dilution kinetics for inactivation. Further, interconversion of phospho-NR between two conformational states which differ in enzymatic activity may be modulated by 5'-AMP. Work continues on the purification of NR-PK and the inhibitor protein as well as the protein phosphatases for SPS and NR in an effort to elucidate the elements of the light-dependent signal transduction pathway that mediates the regulation of these, and perhaps other, cytosolic enzymes. Overall, the models for regulatory phosphorylation of SPS and NR offer new insights into the control and coordination of carbon and nitrogen metabolism.

J6-023 TARGETING OF PROTEINS TO THE PLANT NUCLEUS, Natasha V. Raikhel, Glenn R. Hicks, Stephane Lobreaux, Mark Shieh, Antje Heese-Peck, Harley Smith, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.

The import of proteins into the nucleus is an essential process that has received little attention in plants. In animals, this process involves two steps: nuclear localization signal (NLS) recognition and translocation through the nuclear pore complex (NPC). To characterize NLSs, we have identified targeting signals within the plant transcription factors Opaque2 (O2) and R. To identify factors involved in NLS recognition, radiolabeled peptides to two classes of NLSs, a bipartite signal from O2 and the NLS from the SV40 large T-antigen, were allowed to associate with purified tobacco and maize nuclei. The functional NLSs, which stimulate import in plant cells in vivo, competed for a single low affinity binding site, whereas mutant NLSs, which are inefficient in vivo, were poor competitors. A third class of NLSs, a Mat α-2 like signal found within the R protein, was also shown to compete for the same binding site. Biochemical and localization studies indicate that the binding site is located at the nuclear envelope and NPC. By the use of crosslinking reagents and the radiolabeled O2 NLS peptide under conditions similar to those used for in vitro binding, we have labeled two NLS binding proteins (NBPs) of 50 to 60 kDa and at least two NBPs of 30 to 40 kDa. The biochemistry and affinity of the NBPs indicates that they constitute the NPC binding site. This suggests that some components of NLS recognition reside at the NPC in plants. We are currently identifying NPC proteins in plants in order to purify NBPs and examine the nature of this complex, yet poorly understood structure. Wheat germ aggutinin (WGA), a lectin that specifically binds to *N*-acetylglucosamine (GlcNAc), inhibits the transport of proteins through the NPC in vertebrates suggesting that NPC proteins modified by GlcNAc are involved in import. By EM localization, WGA protein blot analysis and a galactosyltransferase assay, we have shown that plant NPCs also contain proteins modified by GlcNAc. Like vertebrate NPC proteins, the sugars were bound via an O-linkage as demonstrated by β-elimination. Interestingly, unlike vertebrate proteins, which are modified by a single O-linked GlcNAc, plant nuclear proteins contained oligosaccharides that consisted of five or more saccharides. To identify other factors involved in import and functionally test purified NBPs, we developed an in vitro import system using evacuolated protoplasts from cultured cells. In this system, we selectively permeabilize the plasmalemma but not the nuclear envelope. Thus, fluorescent import substrates can be introduced and monitored for nuclear accumulation using fluorescence microscopy. Overall, this combination of approaches will permit us to identify and functionally examine all components of the nuclear import apparatus of plants.

Hormonal Signaling

J6-100 **AUXIN AND HYDROGEN ION CONTROL OF ETHYLENE INDUCED LATERAL GROWTH,** Grant M. Barkley and Kimberly A. Booth, Department of Biological Sciences, Kent State University, Warren, OH 44483.

Induction of lateral growth by ethylene [1-3 µl/l] in epicotyl tissue of *Pisum sativum* involves sequential and complex biochemical changes. Ethylene, within the first hour after application, begins to inhibit normal vertical growth in favor of epicotyl swelling or lateral growth. Lateral growth begins within 150 minutes and reaches a sustained rate of growth of .4-.6 µm per minute after 3 hours. Both the decline in elongation and the swelling response are controlled by coordinated effects of auxin concentration and cell wall acidification. Other changes include increased synthesis of cell wall material, microtubule reorientation, modification of H+ pumping along with increases in rough endoplasmic reticulum and dictyosome formation. Lateral growth is inhibited by high pH buffers and inhibitors of proton pumping. Exogenous auxin, when given to stem tissue in concentrations of 10^{-5} to 10^{-3} M show a 30 minute transition to a new sustained rate of lateral growth, without typical latent period. High auxin concentrations, apparently, can stimulate lateral growth by mechanisms other than involvement with proton pumping. The effects of 2,6-dichlorobenzonitrile, as an inhibitor of cellulose synthesis, and calcium on epicotyl lateral growth will also be examined.

J6-101 **TONIC CONTROL AND ABSCISIC ACID-SENSITIVITY OF K^+ CHANNELS IS MEDIATED BY PROTEIN PHOSPHORYLATION IN GUARD CELLS**
Michael R. Blatt[1], Jerome Giraudat[2], Jeff Leung[2], Fiona Armstrong[1], and Alexander Grabov[1]; [1]Biological Sciences, University of London, Wye College, Wye, Kent TN25 5AH England; and [2]CNRS Institut Sciences Vegetales, Batiment 23, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France.

Regulation of stomatal aperture in response to exogenous as well as endogenous signals is a highly concerted process and depends on coordinate modulation of the several transporters, including two distinct K^+ channels, at the guard cell plasma membrane[1]. Studies with the plant water-stress hormone abscisic acid, (ABA) which closes stomata, have shown that ABA modulates at least three distinct channel activities which are linked to cytoplasmic alkalinization and/or to a rise in $[Ca^{2+}]_i$, the latter probably coupled through inositol-1,4,5-trisphosphate-mediated Ca^{2+}release from intracellular stores. Molecular genetic analyses have now added another dimension, identifying the *abi-1* mutant with a lesion in a novel protein phosphatase[2,3] and supporting the view that protein phosphorylation contributes to ABA-evoked transport regulation. We report that the *ABI-1* gene product confers ABA-insensitivity, and the characteristic wilty phenotype, on transgenic tobacco. Voltage clamp analyses demonstrate that the transgene is associated with a reduction in the activity of one class of K^+ channels that mediate guard cell K^+ loss, and with a loss of ABA response in both K^+ channels. Furthermore, K^+ channel sensitivity to the hormone is recovered with protein kinase antagonists. These, and additional observations point to a role for protein phosphorylation in long-term tonic control of channel activity.

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J6-102 DIFFERENT SIGNALS CONTROL THE INITIATION AND MAINTENANCE OF SEED MATURATION SPECIFIC GENE EXPRESSION IN TOBACCO, Mauricio M. Bustos and Steven J. Gagliardi, Department of Biological Sciences, UMBC, Baltimore, MD, 21228-5398.

Hormonal and nutritional signals required for initiation and maintenance of seed maturation are being studied in tobacco. F2 embryos harboring a GUS gene driven by a maturation (*Mat*) specific promoter (β-phaseolin) were dissected at various stages of development and cultured in liquid media in the presence of ABA (0-10 µM) and mannitol (0-10%). After a 3 to 7-day period of incubation at 28 C in the dark, materials were scored for % germination, size, total protein and starch, GUS expression (enzymatic activity and mRNA) and expression of endogenous markers for maturation (12 S globulin), post-abscision (dehydrin) and germination (malate synthase). In the absence of ABA and mannitol, pre-maturation (11 DAF) embryos germinated at high frequency (100%) and there was no induction of *Mat* expression. Either ABA or high osmoticum (10% mannitol) prevented germination. Only ABA (but not osmoticum) induced *de novo Mat* expression (e.g. GUS), yielding a 300- to 400-fold increase relative to untreated controls. However, *Mat* expression was concomitant with changes characteristic of germination, such as an increase in embryo size, enlargement of the shoot apical meristem, production of leaf primordia and vascular differentiation. These changes were reminiscent of precocious germination in viviparous mutants. Although ABA treatment of maturation-stage embryos (already expressing GUS at the time of excision) repressed germination, it was insufficient to maintain *Mat* gene expression. Therefore, a different signal transduction pathway, possibly requiring attachment to the plant, appears to be involved in maintaining the maturation program after it has been initiated by ABA.

J6-103 **INITIAL MOLECULAR GENETIC ANALYSIS OF THE SIGNALS REGULATING SENESCENCE AND "REJUVENATION" IN SOYBEAN COTYLEDONS** Joel M. Chandlee, Normand E. Allaire and William R. Krul, University of Rhode Island, Dept. of Plant Sciences, Kingston, RI 02881

Senescence is an important developmental process in plants that eventually leads to whole plant, organ, tissue and cell death through endogenously controlled degenerative processes. Understanding senescence at the molecular level should provide fundamental information about plant and cell differentiation and the regulation of cellular events through the action of plant hormones and other signals. Soybean cotyledons usually proceed through senescence and abscission by 16-20d after germination. Symptoms of senescence can be reversed by surgical removal of the apical meristem leading to regreening or "rejuvenation" of the cotyledons. The point at which regreening can no longer be induced by removal of the epicotyl is referred to as "the point of no return, (PONR)" and it occurs around 16d after germination under defined growth conditions. The soybean cotyledon system exhibits regulated changes in gene expression during the processes of senescence and "rejuvenation". An analysis of gene products regulated during both phases of development has been initiated through the production and subsequent screening of cDNA libraries using a differential hybridization protocol based on PCR methods. Specific clones representing up-regulated genes during the developmental processes of senescence and "rejuvenation" have been isolated and initially characterized. In addition, a collection of identified cloned genes from a variety of plants including Arabidopsis, avocado, barley, corn, soybean and tomato have been used as probes on dot blots and Northern blots to determine their patterns of expression during the processes of senescence and "rejuvenation". The probes were selected because of their probable involvement in the cellular and physiological processes occurring during these two developmental phases. Those which display patterns of differential regulation during senescence and "rejuvenation" are to be used in subsequent studies to analyse more precisely the regulation of the genes and eventually the internal regulatory signals involved.

J6-104 BRASSINOSTEROIDS AS SIGNALS IN PLANT GROWTH AND DEVELOPMENT, Steven D. Clouse*, Mark Langford, Trevor C. McMorris, *Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Brassinosteroids are plant growth-promoting natural products with structural similarities to insect and mammalian steroid hormones. The wide distribution of brassinosteroids in the plant kingdom, their marked effect on cell proliferation and elongation at nanomolar levels, and their interactions with other plant hormones, suggest that these compounds play a role as endogenous plant-growth regulators. We have shown that brassinosteroids regulate gene expression in elongating soybean[1] and *Arabidopsis*[2] stems. In order to investigate brassinosteroids as signals influencing plant growth and development, we have identified mutants in *Arabidopsis thaliana* that are insensitive to brassinosteroid action. Inhibition of root elongation by exogenous hormones is a common response in wild-type *Arabidopsis* seedlings, with auxin, ethylene, cytokinin and methyl jasmonate all showing this effect. We found that 10^{-7} M 24-epibrassinolide (a synthetic brassinosteroid) caused approximately 60% inhibition of root elongation in the Columbia ecotype of *Arabidopsis*. EMS-mutagenized seeds of *Arabidopsis* were screened for their ability to elongate roots in the presence of this concentration of brassinosteroid and several putative brassinosteroid-insensitive mutants were identified. One such mutant, BRI-1, has an extremely dwarf phenotype, with loss of apical dominance and almost complete male sterility. Based on the root elongation assay, BRI-1 is insensitive to brassinosteroids but retains complete sensitivity to auxins, cytokinins, and gibberellins. Properties of BRI-1 and its use in dissecting the brassinosteroid signal transduction pathway will be discussed.

1. D.M. Zurek, S. D. Clouse. 1994. Molecular Cloning and Characterization of a Brassinosteroid-Regulated Gene from Elongating Soybean Epicotyls. Plant Physiol 104:161

2. S.D. Clouse, A.F. Hall, M. Langford, T.C. McMorris, M.E. Baker. 1993. Physiological and Molecular Effects of Brassinosteroids on *Arabidopsis thaliana*. J Plant Growth Regul 12:61

J6-105 A G-PROTEIN IS ONE OF THE TRANSDUCING ELEMENTS DOWNSTREAM OF THE AUXIN BINDING PROTEIN, D. Alex Groarke and Paul A. Millner, Department of Biochemistry and Molecular Biology, University of Leeds, LS2 9JT, UK.

One route for the action of auxins on plant cells is postulated to be via a surface localised auxin binding protein (ABP). Since ABP is known to be external to the cell an ABP associated membrane protein has been sought. The C-terminus of *Zm*ABP1 was predicted to be solvent exposed and to offer a likely region of molecular interaction with other proteins. Evidence for the proposed involvement of ABP in signal transduction is shown by the effect of peptides corresponding to the *Zm*ABP1 C-terminus on G-protein activity. Micromolecular concentrations of the peptides A6.1 (DEDCFEAAKDEL) and A6.2 (PFVWDEDCFEAAKDEL) stimulated GTPγS binding to isolated *Zea* microsomal membranes. However, no such activation was seen with peptides A1 to A5 which corresponded to solvent exposed regions of the ABP elsewhere within its sequence. Peptide A6.1n (AAEFCDEDKDEL), which retained the C-terminal KDEL sequence but had the subsequent N-terminal eight residues inverted in sequence compared to A6.1, was ineffective whilst peptide A6.3 which lacked KDEL did stimulate GTPγS binding. *In vivo*, peptide A6.1 has previously been shown to inhibit inward K^+ channels in intact *Vicia* guard cells, a result reinforced by parallel ion imaging studies.

In the above work, GTPγS binding showed considerable variability and it was hypothesised that the G-protein present in this signal transduction pathway may be loosely associated with the membrane and easily lost on membrane isolation. To test this, microsomal membrane preparations were washed with 1.0 M NaCl to bring about the release of loosely attached G-proteins. Size fractionation via gel permeation chromatography and subsequent dye affinity chromatography of the 50 kDa to 30 kDa fraction on Affi-Gel Blue allowed identification of two polypeptides which were recognised by two distinct antibodies directed against Gα subunits. GTPγS binding studies showed that fractions containing immunologically detectable GPα1 or Gαo homologues were able to restore peptide A6.2 dependent binding to the washed microsomal membranes.

J6-106 DIFFERENTIAL RESPONSE OF MAIZE ANTIOXIDANT DEFENSE GENE CATALASES AND SUPEROXIDE DISMUTASES TO SALICYLIC ACID, Lingqiang Guan and John G. Scandalios, Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695-7614

The dose response of the catalase (*Cat*) and superoxide dismutase (*Sod*) genes to salicylic acid (SA) was examined in the scutella of maize during embryo development and during post-imbibition. In 28 days post-pollination scutella, changes in *Cat1* and *Cat2* transcripts as well as in total catalase activity were observed. Total catalase activity was increased about five to seven fold following 1 mM SA treatment. The *Cat2* transcript and CAT-2 protein increased dramatically following a 24 hr treatment of 1 mM SA. CAT-1 activity also increased while no obvious changes in *Cat1* transcript were observed. *Cat3* transcript and activity decreased with the SA treatment. At higher doses of SA (4 and 5 mM) both total catalase activity and transcript levels decreased. In the CAT-2 null mutant, increasing levels of CAT-1 activity following 1 mM SA treatment at 28 days post-pollination compensate for the absence of CAT-2. In post-imbibition scutella, similar changes in total catalase activity were observed; however, transcript accumulation for *Cat1* and *Cat2* was found to be the opposite from that observed for these transcripts during the post-pollination period. During this period, the *Cat1* transcript increased dramatically with 0.5 and 1 mM SA for 24 hrs, while the *Cat2* transcript failed to accumulate following the SA treatment. The response of the different *Cat* genes to SA is developmental stage dependent and each may be regulated at different levels of gene expression. Post-translational regulation may account for the increase in *Cat1* expression in developing embryos and the increase in *Cat2* expression in mature embryos, whereas, the regulation of *Cat2* at 28 days post-pollination may be transcriptional. Differential responses were also observed for the different SOD isozymes after SA treatment. The effect of SA on catalase activity in leaves as well as on purified CAT-2 protein *in vitro* was also examined.

J6-107 **ABSCISIC ACID-INDUCTION OF GENE-EXPRESSION POSSIBLY INVOLVES PROTEIN PHOSPHATASE ACTIVITY**, Heimovaara-Dijkstra S., Nieland T., Van der Meulen R., Wang M. Centre for Phytotechnology RUL/TNO, Department of Plant Molecular Biology, Wassenaarseweg 64, 2333 AL Leiden, the Netherlands

It is generally accepted that protein phosphorylation/dephosphorylation plays an important role in signal transduction cascades. Evidence is now accumulating that for plants the same holds true. To study the role of (de)phosphorylation in ABA signal transduction, we investigated the effect compounds which were reported to inhibit phosphatase action had on ABA-induced gene-expression. Three of these inhibitors: phenylarside oxide (PAO), Calyculin A (CA) and Okadoic Acid (OA) appeared to inhibit ABA-induced Rab gene-expression. PAO brought about complete inhibition, while CA and OA only caused partial inhibition of Rab gene-expression. ABA-unsensitive expression (GAPDH) was not affected by these compounds.

The same three inhibitors were shown to bring about rapid hyperphosphorylation of two approx. 40 kDa proteins, present in the membrane-bound fraction of barley aleurone. Again, the induction of hyperphosphorylation was most effectively affected by PAO, while CA and OA had a less pronounced effect. Possibly these compounds affect different mechanisms in the ABA-signal transduction pathway. Other phosphatase inhibitors we used had no visible effect on either Rab-gene expression nor on the phosphorylation status of barley aleurone proteins in our experimental conditions.

The hyperphosphorylation of the two 40 kDa proteins brought about by PAO coincided with an increase of tyrosine-phosphorylation of two 40 kDa proteins with different pI, as determined with anti phosphotyrosine antibodies. This is consistent with the proposed activity of PAO as a protein tyrosine phosphatase inhibitor (Garcia-Moralez *et al.*, PNAS USA 87: 9255-9259, 1990). A possible role of (tyrosine) activity in ABA-induced gene-expression was further investigated.

J6-108 STRUCTURE AND FUNCTION OF PLANT LEGINSULIN AND LEGINSULIN-BINDING PROTEIN, Hisashi Hirano, Setsuko Komatsu, Yoshihiro Watanabe, Sergei F. Barbashov, Naoko K. Nishizawa*, Hideyuki Kajiwara and Hideji Karibe, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305 and *University of Tokyo, Bunkyo, Tokyo, 113 Japan.

The soybean seed basic 7S globulin named leginsulin-binding protein (LBP) is capable of binding bovine insulin and insulin-like growth factors. Although no amino acid sequence similarity was found between LBP and the animal insulin or insulin-like growth factor receptor, LBP showed structural similarities to the insulin receptor in glycosylation, the presence of a cysteine-rich domain and disulfide-bonded α and β subunits. Both proteins are also synthesized as larger precursor polypeptides which are post-translationally cleaved at the *N*-terminal side of a serine residue to generate α and β subunits. In LBP, we found a consensus sequence for a nucleotide-binding site, indispensable for protein phosphorylation, and a protein kinase activity which corresponds to about two thirds of the tyrosine kinase activity of the rat insulin receptor. Furthermore, it was shown by immunocytochemistry that LBP is localized in the middle lamella of cell walls and the plasma membranes. These results suggest that LBP may have insulin receptor-like functions. The LBP-like proteins were found in seeds and/or roots of several plant species like cowpea, French bean, winged bean, carrot. It was speculated that there may be insulin-like proteins which are capable of binding LBP. To this end, a 4-kDa peptide named leginsulin, which can bind to LBP and compete with insulin for binding to LBP, was isolated from radicles of germinated soybean seeds by affinity chromatography. The leginsulin had a stimulatory effect on the phosphorylation activity of LBP, suggesting that it is involved in cellular signal transduction. The leginsulin consists of 37 amino acid residues with six half-cystines in three disulfide bridges. A portion of the peptide is processed to delete the *C*-terminal glycine like a number of peptide hormones, but not *C*-terminally amidated. The cDNA encoding the leginsulin was cloned and sequenced, and this peptide was considered to be synthesized as a 13-kDa precursor polypeptide. The leginsulin-like peptides were also found to be present in the seeds and/or roots of several plant species like azuki-bean, French bean, pea, carrot. Although there is no sequence similarity between the leginsulin and insulin or insulin-like growth factors, the leginsulin is a possible candidate for hormone-like peptides in plants.

J6-109 GENETIC ANALYSIS OF ABSCISSION-SPECIFIC CELLULASE AND POLYGALACTURONASE GENE EXPRESSION

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Abscission is a plant developmental process that leads to organ separation as a result of cell wall breakdown. This process is stimulated by ethylene but inhibited by auxin. Among many cell wall hydrolytic enzymes, endo-ß-1,4-D-glucanase (cellulase) and polygalacturanase (PG) are known to be involved in abscission. Abscission-specific tomato PG (TAPG) and cellulase (TAC1) cDNA clones have been identified. Three different TAPG cDNAs (TAPG1, TAPG2, and TAPG4) have been identified. TAPG1 and TAC1 are expressed in ethylene-induced leaf and flower abscission zones. TAC1, but not TAPG1, is expressed at low levels in ripening tomato fruit.

The objective of this project is to identify the promoter elements that respond to ethylene and auxin in a tissue-specific manner. With this goal in mind, we constructed a tomato (*Lycopersicon esculentum* cv. Rutgers) genomic library. From this library, we obtained genomic clones that strongly hybridized to TAPG1 and TAC1 probes. Southern blot analysis indicated that an 8.0 kb and 10 kb *Sal*I fragment contain the entire TAPG1 and TAC1 sequence, respectively. The 8.0 kb *Sal*I fragment also hybridized to the TAPG2 cDNA probe. This suggests that TAPG1 is highly homologous to TAPG2. The 8.0 kb and 10 kb fragments have been subcloned into the Deletion Factory (GIBCO BRL) vector to generate nested deletions for sequencing.

J6-110 LIPO-CHITIN-OLIGOSACCHARIDES (LCOs) AS ENDOGENOUS ORGANOGENESIS SIGNALS OF THE PLANT. Dimitris Kafetzopoulos, Eric Kamst, Ben J. J. Lugtenberg, Herman P. Spaink, Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The recent experimental evidence that the rhizobial nodulation signals (1), termed Nod factors, can influence the developmental processes in non-leguminous plants suggests that endogenous lipophilic chitin-oligosaccharides (LCOs) are involved in plant development (2). This hypothesis is consistent with the observation that the expression of several plant chitinases correlates with the normal development of plant organs and embryos, suggesting that these chitinases are involved in the release of endogenous chitin-like signal molecules.

Assuming that the enzyme activities involved in the biosynthesis of the plant chitin-oligosaccharide signals are similar to the biosynthetic enzymes of Nod factors in Rhizobium, we have used the observed significant similarities of the rhizobial NodC and NodB proteins to the eukaryotic chitin synthases and chitin-deacetylase respectively (3), for the isolation of their plant homologues.

Furthermore, in order to isolate and further characterize these potential LCO signal molecules from plant extracts, we have employed the following structure criteria: i) susceptibility to chitinases, ii) binding to chitin-oligosaccharide specific lectins, and iii) acetylation by the rhizobial NodL protein (a specific transacetylase of chitin-oligosaccharides) (4).

1. Spaink et al., (1991), Nature, 354, 125-130.
2. De Jong et al., (1993), Plant Cell, 5, 615-620.
3. Kafetzopoulos et al., (1993), Proc. Natl. Acad. Sci. USA, 90, 8005-8008.
4. Bloemberg et al., (1994), Mol. Microbiol., 11, 793-804.

J6-111 ACTIVATION OF TOBACCO GLUTATHIONE S-TRANSFERASE GENES AND *as-1* TYPE ELEMENTS BY AUXINS, SALICYLIC ACID AND METHYL-JASMONATE: CROSS-TALK OF THREE PHYTOHORMONE PATHWAYS AT A SINGLE CIS-ACTING ELEMENT, Eric Lam*, Chengbin Xiang and Zhong-He Miao, AgBiotech Center, Waksman Institute, Rutgers University, Piscataway, NJ 08854

The *as-1* type cis-acting elements have been shown to respond to exogenous application of auxins, salicylic acid (SA) and methyl-jasmonate (MJ) in transgenic tobacco. This type of element includes the *as-1* site from the -75 region of the CaMV 35S promoter, the *ocs* element and the *nos* element from the T-DNA genes of *Agrobacterium tumefaciens*. These sequences have all been demonstrated to interact with a similar nuclear factor called ASF-1 (activating sequence factor 1). In an effort to characterize the regulatory properties of the *as-1* element, we have examined the effects of a comprehensive set of phytohormones and abiotic stress-inducing agents on transgenic tobacco seedlings containing synthetic promoter constructs. The results showed that ethylene, cadmium, and heat shock were ineffective in the activation of this type of cis-acting elements. This result suggests that the induction of transcription activity by auxin, SA and MJ was not due to a general stress response. In order to biochemically characterize the signal transduction pathways involved, we have established a cell suspension culture from one of our transgenic tobacco plants containing a synthetic promoter with a tetramer of the *nos* element fused upstream of a minimal TATA element. We showed that this reporter gene responded to hormone treatments in the cell suspension in a similar fashion to that of transgenic tobacco. Kinetic and dose reponse analyses demonstrated that the activation of the promoter by 2,4-D, SA and MJ occurred with similar kinetics and sensitivity. Addition of multiple inducers suggests that these phytohormones may function in an additive manner. These observations indicate that the three phytohormones can activate transcription via parallel signal transduction pathways that converge at a single type of cis-acting element. We have previously shown that ASF-1 can bind to an *as-1* type element in the promoter of tobacco GNT35, an auxin inducible gene that encodes a novel type of glutathione S-transferase. We found that this gene is also induced by the same phytohormones that activated the *as-1* type elements. This observation provides support for the involvement of ASF-1 in the control of this class of plant genes.

