



Mid-Atlantic Plant Molecular Biology Society





Twelfth Annual Meeting, 1995




Beltsville, MD



CONTENTS

INTRODUCTION

Welcome to the twelfth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. These meetings are designed to bring some of the best scientific minds to our area and to introduce some of the most interesting advances in plant molecular biology to our area scientists at a reasonable price and at an accessible location. We hope to entice a large number of students, postdocs and senior scientists to attend and actively participate in presentations and discussions. In addition, the meeting is designed to encourage mixing of scientist in an informal atmosphere during on-site lunches, breaks, socials, and banquet to provide each participant the opportunity to meet invited speakers and other members.

The meeting encompasses a large range of important research areas, which we hope will stimulate participants by informing them about advances outside of their own immediate interests. Please contact members of the organizing committee if you have thoughts or comments for consideration in the planning of future meetings or join next year's organizing team and volunteer your services to improve upon what we did this year. All are welcome at every stage of planning and organizing each meeting.

Many people were involved in the organization and planning of the meeting and we give them our hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date product advances and help defray costs. **Please visit our visitors' displays** which are located with the posters. The level of interest you show in their products is a critical factor in their willingness to support future meetings.

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society. Enjoy the meeting!

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Ben Matthews
Co-Organizers

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John Jurek

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Plant Genome Data and Information Center
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Abstracts

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1995 MAPMBS MEETING SCHEDULE

WEDNESDAY, JULY 19

8:00 a.m. Registration and Poster set-up

8:50 a.m. Opening remarks - Ben Matthews

GENE REGULATION I
(Moderator: Joan Gebhardt, USDA-ARS)

9:00 am Ann Callahan, Linda Dunn, Michelle Shipley, Reuben Cohen and Deanna Fishel (USDA-ARS, Kearneysville). Stress proteins in peach fruit ripening.

9:30 am Mark Tucker, Susan Koehler, Gail Matters, Pravendra Nath, and Elizabeth Kemmerer (USDA-ARS, Beltsville; Brown University; and NBRI, Lucknow, India). Analysis of the gene promoter for a bean abscission cellulase.

9:50 am Xiaoying Lin, Gwo-Jiunn Hwang, and Lynn Zimmermann (University of Maryland, Baltimore County). Isolation of a diverse set of cDNA clones from carrot somatic embryos and characterization of gene expression during plant embryogenesis.

10.10 am **COFFEE BREAK/POSTERS (Set-up and viewing)/EXHIBITORS**

GENE REGULATION I (continued)

11.00 am M.L. Sistrunk, W.Xu, M. Purugganan1, D. Antosiewicz, D. Polisensky, S. Fry and J. Braam (Rice University and University of Edinburgh). Regulation and functions of the *Arabidopsis TCH* genes.

11:30 am Mauricio Bustos, Hans Eiben, Maw-Shenq Chern and Andrew Bobb (University of Maryland, Baltimore County). ROM1 is a transcriptional repressor protein that modulates temporal expression of seed storage protein and lectin genes during the transition from late cotyledon to maturation stages of embryogeny.

11:50 am Joan Gebhardt, Gregory Wadsworth and Benjamin Matthews (USDA-ARS, Beltsville). Characterization of a soybean cDNA clone encoding the cytoplasmic isozyme of aspartate aminotransferase.

12:10 pm **LUNCH, EXHIBITORS**

GENE REGULATION II
(Moderator: Cleo Hughes, USDA-ARS)

1:20 pm David E. Somers (University of Virginia). Genetic analysis of circadian rhythyms in *Arabidopsis*.

1:50 pm Caren Chang, Jian Hua, Hajime Sakai and Eliot Meyerowitz (University of Maryland, College Park; and California Institute of Technology). The ethylene response in *Arabidopsis*: a two-component gene family.

2:20 pm Karen Clark and Caren Chang (University of Maryland, College Park). Identifying interacting proteins: the yeast two hybrid system.

2:40 pm **COFFEE BREAK/POSTER VIEWING/EXHIBITORS**

TRANSFORMATION AND TECHNIQUES I
(Moderator: James Saunders, USDA-ARS)

3:30 pm Randall Niedz (USDA-ARS, Orlando). The development of a genetic transformation system for citrus.