J6-112 AUXIN REDISTRIBUTION AND GRAVITROPISM: SUPPORT FOR THE CHOLODNY-WENT THEORY AND REGULATION BY CYTOKININ

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Transport of the plant hormone auxin plays an important role in regulating plant development and responses to environment. The Cholodny-Went hypothesis proposes that lateral transport of auxin is responsible for stem curvature in response to gravity or light stimulus. In the gravitropic response of dicot stems, auxin acts as the transducing signal between the site of gravity perception (the starch parenchyma cells surrounding the vascular tissue) and the site of the asymmetric growth response (the epidermal cells). We have studied lateral auxin redistribution in response to gravity in the *diageotropica* (*dgt*) mutant of tomato. Double labeling studies with ^{3}H-IAA (indoleacetic acid, the major naturally-occurring auxin) and ^{14}C-BA (benzoic acid, an inactive analog) demonstrate that in *dgt* stems, which curve downward much more slowly in response to gravity, auxin redistribution is also greatly retarded. The single gene, recessive *dgt* mutant exhibits other phenotypic characteristics which indicate either increased cytokinin production or sensitivity. We have found that exogenously-applied cytokinin can effectively mimic the *dgt* phenotype, including the slower gravicurvature response, in wild type plants. Lateral auxin transport in response to gravity is also retarded in cytokinin-treated wild type plants. Taken together, these findings 1) support the Cholodny-Went hypothesis and 2) suggest that a second factor, cytokinin, may play a role in gravitropism through controlling lateral auxin transport.

J6-113 THE ARABIDOPSIS HOMEOTIC GENE *AG* REGULATES FLORAL INDUCTION AND SEED DEVELOPMENT,

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The Arabidopsis floral homeotic gene *AG* is known to control the determinacy of floral meristem and the identity of reproductive organs. We have also confirmed the proposed negative interaction between *AG* and another floral gene *AP2* in regulating organ identity, using transgenic plants that ectopically express the *AG* cDNA. After further analysis of these transgenic plants, we have learned that the alteration of *AG* expression affects the timing of flowering as well as the structure of the inflorescence meristem. We have analyzed flowering time of the transgenic plants, and *ag* mutant plants, under different lighting conditions. Our results strongly suggest that *AG* plays a role in regulating floral induction. We are in the process of further studies of this aspect of *AG* function using genetic and molecular approaches, to uncover possible interactions of *AG* with other genes controlling floral induction and meristem function.

In addition to *AG*'s in floral induction, in floral meristem and organ identity, *AG* is also expressed in developing ovules and seeds. We have found that the *AG* transgenic plants behave in a way similar to *ap2* mutants. Both exhibit abnormal ovule development, abnormal seed development, and vivipary. We have performed a number of experiments to charaterize the ovule and seed development in these plants. We have found that the size and shape of ovules, the rate of embryo development, and the size and shape of the seedcoat are abnormal in the *AG* transgenic and *ap2* mutant plants. In addition, the level of some seed storage proteins are altered. Further, the seeds from these plants show abnormal response to the hormones ABA and to a GA biosynthesis inhibitor. It is intriguing that the floral regulatory genes *AG* and *AP2* may play a role in the hormonal control of seed development. It is possible that AG and AP2 regulate hormonal balance during seed development; alternatively, hormonal levels may modulate the activity of AG and/or AP2. We are currently conducting experiments to distinguish these models.

Our studies on *AG* function have revealed that *AG* plays several roles during flower development. In addition to its function in floral meristem determinacy and reproductive organ identity, as suggested by the mutant phenotypes, we have found that *AG* can also affect floral induction and the inflorescence meristem, and it regulates ovule and seed development. Moreover, it is exciting that *AG* may be involved in hormonal regulation of development.

J6-114 **GIBBERELLIN REGULATES FLAVONOID GENE TRANSCRIPTION IN THE DEVELOPING COROLLA OF PETUNIA FLOWERS**

Joseph N.M.Mol, David Weiss, Leon A. Mur, Cornelis Spelt, Rik van Blokland, and Jan M.Kooter, Department of Genetics, IMBW, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

The pigmentation of petunia corollas is under developmental control. The growth of the corolla is accompanied by the synthesis of anthocyanin. When anthers are removed at an early stage of corolla development the corolla fails to elongate and does not become pigmented. This could be restored by the addition of gibberellin (GA_3) which suggests that the anthers produce GA which is translocated to the corolla. Here it induces the anthocyanin biosynthesis pathway and the growth of the tissue.

We have set up an *in vitro* system to study these GA-regulated processes at the molecular level. Detached green corollas incubated in a sucrose medium elongate and become pigmented only when GA_3 is present in the medium. GA induces the accumulation of several mRNAs encoding flavonoid enzymes such as chalcone synthase (CHS), chalcone flavanone isomerase (CHI) and dihydroflavonol 4-reductase (DFR). The GA-inducible ß-glucuronidase (GUS) activity in corollas of transgenic plants carrying the *gus* gene driven by the *chi* or *chs* promoter indicates that GA acts at the level of transcription initiation. Additional evidence for such a role was obtained from the analysis of nascent transcripts in nuclei isolated from *in vitro* grown corollas. The transcription rates paralleled the steady-state *chs* mRNA levels.

Transcription of the *chs* gene requires the continuous presence of GA. However, after the expression had been switched off by an incubation in sucrose medium for 24 hr, it lasted approximately six hours before the effect of GA on gene expression was detectable. This delay in the induction suggests that GA controls flavonoid gene transcription in an indirect manner, via the induced synthesis of other factors, such as regulatory proteins. The inhibitory effect of cycloheximide on the action of the hormone is consistent with such an activation model. Our data therefore support a model in which GA induces the expression of a regulatory gene which in turn is involved in the regulation of flavonoid gene transcription.

[See Weiss et al.(1992) Plant Physiol. 98, 191-197]

J6-115 THE BARLEY 60KD JASMONATE-INDUCED PROTEIN (JIP60) IS A NOVEL RIBOSOME INACTIVATING PROTEIN.

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Methyl jasmonate induces the accumulation of specific gene products in plants (JIPs). cDNAs of jasmonate-induced proteins have been characterized from barley. One of them, JIP60, encodes a 60kD polypeptide described by Becker and Apel (1992). The N-terminal region of this 60kD, jasmonate-induced protein of barley leaves (JIP60) is shown to be homologous to the catalytic domains of plant ribosome inactivating proteins (RIP type II). Western blotting of leaf extracts and *in vitro* reconstitution experiments indicate that JIP60 is synthesized as a precursor which is processed *in vivo*. This is in keeping with in vitro translation experiments indicating that a deletion derivative of the N-terminal region, but not the putative precursor, strongly inhibits protein synthesis on reticulocyte ribosomes. This indicates that JIP60 is a novel ribosome inactivating protein requiring at least two processing events for full activation. JIP60 derivatives do not significantly inhibit *in vitro* protein synthesis on wheat germ ribosomes. These and other results suggest that JIP60 may be involved in plant defense.

Becker, W. and K. Apel (1992) Plant Mol. Biol. **19**, 1065-1067

J6-116 PROMOTER ANALYSIS OF AN ETHYLENE-INDUCIBLE ABSCISSION CELLULASE GENE IN BEAN

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The accumulation of pI 9.5 cellulase and its mRNA in leaf abscission zones is induced by ethylene and inhibited by auxin. In an effort to identify cis-acting regulatory elements in the bean cellulase gene promoter we raised transgenic tomato plants containing bean promoter deletions ligated to a GUS reporter gene. Transcipts of the GUS gene were detected in leaf abscission zones of ethylene-treated transgenic tomato plants containing a -210 bp bean promoter. To further charecterize regulatory promoter elements we developed a transient assay for bean explants using particle gun bombardment. To establish transient assay conditions a chimeric gene was constructed with a luciferase reporter gene fused to a 2.8 kbp bean cellulase upstream sequence and an ~800 bp bean 3' termination sequence. Though highly variable results were obtained, the mean values indicate that reporter gene expression was abscission-specific and inhibited by 2,5 norbornadiene (an ethylene action inhibitor) and 2,4-D (an auxin analog). The homologous abscission cellulase gene from soybean has been cloned and partially sequenced. Comparison of bean and soybean sequence will identify conserved regions that will aid in designing new constructs to be tested in transient assay and transgenic plants.

J6-117 AUXIN INDUCIBLE GENES: TOOLS FOR THE ANALYSIS OF AUXIN SIGNAL TRANSDUCTION PATHWAY(S) IN ARABIDOPSIS THALIANA

Remko Offringa, Dianne A.M. van der Kop, Eric van der Graaff, Leon Neuteboom, Bert van der Zaal and Paul J.J. Hooykaas, Institute of Molecular Plant Sciences, Leiden University, Clusius Laboratorium, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

We wish to isolate genes encoding proteins that are involved in the auxin signal transduction pathway(s). In one successful approach the promoter of the auxin inducible nt103 gene from tobacco was fused to the coding regions of two marker genes present on one T-DNA construct, i.e. a reporter gene (uidA) and a selectable marker gene (nptII). Transgenic Arabidopsis plant lines containing one copy of this construct showed resistance to kanamycin only after treatment with auxin. Seeds of these parental lines were used for EMS mutagenesis and M2 seeds were screened for seedlings that showed resistance to kanamycin without auxin treatment. Several kanamycin resistant individuals were obtained, some of which turned out to have higher GUS activity as well. These mutants probably have a mutation in a gene involved in the auxin signal transduction, resulting in up-regulation of the nt103 promoter. Two selected mutants are presently being characterized.

The induction of the nt103 promoter is not specific for auxins but occurs after treatment with non-active auxin analogues and salicylic acid as well. Via different approaches we are now isolating genes that are more specifically induced by indole-3-acetate and active analogues. By cloning these newly identified auxin inducible promoters in front of the uidA/nptII fusion gene, both their expression pattern as well as their suitability for the mutagenesis approach described above will be determined. In addition the new genes will be useful tools in the analysis of auxin signal transduction at the cellular level.

J6-118 IDENTIFICATION OF NOVEL PROTEINS IN THE ETHYLENE SIGNAL TRANSDUCTION PATHWAY.

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The gaseous hormone ethylene is involved in several physiological and developmental processes in higher plants including germination, leaf and flower abscission and senescence, fruit ripening, and sex determination in some monoecious plants. Ethylene also plays a role in mediating plant responses to environmental stimuli, including various stresses such as chilling, drought and pathogen attack. The molecular mechanisms underlying ethylene perception are beginning to be unravelled using molecular genetic approaches in *Arabidopsis*. However, some components of this pathway may not be easily identified through genetic analysis (for example, duplicated genes). Therefore, we are employing molecular approaches to identify novel elements using the *CTR1* gene as a starting point. *CTR1* was identified by mutations that activate ethylene responses in the absence of exogenously added ethylene (Kieber *et al.* Cell **72**:427-441, 1993). CTR1 encodes a protein with homology to the Raf family of serine/threonine protein kinases. Here we describe the use of the yeast two-hybrid interaction system to identify proteins that interact with CTR1. This system employs two plasmid constructs, the first being a "bait" plasmid containing a lexA DNA-binding domain fused to the gene of interest, and the second being a Gal4 activation domain fused to a library of cDNA clones. Positive interactions in this system are identified by transcription of two reporter genes, His3 and LacZ. We have constructed bait plasmids containing different domains of *CTR1*, as well as the entire *CTR1* gene. We have identified a number of cDNA clones that interact with these baits and are currently characterizing a number of these clones.

J6-119 SOLUBILIZED NAPTHYLPHTHALAMIC ACID BINDING PROTEIN,

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A novel combination of the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) and sodium molybdate has been used to effectively solubilize the N-1-napthylphthalamic acid (NPA) binding protein (NPA-BP) from zucchini hypocotyl microsomal and plasma membranes. Activity of the solubilized NPA-BP has been characterized by monitoring binding to ^{3}H-NPA with a GF/B filtration assay. Binding of ^{3}H-NPA to the solubilized protein is specific, saturable, time dependent, and reversible. Analysis of the data by the program LIGAND indicates a single binding site with a Kd of 4.0 nM and a Bmax of 10.9 pmol/mg solubilized plasma membrane protein. The solubilization procedure yields 23% of the NPA-BP from plasma membranes. The solubilized NPA-BP exists as a large detergent complex based upon its chromato-graphic properties on Superose 6 FPLC. Interestingly, the auxins indole acetic acid (IAA), 1-napthyleneacetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2,4-d) do not displace ^{3}H-NPA binding from the solubilized NPA-BP. The properties of the solubilized protein are similar to the membrane bound form.

J6-120 INDUCTION OF THE *LOX1* GENE DURING GERMINATION: IMPLICATIONS FOR THE BIOSYNTHESIS OF LIPID-DERIVED SIGNALS, [1]T. Kaye Peterman, [2]Melissa Melan and [1]Asha Ramakrishna, Department of Biological Sciences, Wellesley College, Wellesley MA 02181; [2]Department of Biological Sciences, Duquesne University, Pittsburg, PA 15282

Lipoxygenases (LOXs) catalyze a key step in the production of jasmonates and other membrane lipid-derived molecules which may serve as signals in plant cells. The *LOX1* gene of *Arabidopsis* encodes a LOX which is induced in response to the stress-related hormones ABA and methyl jasmonate as well as in response to bacterial pathogen attack. The *LOX1* gene is also transiently expressed during germination. *LOX1* mRNA was not detected in imbibed seed but reached a maximum level within 24 hours. Immunoblot analysis of LOX protein levels and measurements of enzyme activity indicated that the induction of the *LOX1* gene resulted in the production of functional enzyme. LOX protein was first detected after 24 hours of growth and levels remained high for two days before decreasing. LOX activity paralleled the changes in protein levels. *In situ* hybridization studies revealed that the transient expression of the *LOX1* gene occurred in the epidermis during the first day of development. These results suggest that the *LOX1* gene product is important for a function of the epidermis at this precise time during development. They are also consistent with a role for LOX-derived signals in this tissue during germination. Results from "antisense" and "overexpression" transgenic plant experiments, which directly test this hypothesis, will be presented.

J6-121 A NOVEL GENETIC SCREEN FOR ABA- AND OSMOTICUM-INSENSITIVE MUTANTS OF *ARABIDOPSIS* Christopher D. Rock, Terry L. Thomas,* and Ralph S. Quatrano, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, *Department of Biology, Texas A&M University, College Station, TX 77840

Abscisic acid (ABA) controls a myriad of physiological responses to developmental and environmental ques, such as seed protein accumulation, dormancy, root growth, stomatal closure, and stress-induced gene expression (1,2). The ABA-insensitive mutants characterized thus far (*abi1-abi5*; 3,4) have been identified by screening for germination in the presence of inhibitory concentrations of ABA. No ABA-insensitive mutant has yet been identified which is specific for a vegetative response, nor have mutants been found in an osmotic stress pathway which is distinct yet overlapping with ABA-response pathway(s) (5). We have developed a visual screen for ABA- and osmoticum-induced gene expression in roots by utilizing transgenic plants harboring two independent copies of the carrot Dc3 promoter (6) fused with the GUS (ß-glucuronidase) gene. Transgenic seedlings respond to ABA and mannitol in a dose-dependent manner by increasing GUS activity, and ABA and mannitol together have a synergistic effect. ABA- or mannitol-treated seedlings which are developed with X-Gluc substrate specifically stain the root steele blue, and stained seedlings survive the treatment. This screen targets tissue-specific ABA-insensitivity genes in ABA- and turgor-perception pathways; the screen should also identify mutants in ABA metabolism. We are currently screening 50,000 ethylmethane sulfonate (EMS)-generated M2 plants for gain-of-function and loss-of-function mutations. (Funded by NIH grant GM44288 to R.S.Q. and NIH fellowship GM14752 to C.D.R.)

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J6-122 THE SEARCH FOR CIS AND TRANS ACTING TRANSLATIONAL REGULATORS OF CTR1 John Vogel, Wenyan Ma, Joseph Kieber, Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, 840 W Taylor St., Chicago, IL 60607

The plant hormone ethylene plays a role in many developmental and environmental plant responses including leaf abscission, senescence, fruit ripening, drought response and pathogen attack. Mutations in *CTR1* result in seedlings that display a triple response in the absence of exogenously applied ethylene. (Kieber et al. *Cell* **72**:427-441, 1993). CTR1 acts as a negative regulator of the ethylene response pathway. To study the expression of CTR1, transgenic lines of *Arabidopsis* were constructed containing the GUS gene fused to the 5' and 3' flanking regions of *CTR1*. GUS activity in these lines is dramatically induced by ethylene but not in other random promoter-GUS fusions. CTR1 appears to be translationally regulated because the level of CTR1 mRNA is constant in ethylene and air but the GUS activity increases >100 fold in ethylene. The 5' untranslated region of CTR1 mRNA contains three AUG codons before the main open reading frame that may act as cis acting regulatory elements. Several *Arabidopsis* lines that contain the GUS gene fused to modified upstream regions are being constructed to determine the role of the upstream elements in the regulation of CTR1. In order to isolate trans acting factors that are responsible for the translational regulation of CTR1, two additional *Arabidopsis* lines were constructed that contain another reporter gene in addition to the GUS fusion mentioned above. One contains the green fluorescent protein (GFP) gene (Chalfie et al. *Science* **263**:807-805, 1994) and the other contains the luciferase gene. Plants expressing GFP should fluoresce green in the presence of blue light. This should greatly simplify the screening process since no chemical substrate is required and the viability of the plants should not be decreased by GFP. Seed from these lines will be mutagenized and the progeny screened for inappropriate expression of the reporters. The mutants obtained should include mutations in trans acting factors which regulate CTR1. These experiments will tell us more about the mechanisms of translational control and the effects of inappropriate expression of CTR1 on plants.

J6-123 **THE INWARD RECTIFYING POTASSIUM CONDUCTANCE IN BARLEY ALEURONE PROTOPLASTS: MODULATION BY ABA**, M. Wang[3], M.T. Flikweert[2,3] and B. Van Duijn[1,2], 1. Institute of Molecular Plant Sciences, Leiden University, 2. Department of Physiology, Leiden University, 3. Center for Phytotechnology RUL/TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The plant hormone abscisic acid (ABA) is involved in grain dormancy and is able to induce the expression of e.g. Rab genes in barley aleurone cells and protoplasts. The barley aleurone isolated from dormant and nondormant grains show a different responsiveness to ABA-induced gene expression. To study the involvement of ion channels in the ABA-induced ion- concentration and -flux changes we applied the patch-clamp technique to barley aleurone protoplasts. Protoplasts obtained from aleurone of both dormant and nondormant grains (*Hordeum distichem* L. cv Triumph) were used to investigate whether ABA responsiveness differences, like exist for Rab gene expression, are also present for modulation of ion channels. In all types of protoplasts a time and voltage dependent inward directed current through the plasma membrane could be measured. This current was hyperpolarization activated and showed a reversal potential that shifted with the potassium equilibrium potential. We conclude that this current is an inward rectifying potassium current ($I_{K,in}$) as was previously described by Bush et al. (Planta 176:368-377, 1988) for aleurone protoplasts from Himalaya grains. We compared the $I_{K,in}$ in the different types of protoplasts. From this we conclude that, taking the size of the protoplasts into account, the $I_{K,in}$ in protoplasts from dormant Triumph grains showed a much smaller $I_{K,in}$ than in protoplasts from non-dormant grains. Addition of 5 μM ABA resulted, in all types of protoplasts used, in a doubling of the maximal conductance of the $I_{K,in}$. The ABA responsiveness difference between aleurone protoplast from dormant and nondormant grains could also be found in the ABA-induced increase in the $I_{K,in}$. In protoplasts from dormant grains 0.5 μM ABA was still able to induce an increase in the $I_{K,in}$, whilst this concentration did not have any effect on the $I_{K,in}$ of aleurone protoplasts from nondormant grains.

J6-124 TEMPORAL AND SPATIAL REGULATION OF ETHYLENE BIOSYNTHESIS IN *ARABIDOPSIS* ETHYLENE OVERPRODUCING MUTANTS, Keith Woeste, and Joseph Kieber, Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, 840 W. Taylor St., Chicago, IL, 60607

The gaseous hormone ethylene is integral to the regulation of plant growth. Ethylene mediates both developmental changes, including germination and bud-break, and the response to environmental stresses such as chilling and injury. The regulation of ethylene production is critical to an understanding of the effects of ethylene. The ethylene biosynthetic pathway is well characterized and the genes encoding the key regulatory enzyme (ACC synthase) have been cloned from a number of species including *Arabidopsis*. We used the seedling triple response to identify five mutants affected in ethylene biosynthesis in *Arabidopsis*. These mutants, *eto* 1-5 (ethylene overproducing), define elements involved in the regulation of ethylene biosynthesis. Eto mutant seedlings produce from 10 to 100-fold more ethylene than wild-type seedlings. We used gas chromatography to measure ethylene production by wild-type and Eto mutants at several developmental stages and to evaluate the spatial and temporal regulation of ethylene biosynthesis. We determined the amount of ethylene made by double Eto mutants and Eto/Ein (ethylene insensitive) double mutants. We also determined the effects of ethylene elicitors on ethylene production by wild-type and Eto seedlings. These results begin to provide a picture of the circuitry regulating ethylene biosynthesis in *Arabidopsis* and help define the role of ethylene in development.

J6-125 CHARACTERIZATION OF OCS ELEMENTS AND THEIR BINDING FACTORS IN *ARABIDOPSIS*, Bei Zhang, Wenqiong Chen & Karam Singh, Department of Biology, University of California at Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90024

ocs elements are a group of promoter sequences which have been exploited by *Agrobacterium* and certain viruses, to express genes in plants. We examined the activity of ocs elements linked to a minimal plant promoter and a GUS reporter gene in transgenic *Arabidopsis* plants. GUS activity was only detected in callus and in root tips following auxin treatment. Using a sensitive RT-PCR assay, we showed that ocs elements expression was significantly increased in both roots and aerial tissues following auxin treatment, although the absolute levels in aerial parts was substantially lower. Interestingly, salicylic acid (SA), a plant pathogenesis-resistance signal molecule, also induced ocs elements expression in both roots and aerial parts of the plants. Treatment of cycloheximide did not interfere with the auxin and SA induction in ocs elements, suggesting that post-translational modifications may play important roles in these process. In *Arabidopsis*, six related transcription factors that are candidates for mediating auxin and/or SA induction of ocs elements have been isolated by our lab and others. These factors which we call OBFs (ocs elements binding factor) belong to a specific class of plant b-zip proteins and can be classified into two subfamilies; the subfamily members share high homology except for a short region in the amino terminus. The characterization of OBFs including their expression patterns at the RNA and protein levels and their interactions with other proteins are being undertaken and our progress will be presented.

J6-126 MOLECULAR CLONING OF AN ETR1 cDNA HOMOLOG FROM TOMATO

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The phytohormone ethylene elicits a broad range of physiological responses including fruit ripening, abscission, and senescence. Chang et al. (Science 262: 539-544, 1993) cloned and sequenced the *Arabidopsis ETR1* gene which was previously shown to be involved in an early step of the ethylene signal-transduction pathway. The objective of our research is to investigate ethylene signal-transduction in tomato abscission. We have cloned an ETR1 homolog (TAE1) from a tomato leaf abscission zone cDNA library using the ETR1 cDNA as a probe. The sequences of *Arabidopsis* ETR1 and its tomato homolog will be compared. Southern blot analysis at moderate stringency indicated that there is only one gene similar to the tomato TAE1 cDNA. Northern blot analysis indicated that theTAE1 message is constitutively present in fruit, stem, petiole, and leaf and flower abscission zones.

Signaling in Plant-Pathogen Interactions

J6-200 ISOLATION AND CHARACTERIZATION OF THREE BARLEY 14-3-3 HOMOLOGUES INDUCED BY THE POWDERY MILDEW FUNGUS *ERYSIPHE GRAMINIS*.

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As part of our groups work with shotgun cloning of barley leaf cDNA's representing mRNA species accumulating in response to infection by the powdery mildew fungus *Erysiphe graminis* three cDNA clones have been isolated which encode members of the family of signal transduction regulators known as 14-3-3 proteins.
Genomic blot analysis indicated that, in addition to these genes, the barley genome posseses at least one additional gene.
By the use of a polyclonal antisera western blots show that the 14-3-3 proteins accumulate specifically in the epidermis tissue in response to attack by powdery mildew.
In the case of *Arabidopsis thaliana* challenged with *Peronospora parasitica* we have found no induction of any 14-3-3 homologues. However when challenged with barley powdery mildew preliminary results shows induction.
In order to elucidate the role of 14-3-3 proteins in plant defence responses one of the barley 14-3-3 cDNA's has been overexpressed in *Nicotiana tabacum*. The effect on the tobacco response towards attack by *Erysiphe cichoracearum* will be reported.

J6-201 ECTOPIC CONTROL OF VASCULAR DIFFERENTIATION BY A PARASITIC NEMATODE, David M. Bird, Mark A. Wilson and Jennifer S. Becker, Department of Nematology, University of California, Riverside, CA 92521.

Root-knot nematodes (*Meloidogyne* spp.) induce an apparently novel cell type (called a *giant cell*) in the roots of infected plants. Morphologically, giant cells share features both with developing xylem cells and mature transfer cells, including increased cytoplasmic density, high metabolic activity, multiple nuclei and extensively remodeled cell walls. Giant cells serve as the obligate nutritive source for the developing nematode, which ultimately becomes sedentary.

To understand the transcriptional basis for the parasitic interaction, a subtracted cDNA library was generated from a small number of hand-dissected tomato giant cells. This bank contains 287 clones encoding the 3'-ends of tomato genes whose expression is up-regulated (compared to healthy root tissue) during feeding by *M. incognita*. We have analyzed ~125 of these clones by sequencing, and their expression in a range of tomato tissues has been determined. Nearly all the clones define unique or low copy number genes. A global picture is emerging of elevated expression in giant cells of transcripts that also are expressed in young and expanding tissues, including meristematic and vascular precursor cells. Function can be assigned to many of these transcripts, which can be grouped into categories (including signal reception/transduction; differentiated giant cell function; anti-sense transcripts; general metabolic up-regulation).

We have begun to localize giant cell transcripts in infected and healthy plants by *in situ* analysis. Our most extensive data is for the *LeUbc4* gene which encodes a ubiquitin-conjugating enzyme abundantly expressed in giant cells and also in vascular parenchyma cells. This is consistent with a model in which giant cell formation is a modified form of vascular differentiation.

J6-202 THE PR-1a PROMOTER CONTAINS A NUMBER OF REGULATORY ELEMENTS THAT BIND GT-1 LIKE FACTORS WITH DIFFERENT AFFINITY, Annemarie S. Buchel, Richard Molenkamp, John F. Bol and Huub J. M. Linthorst, Institute of Moleculat Plant Sciences, Gorlaeus Laboratories, Leiden University, 2300 RA Leiden, The Netherlands

The 900 bp promoter region of the tobacco PR-1a gene was divided into eight fragments using PCR. The fragments were tested for their ability to bind to nuclear factors isolated from tobacco leaf. Band shift assays demonstrated that all but one of the fragments specifically interacted with nuclear proteins. From competition experiments it was determined that the same nuclear factors bind various promoter fragments with different affinity. Moreover, efficient competition with a synthetic tetramer of box II of the rbcS promoter (Lam and Chua, 1990) indicated that nuclear factor GT-1 is involved in these interactions. Furthermore, relative to nuclear extracts from untreated plants, salicylic acid-treated and tobacco mosaic virus-infected tobacco showed reduced binding capability of active GT-1. The results suggest that one of the steps leading to induction of the PR-1a gene by SA or pathogens involves the preferential removal of GT-1 from far-upstream regulatory elements of promoter.

J6-203 CLONING AND CHARACTERISATION OF THE *Cf-2* DISEASE RESISTANCE GENE, RELATED FAMILY MEMBERS AND THE CORRESPONDING NULL LOCUS, Mark S. Dixon, David A. Jones, James Keddie, Martin Ganal+, Kate Harrison, Colwyn M. Thomas, Steven D. Tanksley* and Jonathan D. G. Jones, Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich, UK, + Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, *Department of Plant Breeding, Cornell University, Ithaca, NY, USA.

The *Cf-2* and *Cf-5* genes that confer resistance to the leaf mould pathogen *Cladosporium fulvum* were originally introgressed from *Lycopersicon pimpinellifolium* and *L. esculentum* var. *cerasiforme* respectively. Previous studies have located these genes to a region close to the centromere of chromosome 6 and indicate that they are allelic or very closely linked to each other, and closely linked to *Mi*, a gene conferring resistance to root-knot nematodes (M. Dickinson *et al.* 1993 MPMI,Vol. 6, pp341-347 and D. A. Jones *et al.* pp348-357). High resolution mapping has identified an RFLP marker (MG112) which identifies a small tightly linked gene family of which *Cf-2* is a member (M. Dixon *et al.* MPMI submitted). Using a *Cf-2/Cf-5* trans heterozygous plant, test crosses were performed to look for allelism. Only one recombinant, that was sensitive to *C. fulvum*, was identified from 12,000 plants. The breakpoint has been mapped and suggests that *Cf-5* may also be identified by MG112. A binary cosmid library representing approximately 6 genome equivalents has been constructed from plants homozygous for the resistance genes *Cf-2* and *Cf-9*. Cosmids identified by MG112 have been isolated and assembled into a contig, and several cosmids have been used for direct complementation of tomato. Two overlapping cosmids conferred resistance to *C. fulvum* and the region in common has been further characterised. Sequence analysis will be presented and compared with other cloned resistance genes, related family members and the corresponding null allele from a sensitive plant. Initial results from large scale mutagenesis of plants carrying *Cf-2* and the function of *Cf-2* in other plant species will also be discussed.

J6-204 GENETIC DISSECTION OF THE SAR SIGNAL TRANSDUCTION PATHWAY(S) IN ARABIDOPSIS

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Systemic acquired resistance (SAR) is a nonspecific defense response in plants that is associated with an increase in the endogenous level of salicylic acid (SA) and elevated expression of pathogenesis-related (PR) genes. To identify mutants involved in the regulation of PR genes and the onset of SAR, we transformed Arabidopsis with a reporter gene containing the promoter of a ß-1,3-glucanase-encoding PR gene (*BGL2*) and the coding region of ß-glucuronidase (*GUS*). The resulting transgenic line (*BGL2-GUS*) was mutagenized, and the M_2 progeny were scored for GUS activity. We report the characterization of two distinctive mutants identified in this screen, *cpr1* (constitutive expresser of PR genes) and *npr1* (nonexpresser of PR genes). The mutant *cpr1* was shown by RNA gel blot analysis to have elevated expression of the endogenous PR genes *BGL2*, PR-1, and PR-5. Genetic analyses indicated that the *cpr1* phenotype is caused by a single, recessive nuclear mutation, and is suppressed in plants producing a bacterial salicylate hydroxylase, which inactivates SA. Furthermore, biochemical analysis showed that the endogenous level of SA is elevated in the mutant. Finally, the *cpr1* plants were found to be resistant to the fungal pathogen *Peronospora parasitica* NOCO2 and the bacterial pathogen *Pseudomonas syringae* pv *maculicola* ES4326, which are virulent on wild-type *BGL2-GUS* plants. Because the *cpr1* phenotype is recessive and associated with an elevated endogenous level of SA, we propose that the *CPR1* gene product acts upstream of SA as a negative regulator of SAR. Contrary to *cpr1*, the *npr1* mutant carries a single recessive mutation that abolishes the SAR-responsive expression of the reporter gene as well as other PR genes. While SA-, INA, or avirulent pathogen-induced SAR protects wild-type plants from *Pseudomonas syringae* infection, the mutant cannot be protected by pretreatment with these inducers. The insensitivity of *npr1* to SA, INA, and avirulent pathogens in SAR induction indicates that these inducers share a common signal transduction pathway. Moreover, in *npr1*, the localized expression of PR genes induced by a virulent *Pseudomonas* pathogen is disrupted and the lesion formed is less confined. These results suggest a role for PR genes in preventing the proximal spread of pathogens in addition to their suggested role in SAR.

J6-205 SERINE/THREONINE PROTEIN PHOSPHATASE IS REQUIRED FOR TMV-INDUCED HYPERSENSITITY IN TOBACCO,

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A major gap in understanding host-pathogen interactions is the knowledge of how molecular signals are passed within infected tissue. We hypothesis that reversible protein phosphorylation is involved in a TMV-activated signal transduction pathway for programmed cell death in N gene-containing tobacco. Tobacco (genotype NN) form local necrotic lesions after infection with TMV at the site of replication. This phenotype is temperature sensitive. Plants must be at a permissive temperature for a minimum of four hours to induced the necrotic phenotype, indicating reversible molecular events are engaged during this early phase of cell death program. We have measured a flux in phosphorylation levels of endogenous proteins which corresponds with a complex shift in serine/threonine protein phosphatase type 1 activity in N gene-containing tobacco during the first 5 hours after temperature induction. Okadaic acid (a specific inhibitor of serine/threonine protein phosphatases) treatment of TMV infected leaves *in vivo* blocks necrotic lesion formation, if added between two hours before and two hours after temperature induction. However, okadaic acid treatment has no effect on necrotic lesion formation if added four hours after temperature induction. Nuclei of cells peripheral to the necrotic tissue have degraded DNA, as demonstrated using *in situ* staining techniques for DNA degradation. This DNA degradation is consist with the hypothesis that theses cells are dying by an apoptotic mechanism. This is the first demonstration that protein phosphatases are necessary for programmed cell death and that their activities are modulated in this process.

J6-206 CYTOPLASMIC pH CHANGE AND POTASSIUM EFFLUX FROM ELICITOR-TREATED SOYBEAN CELLS AND PROTOPLASTS,

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Fluorescence ratio imaging and microspectrophotometry were used to determine cytoplasmic pH in elicitor-treated protoplasts, suspension culture cells, and root cells of soybean. Mean cytoplasmic pH of untreated protoplasts was 7.0, based on *in vivo* calibration curves for intracellular pH. Elicitor-active oligoglucans, derived from *Phytophthora megasperma* f.sp. *glycinea*, changed the cytoplasmic pH within 5 minutes. Ratio imaging revealed that the pH change was spacially complex. At low concentrations (10^{-5}g/ml) of elicitor, cortical alkalinization was accompanied by perinuclear acidification. The cortical alkalinization was transient (gone in 10 min), but the acidification continued for up to 40 min. pH response and potassium efflux were examined in intact suspension culture cells. Increased potassium flux out of the cell accompanied elicitor treatment. The spacial complexity of the cytoplasmic pH changes seen after elicitor treatment may explain the divergent results reported for intracellular pH change in soybean in response to elicitors.

J6-207 SALICYLIC ACID IS INVOLVED IN EXPRESSION OF TOBACCO PR2/GUS GENE IN TRANSGENIC POTATO PLANTS,

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Plants respond to pathogens in a variety of ways. These responses are accompanied by multiple molecular and biochemical changes. Mechanisms that regulate these changes are still not completely known. We are studying the response of potato plants to virus (Potato virus Y [PVY]) or fungus (*Phytophtora infestans*) infections as a model for plant-pathogen interactions. We observed increases in endogenous salicylic acid (SA) level, which we believe plays a role as signaling element in plant response to pathogen attack. Similarly to tobacco plants, SA was also found in a conjugated form (majority of the conjugates appeared to be SA glucoside), whose level increased in parallel with the free SA level. To correlate changes in endogenous level of SA with expression of genes activated after pathogen invasion, the PR2/GUS transgenic approach was used. Histochemical analysis of infected leaves demonstrated strong expression of chimeric gene in tissue surrounding infection sites. Expression of PR2/GUS gene in uninoculated leaves was correlated with immunodetection of viral proteins in the tissue. Applying of exogenous SA to plants was also sufficient to induce the PR2/GUS expression. These results should give clues for better understanding of mechanisms of disease resistance and SA function in this process.

J6-208 HOST-PATHOGEN RECOGNITION: ISOLATION OF cDNAs ENCODING THE PEROXIDASE HOMOLOGUES FROM *STRIGA ASIATICA* AND THEIR EXPRESSION DURING DEVELOPMENT,

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Striga asiatica, a parasitic angiosperm, has two levels of host recognition, one associated with germination, and another with haustorial development, that are critical for its development. The host derived signal molecules for these developmental transitions define the host specificity of the parasite. Parasite-derived oxidative enzymes have been suggested to be involved in processing the haustorial inducing signal from the host root surface. We pursued the identification of this enzyme by molecular biological techniques with the known gene sequences of plant peroxidases. We designed the degenerate primers for the conserved regions to amplify the homologous peroxidase genes from *Striga asiatica* by the polymerase chain reaction. Two peroxidase homologues (Peroxidase-A and -B) were cloned and the deduced amino acid sequences of *Striga* peroxidases were found to have high homology with a peoxidase of *Nicotiana sylvestris* which is highly expressed during tobacco protoplast regeneration. Using quantitative RNA-PCR technology the expression levels of the peroxidase genes were measured during development and appear to be induced within hours following exposure to the germination stimulant. The cloned peroxidase genes were not expressed in the mature plant or in preconditioned seeds. The expression was reduced within hours of the exposure of the seedlings to 2,6-DMBQ, haustorial inducer. The involvement of these genes in host detection and haustorial development will be discussed.

J6-209 ANALYSIS OF THE ROLE OF ETHYLENE IN SYSTEMIC ACQUIRED RESITANCE

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Ethylene is a plant hormone that controls many aspects of plant growth and development, and also plays a role in stress response. Ethylene production increases during the hypersensitive response of Samsun NN tobacco to tobacco mosaic virus (TMV). The hypersensitive response is followed by the induction of the expression of genes encoding pathogenesis related (PR) proteins and the development of a systemic acquired resistance (SAR) against viruses, fungi and bacteria. The ethylene biosynthetic pathway is controlled by two key enzymes, 1-aminocyclopropane-1-carboxylic acid synthase (ACCS) and ethylene forming enzyme (EFE). ACCS converts S-adenosylmethionine into ACC and EFE converts ACC to ethylene. Local and systemic induction of ACCS and EFE gene expression by TMV was analysed. In a time course experiment total RNA was isolated from local and systemic TMV induced leaves. Northern blotting showed that the ACCS and EFE genes are locally induced by TMV. No systemic induction was observed. EFE genes were induced by treatment of the plants with ethephon, but ACCS genes were not induced. The cDNAs for ACCS and EFE were transformed into Samsun NN tobaccoplants in the sense or antisense orientation. Plants were transformed with the single gene or with a combination of the ACCS and EFE genes. The effect of overexpression or silencing of these genes on ethylene production and induction of PR genes and SAR is being investigated.

J6-210 **INITIAL MEMBRANE RESPONSES AND TRANSDUCTION OF *N*-ACETYLCHITOOLIGOSACCHARIDE ELICITOR SIGNAL IN RICE CELLS,**

Kazuyuki Kuchitsu, Munehiro Kikuyama[1], Hiroaki Kosaka[2] and Naoto Shibuya, *Dept. of Cell Biol., Natl. Inst. of Agrobiol. Resources, Tsukuba, Ibaraki 305; [1]Biol. lab., Univ. of the Air, Chiba 261; [2]Dept. of Physiol., School of Medicine, Osaka Univ., Suita, Osaka 565, JAPAN*

Plant cells recognize specific oligosaccharides derived from the cell surface of pathogenic microorganisms and initiate defense responses including gene expression and production of antimicrobial substances. We showed that *N*-acetylchitooligosaccharides, fragments of the backbone polymer of fungal cell wall, elicit the phytoalexin production [1] and specific gene expression in suspension-cultured rice cells. The presence of a putative receptor for this elicitor was found in the microsomal membrane preparation from the rice cells [2]. The oligosaccharide induced rapid membrane responses including transient membrane depolarization [3], transient efflux of K^+ and Cl^-, and influx of H^+ across the plasma membrane, changes in intracellular ion concentration (cytoplasmic pH, *etc.*), transient generation of reactive oxygen species [4], and transient phosphorylation of several specific polypeptides [5].

Detection of spin adducts by ESR spectroscopy directly indicated the release of hydroxyl radical ($^{\cdot}OH$), as well as $^{\cdot}O_2^-$ and H_2O_2 from the elicited cells. The transient changes in membrane potential, ion fluxes and generation of the reactive oxygen species required extracellular Ca^{2+}, suggesting that influx of Ca^{2+} through the plasma membrane might trigger these responses. The generation of reactive oxygen species was also induced by calyculin A, a protein phosphatase inhibitor, and inhibited by a protein kinase inhibitor K-252a, suggesting the involvement of protein kinase/phosphatase regulation in the signal transduction. These rapid and transient responses were induced by only the oligomers with certain degree of polymerization at sub-nM concentrations, while deacetylated chitooligosaccharides were not active. Such strict structural requirement for the oligosaccharides was similar among various responses and phytoalexin production, suggesting the possible involvement of these initial responses in the signal transduction network initiated through a single receptor.

[1] Yamada, A. *et al.* (1993) *Biosci. Biotech. Biochem.* 57:405-409; [2] Shibuya, N. *et al.* (1993) *FEBS Lett.* 329:75-78; [3] Kuchitsu, K. *et al.* (1993) *Protoplasma* 174:79-81; [4] Kuchitsu, K. and Shibuya, N. (1993) *J. Cell. Biochem.* 17A:33; [5] Kuchitsu, K. *et al.* (1994) *Plant Cell Physiol.* 35:s90.

J6-211 CHARACTERIZATION OF GENES CORRELATED WITH SYSTEMIC ACQUIRED VIRUS RESISTANCE

Huub J.M. Linthorst, Monique M. van Oers and John F. Bol, Institute of Molecular Plant Sciences, Leiden University, The Netherlands

Infection of tobacco leaves with tobacco mosaic virus (TMV) induces a systemic resistance against a variety of pathogens, like bacteria, fungi and viruses. This systemic acquired resistance (SAR) is accompanied by the synthesis of so called pathogenesis-related (PR) proteins of which several groups are presently known. Although some of these PR proteins have shown antifungal activity, proteins correlated with resistance against viral infections have not been identified thus far. The aim of our study is to identify plant genes functional in inducing resistance against viruses, either in the signalling pathway or in actual defense mechanisms. The strategy was adopted to compare the mRNA set of tobacco plants showing SAR with the mRNA content of non-resistant plants. SAR was induced either by TMV-infection or by exogenously applied salicylate. mRNA was isolated from the upper leaves of plants that were either inoculated with TMV, treated with salicylate, or mock-inoculated, and cDNA libraries were constructed. To identify and isolate from these libraries those genes that are differentially expressed in resistant and non-resistant tobacco leaves, DNA probes are currently obtained. To this aim, subpopulations of the termed mRNA sets are screened for differences in mRNA content by PCR technology. Differentiallly expressed genes identified with this method will be presented.

J6-212 OXIDATIVE STRESS IN PLANT-PATHOGEN INTERACTIONS. Carlos A. Malpica, Marc van Montagu and Dirk Inzé. Laboratorium voor Genetika. Universiteit Gent. Gent B9000 Belgium.

Regulation studies of superoxide and hydrogen peroxide scavenging activities in *N. plumbaginifolia* have been carried out in our laboratory contributing to the study of oxydative stress caused by the environment in plants.

Tobacco cultivars with the *N* gene from *Nicotiana glutinosa* develop an incompatible interaction with tobacco mosaic virus (TMV) showing a necrotic hypersensitive response few days after inoculation. This biological stress is characterized by the production of free oxygen radicals and toxic oxygen derivatives. Although many plant enzyme activities are potentially involved in modulating this form of oxidative stress, the identity of those actually involved in plant-pathogen interactions, and the importance of their individual contribution are unknown.

TMV is used as a pathogen model for the study of plant-pathogen incompatible interactions in *Nicotiana* species. Superoxide, hydrogen peroxide, or both are proposed to be intimately involved in determining the outcome of the interaction. We have monitored oxidative responsive activities and plant gene transcription levels during early events of virus infection. If either superoxide or hydrogen peroxide were a second messager the changes in these activities could modify their signal transducing capabilities. The results from these experiments will be discussed.

J6-213 MEMBRANE AND CYTOSOLIC RESPONSES INDUCED BY OLIGOGALACTURONIDES : POSSIBLE INVOLVEMENT OF THE PLASMALEMMA PROTON PUMP

Johan Messiaen, Pierre Van Cutsem, University of Namur, Plant Biotechnology, Namur, Belgium

Oligogalacturonides with a degree of polymerization ≥ 9 in a calcium induced-conformation trigger the activation of several defense-related genes in suspension-cultured carrot cells (Messiaen and Van Cutsem, Plant Cell Physiol (1993) 34: 1117-1123). Oligogalacturonides also induce a membrane depolarization, a cytosolic acidification and a cytosolic free calcium mobilisation (Messiaen and Van Cutsem, Plant Cell Physiol (1994) 35: 677-689).

We showed that cytosolic calcium was mobilised within 5 minutes after elicitor treatment by the opening of voltage independent calcium channels. Calcium mobilisation also triggered the observed membrane depolarization and cytosolic acidification. These two responses were inhibited by calmodulin antagonists (chlorpromazine, trifluoperazine and calmidazolium) and fusicoccine, were insensitive to vanadate, and were accompanied by an increase in external pH and by an elevation of the ATP level in elicitor-treated cells. Membrane depolarization and cytosolic acidification were therefore believed to be induced by an inhibition of the plasmalemma proton pump.

Facilitated proton influx was coupled to potassium efflux. Inhibition of this potassium efflux with tetrapentylammonium showed that the K^+/H^+ exchange was only partly reponsable for the cytosolic acidification response. We also showed that both elevated cytosolic calcium and cytosolic acidification (and K^+ efflux) were absolutely necessary for signal transduction and defense gene activation.

J6-214 **CHOLERA TOXIN INDUCES DEFENSE REACTIONS AND PATHOGEN RESISTANCE IN TRANSGENIC PLANTS,** **Ferenc Nagy[1], Roland Beffa[1], Frederick Meins Jr[1], Gunther Neuhaus[2], Jean-Pierre Metraux[3] Friedrich-Miescher Institut, Basel CH-4002 Switzerland[1], ETH, Zurich, CH-2500, Switzerland[2], University of Fribourg, Fribourg CH-1700, Switzerland[3]**

In many plant species, including tobacco, the hypersensitive response (HR) at the site of infection triggers systemic acquired resistance (SAR). In eukaryotic cells, heterotimeric GTP-binding proteins (Gα proteins) are involved in transmitting extracellular signals to specific effectors. In some cases, cholera toxin (CTX) can activate the signalling pathway by blocking the GTPase activity of the G subunit which locks the G protein in its active form. We transformed tobacco plants with a chimeric gene encoding the A1 subunit of CTX regulated by a light-inducible wheat Cab promoter. The transgenic plants were far less susceptible to infection by the bacterial leaf pathogen *Pseudomonas tabaci* than were untransformed plants. The transgenic plants constitutively expressed the pathogenesis-related (PR) proteins PR-1 and the class II extracellular isoforms of PR-2 (β-1,3-glucanase) and PR-3 (chitinase); the elicitor-inducible sesquiterpene cyclase (EAS); and they accumulated free and conjugated salisylic acid (SA). Therefore, uninfected CTX transgenic plants mimic the SAR state normally triggered by pathogen infection. Our results suggest that CTX sensitive G-proteins are involved in SAR and in SAR-associated induction of class II isoforms of PR-2 and PR-3, but not in the induction of the class I isoforms. Microinjection and grafting experiments confirmed these data and resulted in the identification of several key components of the signalling pathway required for the induction of SAR related genes.

J6-215 CHARACTERIZATION OF A DISEASE RESISTANCE LOCUS USING MUTANTS, Pat Okubara, Peter Anderson, Rosa Arroyo, Adrie Frijters, Dean Lavelle, Oswaldo Ochoa, Guo-Liang Wang, Zhen Zhang and Richard W. Michelmore, Department of Vegetable Crops, University of California, Davis, CA 95616

Fifteen genes (*Dm*) conferring resistance to the downy mildew fungal pathogen *Bremia lactucae* have been mapped to four separate linkage groups in *Lactuca sativa* (lettuce). We have used mutants lacking *Dm1*, *Dm3*, *Dm5/8* or *Dm7* activity to identify novel RAPD, AFLP and cDNA markers closely linked to these loci. The major cluster contains at least 9 *Dm* genes, including *Dm3*, and spans over 16 cM. Deletion mutants and pulsed-field gel electrophoresis were used to estimate the physical size of the *Dm3* region. Conventional Southern blot analysis revealed several classes of repeated sequences, some specific to the *Dm3* locus. Additional *Dm* mutants are being identified from both untreated populations and from M_2 families derived from seed treated with ethylmethane sulfonate (EMS). In contrast to the deletion mutants, spontaneous and EMS mutants show a range of susceptible phenotypes, including profuse sporulation, sparse sporulation accompanied by local necrosis, and local or regional necrosis without sporulation. We have focused our efforts on the isolation of *Dm3*. Molecular markers saturating the *Dm3* region are being used to construct fine-resolution genetic and physical maps for map-based cloning. Inverse polymerase chain reaction and T-DNA-specific primers (J. Jones, Sainsbury Laboratories, Norwich) were used to obtain lettuce DNA sequences that were adjacent to a T-DNA in a transgenic *dm3* plant. One copy of the flanking DNA was found to be missing in all *Dm3* deletion mutants. We are currently constructing and screening bacterial artificial chromosome (BAC), conventional genomic, size-selected and cDNA libraries for DNA and mRNA sequences linked to *Dm3*.

J6-216 THE ELICITATION AND SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN THE TWO-PHASED ACTIVE OXYGEN RESPONSE DURING PLANT/BACTERIA INTERACTIONS, Elizabeth W. Orlandi[1], Norton M. Mock[2], and C. Jacyn Baker[2], [1]Department of Botany, University of Maryland, College Park, MD, 20742. [2]USDA, ARS, Molecular Plant Pathology Laboratory, BARC-W, Beltsville, MD 20705.

Two phases of active oxygen (AO) production occur during incompatible plant/bacteria interactions which result in a hypersensitive response (HR). The first phase occurs immediately after addition of compatible, incompatible, and saprophytic bacteria to plant cell suspensions. The second phase follows about 1.5 hours later only after treatment of cells with incompatible bacteria. The two phases were compared in studies on the bacterial products that elicit them and on the plant cell signal transduction processes involved. Loosely-bound extracellular bacterial products which elicited the phase I AO response in tobacco suspension cells were isolated from *P.s.* pv *syringae* and *P. fluorescens*. Activity of the first phase elicitor was compared to harpin, the reputed elicitor of the HR and the second phase AO response. Studies with inhibitors of Ca^{++} transport and protein phosphorylation indicated that both phases of AO require similar signal transduction pathways for activation. Simultaneous treatment of cells with both elicitors resulted in a synergistic enhancement of AO production. However, pretreatment of cells with phase I elicitor resulted in a greatly diminished AO response to later treatments with either first phase or second phase elicitors. This may, in part, be due to the elicitation of increased AO-scavenging activity. Despite the production of relatively high amounts of active oxygen by elicitors of both phases, treatment of suspension cells with first phase elicitors did not cause hypersensitive cell death. This indicates that AO production alone is not sufficient to be either the direct cause or the indirect elicitor of the hypersensitive response.

J6-217 PAL-SUPPRESSED TOBACCO ARE UNABLE TO DEVELOP SAR IN RESPONSE TO TMV INFECTION Jacqueline A. Pallas[1], Nancy L. Paiva[1], Christopher J. Lamb[2], and Richard A. Dixon[1]. [1]Plant Biology Division, Noble Foundation, P.O. Box 2180, Ardmore, OK 73402; [2]Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037.