4:00 pm John Wallace (Bucknell University). Recovery of plasmid DNA from transformants of the moss, *Physcomitrella patens*.

4:20 pm **SOCIAL HOUR**

KEYNOTE ADDRESS
(Introduction: Ben Matthews, USDA-ARS)

5:30 pm Chris Lamb (Salk Institute for Biological Studies). Mechanism and function of the oxidative burst in plant disease resistance.

6:45 pm **DINNER**

THURSDAY, JULY 20

PLANT-MICROBE INTERACTIONS
(Moderator: John Hammond, USDA-ARS)

8:45 am Donald Nuss (University of Maryland Biotechnology Institute, College Park). Using mycoviruses to understand and control fungal pathogenesis.

9:15 am Rosemarie Hammond and James Crosslin (USDA-ARS, Beltsville; and Washington State University). Genetic relatedness of Prunus necrotic ringspot virus isolates correlates with disease severity.

9:35 am Dawn Gundersen-Rindal, I-M. Lee, S. Rehner, R. Davis and D. Kingsbury (USDA-ARS, Beltsville; and Johns Hopkins University). Approach to taxonomy for unculturable mycoplasmalike organisms.

9:55 am **COFFEE BREAK/POSTER VIEWING/EXHIBITORS**

PLANT-MICROBE INTERACTIONS (continued)

10:50 am Ramon Jordan (USDA-ARS, Beltsville). Antibody expression in transgenic plants: from production to protection.

11:20 am Gary Kinard (USDA-ARS, Beltsville). Molecular detection of apple chlorotic leafspot and apple stem grooving viruses.

11:40 am John Hammond (USDA-ARS, Beltsville). Transcapsidation in potyvirus-inoculated transgenic plants expressing various forms of potyvirus coat protein.

12:00 **BUSINESS MEETING**

12:15 pm **LUNCH**

TRANSFORMATION AND TECHNIQUES II
(Moderator: Gordon Snyder, USDA-ARS)

1:30 pm Jhy-Jhu Lin (Agricultural Biotechnology and Molecular Biology R&D, Life Technologies). AFLP: a novel PCR-based assay for plant and bacterial DNA polymorphism.

2:00 pm Jia Li and Elizabeth Grabau (Virginia Polytechnic Institute and State University). Overexpression of a fungal phytase gene in soybean suspension culture cells transformed by particle bombardment.

2:20 pm Gordon Snyder, John Ingersoll and Lowell Owens (USDA-ARS, Beltsville). Biolistic-mediated transformation of sugarbeet with novel plant pathogen defense genes.

2:40 pm **CLOSING REMARKS**

1995 MAPMBS POSTER SESSIONS
Wednesday 10:10 am - 11:00 am and 2:40 pm - 3:30 pm
Thursday 9:55 am - 10:50 am

POSTER (Page)

1 (31) Gwo-Jiunn Hwang and J. Lynn Zimmermann (University of Maryland, Baltimore County). Post-transcriptional regulation of gene expression during carrot somatic embryogenesis.

2 (32) Camellia Moses Okpodu and Ruth G. Alscher (Virginia Polytechnic Institute and State University). Glutathione reductase isoforms in isolated chloroplasts from pea (*Pisum sativum* L., cv. Nugget) protoplasts change in response to oxidative stress.

3 (33) Dingbo Zhou, Autar Mattoo and Mark Tucker (University of Maryland, College Park, and USDA-ARS, Beltsville). Constitutive expression of an ETR1 homolog from tomato.

4 (34) Yuval Shimoni and Gad Galili (Weizmann Institute of Science, Rehovot, Israel). Assembly of wheat storage proteins into protein bodies.

5 (35) Andrew Kalinski, R, Shyam Dwivedi, Daniel Rowley and Eliot Herman (USDA-ARS, Beltsville). Developmental regulation of expression and accumulation of soybean vegetative cell thiol protease.

6 (36) Janet L. Donahue, Camellia Moses Okpodu, Carole L. Cramer, Elizabeth A. Grabau and Ruth Grene Alscher (Virginia Polytechnic Institute and State University). Sensitivity to paraquat in pea (*Pisum sativum* L.) is related to development and correlates with the behavior of scavenging enzymes.

7 (37) Jonathan Kuo and Jhy-Jhu Lin (GIBCO/BRL, Life Technologies). Polymorphic analysis of *Arabidopsis thaliana* and *E. coli* strains using Amplified Fragment Length Polymorphism (AFLP).