The phenomenon of systemic acquired resistance (SAR) has been well characterised in tobacco where an initial infection by tobacco mosaic virus (TMV), producing a hypersensitive response, can protect the plant against subsequent infections by TMV or fungal pathogens such as *Cercospora nicotianae*. The response of tobacco (Xanthi-nc) plants suppressed for phenylalanine ammonia-lyase (PAL) activity to infection by tobacco mosaic virus has been studied. These plants, which contain a bean PAL2 transgene in the sense orientation, exhibit suppressed production of phenylpropanoid products and have been shown to have reduced endogenous tobacco PAL mRNA (Elkind et al., 1990, PNAS 87:9057-9061). Previous work has shown that lesions produced by *Cercospora nicotianae* infection develop more rapidly in PAL-suppressed plants than in wild type plants (Maher et al., 1994, PNAS 91:7802-7806). Lesions produced by TMV infection have a marked difference in appearance with a reduced deposition of phenolics. The lesions however, do not develop at a faster rate than lesions on wild type tobacco, and PR proteins are induced in the transgenics upon primary infection with TMV. Examination of systemic leaves showed that pathogenesis-related (PR) proteins are not induced in PAL-suppressed plants and secondary infection with TMV does not result in the restriction of lesion size and number seen in wild type plants. Treatment of PAL-suppressed plants with isonicotinic acid (INA) will induce PR protein expression and also protection against TMV infection. However treatment of the plants with salicylic acid (SA), while inducing PR proteins, will only partially compensate for the lack of SA in the PAL-suppressed plants. Initial studies have shown that these sense-suppressed plants do show a lower amount of free SA than wild type.

J6-218 PURIFICATION OF AN ISOFLAVONE PRENYLTRANSFERASE IN *LUPINUS ALBUS*, Jenny Saleeba, Pierre Laflamme, Jacynthe Seguin, Patrick Gulick and Ragai Ibrahim, Biology Department, Concordia University, Montreal, Quebec, Canada H3G 1M8.

A wide range of methylated, sulphated, prenylated and glycosylated flavonoid compounds are present in higher plants. Various flavonoid compounds have been shown to play significant roles in pigmentation, UV protection, fertility, plant structure, and plant-microbe interactions including defense from microbial attack. Enzymatic prenylation of isoflavones, for example, increases the hydrophobicity and fungitoxicity of the compounds. *Lupinus albus* is noted among the legumes for the wide variety of prenylated isoflavones that can be found in the root tissue. Furthermore, *L. albus* produces these compounds in a constitutive manner whereas most legumes synthesize fungitoxic isoflavones only upon elicitation. Isoflavone prenyltransferase in *L. albus* is a membrane associated enzyme which catalyses the 6, 8, and 3' monoprenylation and 6,3' and 3',5' diprenylation of genistein and 2'-hydroxygenistein. We present data on the characterization and partial purification of this enzyme.

J6-219 NEMATODE RESISTANCE GENES FROM *BETA PATELLARIS* AND *BETA PROCUMBENS* Niels N. Sandal[1], Daguang Cai[2], Christian Jung[2], Rene Klein-Lankhorst[3], Michael Kleine[2], Elma Salentijn[3], Willem Stiekema[3] and Kjeld A. Marcker[1]

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The beet cyst nematode (bcn) (*Heterodera schachtii Schm.*) is an important pest in sugar beet (*Beta vulgaris* L.) cultivation.
In our project we use various fragment addition and introgression sugar beet lines carrying a nematode resistance gene from chromosome 1 of *Beta patellaris* or *Beta procumbens*. We use a map-based cloning strategy to identify the bcn gene. The plant lines containing the smallest area of wild beet DNA are the introgression lines B883, AN1-65-2 and A906001 with the bcn resistance locus from *B. procumbens* chromosome 1.
Several copies of a repetitive marker have been found in these lines. A RFLP marker also shows hybridization to the introgression lines. Finally bulked segregant analysis of pools of resistant and susceptible sugar beets from the backcrossing of the introgression lines identified 3 RAPD markers present in all resistant introgression lines.
In analysis of backcross plants we have identified at least one line where there is a crossover between the repetitive marker and the bcn resistance locus. The genetic distance between the repetitive marker and the bcn resistance locus is less than 1 cM. Long range mapping experiments indicate that the bcn resistance locus is contained within only 300kbp of wild beet DNA. From a YAC library of the A906001 line much of the 300 kb area has been isolated with the repetitive marker. At the moment other YAC clones are being isolated with the help of the other markers and YAC end specific probes.

J6-220 THE OCTADECANOID SIGNALLING PATHWAY MEDIATES DEFENCE RESPONSES AGAINST PATHOGEN ATTACK IN RICE PLANTS, Patrick Schweizer, Antony Buchala and Jean-Pierre Métraux, Institut de biologie végétale, University of Fribourg, CH-1700 Fribourg

The plant growth regulator jasmonic acid (JA) has been implicated in plant reactions to stresses like wounding and dessiccation, while its potential role in defence against pathogens is still speculative. - We are examining the potential of JA to induce defense-related genes as well as local or systemic acquired resistance in rice against the rice blast fungus *Magnaporthe grisea*. Exogenously applied JA or its methyl ester (MeJA) induced local accumulation of extracellular pathogenesis-related (PR) proteins in rice, including PR1. While JA was not sufficient to induce local acquired resistance to *M.grisea*, systemic acquired resistance was expressed in new leaves growing after JA treatment of older leaves. Exogenously applied JA furthermore enhanced systemic acquired resistance obtained by drench application of low doses of 2,6-dichloroisonicotinic acid (INA), a well established inducer of resistance in plants. Support for a role of jasmonates in mediating plant defence reactions comes from experiments using diethyldithiocarbamic acid (DIECA) as inhibitor of JA biosynthesis (Farmer et al., Plant Physiology 106 (in press)). DIECA inhibited PR1 induction by INA, whereas induction of PR1 by jasmonic acid was not impaired by this inhibitor. Moreover, low doses of exogenously applied JA restored PR1 induction by INA in the presence of DIECA. Simultaneous application of INA and JA resulted in synergistic induction of the accumulation of PR1 and another major, INA-induced protein of unknown function that was not induced by JA alone. On the other hand, INA repressed induction of an abundant jasmonate-induced protein, indicating a complex interaction between the octadecanoid and another, still unknown, signalling pathway in rice that is expressing acquired disease resistance. Data on endogenous JA levels in pathogen-attacked, susceptible or acquired resistant rice will also be presented.

J6-221 CHARACTERIZATION OF A *cis*-ELEMENT INVOLVED IN THE RESPONSE OF THE TOBACCO ß-1,3 GLUCANASE GENE TO SALICYLIC ACID, Jyoti Shah and Daniel Klessig, Waksman Institute, Rutgers University, P.O. Box 759, Piscataway, NJ 08855.

Salicylic acid (SA) is an endogenous signal for the induction of certain plant defense responses including the expression of various pathogenesis-related (PR) genes. We have identified a SA-responsive region (-364 to -288 from the transcription start site) in the 5' untranscribed region of the ß-1,3 glucanase (PR-2d) gene of *Nicotiana tabacum* which confers SA inducibility to a heterologous promoter. Gel retardation and southwestern analyses indicate the binding of at least two nuclear protein factors to a segment of this region (-348 to -324). Binding of these factors is specific. This was further confirmed by methylation interference analysis. However, no differences in the binding of these factors were observed in nuclear extracts made from water-treated or SA-treated plants. We are currently in the process of cloning these factors with the hope of working backwards from the PR-2d promoter to define the other components of the SA-signal transduction pathway.

In parallel, using SA-dependent expression of the *Agrobacterium tumefaciens tms2* gene driven from the tobacco PR-1a promoter, we have devised a genetic screen to identify mutations in SA-signal transduction pathway. Results on the preliminary characterization of some of these mutants will be presented.

J6-222 A PATHOGEN-INDUCED ANTIFUNGAL PLANT DEFENSIN FROM RADISH LEAVES, Franky R.G. Terras and Willem F. Broekaert, F.A. Janssens Laboratory of Genetics, University of Leuven, Belgium

We recently characterized a novel type of 5 kDa cysteine-rich antifungal peptides from radish seeds which we termed 'plant defensins'. *In vitro*, the two seed defensin isoforms alter the tubular hyphal growth into a dense cluster of short and highly branched hyphae. *In vivo*, these peptides are eluted into the surrounding medium during germination of the radish seeds, thus creating a zone around the seedling wherein fungal growth is inhibited. Furthermore, at least one other defensin isoform is constitutively expressed in radish leaves, albeit at low levels. Inoculation of a single leaf with spores of the fungi *Alternaria brassicola* or *Botrytis cinerea* results in the accumulation of transcripts cross-hybridizing with a seed defensin cDNA derived probe throughout all leaves. A systemic induction of the expression of the radish leaf defensin is also triggered by mercuric chloride. The radish leaf defensin was isolated and displayed antifungal characteristics comparable to those of the seed defensin isoforms. The inducibility of the radish leaf defensin by other treatments (e.g. wounding, salicylic acid) is currently under evaluation, as well as the kinetics of the production of the putative systemic signal.

J6-223 THE CO_2 SIGNAL TRANSDUCTION CHAIN IN STOMATAL GUARD CELLS, Alex A. R. Webb, Martin R. McAinsh, Terry, A. Mansfield and Alistair, M. Hetherington, Division of Biological Sciences, IEBS, Lancaster University, Bailrigg, LANCASTER, LA1 4YQ, U.K.

The guard cell has been used as model system for studying signalling mechanisms in plant cells. Much has been learnt about the pathways by which abscisic acid (ABA) brings about stomatal closure. However, little is known about the mechanisms by which CO_2 promotes stomatal closure and inhibits stomatal opening.

We have been testing the hypothesis that CO_2 induces stomatal closure via a calcium-based signal transduction pathway which shares common components with the ABA transduction pathway. This has been achieved by examining the effect of chelators of Ca^{2+} on the response of stomata in detached epidermis of *Commelina communis* to CO_2. The response of the guard cell cytosolic free calcium concentration ($[Ca^{2+}]_i$) to challenge with 700 ppm CO_2 has been monitered using fura-2 microinjected into the cytosol. The dependency of CO_2-induced stomatal closure on an increase in $[Ca^{2+}]_i$ is currently being investigated by buffering $[Ca^{2+}]_i$ at resting values prior to challenge with 700 ppm CO_2.

Cell Cycle

J6-224 CYCLIN EXPRESSION DURING LATERAL ROOT MERISTEM INITIATION IN *ARABIDOPSIS*, Adán Colón Carmona, Christopher J. Lamb and Peter Doerner, Plant Molecular and Cellular Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

Cyclins are important components of the regulatory machinery controlling plant growth. Cyclins and cyclin-dependent kinases (cdks) associate to regulate cell cycle progression. While cdks are expressed throughout the cell cycle, expression of cyclins oscillates. In the yeast cell cycle, for example, cyclin levels increase before DNA synthesis and cell division, and these increases are prerequisites for cell cycle progression. In pericycle cells, a checkpoint at the G2 to M phase transition regulates the post-embryonic formation of lateral root meristems. In response to a mitogenic signal, G2 arrested pericycle cells proceed to divide and initiate lateral root formation.

Cyclin levels in auxin-treated *Arabidopsis* roots increase several-fold, suggesting that cyclins play a role in the initiation of lateral roots. *Atcyc1* mRNA abundance was previously shown to be induced by auxin prior to the first visible cell division in the developing lateral root meristem. We examined the expression of eight *Arabidopsis* cyclin genes in roots at various time points after auxin treatment. Most cyclin genes are expressed at several-fold higher levels eight hours after auxin treatment when compared to non-induced roots. However, thirty hours after treatment, their expression has subsided. In addition, we examined the effects of colchicine and cycloheximide on the induction of cyclin genes and lateral root formation.

J6-225 SYMBIOTIC INDUCTION OF MADS-BOX GENES DURING DEVELOPMENT OF ALFALFA ROOT NODULES. Kathleen Dunn and Jacqueline Heard. Department of Biology, Boston College, Chestnut Hill, MA 02167

Floral homeotic genes from several plant species have been shown to share considerable sequence similarity with transcription factors from yeast (MCM1) and humans (SRF). All of these putative transcription factors contain a highly conserved DNA binding domain called the MADS box and are responsible for transducing environmental signals influencing organ development. To address the possibility that a member of this class of transcriptional regulators may be active in alfalfa (*Medicago sativa*) root nodule development, degenerate oligonucleotides were designed to conserved sequences flanking the MADS box domain of the *Arabidopsis thaliana ap3* homeotic gene. PCR amplification from an alfalfa nodule cDNA library resulted in one product sharing significant sequence and amino acid homology with MADS- box containing genes from several plant species as well as the transcription factors from yeast and human. Subsequently, two clones were isolated from nodule cDNA, *nmh7* and *nmhC5*. RT-PCR analysis suggests that both of these clones are nodule specific and distinct from other MADS-box transcripts present in flower tissue. *In situ* hybridization using the conserved MADS-box domain showed expression is localized primarily to the infected cells of the nodule. No signal was found in the nodule meristem. To determine whether intracellular infection is required for the induction of *nmh7* and *nmhC5*, their expression was analyzed in uninfected or "empty" alfalfa root nodules which are formed in response to an exo- mutant of alfalfa's endosymbiont *Rhizobium meliloti*. Neither *nmh7* nor *nmhC5* was expressed in nodules that lack intracellular bacteria suggesting induction of these genes is likely to be part of a signal transduction pathway initiated by the infecting bacterium. Interestingly, RT-PCR analysis did reveal the presence of a unique MADS-box transcript found "empty" nodules, but not in infected nodules. The relationship of this MADS-box transcript to nodule development, to MADS-box genes expressed during flower development or to a host response to an aborted symbiotic infection is currently being investigated.

J6-226 **FACTORS THAT INFLUENCE *LUFFA CYLINDRICA* PROTOPLAST DIVISIONS**

Volodymyr P. Duplij[1], Stanislav Ya. Ledovskiy[2], Vladimir A. Sidorov[1]
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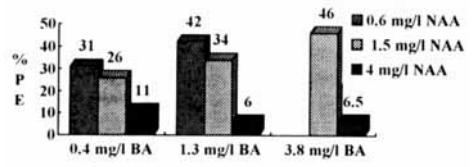
Dish-rag gourd (*Luffa cylindrica* Roem.) has been grown for a long time for food, medicine, or ornament. Today it is known as a source of industrial fibres. Its resistance to diseases of *Cucurbitaceae* and unfavourable factors of environment makes it useful for involving in somatic hybridization with other crop plants of this family.

Our previous research on this species stated that immobilization significantly enhanced the rate of cell divisions. *L. cylindrica* mesophyll protoplasts were embedded in Ca-alginate at a density of 2.5×10^5 /ml and cultivated in modified K_3-NM nutrient medium with various contents of growth regulators. Plating efficiency (PE) was estimated as a percentage of cells divided at least twice within 14 days. PE was the highest (46, 42%) when 1.5 mg/l NAA, 3.8 mg/l BA and 0.6 mg/l NAA, 1.3 mg/l BA were used. The effect of different concentrations of NAA and BA on colony formation is shown on the diagram. Protoplasts divided only occasionally (PE<0.5%) in the media containing from 0.4 to 1.2 mg/l 2,4-D and 1.2 mg/l 2iP.



J6-227 AXILLARY MERISTEM DEVELOPMENT IN Arabidopsis thaliana. Vojislava Grbic and Kathy Barton, Department of Genetics, University of Wisconsin-Madison, Madison, WI 53705.

Axillary shoot meristems in Arabidopsis activate at the transition of the primary shoot apical meristem from the vegetative to the reproductive state. They have developmental continuity with the primary shoot apical meristem and are initiated in the axils of the leaves. We are interested in the signaling pathway leading to the activation of the axillary shoot meristems. We have examined axillary meristem in axl1, axl2 and Zu ecotype, Arabidopsis variants which have branchless primary inflorescence due to the lack of activation of axillary meristems. In addition, we studied axillary meristem development in Sy-0 ecotype, in which they form a rosette of leaves at stem nodes. Also, axillary meristems in late flowering strains associated with the rosette leaves activate independently of the developmental state of the primary shoot apical meristem. Genetic and structural analysis of axillary meristem development in these strains will be presented.

J6-228 MBP1, A PLANT MAR-BINDING PROTEIN WITH STRUCTURAL SIMILARITY TO MYOSIN, Iris Meier[1], Wilhelm Gruissem[2], and Dagmar Schneider[1], Institute for General Botany, AMP I, University of Hamburg, D-22609 Hamburg, Germany ([1]), Department of Plant Biology, University of California, Berkeley, CA 94720, USA ([2])

A variety of observations indicate that the interactions between the nuclear protein matrix and matrix attachment regions (MARs) on the DNA define domains within the eucaryotic chromatin and play an important role in regulating gene expression, possibly by shielding genes from the influence of flanking regulatory regions. MAR elements have been roughly defined as AT-rich DNA sequences of 300 to more than 1000 bp length, which are located exclusively in noncoding regions. The amino acid sequence of a small number of animal MAR-binding proteins is known, yet their structures do not provide evidence about how they act in binding DNA and attaching chromatin to the nuclear matrix. We have isolated the cDNA for a DNA-binding protein from tomato (MBP1) with the characteristics of a MAR-binding protein. MBP1 was expressed in *E. coli* and, in a "south-western" binding assay, shown to bind with high affinity to MAR elements of chicken, *Drosophila* and soybean, but with much reduced affinity to a non-MAR DNA fragment of equal AT content. MBP1 is a novel protein with highest homology to the a-helical rod domain of myosin as well as other animal filament proteins. Secondary structure analysis showed that MBP1 is more than 80% a-helical and that the a-helices are amphipathic, with hydrophobic residues mainly at positions 4 and 7. A deletion analysis of MBP1 demonstrated that a defined DNA-binding domain is localized at the C-terminus of the protein. The structure of MBP1 immediately suggests a mechanism for chromatin-matrix interaction. The protein may anchor to MAR-elements via the C-terminus and to nuclear structural filament proteins (e.g. the lamins) through the amphipathic residues.

J6-229 HIGH DENSITY AEROPONIC CULTURE FOR THE STUDY OF EARLY NODULATION IN *MEDICAGO TRUNCATULA*. A.-U. B. van Tilburg and Douglas R. Cook. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Medicago truncatula, an annual forage crop, forms a symbiotic association with *Rhizobium meliloti* under limiting nitrogen conditions. Indeterminate nodules are formed in which the bacteria receive photosynthate from the plant and fix atmospheric nitrogen that the plant utilizes as ammonium. Our laboratory has previously discovered an mRNA encoding a *Rhizobium*-induced peroxidase, *rip*1, that accumulates very rapidly after *M. truncatula* is inoculated with *R. meliloti*. *rip*1 mRNA reaches maximum accumulation between 3 to 6 hours post inoculation. The development of a high-density aeroponic culture system for *M. truncatula* allows the efficient isolation of RNA, DNA, and protein from extremely young root tissue. Seedlings are planted in individual containers at a density of 6×10^3 M^{-2}. Analysis of root tissue has been performed as early as 100 hours after seed germination treatments. This system is being used to study DNA/protein interactions between upstream promoter sequences of early nodulation genes (including *rip*1, *ENOD*11 and *ENOD*12) and nuclear proteins under inoculated and uninoculated conditions.

J6-230 A DEVELOPMENTALLY-REGULATED RIBOSOMAL DNA-BINDING FACTOR IN PEA LEAVES Liwen Wang, Ken Piller, Kevin Folta, Lon S. Kaufman. Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607

In the common garden pea, Pisum sativum L. cv Alaska, ribosomal RNA gene transcription rates become elevated during the first four days of leaf development. This increased ribosomal RNA gene expression is actively regulated by mitosis. Mobility shift analysis, using nuclear extracts derived from leaf tissue at different developmental stages, indicates that a protein complex binds to the core promoter region after the onset of leaf development, coincident with the onset of rDNA transcription. This binding can be induced by mitosis. This complex only binds to the bottom strand of the promoter as well as to 18s rRNA, but with a 10-fold lower affinity. This complex does not recognize the top strand of the promoter nor other ssDNA. This complex contains three proteins--24KD, 35KD and 44KD. Clones of the 44KD polypeptide suggest that it is a eukaryotic ribosomal protein S1 homolog. Clones of the 44KD polypeptide do not code for the 20KD C-terminal portion of the prokaryotic S1, which is responsible for mRNA binding. The dual binding ability of this complex and the identification of a modified ribosomal protein as part of the complex suggests a feedback mechanism for regulating rDNA transcription.

J6-231 EXPRESSION AND BIOCHEMICAL FUNCTION OF *NODPQ*$_1$ AND *NODPQ*$_2$ IN *RHIZOBIUM MELILOTI*, Michael G. Willits and Sharon R. Long, Department of Biological Sciences, Stanford University, Stanford, California, 94305

We are currently studying genes in *Rhizobium meliloti* that convert inorganic sulfate to the high energy sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS). The enzymes involved are ATP sulfurylase and adenosine-5'-phosphosulfate (APS) kinase. In *Escherichia coli* PAPS is the sulfate donor for cysteine synthesis, and strains with mutations in the genes encoding ATP sulfurylase (*cysDN*) or APS kinase (*cysC*) are cysteine auxotrophs.

R. meliloti has three loci homologous to *cysDNC*. One locus, *saa*, appears to be involved with cysteine and methionine synthesis. The second two loci are *nodPQ*$_1$ and *nodPQ*$_2$, and they are 98% identical to each other at the nucleotide level. We know that *nodPQ*$_1$ is regulated along with the *nod* regulon and produces PAPS used in Nod Factor synthesis. Since *nodPQ*$_2$ is not regulated with the *nod* genes, we set out to study its regulation using a *gusA* promoter fusion. We found that *nodPQ*$_2$ shows growth-rate dependent regulation, leading to a thirty-fold increase in expression as the cells enter stationary phase. *nodPQ*$_1$ shows similar regulation with a four-fold increase in expression. We have mapped the transcription start site of *nodPQ*$_1$ and found that the sequence upstream of this site is homologous to the growth-rate regulated gearbox promoters seen in *E. coli*. We are now trying to gain a more detailed understanding of the sequences necessary for growth-rate dependent regulation in *Rhizobium* using mutagenized *nodPQ*$_1$ and *nodPQ*$_2$ promoters fused in frame to a *gusA* gene. We are also investigating the role this regulation may play by analyzing various strains containing mutations in the *nodPQ* loci. The major feature we are studying is the lipopolysaccharide (LPS), because *R. meliloti* is unique in having sulfated LPS. Therefore we are looking at the degree the LPS is sulfated in *nodPQ* mutants versus wild type.

Signaling By Light

J6-300 FUNCTIONALLY REDUNDANT DNA:PROTEIN INTERACTIONS CONTRIBUTE TO PHYTOCHROME- AND CIRCADIAN CLOCK-REGULATED EXPRESSION OF THE *ARABIDOPSIS CAB2* GENE, Shawn L. Anderson, Graham R. Teakle, Steve A. Kay, NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903

We have used an *in vivo cab::luciferase* bioluminescent marker that has allowed us to assay the expression of chimeric constructs with unprecedented sensitivity and time resolution in living seedlings. Dissection of -322 to +1 of the *cab2* promoter has revealed a minimal 73 bp domain confers both circadian- and phytochrome-regulation upon a heterologous promoter. A conserved repeated GATA sequence within the 73 bp regulatory domain is specifically bound by a protein factor designated CGF-1. Analysis of *luc* expression in constructs containing site-directed mutation of the CGF-1 binding site demonstrate that CGF-1 binding is required for the generation of the high amplitude oscillation in *cab2::luc* expression in green tissue, and the pattern of phytochrome-regulated *cab2::luc* expression in etiolated plants. Binding of CGF-1 to the -111 to -38 region of the *CAB2* promoter is competed with upstream fragments of the *CAB2* promoter in gel retardation assays, demonstrating redundant binding of CGF-1 to the *CAB2* promoter *in vitro*. *CAB2* 3' deletions fused to *luc* are also clock and phytochrome regulated, demonstrating functional redundancy within the *CAB2* promoter, as well. We discuss the role of *CAB2* promoter-protein interactions in terms of clock and phytochrome regulation and present a model of the *CAB2* promoter in which the binding of CGF-1 at multiple sites in the promoter is required for the phytochrome-regulated high amplitude oscillation in *CAB2* expression.

J6-301 VIOLAXANTHIN DE EPOXIDASE

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The xanthophyll cycle consists of light - dependent conversions of three xanthophylls in a cyclic reaction involving a deepoxidation sequence from the diepoxide violaxanthin via the monoepoxide antheraxanthin to the epoxide-free form zeaxanthin, and an epoxidation sequence in the reverse direction. These two reaction sequences are catalysed by two different enzymes. The xanthophyll cycle is present in the thylakoid membranes of all higher plants, ferns, mosses, and several algal groups. Since the xanthophyll cycle is believed to be involved in the photo protection due to high light, it is of great importance to characterize this process. This work presents identification and purification of one of the enzymes regulating the xanthophyll cycle, namely the Violaxanthin de-epoxidase.

J6-302 A NOVEL PHYTOCHROME MEDIATES THE FAR-RED INDUCTION OF THE *Athb-2* GENE IN *Arabidopsis thaliana*, Monica Carabelli[1], Giorgio Morelli[2], Garry Whitelam[3] and Ida Ruberti[1], [1]Centro di Studio per gli Acidi Nucleici, CNR Roma, Italy, [2]Unità di Nutrizione Sperimentale, INN Roma, Italy and [3]Department of Botany, University of Leicester, UK.

The *Arabidopsis Athb-2* gene encodes a 32 kDa protein characterized by the presence of a homeodomain (HD) with a closely linked leucine zipper (Zip) motif (Ruberti et al., 1991; Carabelli et al., 1993).

DNA binding studies showed that the Athb-2 HD-Zip domain forms a dimeric complex which is able to bind a 9 bp dyad-symmetric sequence (Sessa et al., 1993).

Athb-2 mRNA analysis showed that this gene is expressed during the vegetative and reproductive phases of plant growth. A significant increment in the amount of *Athb-2* transcripts was observed in flowering plants. A higher steady-state level of the *Athb-2* mRNA was also found in dark-adapted plants. Remarkably, far-red-rich light treatment of *Arabidopsis* plants results in a rapid and strong induction of the *Athb-2* expression (Carabelli et al., 1993).

Light induction experiments in wild type plants and mutants deficient either in phytochrome A or in phytochrome B or in both phytochrome A and B have now established that the far-red induction of the *Athb-2* gene is mediated by a phytochrome other than A and B.

Ruberti et al., (1991) EMBO J. **10**, 1787-1791
Carabelli et al., (1993) Plant J. **4**, 469-479
Sessa et al. (1993) EMBO J.**12**, 3507-3517

J6-303 SIGNAL TRANSDUCTION IN THE UV-B AND BLUE-LIGHT-REGULATION OF CHALCONE SYNTHASE GENE EXPRESSION IN *ARABIDOPSIS*, John M. Christie and Gareth I. Jenkins, Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

Chalcone synthase (CHS) catalyses the first committed enzymatic step of the flavonoid biosynthesis pathway in plants. In *Arabidopsis*, *CHS* gene expression is under the control of endogenous and environmental signals including UV-B, UV-A and blue light. There is evidence that these wavelengths are detected by separate photoperception systems that can interact synergistically to induce *CHS* gene expression. We have used a photomixotrophic *Arabidopsis* cell suspension culture to investigate the light-regulation of *CHS* gene expression. Our results demonstrate that *CHS* transcript levels in the cell culture are regulated by UV-B, UV-A and blue light, similar to the intact plant, and we have examined possible signal transduction events involved in the regulation of *CHS* expression using various signalling agonists and antagonists.

The increase in *CHS* transcript levels following exposure to UV-B or blue irradiation occurs within 4-6 hours and is not transient at least over 24 h. The increase in *CHS* transcripts induced by UV-B is inhibited by the calmodulin antagonist W-7, whereas the blue light-induction of *CHS* expression is unaffected by an equivalent amount of the compound. This indicates that calmodulin plays a role in the UV-B signal transduction pathway but probably not in the blue light-specific pathway. Further experiments implicate reversible protein phosphorylation in both the UV-B and blue light response pathways, in that the kinase inhibitor staurosporine and the phosphatase inhibitor okadaic acid inhibit the increase in *CHS* transcript levels.

J6-304 INOSITOL 1,4,5-TRISPHOSPHATE MAY MEDIATE CLOSURE OF K^+ CHANNELS BY LIGHT AND DARKNESS IN *SAMANEA SAMAN* MOTOR CELLS, Gary G. Coté, Hak Yong Kim, and Richard C. Crain, Department of Molecular and Cell Biology, The University of Connecticut, Storrs, CT 06269-3125

Leaflet movements of *Samanea saman* depend in part upon circadian-rhythmic, light-regulated K^+ fluxes across the plasma membranes of extensor and flexor cells in opposing regions of the leaf-moving organ, the pulvinus. We previously showed that blue light closes open K^+ channels in flexor protoplasts during the dark period (subjective night), while transfer to darkness closes open K^+ channels in extensor protoplasts during the light period (subjective day). We now report that both these channel-closing stimuli increase inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] levels in the appropriate protoplasts. Neomycin, an inhibitor of polyphosphoinositide hydrolysis, inhibits both blue light-induced Ins(1,4,5)P_3 production and K^+ channel closure in flexor protoplasts and both dark-induced Ins(1,4,5)P_3 production and K^+ channel closure in extensor protoplasts. The G protein activator mastoparan mimics blue light and darkness in both increasing Ins(1,4,5)P_3 levels and closing K^+ channels in the appropriate cell type at the appropriate time. These results indicate that phospholipase C-catalyzed hydrolysis of phosphoinositides, possibly activated by a G protein, is an early step in the signal transduction pathways by which light and darkness close K^+ channels in *S. saman* pulvini.

J6-305 *ARABIDOPSIS* MUTANTS INVOLVED IN SUCROSE REPRESSION OF PLASTOCYANIN GENE EXPRESSION. Paul Dijkwel, Patricia Kock†, Rex Bezemer†, Peter Weisbeek†, Sjef Smeekens† and Nam-Hai Chua. Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021. †Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Plastocyanin (PC) functions as an electron carrier in the photosynthetic electron-transfer chain in the chloroplast. Expression of the nuclear-encoded *PC* gene was studied in transgenic *Arabidopsis* plants carrying a *PC*-promoter firefly-luciferase fusion gene. Luciferase does not accumulate in the presence of its substrate luciferin and luciferase activity can be measured *in vivo*, using a sensitive video-imaging equipment. These properties make it possible to measure real time promoter activity in a large number of individual plants without destruction of the plants.

Luciferase activity in etiolated seedlings was increased after 2 days of growth in darkness. This increase could be repressed in a concentration-dependent manner by growing the seedlings on sucrose-containing medium. Growth on non-metabolizable sugars did not result in repression of luciferase activity. Also, *PC* mRNA levels were increased after 2 days of growth in darkness and then gradually decreased to basal levels after 6 days of growth. This transient increase in *PC* mRNA abundance could be repressed in seedlings grown on sucrose-containing medium. Carbohydrate analysis in seedlings grown in the presence of sucrose and glucose revealed an inverse relation between seedling carbohydrate content and *PC* gene expression. In light-grown seedlings transient activation of the *PC* gene was observed as in dark-grown seedlings but expression levels were 15-fold higher. This increase in the light could also be repressed by growth on sucrose. These findings suggest the presence of a sucrose-sensitive, developmentally-controlled expression mechanism that operates independently from light.

A mutant screen was developed to identify components involved in this expression mechanism. *In vivo* luciferase activity was measured in EMS-mutagenized transgenic seedlings carrying a *PC*-luciferase fusion gene. Following this approach, several mutants were isolated in which the luciferase activity is no longer repressed when grown on a sucrose-containing medium. Genetic and biochemical characterization of the mutants will be presented.

J6-306 INTERACTION BETWEEN THE PHYTOCHROME AND GRAVITY SENSORY-RESPONSE SYSTEMS IN *ARABIDOPSIS*, Roger P. Hangarter, Jae-Hyeon Kim and Bhaju Tamot, Department of Plant Biology, The Ohio State University, Columbus, OH 43210

Hypocotyls of dark-grown *Arabidopsis* seedlings exhibit strong negative gravitropism, growing away from the gravitational vector in a unidirectional manner. However, when grown in red light, the growth direction is randomized with respect to the gravity vector. This response has been shown to be mediated by phytochrome and, through the analysis of various phytochrome-deficient mutant strains of *Arabidopsis*, it was found that the red light-absorbing form (Pr) of phytochrome B regulates normal hypocotyl gravitropism in darkness. Far-red light was also found to cause a similar, though less extreme, disruption of growth orientation. However, it appears that the phytochrome A effect is mediated by the Pfr form. In addition, when dark-grown seedlings are transferred to red or far-red light, their growth pattern shifts from negative gravitropic to plagiotropic and the effect is dependent on phytochromes A and B. These phytochrome-dependent effects on the gravity response are apparent only in the shoot since roots continue to exhibit normal positive gravitropism. Thus, the phytochrome-mediated signal transduction systems interact with part of the gravity sensory-response system in *Arabidopsis*.

J6-307 **CIRCADIAN CLOCK CONTROLLED *Lhc* GENE EXPRESSION: ANALYSIS OF CIS- AND TRANS-REGULATORY ELEMENTS, Nicole Merforth, Daniel Dreesmann, Sabine Riesselmann*, Birgit Piechulla, Institut für Biochemie der Pflanze, Untere Karspüle 2, 37073 Göttingen, Germany, *Present address: LG Molekulargenetik, Universität Hannover, Herrenhäuserstr.2, 30419 Hannover, Germany**

All nineteen genes encoding light harvesting complex proteins in tomato exhibit a characteristic circadian expression pattern. Two strategies were applied to analyse possible regulatory components. i) trans-regulatory elements: Band shift experiments demonstrate the binding of several distinct proteins to the 5'upstream regions of three *Lhc* genes (cab 1B, cab 8, cab 11). ii) cis-regulatory elements: Sequence comparison of 5'upstream regions of the *Lhc* genes does not exhibit a conserved DNA motif present in all *Lhc* genes, except for the 'CCAAT'- or 'TATA'- motif. Deletion mutant analysis in transgenic tobacco plants demonstrates that 5'upstream sequences of either 68 or 148 nucleotides upstream of the transcription start site are sufficient for mediating the circadian *Lhc* mRNA accumulation. These results suggest that the regulatory components of the 'circadian clock' are most likely localized within or closely adjacent to the polymerase initiation complex (PIC).

J6-308 ANALYSIS OF SIGNAL TRANSDUCTION PATHWAYS CONTROLLING DEVELOPMENTAL TIMING IN *ARABIDOPSIS*, Karen A. Hicks[1], Steve A. Kay[2], and D. Ry Meeks-Wagner[1], Institute of Molecular Biology[1], University of Oregon, Eugene, OR 97302 and NSF Center for Biological Timing[2], University of Virginia, Charlottesville, VA 22901.

The timing of the transition from vegetative to reproductive development in plants is controlled in part by photoperiod, or daylength. Previous work in the Meeks-Wagner lab led to the isolation of the *early flowering3* (*elf3*) mutant of *Arabidopsis*, which is photoperiod insensitive with respect to the timing of floral initiation. In addition, the *elf3* mutant displays the characteristic long-hypocotyl phenotype of plants defective in light reception or transduction of light signals.

Photoperiodic responses are mediated by the cycling of light/dark periods, and have long been thought to be tied to a biological clock. We have found that the *elf3* mutant exhibits a striking defect in endogenous rhythmic expression from the circadian-regulated promoter *cab2*. Expression from a *cab-luc* reporter gene was followed by imaging single transgenic seedlings using a low-light video camera and a photon-counting image processor. A significant defect in *cab-luc* cycling was observed in the *elf3* mutant as compared to a wild type control under constant light conditions. Further experiments which address the entrainment of *cab* cycling in light/dark cycles are underway, the results of which may provide a link between the defect in photoperiodic induction of flowering and *cab-luc* expression in the *elf3* mutant.

ELF3 likely functions in a signal transduction pathway required for light regulation of floral development. In order to further define the genetic pathways that regulate floral initiation, a genetic screen is being used to isolate suppressor and enhancer mutations of the EMS-induced allele of *elf3*, *elf3-1*. This screen should facilitate the identification of important genes that interact with *ELF3* to regulate floral induction. Ultimately, a complete understanding of the wild type function of *ELF3* will require a detailed molecular analysis. Towards this end, we are using chromosomal walking techniques in order to isolate the *ELF3* locus.

J6-309 ISOLATION OF *ARABIDOPSIS* MUTANTS ALTERED IN THE LIGHT-REGULATION OF CHALCONE SYNTHASE GENE EXPRESSION USING A TRANSGENIC SCREENING APPROACH, Gareth I. Jenkins, Jennie A. Jackson, Geeta Fuglevand and Morgan J. Shaw, Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

We produced transgenic *Arabidopsis* plants expressing β-glucuronidase (GUS) driven by a chalcone synthase gene (*CHS*) promoter. A single diploid individual, homozygous for the transgene, was selfed to produce an isogenic line. GUS activity in this line increases with increasing fluence rates of white light and is induced by UV-B, UV-A and blue light but not significantly by red light, similar to the endogenous *CHS* transcripts. Seed from this line was mutagenised and M2 seedlings screened for mutants with altered light-regulation of the transgene. Putative mutants with low GUS activity are not altered in the light-induction of endogenous *CHS* transcript levels and hence are not regulatory mutants. Experiments with 5-azacytidine suggest that methylation of the transgene contributes to the low GUS activity in these lines. Two mutant lines with enhanced light-stimulation of GUS activity show a corresponding increase in endogenous *CHS* transcript levels. These mutants are diploid and not altered in the organisation of the transgene in genomic DNA. One of these, designated *icx1* (increased chalcone synthase expression), has been studied in detail. In *icx1*, enhanced light-induction is also observed for transcript levels of chalcone isomerase and dihydroflavonol reductase and for anthocyanin synthesis. In darkness the level of GUS activity is very low, similar to the wild-type, and the morphology is normal and not like the *det* or *cop* mutants. The *icx1* phenotype segregates independently of the transgene but appears to co-segregate with several other phenotypic characteristics, including fewer trichomes and narrow leaves at higher fluence rates of white light. On the basis of these data and comparison with the *Arabidopsis ttg* (transparent testa glabra) mutant, we suggest that the *ICX1* gene product may be concerned with both the light-regulation of gene expression and developmental processes occurring in the epidermis.

J6-310 IDENTIFICATION OF SEQUENCE SPECIFIC DNA BINDING PROTEINS THAT INTERACT WITH THE CHLOROPLAST LIGHT RESPONSIVE *psbC-psbD* PROMOTER Minkyun Kim and John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

The chloroplast genes *psbD* and *psbC* are cotranscribed from a complex chloroplast transcription unit. These genes encode chlorophyll binding proteins that are located in, Photosystem II, a protein complex that mediates oxygen evolution. We have previously demonstrated that two of the *psbD-psbC* mRNAs is regulated at the transcriptional level by blue and UVA light (EMBO J. 8:2785-2794). In this study, transcription of *psbD-psbC* was analyzed in vitro using high salt extracts from etioplasts isolated from dark grown barley and chloroplasts isolated from illuminated barley. DNA containing 185 bp of the light modulated *psbD-psbC* promoter was transcribed with fidelity in vitro. Gel retardation, competition and DNAse I footprinting experiments identified two major sequence specific DNA binding sites/DNA binding proteins that interact with this promoter. One of the binding sites was similar in sequence to GT-1 binding sites found in the promoters of nuclear light regulated genes. The interaction of proteins with the *psbD-psbC* GT-1 like sequence was strong in extracts of dark grown plants and reduced in extracts of light grown plants. Phosphorylation of proteins in the extracts from dark-grown plants reduced binding. We speculate that phosphorylation of the GT-1 like protein may derepress transcription from the *psbD-psbC* promoter in vivo. This research was supported by NIH grant GM37987.

J6-311 **A blue-light photoreceptor regulates different aspects of plant development: an analysis of transgenic Arabidopsis plants overexpressing *HY4* gene.**

Chentao Lin, and Anthony R. Cashmore,

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Light regulates many aspects of plant development via collective action of different photoreceptors . In addition to the well characterized responses mediated by red/far red light, blue light also has a profound effect on plant development. The Arabidopsis gene *HY4* is known to encode a flavoprotein mediating the blue light-dependent inhibition of hypocotyl elongation. Several lines of evidence indicated that HY4 is a blue light photoreceptor (see Cashmore et al., this meeting). The effects of HY4 on plant development were further studied in transgenic Arabidopsis plants overexpressing HY4. It was found that overexpression of HY4 resulted in increased sensitivity of the transgenic plants to blue, green, and UV-A light in the hypocotyl elongation response. Under these light conditions, transgenic Arabidopsis showed substantially shorter hypocotyl than that of the wild type plants, this phenotype had been previously demonstrated in treansgenic tobacco plants overexpressing HY4. In contrast to the transgenic tobacco which had few phenotypic alternations in the adult plants, adult Arabidopsis transgenic plants were dwarf when grown under normal light condition. In addition to the effect on stem elongation, overexpression of HY4 in Arabidopsis had other effects on plant development, such as anthocyanin biosynthesis, and flowering time. The possibility that HY4 may regulate different aspects of plant development will be discussed.

J6-312 ROLE OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASES IN MEDIATING LIGHT-REGULATED ROOT GRAVITROPISM IN MAIZE, Ying-Tang Lu and Lewis J. Feldman, Department of Plant Biology, University of California, Berkeley, CA 94720

Light regulates many aspects of plant growth and development, yet relatively little is known about the steps involved in transducing the light signal into a developmental response. A model system for studying light signal transduction is shown by roots of certain cultivars of corn which respond to gravity only if illuminated. This light-regulated root gravitropism involves phytochrome and is mediated by calcium, but very little else is known about the mechanism of light transduction. Towards further characterizing light signal transduction in this system we isolated two isomers of calcium/calmodulin-dependent protein kinase (both cDNAs and genomic DNAs) from *Zea mays* (cv. Merit) root caps, the site for both gravity and light perception in roots. Sequencing analyses show that these isomers have over 90% identity in amino acid sequence, and that both contain kinase catalytic and calmodulin-binding domains. Though their expressions appear to be differentially regulated, both isomers have similar genomic structures. Further analyses indicate that the *E. coli* -expressed fusion protein binds to calmodulin (CaM) in a calcium-dependent manner. However, this binding is abolished in the presence of 50 μM of KN-93, a specific inhibitor of animal CaM kinase II. Interestingly, light-regulated root gravitropism is also inhibited by this drug at this concentration. We have previously shown that KN-93 acts by interrupting light signal transduction, and does not affect either the light perception or root elongation. These data suggest that CaK kinase may be involved in light-regulated root gravitropism by mediating light signal transduction. An investigation of how the CaM kinase acts in light transduction is under the way via studies of expression regulation, substrate identification and analysis transgenic plants.

J6-313 LIGHT SIGNAL TRANSDUCTION MUTANTS OF *ARABIDOPSIS*. Raphael Mayer[1], Andrew Millar[1], Steve Kay[2] and Nam-Hai Chua[1], [1] Rockefeller University, 1230 York Ave., New York, NY 10021, USA. [2] NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903.

Genetic studies in *Arabidopsis* offer a promising approach to elucidate the mechanism of light signal transduction. In the past, several putative light signaling mutants have been isolated based on their aberrant morphological phenotypes in the dark. However, such mutants appear to have pleiotropic effects upon several different developmental pathways. To isolate mutants that are more specifically affected in light signal transduction pathways, we have designed a screen based on the *in vivo* response of a molecular marker to light. We have used a video imaging technique to follow the luciferase activity of mutagenized *Arabidopsis* plants carrying a transgene composed of a light responsive promoter (*cab2*) fused to a luciferase coding sequence. The mutants were selected based on their altered expression pattern of the *cab* promoter in response to specific light intensities or wave lengths. The *cab*:luciferase activity of each mutant was also used as a genetic marker for segregation analysis and mapping of the mutations. Several mutants altered in their *cab*:luciferase has been isolated, one of them termed phytochrome signaling 1 *(psi1)* has been characterized in details: *psi*1 overexpresses luciferase activity in the dark and also in response to far-red light and light of other wave-lengths. In contrast to the *cop/det* mutants which are epistatic to *phy* mutants, studies on the double mutant *phyA/psi*1 revealed that *phy*A is epistatic to *psi*1. Our data suggest that PSI1 functions in PHYA signal transduction chains. The *psi*1 mutant shows normal chloroplast development and phytochrome levels, but has a unique subset of physiological phenotypes including wave-length dependent deetiolation, gravitropism and late flowering.

J6-314 EXPRESSION PATTERNS OF THE *Arabidopsis* PHYB, PHYD, AND PHYE PHYTOCHROMES, Bob Sharrock, Lakshmi Palecanda, and Sarah Mathews, Department of Biology, Montana Sate University, Bozeman, MT 59717

The apoprotein portions of the red/far red light-sensing photoreceptor phytochrome are encoded by gene families in all angiosperm plants that have been analyzed. We have previously determined the cDNA and/or genomic nucleotide sequences coding for five phytochrome apoproteins from *Arabidopsis*, *PHYA-PHYE*. Phylogenetic analysis of these sequences indicates that the PHYB, PHYD, and PHYE apoproteins constitute a subgroup of more closely related and more recently derived structures compared to PHYA and PHYC. In order to characterize the upstream DNA regulatory sequences of the B, D, and E genes and to identify potential differences in their patterns of expression, the putative promoter regions for these genes have been sequenced, their transcription start sites identified, and translational fusions of these sequences to the GUS gene have been constructed and introduced into *Arabidopsis*. Results of histochemical and quantitative GUS assays on these transgenic lines indicate that the *PHYB*, *D*, and *E* genes are differentially expressed in various plant organs, at different developmental stages, and in response to light. This suggests that the individual members of the PHYB/D/E phytochrome subgroup may play distinctive roles in plant photoregulation at least in part as a consequence of their respective expression patterns.

J6-315 THE LONG-HYPOCOTYL (*lh*) MUTATION IN CUCUMBER DOES NOT ELIMINATE RESPONSES TO CONTINUOUS RED LIGHT: POSSIBLE ROLES FOR phyA AND phyB IN REGULATION OF STEM ELONGATION AND HOOK OPENING, James R. Shinkle, Department of Biology, Trinity University, San Antonio, TX, 78212-7200

Cucumber seedlings carrying the *lh* mutation lack phyB, and are thus phenotypically similar to *Arabidopsis hy-3* mutants. Studies with the latter species suggest that there may be a phyA-regulated inhibition of stem elongation, and that phyB does not regulate early events in red-light-induced hook opening. Because cucumber seedlings are much larger than *Arabidopsis* seedlings, it has been possible to more clearly characterize the two growth responses in terms of kinetics and fluence-dependence. Dim Red Light (DRL, 0.5 μmol m^{-2} sec^{-1}) induces identical transient, inhibition of hypocotyl elongation in both wild-type and *lh* seedlings, suggesting control by phyA. When the fluence rate is increased 10-fold (10X DRL), wild-type seedlings show a persistent inhibition of elongation, while the *lh* mutants show a transient inhibition of shorter duration than that seen in response to DRL. Hence, phyB appears to regulate stem elongation in a fluence-rate-dependent manner. The fact that higher fluence red light causes less inhibition of elongation in *lh* seedlings than does DRL suggests that phyA or other phytochromes either interact with phyB, or are themselves differentially affected by different red light fluences. In contrast to *hy-3*, the *lh* mutation does affect hook opening in the first 24h of irradiation with continuous red light. *lh* seedlings show no hook opening in DRL, and only partial hook-opening in 10X DRL, while wild-type seedlings show some response to DRL and a greater response to 10X DRL. This is the reverse of the situation in light-regulated stem elongation where phyB is not involved in DRL-induced responses. These results indicate that transduction of phyA and phyB signals can vary between two fairly similar growth responses.

J6-316 DISTINCT ACTION SPECTRA FOR PHYTOCHROME A- AND PHYTOCHROME B-DEPENDENT SEED GERMINATION IN *ARABIDOPSIS THALIANA*

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In *Arabidopsis thaliana*, germination of wild type (WT) seeds imbibed for 1-14h is photo-reversibly regulated by phytochrome B (PhyB) stored in dormant seeds, whereas phytochrome A (PhyA) is newly synthesized during imbibition and induces seed germination after imbibition for 48h or longer (Shinomura et al., 1994). With this system and PhyA-null mutant *fre1, phyA-201*, and the PhyB-null mutant *hy3, phyB-1*, we have determined *in vivo* action spectra for PhyA- and PhyB-dependent induction of seed germination separately, using Okazaki large spectrograph.

Fluence-response curves showed that PhyB-dependent germination was induced by irradiation with 667 nm light of 10-10^3 μmol m^{-2}, and photo-reversibly prevented by 726 nm light of similar fluence. On the contrary, PhyA-dependent germination was photo-irreversibly induced by very low fluence of both 667 nm light of 10^{-3}-10^{-1} μmol m^{-2} and 726 nm light of 10^{-1}-10 μmol m^{-2}. Action spectra for PhyB response showed that monochromatic lights ranged from 550 to 690 nm induce germination, while those from 700 to 800 nm suppress it. In contrast, PhyA response was induced by any monochromatic lights from 300 to 770 nm, and we never observed photo-reversible effect with any fluence of all wavelength tested. The PhyA-dependent germination response obeyed the reciprocity low within a fluence range from $2x10^{-3}$ to $2x10^{-1}$ μmol m^{-2} red light and a time range of 1 s - 5 min.

This is the first *in vivo* action spectra for PhyA and PhyB functions, demonstrating that, though the primary and secondary structures of PhyA and PhyB are very similar, the modes of light-signal perceptions with PhyA and PhyB are essentially different.

J6-317 CHARACTERIZATION OF A CHLAMYDOMONAS GENE ENCODING A PROTEIN OF THE DNA PHOTOLYASE/ BLUE LIGHT-PHOTORECEPTOR FAMILY, Gary D. Small[1], Byeongyong Min[1] and Paul A. Lefebvre[2], Dept. of Biochem. and Mol. Biology, Univ. of South Dakota, Vermillion, SD 57069[1], Dept. of Genetics and Cell Biology, Univ. of Minnesota, St. Paul, MN 55108[2]

The organization and nucleotide sequence of a gene from Chlamydomonas reinhardtii encoding a member of the DNA photolyase/blue light photoreceptor protein family is reported. A region of over 7 kb encompassing the gene was sequenced. Northern analysis detected a single 4.2 kb mRNA. A cDNA structure was derived by a combination of a partial cDNA from a cDNA library and by RNA-PCR followed by sequencing and comparison to the genomic sequence. The 5' end was determined by the RACE protocol. The gene consists of eight exons and seven introns. The gene is predicted to code for a protein of 867 amino acids. The first 500 amino acids exhibit significant homology with previously sequenced DNA photolyases, showing the closest relationship to mustard (Sinapis alba) photolyase with 43% identity. An even higher identity, 49%, is obtained when the Chlamydomonas gene product is compared to the putative blue-light photoreceptor from Arabidopsis thaliana. Both the Chlamydomonas and the Arabidopsis proteins differ from the well characterized DNA photolyases in that they contain a carboxyl terminal extension of 367 and 181 amino acids, respectively. However, there is very little homology between the two proteins in this region. A previously isolated Chlamydomonas mutant, phr1, that is deficient in DNA photolyase activity, especially in the nucleus, was shown by RFLP analysis not to be linked to the gene we have isolated. We propose that the gene that we have isolated is a candidate Chlamydomonas blue light photoreceptor gene.