(38) Wen-Ling Hsieh and Stephen M. Wolniak (University of Maryland, College Park). Isolation and characterization of cyc*ZmW*, a functional cyclin homologue in *Zea mays*.

(39) José J. Pueyo, Maarten J. Chrispeels and Eliot M Herman (University of California, San Diego and USDA-ARS, Beltsville). Degradation of a secretory protein with a destabilizing epitope occurs in the vacuole.

(40) Jin Ma, Tomoko Fukusawa-Akada, Rajnish Khanna, Grald F. Deitzer and John C. Watson (University of Maryland, College Park and Indiana University/Purdue University at Indianapolis). Molecular cloning of an *Arabidopsis* homolog of the PsPK3 protein kinase from *Pisum sativum* L.

(41) N. Assad-Garcia, R. Fike and J.-J. Lin (GIBCO/BRL, Life Technologies). Improved regeneration of plant tissues: a novel medium format and membrane-based liquid culture system.

(42) John C. Ingersoll, Thomas M. Heutte and Lowell D. Owens (USDA-ARS, Beltsville). Effect of promoter-leader sequences on transient expression of reporter gene chimeras biolistically transferred into sugarbeet suspension cells.

(43) C.J.M. Maroon, P.A. Feldstein, R.W. Hammond and T.O. Diener (Center for Agricultural Biotechnology, University of Maryland, College Park and USDA-ARS, Beltsville). Constructs for identifying promoter activity in the potato spindle tuber viroid (PSTVd) RNA.

STRESS PROTEINS IN PEACH FRUIT RIPENING

Ann Callahan, Linda Dunn, Michelle Shipley, Reuben Cohen and Deanna Fishel.
USDA-ARS, Appalachian Fruit Research Station, 45 Wiltshire Rd, Kearneysville, WV 25430

We have isolated a number of genes that are involved in the process of peach fruit development with the final goal of further understanding fruit ripening and/or being able to manipulate fruit quality in a desirable manner. Most of the genes were initially identified through a differential screen of fruit cDNA libraries. They were then further characterized through developmental RNA blot analyses and sequence analysis. In this manner we have isolated two cDNA clones from our ripe cDNA library that are related to stress proteins in other plant systems. By sequence analysis, one of these clones, (pch348), encodes a low molecular weight heat shock protein, (LMW-HSP) while the other, (pch205), encodes a protein very much related to a desiccation-induced protein (ATRD22).

Our initial RNA blot experiments suggested that the genes we have isolated are involved in fruit ripening in that RNA accumulates during fruit ripening. Further work with the LMW-HSP protein suggests that the protein accumulation seen is specific to fruit ripening and not just to a heat shock response. Whether or not these genes are essential to fruit ripening or induced by unrealized stresses, we do not know.

We have begun to follow-up on those experiments by looking at the RNA accumulation of pch348 and pch205 during stress situations and at known stress related genes during fruit development. Potentially the manipulation of some of these stress genes may be a means of regulating ripening on the tree and may even prolong the shelf-life of peach .

ANALYSIS OF THE GENE PROMOTER FOR A BEAN ABSCISSION CELLULASE

Mark L. Tucker[1], Susan M. Koehler[1], Gail L. Matters[2], Pravendra Nath[3] and Elizabeth C. Kemmerer[1], [1]USDA, ARS, Plant Molecular Biology Laboratory, Beltsville, MD 20705, USA, [2]Division of Biol. and Medicine, Brown University, Providence, RI 02912, USA & [3]National Botanical Research Institute, Lucknow, 226-001, India

Bean leaf abscission (organ separation) correlates with the *de novo* accumulation of a pI 9.5 cellulase and its mRNA. Overlapping genomic clones encoding the bean abscission cellulase (BAC) were isolated and partially sequenced. In addition, a genomic clone for a soybean abscission cellulase (SAC) was identified and the sequence compared to the bean genomic sequence. Several regions in the BAC 5′ upstream sequence are highly conserved in the soybean 5′ sequence. Increasing lengths of the 5′ upstream sequence of the BAC gene were fused to the reporter genes luciferase and β-glucuronidase (GUS). Transient expression of luciferase activity in bean using particle gun bombardment resulted in 5 to 10-fold enhancement of luciferase activity in abscission zones over stems and petioles. Tomato plants were stably transformed using *A. tumefaciens*. A BAC promoter construct containing 210 bp of sequence 5' to the transcription start site was sufficient to drive low levels of ethylene-inducible GUS expression in tomato leaf abscission zones and adjacent petioles. GUS expression was not observed in ethylene treated stem tissue or fruit. Expression from the -210 promoter was inhibited by silver thiosulfate, an ethylene action inhibitor. However, unlike expression in bean, expression from the -210 promoter in transgenic tomato was less sensitive to inhibition by auxin.