J6-318 LIGHT-REGULATED TRANSCRIPTION FACTOR GENES FROM *ARABIDOPSIS THALIANA*, Sjef Smeekens, Nicolette Quaedvlieg, Jan Dockx, Fred Rook, Marcel Proveniers and Peter Weisbeek. Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Light as a signal initiates developmental processes in germinating *Arabidopsis* plants, collectively called photomorphogenesis. Most notable among these processes is the cellular differentiation process which lead to the formation of true leaves. We are interested in investigating what type of processes need to be activated by light to initiate cellular differentitation. Our approach has been to identify transcription factor (TF) genes which are activated by light immediately after transfer of etiolated seedlings to light and to study the processes controlled by such transcription factors. Light regulated TF factor genes have been isolated and three were selected for further study. These TF encode a homeodomain protein (H1), a myb protein (M4) and a basic leucine zipper protein (B2), respectively. These three TF genes are activated within one hour of transfer of etiolated seedlings to the light. The H1 gene shown a normal light/dark adaptation response but the M4 gene is only transiently expressed upon shift from dark to light. Mutants (*det1, cop1*) have been isolated which show photomorphogenetic development in the dark. We tested whether the three TF genes were expressed in these mutants. The H1 and B2 genes are derepressed in etiolated *det1* and *cop1* seedlings whereas the M4 gene is not. This suggests that H1 and B2 may be required for regulation of presently unknown developmental steps leading to the dark phenotype of these mutants. H1 and B2 have long and complex 5' untranslated regions in which uORFs are present. A transgenic approach is now being taken to localize expressing tissues and to uncover the biological function of these genes.

J6-319 PHOTOMORPHOGENIC MUTATIONS AFFECT THE CIRCADIAN PERIOD OF *CAB2*::LUCIFERASE EXPRESSION IN *ARABIDOPSIS*. David E. Somers, Andrew J. Millar and Steve A. Kay, NSF Center for Biological Timing, Department of Biology, University of Virginia, Charlottesville, VA 22903.

Light can entrain a wide range of biological processes to the central timekeeping oscillator(s) of plants. Since light is a primary input to the entrainment progam, mutations which cause light-dependent morphological defects may also disrupt or modify components in the signal transduction pathway leading to and from the clock. By testing if known photomorphogenic mutations correlate with aberrant circadian function, we can begin to relate clock regulation to the proposed hierarchy of phototransduction pathways. To this end we have examined the circadian oscillation in the expression of the *cab2* promoter fused to the luciferase coding region (*cab2::Luc*) in the background of known and putative light transduction pathway mutants. This unique gene fusion allows non-destructive *in vivo* monitoring of bioluminescence in living tissue. Preliminary results indicate that light inputs via both phytochrome and blue light receptor pathways are important in maintaining a normal circadian oscillation of bioluminescence in continuous light. A more detailed disssection of the relative contribution of both pathways using blue light mutants (*blu1, blu2, blu3, hy4*) and phytochrome mutants (*phyA, phyB*), alone and in combination, will be presented.

J6-320 SHIELDING AND REPAIR: RESPONSES OF MAIZE TO ULTRAVIOLET RADIATION, Ann E. Stapleton and Virginia Walbot, Department of Biological Sciences, Stanford University, Stanford CA 94305-5020
Plants use light for photosynthesis, and are unavoidably exposed to the ultraviolet radiation that is present in sunlight. Because plants lack the behavioral mechanisms that mobile animals use to respond to excess radiation, they may have evolved novel and effective mechanisms to protect essential functions from radiation damage. Plants have two basic mechanisms for coping with UV damage--shielding and repair. Approximately two-thirds of incoming UV radiation does not penetrate through the epidermal layer of a maize leaf. Much of this UV shielding is due to absorption by epidermal flavonoid compounds. We show that anthocyanins synthesized under the control of the regulatory genes *B* and *Pl* can protect maize leaf DNA from damage induced by UV radiation *in vivo*. Thus the interior of the plant is protected from the induction of UV radiation damage.

Shielding, however, does not provide complete protection from UV damage, and is not sufficient to explain differences in UV tolerance. Repair of UV-induced damage is essential for normal growth and development. We demonstrate that there is photoreactivation repair of UV-induced DNA damage in nuclear, mitochondrial and chloroplast genes in maize seedlings, suggesting that all three compartments contain photolyase. Developmental and molecular characterization of maize photolyase will be presented.

J6-321 ISOLATION OF GENES IN THE LIGHT SIGNALING PATHWAY BY mRNA DIFFERENTIAL DISPLAY, Lin Sun and Howard M. Goodman, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114
Most higher plants undergo two distinct developmental pathways in response to the presence or absence of light: In the dark, they develop an etiolated morphology with characteristics such as elongated hypocotyls and unexpanded cotyledons forming the apical hook. In the light, however, plant development is much different. The hypocotyls are shorter, cotyledons expanded and green, and true leaves developed. Dark-grown seedlings can switch to the light developmental program once light is provided. We are interested in finding genes important in initiating or maintaining these two distinct developmental pathways. We have used the mRNA differential display technique (Science 257, 967-971) to isolate genes whose expression is higher either in the dark or in the early transition from dark-growth to light-growth. A number of clones have been isolated this way and some show strong homology to genes known to play a role in signal transduction, including genes encoding receptor kinase, GTP-binding protein, and calmodulin. Characterization and analyses of the expression of the isolated clones will be presented.

J6-322 LIGHT-STIMULATED GROWTH OF LEAF CELLS, Elizabeth Van Volkenburgh[1], J. Theo M. Elzenga[2], and Michael M. Neff[1], [1]Department of Botany AJ-30, University of Washington, Seattle, WA 98195 and [2]Department of Plant Biology, University of Groningen, The Netherlands.
Dicotyledonous leaves, unlike stems, are stimulated to grow by bright light acting through phytochrome, a separate blue-light system, and photosynthesis. Light-induced apoplast acidification, wall loosening, and ion uptake are preceded by a transient depolarization of the plasmamembrane in response to light. The nature of this early cellular response to light has been investigated separately in epidermal and mesophyll cells of the argenteum mutant of pea (*Pisum sativum*), and in phytochrome deficient cotyledons of *Arabidopsis* mutants. The bulk of the membrane depolarization observed in mesophyll cells is most likely a result of chloride efflux passing through ATP-modulated Cl-channels, and is partially dependent on photosynthesis. Light stimulates channel activity on cell-attached patches of mesophyll protoplasts; excision of light-sensitive patches yields the above characterized Cl-channel. On the other hand, the transient depolarization of epidermal cells is more likely due to calcium influx, and is not dependent on photosynthesis. In *Arabidopsis* cotyledons, light-induced depolarization is evident in photosynthetic cotyledons of wildtype seedlings, but is diminished by the absence of phytochrome B. Ion substitution studies indicate that phytochrome B affects calcium, not chloride flux. Previous work showed that phytochrome B is essential for the full growth response of cotyledon cells to light. These results imply that phytochrome B is necessary for initial membrane transport responses of growing cells to light, and that there is an interaction between phytochrome and photosynthetic activity of these cells.

J6-323 A NOVEL *MYB*-RELATED DNA BINDING PROTEIN BINDS TO THE *Lhcb1*3* GENE PROMOTER *IN VITRO* AND AFFECTS ITS EXPRESSION *IN VIVO* IN *ARABIDOPSIS*, Zhi-Yong Wang, David Kenigsbuch, Lin Sun, Eitan Harel, May S. Ong, and Elaine M. Tobin, Department of Biology, University of California, Los Angeles, CA 90024
CA-1 is a nuclear protein that binds to a region of *Lhcb1*3* promoter that is necessary for phytochrome regulation of expression (Sun et al, 1993, Plant Cell 5: 109-121; Kenigsbuch and Tobin, unpublished data). We have isolated two overlapping cDNA clones by southwestern screening that encode a protein with DNA binding specificity similar to CA-1. We have designated this gene cCA-1. The corresponding genomic clone contains 8 exons interrupted by 7 introns. The cCA-1 protein has a DNA binding domain containing a sequence with some similarity to one of the repeat sequences of the myb DNA binding domain. We have shown that cCA-1 protein is transported into nuclei. Southern analysis at both high and low stringency showed that cCA-1 is a single copy gene in the *Arabidopsis* genome with no closely related sequences. Some differences between the cCA-1 protein expressed in *E. coli* and the nuclear CA-1 were found. In contrast to the CA-1 DNA binding activity, which was absent in det1-1 mutant seedlings, the cCA-1 mRNA was detected in dark and light grown seedlings of both wild type and det1-1 mutant *Arabidopsis*. Transgenic *Arabidopsis* plants over expressing antisense RNA of cCA-1 showed reduced expression of the *Lhcb1*3* gene, demonstrating that the cCA-1 protein can affect the expression of this gene *in vivo*.

J6-324 ANALYSIS OF PHOTOSYNTHETIC DEFICIENT MUTANTS: IMPLICATIONS IN LIGHT-REGULATED TRANSLATION Christopher B. Yohn, Avihai Danon, Amybeth Cohen and Stephen P. Mayfield. Dept. of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

Genetic analysis of nuclear mutants of the green algae *Chlamydomonas reinhardtii* deficient in photosystem II (PS II) proteins has revealed that nuclear-encoded factors are required for the translation of specific chloroplast mRNAs. Isolation and characterization of these translational activators has shown that they specifically interact with stem-loop structures in the 5'-untranslated region (5' UTR) of the mRNAs encoding PS II polypeptides. RNA-protein interaction and translational activation are regulated in response to light by altering the binding activity of these translational activators. Binding activity to *psbA* is modulated by redox potential in the chloroplast via the thioredoxin system. Additionally, a threshold level of ADP inhibits the RNA binding activity by phosphorylation of one member of the protein complex. Analysis of nuclear mutants that are deficient in translation of specific PSII polypeptides can reveal the components and pathways of this translational activation system. Characterization of nuclear mutants nac 1-18a and nac 1-11, which have no synthesis of D1 (*psbA*) and D2 (*psbD*), shows aberrant binding of these translational activators to the 5' UTRs of the *psbA* and *psbD* messages as compared to wild type. A major protein component of the wild type RNA-protein complex (Rb47) shows altered mobility on Western blots, indicating modification to this translational activator in the mutant cells. An investigation of the ribosomal association of these messages in the mutants shows that *psbA* and *psbD* mRNAs are bound to polyribosomes *in vivo*, but the mutation present in nac 1-18a and nac 1-11 prevents the completion of translation and/or production of stable gene products (D1 and D2). Tagged insertional mutagenesis of *C. reinhardtii* has produced additional nuclear mutants with specific defects in translation of PS II proteins. Further characterization of these mutants, including cloning, will enable us to gain additional insights into how translation is used as a controlling point in specific regulation of light-activated expression.

Stress Signaling

J6-400 SODIUM COMPARTMENTALIZATION IN THE HALOPHYTE *MESEMBRYANTHEMUM CRYSTALLINUM* : REGULATION OF TONOPLAST Na^+/H^+ ANTIPORT AND H^+-ATPASE ACTIVITY. Bronwyn J. Barkla and Omar Pantoja, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, 62271, Mexico

In the halophyte *Mesembryanthemum crystallinum*, CAM is induced as a response to salt and water stress. The kinetics of induction suggest this is a long-term adaptation to reduced water availability, and not a mechanism for conferring salt tolerance. Upon exposure to NaCl, before the CAM pathway is fully functioning, cells of *M. crystallinum* are able to accumulate high levels of Na^+ and Cl^- within their vacuoles as a tolerance response. This suggests that the cellular mechanisms for salt tolerance can be separated from those related to CAM induction and indicates that signalling events leading to the expression of CAM and salt-tolerance traits in M. crystallinum occur through separate and distinct cellular pathways. In order to examine mechanisms of physiological importance in the ability to tolerate salt, the function and regulation of two key transport proteins implicated in the accumulation and compartmentalization of Na into the vacuole were studied. The active transport of Na ions across the tonoplast occurs through the operation of a Na^+/H^+ antiport driven by the electrochemical gradient of protons generated by the activity of the tonoplast H^+-ATPase. The activity of both these enzymes is induced two-fold in the tonoplast of leaves of M. crystallinum plants treated with 200 mM NaCl as compared to control plants of the same age indicating a possible coordinated regulation by salt. Recently it has been show at the molecular level that there is an increase in the steady state transcript level for the 16 kDa subunit of the H^+-ATPase which is induced by NaCl and not by mannitol, indicating an ionic rather than an osmotic response and, this induction of the 16 kDa message is also seen upon exposure of the plants to ABA (Tsiantis et al. 1994). The effects of osmotic stress and ABA treatment on the activity of the Na^+/H^+ antiport and H^+-ATPase will also be discussed.

J6-401 A ROLE OF NICOTINAMIDE, TRIGONELLINE AND POLY(ADP-RIBOSE)POLYMERASE IN STRESS SIGNALLING IN PLANTS?

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It has been suggested that poly(ADP-ribose)polymerase (PADPRP) mediated nicotinamide (NIC) release from NAD functions as a stress signal in plants and other eukaryotes (1). We show that agents known to cause oxidative stress, e.g. $VOSO_4$, cause an increase in phenylalanine ammonia-lyase (PAL) activity and glutathione levels. Pretreatment of plant tissue culture with PADPRP inhibitor counteracted this increase in PAL activity, while the same level of inhibitor did not influence the PAL activity *per se*. This supports a role of PADPRP in defense signalling. However, the PADPRP inhibitor did not counteract the strong $VOSO_4$ induced increase in reduced glutathione. It is known that NIC can induce defensive and secondary metabolism in plant tissue cultures, such as increased levels of reduced (GSH) and oxidized (GSSG) glutathione, increased PAL activity and accumulation of mRNA encoding defense-involved enzymes like CHS and GR (2,3). Here it is also demonstrated that NIC may protect the activity of oxidative-stress sensitive enzymes within the primary metabolism, e.g. aconitase. In plants NIC is metabolized to trigonelline (TRIG). In a dark grown root differentiated *Pisum sativum* tissue culture TRIG induced a rise in the level of GSH and GSSG and a rapid increase in mRNA accumulation of defensive genes like those encoding chalcone synthase (CHS). Results regarding differential effects of NIC and TRIG will be presented and discussed.

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2. Berglund, T., A.B. Ohlsson and J. Rydström: J. Plant Physiol. 141, 596-600 (1993).
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J6-402 HOW DO ENVIRONMENTAL FACTORS ALTER COTTON FIBER MORPHOLOGY? Judith M. Bradow, Gretchen F. Sassenrath-Cole, Philip J. Bauer, Oscar Hinojosa, and Lynda H. Wartelle, USDA, ARS, SRRC, Box 19687, New Orleans, LA 70179 [GSC, Mississippi State, MS, and PJB, Florence, SC] Cotton fibers, hyperelongated, unicellular outgrowths of the outer integument of *Gossypium* species ovules, go through four distinct, but overlapping, developmental processes. Fiber initiation, elongation, secondary cell wall deposition, and maturation are each sensitive, in varying degrees, to water deficits, insolation reductions by cloud cover, and other environmental factors that increase competition for the physiological resources needed for fiber growth and maturation. Physical and biochemical measurements of cotton fiber of differing maturities [provided by Advanced Fiber Information System or AFIS and x-ray fluorescence] indicated that suboptimal environmental conditions reduced the rates of secondary wall deposition and fiber maturation and prevented realization of full genotypic potential for fiber yield and quality. Maturation rates and elongation also varied according to fruiting position, and fiber lengths and thicknesses at harvest depended on boll position. Rainfall, irrigation, and insolation levels significantly altered these distributions of fiber maturities and morphologies. The observed effects of environmental factors on fiber morphology are also related to species and, to a lesser extent, to variety. Some external causes of significant shifts in fiber morphology have been identified, and the effects have been quantified and localized. The search has begun for the relevant environmental signals and a "unification" of the causes and effects that determine the quality and, therefore, price and end-uses of cotton fiber, an important commodity in the global economy.

J6-403 A MYB-LIKE GENE EXPRESSED IN RICE DURING ANOXIA: IS IT POST-TRANSCRIPTIONALLY REGULATED? Immacolata Coraggio°, Flavio Maragaggia°, Giovanna Giovinazzo*, °Istituto Biosintesi Vegetali, CNR, via Bassini 15, 20133 Milan Italy,* IRBA, CNR, via Provinciale Lecce-Monterone, Lecce Italy

A cDNA coding for a myb-like protein expressed in anoxic rice coleoptiles was isolated. The analyse of the sequence features indicates post-transcriptional regulation. Namely the presence of a 40 aa coding uORF in the long (450 bp) mRNA leader and the possibility of two mRNA deriving from alternative splicing of the same transcript. Both these forms of regulation are known to work in several pathway; for example the alternative splicing of the mouse c-myb, results in two mRNA coding for proteins with antagonist action (a transcriptional activator and one transcriptional repressor acting on the s genes). uORFs regulate the expression of some trans ional factor of the Leucin-zipper class: GCN4 of t and Opaque2 of maize. Moreover the anoxia, as r stress, influences both the splicing and the translatic f some pre-existing mRNA. We are indagating on the tion of both mechanisms in the regulation of myb7 expression. The uORF containing leader of myb7 cDNA was inserted, both wild type and mutagenized, in the pCaCat vector, in the leader of CAT gene; the constructs were used in *in vivo* transient assay in dicotiledones protoplasts and the negative influence of the uORF on CAT activity was measured. PCR analysis demonstrated the *in vivo* existence of both form of spliced mRNA.

J6-404 CHARACTERIZATION AND COMPLEMENTATION OF *CHLAMYDOMONAS* MUTANTS WITH ABBERANT RESPONSES TO SULFUR DEPRIVATION, John P. Davies, Fitnat Yildiz, and Arthur R. Grossman, Carnegie Institution of Washington, 290 Panama St. Stanford, CA 94305

We are using the unicellular green alga *Chlamydomonas reinhardtii* to study how photosynthetic eukaryotes acclimate to nutrient stress. When *C. reinhardtii* is deprived of sulfur it increases its ability to import sulfate and stnthesizes several periplasmic proteins. One sulfur-stress induced periplasmic protein is an arylsulfatase (Ars) which cleaves sulfate from aromatic compounds. To elucidate how *C. reinhardtii* perceives the sulfur status of the medium and controls expression of sulfur-responsive genes, we have screened for mutants that synthesize little or no Ars when grown under sulfur-limiting conditions or that constitutively synthesize Ars under sulfur-replete conditions. Several mutants in both catagories have been isolated. These mutants are being placed in complementation groups and characterized for Ars activity, periplasmic protein accumulation, and sulfate import. Based upon the phenotypes, some of these mutants appear to have lesions in genes that regulate the cell's response to sulfur-limitation. We have cloned both genomic and cDNA sequences for two of these "regulatory" genes. The sequences of the cDNAs may help us elucidate the signal transduction pathway that links the perception of sulfur-stress to the physiological changes that occur during the acclimation process.

J6-405 MOLECULAR BASIS OF OZONE-INDUCED RESPONSES IN *ARABIDOPSIS THALIANA*, Keith R. Davis and Yogesh K. Sharma, Department of Plant Biology and Ohio State Plant Biotechnology Center, The Ohio State University, Columbus, Ohio 43210

We are studying the effects of ozone, an inducer of oxidative stress, on *A. thaliana* growth and the expression pattern of several stress-related genes. We have used RNA blot analysis to show that exposure to 300 ppb ozone resulted in the accumulation of mRNAs encoding a glutathione S-transferase (*GST1*), phenylalanine ammonia-lyase, a neutral peroxidase and a cytosolic Cu/Zn superoxide dismutase to higher levels than ambient air treated control plants. Interestingly, these mRNAs are also known to accumulate in plants inoculated with avirulent pathogens that induce a hypersensitive respones. We are exploring the possible role of reactive oxygen species that are generated in response to both pathogen attack and ozone stress as the signaling molecules in the chain of events leading to the observed overlapping patterns of defense gene expression. We are currently pursuing studies aimed at identifying the cis-elements and trans-acting factors involved in *GST1* gene regulation by ozone and pathogen stress. In addition, we are using the available ethylene mutants to test the hypothesis that ethylene, that is produced in plants exposed to ozone, play a causal role in mediating ozone induced damage.

J6-406 THE EFFECT OF FREEZING AND COLD ACCLIMATION ON POLYSOMES FROM WINTER CEREAL SEEDLINGS.

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No changes in polysome sedimentation profiles or polysome translational activities were observed during cold acclimation of winter wheat (*Triticum aestivum L.*) or winter rye (*Secale cereale L.*) seedlings (1). Nonetheless, polysome behaviour changed radically as a result of acclimation. After one-day seedling freezing at -5°C polysomes from acclimated seedlings desegregated into monosomes, but after tissue thawing, monosomes reaggregated rapidly into polysomes. In the course of these transformations polysome translational activity remained constant. Similar treatments of nonacclimated seedlings did not change polysome sedimentation profiles but significantly decreased their cell-free translational activity. Seedling freezing supposed a slightly destabilization of ribosomes, and as a consequence of it, 0.5 M KCl extracted integral ribosomal proteins more easily from polysomes of both acclimated and nonacclimated seedlings. Most of the high molecular weight proteins associated with polysomes were lost during polysome isolation from frozen and thawed nonacclimated seedlings, whereas polysomes from acclimated seedlings retained high molecular weight proteins. It was suggested that these proteins were necessary to optimise a cell-free translation.

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J6-407 WOUND INDUCIBLE DEFENCE IN *BRASSICA*

Lars-Göran Josefsson, Jan Taipalensuu, Anders Falk, Mette Jørgensen, Johan Meijer and Lars Rask, Dept of Cell Research, Swedish University of Agricultural Sciences, Box 7055, S-750 07 Uppsala, Sweden

The myrosinase- glucosinolate system of *Brassicaceae* plants is thought to function as a wound activated defence against insects and pathogens. This bimolecular system becomes activated when differentially stored enzyme and substrate come in contact upon tissue damage or wounding. Numerous potentially toxic or deterring compounds are then produced, although *Brassica* specialists among the insects may rather percieve these as feeding or egg-laying cues.

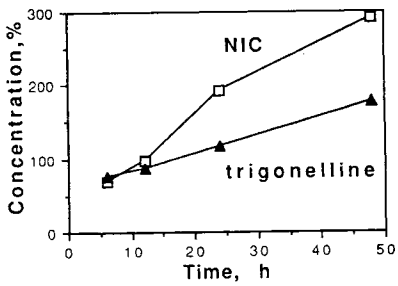
A set of novel proteins can be isolated in complex with, or by other means be shown to interact with myrosinases. Whether the formation of the complexes depends on tissue disruption is presently not known. Protein and cDNA sequences show that they can be grouped into two distinct families. One of these can be further divided into two subfamilies that share certain sequence features. Neither of the families has sofar been ascribed a definite function. Nor do they relate to previously known proteins.

Based on the observation that mRNAs for the myrosinase complexed proteins are inducible by jasmonate and/or mechanical wounding, it is tempting to speculate that their functions may also be in plant defence. Their physical interaction with a distinct but likewise wound activated defence system (myrosinase/glucosinolate) may serve an as yet uncharacterized purpose of regulation. If on the other hand the novel proteins turn out to be defensive proteins "in their own right" the effect of several distinct proteins could possibly be boosted by their delivery as a ready made cocktail.

In order to further clarify the role of jasmonate in this context, we are directing attention to the induction characteristics of these proteins. We are also exploring the possibilities of cloning jasmonate binding proteins, with the hope of identifying a putative receptor involved in jasmonate signal transduction. An initial approach that is being pursued is selection of jasmonate binding phages from a random plant cDNA display library expressing plant protein sequences on the surface of filamentous phage particles.

J6-408 EFFECT OF UV-B AND OXIDATIVE STRESS ON INTRACELLULAR CONCENTRATIONS OF NICOTINAMIDE AND TRIGONELLINE IN PLANTS, Georgi Kalbin, Åke Strid, Anna B. Ohlsson*, Jan Rydström and Torkel Berglund*, Department of Biochemistry and Biophysics, University of Göteborg, S-413 90, Göteborg, *Department of Biochemistry and Biotechnology, Royal Institute of Technology, S-100 44 Stockholm, Sweden.

Plant tissue cultures (*Catharanthus roseus* and *Pisum sativum*) and intact plants (*P. sativum*) were exposed to various conditions giving rise to oxidative stress: supplementary UV-B radiation, treatment with vanadyl sulphate or 2,2´-azobis(2-amidinopropane)dihydrochloride (AAPH). Contents of nicotinamide (NIC) and its metabolite trigonelline were determinated in water extracts from tissue by HPLC. The graph below shows changes in contents of free NIC and trigonelline in *Pisum sativum* leaves during illumination with supplementary UV-B. The increase in the NIC-content (48 h) was about 3-fold by comparison with the initial level. The trigonelline content increased more slowly, which illustrates its synthesis from NIC. These changes in NIC and trigonelline concentrations are consistent with the proposed role of NIC as a stress signal [1] initiating an increased levels of secondary metabolites and glutathione [2]. Both NIC and UV-B gives rise to increased transcription of protective genes, and decreased photosynthetic mRNA [3,4].



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3.T. Berglund, A. B. Ohlsson, J. Rydström, B. R. Jordan and Å. Strid, J. Plant Physiol. 142, 676-684, (1993)
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J6-409 THE STOMATAL SIGNAL TRANSDUCTION SYSTEM IN *Arabidopsis thaliana*, Ellen V. Kearns and Ethan R. Signer, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Genes involved in the stomatal signal transduction (SST) system may one day be used to engineer crops for drought resistance and adaptation to global change. These genes may also prove interesting in their interactions within the SST system, in their regulation, and in their structure. Genes in other plant signal transduction systems have already proven to be unique in structure, combining aspects of different mammalian signal transduction proteins. Despite the importance of SST, this signalling system remains unsolved. *Arabidopsis* is an obvious choice for isolating genes and studying their interactions in pathways, but it is difficult to study on the biochemical and physiological level. This project combines the power of *Arabidopsis* genetics with new stomatal response screens and porometry techniques to investigate the molecular underpinnings of low CO_2 response. EMS-, γ-, and T-DNA-mutated *Arabidopsis*, which have undergone two screens for aberrant low CO_2 response, are now being tested by porometry to determine if they are true SST mutants. T-DNA-tagged genes are now being cloned, mapped, and analyzed. Current progress will be presented.

J6-410 MEJA RESPONSE OF THE TD GENE UNCOVERS A PARENCHYMA-SPECIFIC SYSTEM SHARED BY FLOWERS AND LEAVES OF TOMATO, Eliezer Lifschitz, Limor Broday, Tamar Guthfinger, Dana Hareven and Alon Samach, Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel.

The biosynthetic threonine deaminase (TD), which catalyses the first committed step in the biosynthesis of isoleucine was isolated from tomato as a consequence of its unusual, 500-fold, upregulation in floral organs (Samach et al 1991). Hildmann et al (1992) have shown that TD is induced in potato leaves in response to wounding, abscisic acid and methyl jasmonate (MeJa) To understand the role of TD in the context of its developmental regulation and the response to wounding, we have monitored the evolution of its expression pattern in tomato flowers, examined its putative regulatory sequences in transgenic tomato plants and compared the control of its developmental expression with that of the MeJa response.

We show that in addition to leaves, the TD gene is induced by MeJa in the flowers, irrespective of preexisting high developmental expression. Induction in flowers and leaves is regulated by the same promoter region and follows identical kinetics. Only parenchymatous tissues are capable, subject to tight developmental control, or following application of MeJa, of expressing high level of TD. TD is neither expressed nor induced in meristematic, epidermal, vascular or sporogenous tissues. The conditioning of upregulation in flowers, the response to MeJa and the parenchyma-specific expression, are all regulated by cis-elements within the proximal 192 bp long region of the promoter. Developmentally-correct organ-specific expression is regulated further upstream. It is suggested that developmental expression and MeJa response in parenchyma cells are mediated by a common hypothetical two component system, but that the translation products of the two events play different physiological roles.

J6-411 DEVELOPMENT OF AN ELECTRODE SYSTEM FOR THE QUANTITATIVE APPLICATION OF ELECTRIC SIGNAL TO A SINGLE CELL OF PLANT IN VIVO, Hideaki Matsuoka, Mikako Saito, Kazuo Nakajima and Tomoo Homma, Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

As well as various chemical signals, electric signal is potentially a cell-active signal transmitted between plant cells. In order to elucidate its effects on cell functions, it is important to estimate precisely the electric signal intensity actually working on the cell (Vact). A microelectrode generally applicable to this purpose, however, has inevitably high electric impedance. Therefore, we intended to develop a microelectrode with lower electric impedance useful for the transmission of electric and electrochemical signals. A glass capillary was pulled and a Pt wire was inserted from the thick end. The inside was filled with 3M KCl. The diameter of the other thin end was 1μm as determined from the SEM photograph. When electric potential was applied externally (Vext) to the Pt electrode versus the other Pt wire (counter electrode) in a phosphate buffer solution (0.1M, pH 7.0), the bubble generation occurred at the Pt electrode surface in the glass capillary at Vext=-200(V). This phenomenon was speculated as the H_2 generation due to the water splitting which should occur theoretically at -1.23(V) (*i.e.* Vact≒-1.23(V)). This considerable difference of Vact and Vext was suspected due to the high impedance of the thin part of the capillary. Then the electrolyte within the capillary was replaced by Pt salt solution, the bubble generation occurred at Vext=-100(V). Therefore the electric impedance was lowered. At the same time, it was found that the bubble generation occurred at the tip of the thin end and not at the Pt electrode surface. This indicated that the electron transport was facilitated by Pt salt and thus the redox reaction could occur at the tip of the thin end of the capillary. From these results, it was concluded that the microelectrode containing Pt salt as the electron mediator was applicable to the transmission of the electrochemical signal as well as electric signal. In fact, the application of quantitative electric signal and electrochemical signal to a single cell of plant leaf was demonstrated in this study.

J6-412 EVIDENCE FOR CROSS-PATHWAY REGULATION OF METABOLIC GENE EXPRESSION IN PLANTS, David Patton, David Guyer and Eric Ward, Ciba Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, NC 22709

Our laboratory has been studying the effects that histidine starvation has on gene expression in *Arabidopsis*. In this experimental system seedlings were treated with IRL 1803, a specific inhibitor of imidazoleglycerolphosphate dehydratase, an enzyme of histidine biosynthesis. We have recently discovered that IRL 1803-induced histidine starvation leads to significant induction of at least 8 genes which encode enzymes from diverse metabolic pathways. These pathways include the biosynthesis of purines, histidine, lysine, as well as brached chain and aromatic amino acids. In many cases, a concomitant increase is also seen in the end products of these pathways. Under these same histidine starvation conditions, expression of one other gene is repressed, while two additional genes show little or no change in expression. All IRL 1803-induced changes in gene expression were eliminated by simultaneous addition of histidine. These results demonstrate that plants have a mechanism for sensing and responding to amino acid starvation in a manner similar to the general control phenomenon seen in the yeast *Saccharomyces cerevisiae*.

J6-413 Pin2 GENE EXPRESSION IS INDUCED IN TOMATO LEAVES FOLLOWING ELECTRIC CURRENT TREATMENT. Hugo Peña-Cortés, Joahim Fisahn, Oliver Herde. Institut für Genbiologische Forschung. Ihnestr. 63, 14195 Berlin, Germany.

Mechanical damage and heat stimulation were used to activate proteinase inhibitor II (Pin2) gene expression in tomato plants in both treated (local induction) and non-treated tissues (systemic induction). Both stimuli have been shown to generate electrical signals, leading to a systemic activation of gene expression. Treatment of tomato leaves with electrical current result in the accumulation of Pin2 mRNA in the local and systemic leaves. Additionally, all treatments inducing Pin2 gene activity gave rise to a significant alteration of photosynthetic parameters (e.g. assimilation- and transpiration-rate). However, heat stimulation provoked a different response in the photosynthetic parameters than mechanical wounding or electric treatment. Both mechanical damage and electrical stimulation activated two characteristic time constants in the gas exchange relaxation kinetics. Conversely, heat stimulation resulted in only one major time constant. The results clearly show that direct current application to tomato leaves initiates Pin2 mRNA accumulation locally and systemically. In addition, they suggest the participation of a second slow electrical component in the wound response mechanism of tomato plants and a possible alternative pathway regulating heat-induced Pin2 gene expression.

J6-414 EFFECTS OF ENVIRONMENTAL STRESS ON DAILY RHYTHMS IN *EUGLENA GRACILIS*

Silja K. Petersen-Mahrt, Susanne Widell, Department of Plant Physiology, Lund University, Sweden

The flagellate *Euglena gracilis* shows daily rhythms in several processes, such as phototaxis, cell shape and photosynthesis (1), as well as in enzyme activities in isolated plasma membranes (ATPase, 5'nucleotidase and adenylyl cyclase) (2). Stress e.g., nitrogen deficiency or UV-B radiation influenced these rhythms but differently, indicating that more than one pacemakers was involved (1,2). However, some of the processes seemed to be affected similarly, eg., that of cell shape and that of adenylyl cyclase, which could be interpreted as that the cell shape somehow is connected to adenylyl cyclase activity. Changes in cell shape involve dynamics in the cytoskeleton, and probably also in its connection to the plasma membrane. As a step towards understanding whether adenylyl cyclase could be involved in these processes, we now analyse the plasma membrane-associated cytoskeleton, using preparations obtained from differently stressed *Euglena* cells.

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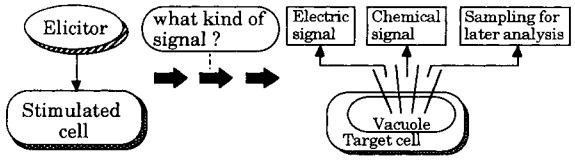
2. Petersen-Mahrt, S.K., Ekelund, N.G.A. and Widell, S. Plasma membrane purification from *Euglena gracilis* using an aqueous two polymer phase system: Enzyme activity distribution in control, UV-B irradiated and nitrogen-deficient organisms. - submitted.

J6-415 *SWIN 1*, A WOUND-INDUCIBLE GENE FROM *SALIX VIMINALIS* L WITH HOMOLOGY TO KUNITZ PROTEINASE INHIBITORS, P. Saarikoski, D. Clapham and S. von Arnold., Uppsala Genetic Centre, Dept. of Forest Genetics, Swedish University of Agricultural Sciences, Box 7027, S-75007 Uppsala, Sweden. A gene coding for a proteinase inhibitor, with homology to Kunitz inhibitors, whose expression is induced in willow leaves(*Salix viminalis* L) in response to wounding, was isloated from a genomic library and characterized. The sequence revealed that the gene is complete and has all the motifs for functional eukaryotic gene.

The region 5' from the transcription start site includes a regulatory sequence common to other eukaryotic genes, TATAAA. The 3' noncoding region contains the sequence AATAAA, a typical sequence for poly(A) addition. Two other positive genomic clones showed a different band pattern after restriction mapping than the sequenced one, indicating the presence of a multigene family. A trypsin inhibitor was purified and N-terminal sequenced. The molecular weight is estimated by SDS-PAGE to be approximately 14 kDa. Significant similarities was found to a cDNA (*win* 3) clone from populus. Homologies started at aminoacid 30 for both win3 and the Salix gene(designated *Swin* 1, for Salix wound induced), indicating that a signal peptide is cleaved off. The gene has been expressed in E-coli, and the protein been purified in relative large amounts and showed activity against trypsin.

J6-416 REAL TIME MEASUREMENT OF SIGNAL TRANSMISSION BETWEEN PLANT CELLS WITH A MICROELECTRODE SYSTEM, Mikako Saito, Hideaki Matsuoka, Naoto Shibuya* and Yoko Nishizawa*, Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan and Laboratory of Applied Microbiology, National Institute of Agrobiological Resources, Tsukuba Science City, Ibaraki 305, Japan*

In order to analyze the signal transmission between plant cells in vivo, we have developed a multifunctional microelectrode system. Based on preliminary experiments, we found that the vacuole was useful locus for obtaining a stable signal of a particular single cell with the microelectrode system. A triple-barreled microelectrode was devised and inserted in a single cell of a *Tradescantia virginiana* L. leaf and the simultaneous measurement of electric signal (intra-vacuolar potential) and chemical signal (H^+, K^+, or Cl^- concentration) was performed. When H^+ (or K^+, Cl^-) was injected directly in the same vacuole with another microelectrode, the concentration change of H^+ (or K^+, Cl^-) and associated change of potential could be measured. When enzyme (urease, luciferase) and its specific substrate were injected, the pH change due to the enzyme reaction was detected. Then this system was applied to the real time measurement of the response of rice cells (*Oryza sativa* L.) to externally applied chemical signals. These signals included an elicitor which could stimulate the gene expression of chitinase. The rice cells which had been stimulated by the elicitor beforehand were also applied as a chemical signal in order to speculate what signal should be transmitted from a stimulated cell to a target cell.



J6-417 DIRECT ELECTRICAL INDUCTION OF GENE EXPRESSION IN TOMATO PLANTS

Bratislav Stankovic and Eric Davies, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

Tomato plants respond to localized wounding by local and systemic activation of proteinase inhibitor (*pin*) genes. Controversy reigns as to whether the wound signal is a hormone transported in the phloem or in the xylem, whether it is electrical, hydraulic, or some combination. A recent report (*Nature* 360, 62-65) furnished convincing evidence against the signal being a hormone transported in the phloem and provided support for a major role for electrical signals. This work did not, however, clearly rule out hydraulic signals and their electrical aftermath, the variation potential (VP).

Here we directly test the hypothesis that electrical signals are (one of) the unidentified wound signals by electrically stimulating one petiole and monitoring for passage of an action potential (AP) directly into the leaf analyzed for *pin1* and *pin2* mRNA. The results clearly demonstrate that a genuine electrical signal (AP), is capable of evoking systemic *pin1* and *pin2* expression, as is a VP, while only a VP is capable of evoking systemic expression of calmodulin mRNA.

Second Messengers

J6-418 **Ca^{2+}-DEPENDENT AND -INDEPENDENT INTERACTIONS OF CALMODULIN WITH PLANT GLUTAMATE DECARBOXYLASE,** [1]Hillel Fromm, [1]Gideon Baum, [2]Wayne A. Snedden, [2]Barry J. Shelp and [1]Tzahi Arazi. [1]Department of Plant Genetics, Weizmann Institute of Science, 76100, Rehovot, Israel; [2]Department of Horticultural Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Plant glutamate decarboxylase (GAD) was cloned and identified as a Ca^{2+}-dependent CaM-binding enzyme in our lab (1). A purified peptide of 32 amino acids corresponding to the GAD carboxyl-end is sufficient to form a stable complex with CaM in the presence of Ca^{2+}. Amino acid substitutions, as well as N-terminal and C-terminal deletions, were used to identify structural motifs which are important for CaM binding. Our study revealed that Trp^{485} is crucial for Ca^{2+}-dependent CaM binding. Substitution of Trp^{485} by a charged residue (Glu or Arg) abolished CaM binding. Among other substitutions, those with larger hydrophobic side chains (e.g. Leu and Phe) restored binding better than residues with short hydrophobic side chains (e.g. Ala) but not as efficient as Trp. Crosslinking experiments revealed specific GAD/CaM interactions *in vitro* even in the absence of calcium. We conclude that calcium-dependent GAD/CaM binding occurs mostly through hydrophobic forces, whereas calcium-independent GAD/CaM interactions are essentially electrostatic and involve a stretch of lysine residues at the carboxyl-side of the domain. Furthermore, we demonstrate that the petunia petal GAD is activated in vitro by Ca^{2+}/CaM. Finally, we have been using transgenic plants to study GAD/CaM interactions. Plants that overexpress the CaM-binding GAD possess high GAD activity in vivo and high steady-state levels of γ-aminobutyric acid (GABA), but are not different in their morphology from control plants under normal growth conditions. By contrast, plants that express GAD lacking its CaM-binding domain have severe morphological aberrations. Analysis of these plants will be presented.

[1]Baum G, Chen Y, Arazi T, Takatsuji H and Fromm H (1993) A plant glutamate decarboxylase containing a calmodulin-binding domain: Cloning, sequence and functional analysis. J. Biol. Chem. 268, 19610-19617.

J6-419 INOSITOL MONOPHOSPHATASE GENES FROM TOMATO. Glenda E. Gillaspy, Kenji Oda, James Keddie and Wilhelm Gruissem, Department of Plant Biology, University of California, Berkeley, CA 94720.

All cells require the ability to respond to external signals. Animal cells often utilize the PI signaling pathway wherein membrane bound receptors are coupled to the production of the second messenger IP_3. Plant cells have been shown to respond to microinjected IP_3 and some of the enzymes involved in the plant PI pathway have been characterized. We have previously described a gene encoding an active, lithium sensitive, calcium regulated inositol monophosphatase (IMP) from tomato (TIM-1). IMP is an enzyme which catalyzes the last step in the breakdown of IP_3 and is proposed to be the pharmacological target of lithium in treatment of manic depression disorder in humans. As well, lithium inhibiton of the enzyme in Xenopus oocytes results in altered development, which can be rescued by inositol addition. The expression of TIM-1 was found to occur in tissues undergoing active cell division and/or expansion. We now describe two more genes which together with TIM-1 make up a small gene family encoding different isoforms of IMP. Expression of TIM-2 and TIM-3 is an order of magnitude lower than TIM-1. RNase protection asays are being used to detect all threee messages simultaneously. We have generated a polyclonal antisera which recognizes all three proteins which is being used to determine the protein expression pattern in western blots and in cytological sections of various tomato tissues. Lastly we describe tobacco plants overexpressing the tomato TIM-1 cDNA under control of the CaMV 35S promoter. These plants express 5-10 fold more IMP protein as compared to controls. The IMP activity levels are increased about 5 fold in these plants. These plants are expected to generate more free inositol as a result of increased IMP activity. The responses of these plants to various stresses related to inositol metabolism will be discussed.

J6-420 A bZIP FACTOR BINDING TO THE G-BOX OF LIGHT- RESPONSIVE GENES IN A PHOSPHORYLATION-INDEPENDENT FASHION, Giovanni Giuliano, Ugo Borello, Donatella Ponti., ENEA, Casaccia Res. Ctr., PO Box 2400, Roma 00100AD, Italy.

A cDNA encoding the GBF1 factor of tomato has been cloned and sequenced completely. It is distinct from the previously characterized GBF factors from the same plant (Meyer I, Gruissem W (1994) Nucl. Acids Res. 22, 470) and shows sequence homology to TAF-1 (Oeda K et al (1991) EMBO J 10, 1793). The GBF1 mRNA shows high levels of expression in mature green fruits. The protein, expressed in *E. coli*, binds a short version of the G-box, and does so in a fashion independent from phosphorylation by casein kinase II, unlike tomato leaf GBF (unpublished) and the previously characterized GBF1 factor from Arabidopsis (Klimczak LJ et al (1992) Plant Cell 4, 87). Nevertheless, GBF1 is a CKII substrate. A model concerning the binding of phosphorylation-dependent and -independent GBF factors to the *RbcS* promoter in various tissues and in response to light will be discussed.

J6-421 ***AtPLC1*, a gene encoding phospholipase C, is induced by drought and salt stress in higher plant, *A. thaliana*.**

Takashi Hirayama, Chikara Ohto[1], Tsuyoshi Mizoguchi and Kazuo Shinozaki.

Laboratory of Plant Molecular Biology, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki, 305, Japan. 1: Bio Research Lab., Research Div., TOYOTA Motor Corporation, 1 Toyota-cho, Toyota, Aichi, 471, Japan.

A cDNA corresponding to a putative phosphoinositide-specific phospholipase C (PI-PLC) in higher plant, *Arabidopsis thaliana*, was cloned using polymerase chain reaction technique. The *AtPLC1* cDNA encoded a putative polypeptide with a calculated molecular weight of 64 kDa which had putative X and Y domains conserved among PI-PLCs identified so far. The overall structure of the putative AtPLC1 protein was the most similar to that of δ type PLCs although the size of AtPLC1 protein was much smaller than those of other organisms. The recombinant AtPLC1 protein synthesized in *E. coli* exhibited phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis activity which was completely dependent on Ca^{2+} concentration as observed with mammalian PI-PLCs. These results suggest that the *AtPLC1* gene encodes a genuine PI-PLC of higher plant. Northern blot analyses showed that *AtPLC1* is expressed at very low level in the plants under normal conditions but are induced significantly under environmental stresses, such as drought, salinity and cold stress. These observation suggest that AtPLC1 protein is involved in the signal transduction pathways of environmental stresses and that the increase in the level of AtPLC1 may amplify the signal pathway, which probably contributes in the adaptation to these stresses.

J6-422 FUNCTIONALLY DIFFERENT MAP KINASES IN *MEDICAGO SATIVA*

Claudia Jonak, Stefan Kiegerl and Heribert Hirt
Institute of Microbiology and Genetics, Vienna Biocenter, Dr. Bohrgasse 9 A-1030 Vienna, Austria

MAP (mitogen activated protein) kinases are pivotal regulatory elements of different complex signal transduction pathways in eukaryotes. They link various extracellular signals to different cellular targets. We have isolated cDNAs encoding four distinct MAP kinases from *Medicago sativa*. The catalytic domains of these kinases are highly homologous whereas the N-termini share little similarity. Several lines of evidence indicate that the different alfalfa MAP kinases are involved in different signal transduction pathways in plants. They show differential expression patterns in various organs. Expression of one cDNA clone is restricted to floral stages and seed pods. The bacterially expressed kinases show autophosphorylation but have highly different phosphorylation rates for various substrates tested. Yeast complementation analysis revealed that one of the alfalfa kinases can specifically suppress growth defect of cells mutant for mpk1 (MPK1 MAP kinase acts downstream of PKC in *S. cerevisiae*). Functional replacement is dependent on an intact upstream MPK1 signal transduction pathway.

J6-423 CLONING AND CHARACTERIZATION OF AN INOSITOL PHOSPHOLIPID-SPECIFIC PHOSPHOLIPASE C FROM SOYBEAN, Jinrui Shi, Robert Gonzales and Madan K. Bhattacharyya, Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401

Inositol phospholipid-specific phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate inositol 1,4,5-trisphosphate and diacylglycerol, which serve as second messengers for intracellular Ca^{++} mobilization and protein kinase C activation, respectively, in mammalian cells. Although there is an enzymatic basis for signal transduction through the hydrolysis of phosphoinositides in higher plants, it is not known whether the signal transduction pathway functions, in part due to the poor knowledge about plant phospholipase C. We report here the cloning of a soybean phospholipase C by immunoscreening. An antiserum against purified soybean plasma membranes was used to screen a cDNA expression library. A positive clone was identified to be phospholipase C by sequencing and database search. The cDNA insert is 2108 bp with an ORF that encodes a protein composed of 600 amino acids. The soybean PLC shows strong homology to *Dictyostelium* PLC and mammalian PLC-δ isoform. A fragment of 246 amino acids from the C-terminal was expressed in *E. coli* and purified to produce antibodies. To investigate the physiological function of the PLC, chimeric gene constructs that contain sense and antisense PLC gene under control of the CaMV 35S promoter were made and introduced into tobacco. Subcellular localization in transgenic tobacco using soybean PLC fused to a reporter epitope is in progress.

J6-424 CHLOROPHYLL FORMATION IN THE LIP1 PEA MUTANT. Christer Sundqvist, Michael P. Timko, Botanical Institute, Carl Skottsbergs Gata 22, S-413 19 Göteborg Sweden, and Department of Biology, University of Virginia, Charlottesville, VA 22901, USA.

When grown in darkness the lip1 mutant of pea cultivar Alaska has many characteristics normally found in light-grown pea seedlings. The leaves are expanded and the internode length is reduced. The plastids lack regular prolamellar bodies but have extended agranal thylakoids. Some areas with membrane accumulations resembling transformed prolamellar bodies are found. The protochlorophyllide concentration is higher in the lip1 mutant than in the wild-type Alaska pea. Only minor amounts of the long-wavelength form of protochlorophyllide is found in the lip1 mutant whereas the wild-type has about equal amounts of short-wavelength and long-wavelength protochlorophyllide in their leaves. With flash irradiation only the long-wavelength form is converted to chlorophyll(ide) but with continuous light there is a decrease also in the content of short-wavelength protochlorophyllide. The lip1 mutant is known to have a low content of the PHYA polypeptide and it has been suggested that the lip1 gene product affects an early step in phytochrome signal transduction (Frances et al., The Plant Cell 4 1519, 1992). We are currently investing the effect of the lip1 mutation on the expression of various enzymes of the chlorophyll biosynthetic pathway to determine whether their expression is specifically altered in a developmental or tissue specific manner.

J6-425 **METHYL JASMONATE INDUCES ANTHOCYANIN SYNTHESIS AND FLAVONOID GENE TRANSCRIPTION IN PETUNIA COROLLAS.** **Guy Tamari, Amihud Borochov and David Weiss.** The Kennedy Leigh Centre for Horticulture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel;

Jasmonates are relatively newly discovered plant signaling molecules that are involved in the regulation of various developmental processes, most of which are related to plant stress responses. Here we show that methyl jasmonate (MeJA) induces anthocyanin biosynthesis and chalcone synthase (*chs*) and dihydroflavonol 4-reductase (*dfr*) gene expression in detached petunia corollas. MeJA promoted β-glucuronidase (GUS) activity in corollas of transgenic plants carrying the *gus* gene driven by a *chs* promoter, indicating that it acts at the level of transcription initiation. We have previously shown that gibberellic acid (GA) plays an important role in the natural regulation of *chs* gene transcription in developing corollas. Whereas *chs* re-induction by GA_3 only started after 5 h but was durable, that of MeJA was more rapid but transient. Therefore, MeJA does not seem to play a role in the natural developmental regulation of the gene. Anthocyanin synthesis was induced by wounding and this effect was enhanced by MeJA. Wounding also induced an increase in jasmonate content in detached corollas. The kinetics of *chs* and *dfr* mRNAs accumulation following wounding was similar to that caused by MeJA. The above results suggest that MeJA plays a role in the mediation of wound-induced changes in flavonoid gene transcription.

J6-426 TRANSGENIC PLANT AEQUORIN REPORTS STIMULUS-INDUCED CHANGES IN CYTOSOLIC FREE CALCIUM IN EPIDERMAL PEELS. Nicola T. Wood, Andrew C. Allan and Anthony J. Trewavas, Institute of Cell and Molecular Biology, The University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland.

Calcium plays a central role in the signalling pathways of many environmental stimuli in plants. Transformation of *Nicotiana plumbaginifolia* with a construct containing the gene for aequorin, fused to the CaMV 35S promoter (pMAQ2), has produced plants whose luminescence directly reports cytosolic calcium ($[Ca^{2+}]_{cyt}$). Previous work with these plants indicated that elevation of cytosolic calcium is involved in plant responses to wind, temperature, light and fungal elicitors.

The majority of stimulus-induced luminescence appears to originate in the epidermis, with reduced contributions from sub-epidermal cells. We have therefore developed an assay for the measurement of $[Ca^{2+}]_{cyt}$ in epidermal peels of transgenic tobacco.

Using this system, elevations of $[Ca^{2+}]_{cyt}$ have been detected in response to various external stimuli including cold shock, mechanical perturbation and the plant growth regulator abscisic acid. Preliminary observations suggest the responsive cell type(s) in each case. The effect of plant growth temperature on stimulus-induced Ca^{2+} transients will also be discussed.

The location of Ca^{2+} pools mobilized into the cytosol upon stimulation has been investigated using various calcium channel inhibitors and transgenic plants containing aequorin targetted to sub-cellular locations.

Phosphorylation

J6-427 CLONING AND BIOCHEMICAL CHAR ACTERIZATION OF DUAL-SPECIFICITY PROTEIN KINASES FROM ARABIDOPSIS AND THE USE OF REVERSE GENETICS TO IDENTIFY MUTANT PLANTS FOR THESE GENES, Nazeem Ali and Kenneth A. Feldmann, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Protein kinases are known to play an important role in many signal transduction processes. In animals, protein serine/threonine and protein tyrosine kinases have been shown to be involved in receiving, amplifying and transmitting signals from the cell surface to the nucleus. In plants, even though an increasing number of protein serine/threonine kinases has been cloned, protein kinases with tyrosine phosphorylation activity remain elusive. Using a functional screening method, namely by screening an expression library with anti-phospho-tyrosine antibodies, we have been able to clone protein kinases with tyrosine phosphorylation activity from Arabidopsis. In particular one of these kinases, ADK1 (Arabidopsis dual specificity kinase 1) has been shown to autophosphorylate on serine, threonine and tyrosine residues. Furthermore ADK1 also phosphorylates poly(Glu/Tyr) *in vitro*, thus providing additional evidence for tyrosine phosphorylation activity. In addition, data will be presented which show that ADK1 is regulated by second messengers. We are currently establishing a "reverse genetics" system for Arabidopsis, which will allow us to identify plants mutant for ADK1 and other genes in large T-DNA transformed populations by using PCR and/or Southern blot hybridizations; the progress of this work will also be presented.

J6-428 **CHEMOPERCEPTION OF MICROBIAL SIGNALS AND OF SYSTEMIN**, Thomas Boller[1,2], Georg Felix[1], K. Baureithel[1], Angelika Fath[1] and Martin Regenass[1], [1]Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, and [2]Botanisches Institut, Universität Basel, Hebelstrasse 1, CH-4056 Basel, Switzerland.

Plants have exquisitely sensitive chemoperception systems for specific exogenous signal substances derived from micro-organisms. For example, suspension-cultured tomato cells perceive characteristic fungal molecules such as chitin fragments, ergosterol, and *N*-linked glycopeptides with fungal-type mannosyl linkages, at threshold concentrations of $\sim 10^{-12}$ - 10^{-10} M. Some of these perception systems for "non-self" molecules show rapid desensitization, leading to a "refractory state" reminiscent of smell perception. Interestingly, the perception system for chitin fragments present in tomato cells also recognizes Nod factors secreted by rhizobia.

Systemin, an endogenous signal substance, produces very similar reactions as the exogenous "non-self" signals in cells of a wild tomato species (*Lycopersicon peruvianum*), inducing alkalinization and ethylene production at a threshold of $\sim 10^{-11}$ M.

To study perception of some of these molecules, they were derivatized with [^{35}S]methionine to yield ligands with specific radioactivities of ~1000 Ci/mmol. Specific binding sites for these ligands were found on intact cells as well as on isolated plasma membranes. Their binding specificities correlated with the specificities of the perception systems in bioassays, indicating that the binding sites function as receptors.

The possible involvement of protein phosphorylation in signal transduction was studied by *in vivo* pulse-labelling with [^{33}P]phosphate, followed by SDS-PAGE and autoradiography. Microbial signals as well as systemin induced similar changes in the pattern of protein phosphorylation. These changes could be prevented by an inhibitor of protein kinases, K-252a. Furthermore, calyculin A, an inhibitor of protein phosphatases, induced the same changes in the pattern of protein phosphorylation as the signals mentioned, but did so even more rapidly. These data indicate that protein phosphorylation/dephosphorylation is essential for signal transduction of microbial signals as well as systemin.

J6-429 FUSICOCCIN SIGNAL TRANSDUCTION INVOLVES A 14-3-3 RECEPTOR PROTEIN AND A CALCIUM DEPENDENT PROTEIN KINASE. Albertus H. De Boer, Henrie A.A.J. Korthout, Paulus C.J. Van der Hoeven, Department of Plant Physiology and Biochemistry, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Fusicoccin (FC) is a phytotoxin produced by the fungus *Fusicoccum amygdali* Del. FC drastically alters plasma membrane traffic, cell growth and cell metabolism. A receptor protein (FCBP), located in the plasma membrane, binds FC with high- and low-affinity. The purified FCBP from oat roots is made up by two subunits of 30- and 31-kD.

We digested the purified 31-kD polypeptide with trypsin and obtained amino acid sequences of three peptides. A search in the EMBL databank, as well as subsequent immunological analysis showed that both FCBP subunits are members of the eukaryotic 14-3-3 family [1]. We proved that the FCBP is a 14-3-3 dimer, by means of affinity chromatochraphy using biotinylated FC.

In mammalian cells 14-3-3 proteins regulate the activity of protein kinase C and Raf kinase. In oat root plasma membranes we identified a kinase which phosphorylates a PKC specific substrate peptide and which is inhibited by calphostin C (IC_{50} 0.4μM), an inhibitor of PKC acting at the regulatory domain. The kinase is Ca^{2+} dependent and is activated by cis-unsaturated fatty acids, like arachidonic acid. A link between FC and this kinase has been made in two ways. (1) Calphostin C inhibits the FC-induced acidification of the apoplast in oat coleoptiles (IC_{50} 0.5μM) and (2) pretreatment of intact roots with FC alters the Ca^{2+}-dependence of the kinase. A similar change in Ca^{2+}-dependence of the kinase was observed in isolated plasma membranes, treated with arachidonic acid. The modification of the kinase and the H^+-ATPase only seems to occur when FC is added *in vivo*; *in vitro* we may loose an as yet unidentified factor. We will present a model for the FC signal transduction pathway and discuss the role of some components for the IAA signal transduction.

[1] Korthout H.A.A.J., De Boer A.H. (1994) The Plant Cell (in press).

J6-430 **EXPRESSION AND POTENTIAL MYRISTOYLATION OF A CALCIUM-DEPENDENT PROTEIN KINASE.** Paul K. Farmer and Jung H. Choi, School of Biology, Georgia Institute of Technology, Atlanta, GA 30332.

A partial cDNA corresponding to the carboxy end of a calcium-dependent protein kinase (CDPK) was previously identified by screening a carrot (Daucus carota) cDNA library. This kinase contains 4 EF-hand motifs near the C-terminus which have been shown to bind calcium. We have employed 5' RACE to identify the missing 320 nucleotides corresponding to the open reading frame in this 60kD protein. DNA sequencing indicates the first eight amino acids in this kinase are M-G-G-C-F-S-K-K. This sequence resembles N-terminal sequences found in other proteins which are N-myristoylated. Since the addition of myristate could anchor this kinase to intracellular membranes, facilitate protein-protein interactions, or alter the biological activity of the enzyme, ongoing studies will examine if this CDPK is myristoylated *in vivo*. The N-terminal amino acid sequence preceding the kinase region has little homology with similar regions found in a soybean CDPK, an Arabidopsis CDPK, or in a second protein kinase isolated from carrot tissue which contains degenerate EF-hands. Therefore, a 20kD protein fragment located at the extreme N-terminus of this CDPK has been overexpressed in E. coli, purified by SDS PAGE, and used to raise polyclonal antibodies. These antibodies will be used to examine (1) *in vivo* myristoylation of the kinase, (2) subcellular localization of this CDPK, and (3) both tissue-specific and developmental expression. Overlapping cDNA fragments are currently being combined to provide a full length CDPK cDNA.

J6-431 CALMODULIN (CAM) AND Ca^{2+}/CAM-DEPENDENT PROTEIN KINASES FROM THE MOSS *FUNARIA* M.M. Johri, M.P. Darokar and J.S. D'Souza, Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay 400 005, India

The moss protonema responds to phytohormones and a variety of other stimuli. We are characterizing phosphoproteins, CAM and Ca^{2+}-dependent protein kinase (PK) activity from chloronema and caulonema cell types. The pattern of endogenous polypeptides, phosphorylated *in vitro* was cell type specific. About 20 of these were phosphorylated; 1 was chloronema-specific and Ca^{2+}-dependent while 10 were phosphorylated more in chloronema than in caulonema and Ca^{2+} enhanced phosphorylation of 8 of these. Two were phosphorylated more in caulonema. CAM levels were 1-2 μg/gm FW in the exponentially growing chloronema cells. Moss CAM has a M_r of about 18 KDa and exhibited total amino acid composition similar to other CAMs. The methylated-CAM was found to be a very potent immunogen and the polyclonal sera contained high titer of antibodies directed against the species-specific and conserved epitopes of CAM. The maximum stimulation of PK by Ca^{2+} was observed in 60-70% ammonium sulphate saturated fraction (F_4). Upon separation by PAGE on native gels, F_4 showed a PK of about 62-68 KDa which was autophosphorylated at ser/thr. Further fractionation on DEAE-cellulose indicated that at least two different PKs are present. The activity of one of them was stimulated 10-20 fold by 60 μM free Ca^{2+} while that of other was stimulated by nanomolar levels of CAM. The chloronema cells thus contain multiple Ca^{2+}/CAM-dependent PKs which could play a role in signal transduction process. A 63-67 KDa polypeptide (PK?) reacted with anti-CAM antibodies and it is being determined if it is a novel type of PK similar to that reported from soybean.

J6-432 RECONSTITUTION OF ARABIDOPSIS CASEIN KINASE II FROM RECOMBINANT SUBUNITS AND PHOSPHORYLATION OF TRANSCRIPTION FACTOR GBF1. Leszek J. Klimczak[1], Margaret A. Collinge[2], Donatella Farini[3], Giovanni Giuliano[3], John C. Walker[2], and Anthony R. Cashmore[1] [1]Plant Science Institute, University of Pennsylvania, Philadelphia, PA 19104-6018 [2]Division of Biological Sciences, University of Missouri, Columbia, MO 65211 [3]ENEA, CR Casaccia, P.O.Box 2400, Roma 00100 AD, Italy

Casein kinase II (CKII) is a multifunctional protein kinase which has been implicated in regulation of central cellular functions such as cell division and growth, gene expression, and DNA replication. Recent data suggest also its involvement in signal transduction, possibly by counteracting the action of a regulated protein phosphatase. We are interested in a detailed characterization of plant CKII because of its phosphorylation of transcription factor GBF1, which results in stimulation of the DNA binding activity. In contrast to the well-defined tetrameric structure of animal and yeast CKII, plant CKII is found in two forms: a monomeric form and an oligomeric form whose subunit composition is unclear. The Arabidopsis homologs of the catalytic subunit α (CKA1) and the regulatory subunit β (CKB1) of CKII were expressed in *Escherichia coli* and examined with regard to their ability to form complexes, the effect of CKB1 on the catalytic activity, and the relationship of the recombinant enzymes to those isolated from plant material. Both subunits were observed to be present in large amounts in the inclusion body fraction and were solubilized and renatured with recovery of catalytic (CKA1) and stimulatory (CKB1) activities. Combination of purified CKA1 and CKB1 proteins resulted in up to 100-fold stimulation of casein kinase activity compared to the CKA1 activity alone, showing that CKB1 has biochemical properties similar to the β subunit from animals. CKA1 and CKB1 spontaneously assembled into a tetrameric complex, $CKA1_2CKB1_2$, which showed very similar properties to those of the oligomeric CKII form isolated from broccoli. However, the properties of the catalytic subunit CKA1 alone differed from those of broccoli monomeric form of CKII-like activity. Phosphorylation of transcription factor GBF1 with the reconstituted $CKA1_2CKB1_2$ enzyme resulted in stimulation of its DNA-binding activity and retardation of the protein-DNA complex, identical to the effects obtained previously with isolated nuclear CKII from broccoli. This result represents the first demonstration in plants of a regulatory phosphorylation entirely reconstituted from homologous bacterially-expressed components.

J6-433 OVER-EXPRESSION, PURIFICATION AND CHARACTERIZATION OF RECOMBINANT PvSF1, A b-ZIP PROTEIN FROM BEAN, FOR USE AS SUBSTRATE IN PHOSPHORYLATION STUDIES, Lauren McHenry, Department of Biology, Loyola College, Baltimore, MD 21210-2699 and Mauricio Bustos, Department of Biological Sciences, University of Maryland at Baltimore County, Baltimore, MD 21228-5398

Reversible phosphorylation of many animal and yeast transcription factors is known to modulate their activities or sub-cellular location, and thus to play a significant role in the terminal steps of signal transduction pathways resulting in *de novo* gene expression. Attempts to determine if phosphorylation of plant basic-leucine zipper (b-ZIP) proteins alter the ability of this type of transcription factor to form sub-unit dimers, to bind to target DNA sequences, or to activate transcription have begun using a b-ZIP protein whose cDNA (*PvSF1*) has recently been isolated from *Phaseolus vulgaris* by 3' RACE (M.-S. Chern and M. Bustos, submitted) PvSF1 has high and specific affinity for seed storage protein gene promoter elements, and thus it is an attractive protein with which to conduct studies of bean (seed) maturation specific signal transduction phenomena. Specifically, the present studies seek to determine the potential role of phosphorylation in modulating the activities of PvSF1. A bacterial clone containing a full-length cDNA encoding PvSF1 protein tagged at the N-terminus with tandem histidine residues was obtained and used to over-express the fusion protein. Presence of the histidine tag was used to purify the recombinant protein by Nickel-affinity chromatography, followed by anion exchange chromatography. The recombinant PvSF1 (rPvSF1) co-migrates on SDS-PAGE with ^{35}S-labeled PvSF1 generated by in vitro transcription/translation (lPvSF1). Both lPvSF1 and rPvSF1 bind to an element (GCCACCTCA) present in the promoter of a bean phytohemagglutinin gene. rPvSF1 can be phosphorylated using a class II casein kinase, a type of kinase isolated from other plants and implicated in signal transduction involving plant transcription factors. Nuclear extracts prepared from developing bean (seeds) are capable of phosphorylating a substrate that co-migrates with rPvSF1 on SDS-PAGE. rPvSF1 is presently being used to raise antibodies in rats for the purpose of determining the temporal expression pattern of PvSF1 during bean (seed) development and as a phosphorylation substrate for identification and purification of bean (seed) kinases as potential participants in regulating specific transcriptional responses during seed maturation.

J6-434 **Protein kinases specifically expressed in tomato pollen: implications in pollen-pistil interactions.** Jorge Muschietti, Yoram Eyal and Sheila McCormick. Plant Gene Expression Center, UC-Berkeley/USDA-ARS. 800 Buchanan St., Albany, CA 94710

Many reproductive barriers in flowering plants are controlled by pollen-pistil interactions, presumably mediated through a cell-cell communication mechanism. A biochemical signal occurring in pollen or in the style leads to either an acceptance response, where the pollen germinates and fertilizes the ovule, or to a rejection response that prevents pollen germination or leads to pollen tube arrest. Several receptor-like kinases have been implicated in cell-cell interactions, e.g. in the sporophytic self-incompatibility system in *Brassica* (Plant J., 5;373-384), where the receptor-like kinase (SRK) in the female side presumably initiates a signal transduction cascade to block pollen hydration and germination. In other reproductive barriers, such as gametophytic self-incompatibility (GSI) or in unilateral incongruity (UI) where the cross between two species is successful only if the wild species is the male, it is reasonable to assume that a protein kinase mediated signal transduction pathway could also occur in the pollen in response to a signal from stylar tissues. For example in the GSI system of *Papaver*, a change in phosphorylation pattern as well as an intracellular pulse of calcium was observed in pollen after interaction with self-incompatible styles (Trends Cell Biol., 3;340-345).

In order to isolate potential pollen components of the pollination signal transduction pathway occurring in the reproductive barrier of UI, we have designed a set of degenerate oligonucleotides based on regions conserved in many protein kinases or specifically in plant receptor kinases, such as the recently described pollen-expressed receptor-like kinase in *Petunia* (Plant Cell, 6;709-721). These oligonucleotides were used for RT-PCR on tomato pollen poly-A+ RNA; 4 different classes of clones obtained were similar to protein kinases and by RNA blots we determined that only 2 of these putative kinase transcripts were pollen specific. Full length clones were obtained from a tomato mature anther cDNA library. The expression and structural characteristics of these putative kinase genes will be discussed.

J6-435 CHARACTERIZATION OF THE B REGULATORY SUBUNIT OF *ARABIDOPSIS* PROTEIN PHOSPHATASE TYPE 2A, Sabine J. Rundle, Andrew Hartung, John W. Corum, Department of Biology, Western Carolina University, Cullowhee, NC 28723

Protein phosphorylation is an important regulatory component of many signal transduction pathways and is regulated by the opposing action of protein kinases and protein phosphatases. We are studying the role of serine/threonine protein phosphatases (PP) in signal transduction during plant development. Specifically, we are interested in finding regulators of plant type 2A PP. We used the polymerase chain reaction to isolate a segment of an *Arabidopsis* PP2A B regulatory subunit (RS). The amplified DNA fragment of 372 nucleotides encodes a part of the *Arabidopsis* B RS protein that is 59% identical and 83% similar with the *S. cerevisiae* PP2A RS protein (*CDC55*). Genomic Southern blots indicate the *Arabidopsis* genome contains one or two genes encoding the B RS. We used the amplified PCR product to screen an *Arabidopsis* cDNA library and isolated a number of clones encoding the B RS. Information regarding these clones will be presented. This is the first report of the existence of the B RS in plants, indicating plant type 2A PP are regulated in a similar manner to their animal and yeast counterparts. This information provides the groundwork for future experiments concerning the role of PP2A in plant signal transduction.

Late Abstracts

MOLECULAR GENETIC DISSECTION OF THE *RPS2* DISEASE RESISTANCE PATHWAY OF *ARABIDOPSIS*. Andrew Bent[1,2], I-ching Yu[1], Xiaochun Zhang[1], Barbara Kunkel[2], Doug Dahlbeck[2], Brian Staskawicz[2], J. Scott Schmidt[1]. [1]Department of Agronomy, University of Illinois, Urbana, IL 61801 ([2]Department of Plant Biology, University of California, Berkeley, CA 98502).

Plant disease resistance typically requires highly specific recognition of an invading pathogen. This agriculturally important process is dependent on pathogen avirulence genes and plant resistance genes. We recently cloned and characterized *RPS2*, an *Arabidopsis* resistance gene specific for *Pseudomonas syringae* bacteria that express avirulence gene *avrRpt2*. (Bent, Kunkel *et al.* Science 265:1856, 1994). *RPS2* is representative of a large class of resistance genes with specificities for fungal, bacterial and viral pathogens. Protein motifs encoded in *RPS2* include leucine-rich repeats and postulated membrane spanning, nucleotide triphosphate binding, and leucine zipper domains, suggesting possible mechanisms of action.

Complex sets of responses are induced upon activation of *avrRpt2/RPS2* defense signal transduction, and current studies seek to identify and characterize additional components of these pathways. We will discuss progress on our current research objectives, which include: 1) Mapping, isolation, and initial characterization of a provisionally identified gene we are calling *SPR1* (this gene is appears to be required for resistance of *Arabidopsis* to *P. syringae* strains that express *avrRpt2*). 2) Isolation of the genes and gene-products for proteins that interact with the leucine zipper and adjoining domains of the Rps2 protein. 3) Mutational analysis to identify new loci involved in *avrRpt2/RPS2* defense signal transduction.

DIFFERENTIAL DNA-BINDING ACTIVITY TO PROMOTER ELEMENTS OF THE *gnl* ß-1,3-GLUCANASE GENE IN HYPERSENSITIVE REACTING TOBACCO PLANTS, Carmen Castresana and Elena Alonso, Centro Nacional de Biotecnología, C.S.I.C., Canto Blanco, Madrid E-28049, Spain

In a hypersensitive reaction to pathogen infection expression of the ß-1,3-glucanase *gnl* gene is induced in cells surrounding the necrotic lesions. To investigate the molecular basis controlling activation of gene expression during this plant defense response the interaction of the *gnl* regulatory elements with putative regulatory proteins has been examined. We demonstrated, by electrophoretic mobility shift assays, that nuclear proteins obtained from both healthy and hypersensitive reacting tobacco leaves interact with DNA sequences involved in induction of *gnl* expression. Among the binding sequences characterized, the promoter region extending from -250 to -217 bp contained the DNA motif -GGCGGC- found to be conserved in most if not all promoters of basic PR proteins encoding genes. The activity bound by this promoter sequence was stronger in hypersensitive responding tissues that in healthy untreated tobacco leaves.

STUDYING THE ROLE OF TWO NEWLY IDENTIFIED GENES THAT EXHIBIT DIFFERENT PATTERN OF DIURNAL EXPRESSION AND LIGHT RESPONSE IN GROWTH AND DEVELOPMENT OF CITRUS, Doron Holland and Mohamad Abu-Abied, Department of Fruit-Tree Breeding and Genetics, The Volcani Center, Bet Dagan, Israel.

Two genes that exhibit different pattern of diurnal expression and light response were cloned from citrus. One of these genes (*c-ino1*) is highly homologous to inositol phosphate synthase from yeast and spirodella. In addition to the diurnal pattern of expression of *c-ino1* it is strictly regulated by light and by ABA. Treatment with ABA changes the diurnal pattern of *c-ino1*. Inositol and its derivatives play a major role in signal transduction and in plant development, particularly in the process of cell wall synthesis. Therefore, growth rate and development of plants are expected to be dependent on *c-ino1* activity. In accordance with these findings it was found that *c-ino1* is abundant in leaves of juvenile plants. Juvenile plants are known to be more vigorous than mature plants and it could be that *c-ino1* activity is one of the functions that are responsible for this trait. In addition to *c-ino1*, we cloned an additional gene (*ccr*) whose mRNA levels show a circadian rhythm. This gene is a novel circadian gene that its function is unknown and is highly homologous to *lir1*, a gene recently isolated from rice. *Lir1* was considered to be present only in monocots until our findings. We have established transgenic plants that have the sense and antisense configuration of *ccr*. Transgenic tobacco plants are now being studied in order to find out the function of *ccr*.

TENTOXIN CAUSES CHLOROSIS BY INTERFERING WITH REGULATORY PROCESSES THAT CONTROL CHLOROPLAST DEVELOPMENT

Neta Holland[1], Yoav Evron[2], Uri Pick[3] and Marvin Edelman[1], Departments of Plant Genetics[1] and Biochemistry[3], Weizmann Institute of Science. Rehovot; Department of Fruit-Tree Breeding and Genetics[2], The Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.

Tentoxin is a phytotoxin that is produced by the fungus *Altenaria alternata* and affects seedlings of sensitive species by inducing chlorosis (inhibition of the accumulation of chlorophylls and carotenoids). Plant sensitivity to tentoxin-induced-chlorosis is determined by codon 83 of the chloroplastic atpB gene (Avni et al. 1992) but inhibition of ATP synthesis by tentoxin is not correlated to chlorosis. Tentoxin was found to increase thylakoid energization *in vivo* and *in vitro* in the presence of nucleotides (Evron et al. 1994). Such over energization and production of free radicals might serve as a signal for chlorosis induction. Indeed, chlorosis is inhibited in the presence of the uncoupler gramicidin or the free radical scavenger ascorbic acid. Tentoxin induced chlorosis is limited to a specific time window during chloroplast development and active plant metabolism is required for its induction. Based on these results, we suggest that tentoxin induces chlorosis by interfering with regulatory processes that control chloroplast development.

"DETECTION OF PHOSPHORYLATION IN TYROSINE RESIDUES OF PROTEINS DURING THE DEVELOPMENT OF COCONUT (*Cocos nucifera* L.) ZYGOTIC EMBRYOS". Islas-Flores, I., Oropeza, C. and Hernández-Sotomayor, S.M.T. Centro de Investigación Científica de Yucatán. Apdo Postal 97, Cordemex 97310, Mérida, Yucatán, México.

Coconut (*Cocos nucifera* L.) regeneration *in vitro* has proved to be very difficult. It has been achieved through somatic embryogenesis, but the process is still inefficient. It is believed that basic research to understand the regulation of differentiation leading to embryogenesis is required to improve current micropropagation protocols. The present paper reports evidence of the occurrence of protein phosphorylation in tyrosine residues in coconut zygotic embryo extracts. Proteins were resolved by SDS-PAGE electrophoresis and phosphorylation in tyrosine residues was detected with monoclonal antibodies. The phosphorylation pattern changed during the embryo development, along with changes in form and size of the embryo. Phosphorylation was not detected in extracts of tissues surrounding the embryo.

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MOLECULAR GENETIC ANALYSIS OF DISEASE RESISTANCE IN ARABIDOPSIS. Barbara N. Kunkel, Michelle Verbsky, Juan Carlos Lopez and Vasantha Aaron. Department of Biology. Washington University, St. Louis, MO 63105.

Interactions between plant pathogens and their hosts are highly specific, suggesting that plant-pathogen interactions involve a continual exchange of information between the two organisms. For example, initiation of pathogenic interactions is dependent on a pathogen's ability to recognize, invade and grow in suitable host plants. Super-imposed on the establishment of these interactions is the potential for the host plant to detect the presence of a specific pathogen and, as a result, rapidly induce expression of resistance responses. We are using a molecular genetic approach to identify and characterize plant genes that control pathogen recognition and the subsequent expression of disease resistance. Mutational analyses of resistance in *Arabidopsis thaliana* to *P. syringae* has resulted in the identification of many susceptible mutants, including several that define a locus, *RPS2*, that is required for recognition of *P. syringae* strains expressing the avirulence gene *avrRpt2*. The *RPS2* locus has recently been cloned, and sequence analysis has revealed several motifs that suggest that the RPS2 protein may interact with other protein components of the cell. However, the role of *RPS2* in mediating disease resistance still remains unclear. We are using a combination of molecular, genetic and biochemical approaches to investigate how *RPS2* mediates recognitional specificity and to identify other components of the defense response pathway. Our progress towards elucidating *RPS2* function and towards identifying additional plant genes involved in the expression of disease resistance in Arabidopsis will be presented.