Isolation of a diverse set of cDNA clones from carrot somatic embryos and characterization of gene expression during plant embryogenesis

Xiaoying Lin, Gwo-Jiunn H. Hwang and J. Lynn Zimmerman
Department of Biological Sciences, University of Maryland Baltimore County
Baltimore, MD 21228

The early events in plant embryogenesis are critical for pattern formation, since they set up the primary apical meristems and the embryo polarity axis. However, very little is known about the molecular events that are unique to the early stages of embryogenesis. The focus of this study is to understand molecular events underling plant embryogenesis. This is addressed by identifying molecular markers from carrot somatic embryo system and characterizing their expression and regulation through embryo development. With a strategy including a cDNA library constructed from polysomal mRNA of globular embryos and a subtracted probe, 50 clones have been isolated and preliminarily characterized. Sequence analysis reveals a large set of genes, including many new genes. Northern blot analysis shows they are expressed in several different patterns and may be regulated at different levels through embryo development. The expression of various genes in somatic embryos indicates that different cellular, metabolic, and morphogenic pathways are activated during plant early embryogenesis.

Twelve clones showed greatly enhanced expression in heart stage embryos. A clone from this group, Gea8 was further characterized through plant development. Its expression during zygotic embryogenesis was investigated. The expression of this gene shows that somatic embryogenesis and zygotic embryogenesis are very similar at the early stages, but differ at late stages, likely due to the lack of induction of a complete maturation program and the initiation of germination in somatic embryos; the level of Gea8 expression is still maintained in zygotic embryos at the cotyledon stage, but much reduced in morphologically similar somatic embryos, plantlets. We have shown that ABA can enhance the expression of Gea8 and may be an endogenous signal for the regulation of Gea8 gene. The promoter regions important for Gea8 expression and regulation are being identified by deletion analysis.

Regulation and Functions of the *Arabidopsis TCH*Genes

M.L. Sistrunk[1], W.Xu[1], M.M. Purugganan[1], D.M. Antosiewicz[1], D.H. Polisensky[1], S.C. Fry[2] and J. **Braam**[1]

[1] Department of Biochemistry and Cell Biology, Rice University , Houston, TX 77251-1892

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TCH3 and *TCH4* are rapidly and strongly upregulated in response to mechanical stimuli, such as touch and wind. *TCH* gene expression is also regulated by cold shock and correlates with cytoplasmic Ca^{2+} increases, as monitored by aequorin luminescence in transgenic plants. The necessity of Ca^{2+} influx for *TCH* gene regulation has been assessed using Ca^{2+} transport inhibitors while simultaneously measured cytoplasmic Ca^{2+} changes. Sequences that confer *TCH3* and*4* regulation have been localized to short 5' regions of the genomic loci. TCH3 is a Ca^{2+} binding calmodulin (CaM)-related protein; its sequence divergence from CaM suggests that potential targets of TCH3 are likely distinct from that of CaM. Immunodetection indicates that TCH3 localizes to cells undergoing expansion, vascular tissue, the root columella and pericycle cells in lateral root primordia. TCH3 accumulation at branch points, especially in wind-challenged plants, suggests a role in reinforcing tissues experiencing mechanical strain. TCH4 shows significant sequence identity to xyloglucan endotransglycosylases (XETs) and TCH4 protein, produced in *E. coli,* has XET activity. XETs are recently discovered enzymes that can cleave and rejoin xyloglucan polymers. Because xyloglucans crosslink cell wall cellulose microfibrils, XETs have been proposed to be involved in cell expansion. Expression of *TCH4* is regulated by hormones that promote expansion and, during development, *TCH4* is expressed in tissues undergoing expansion and/or cell wall modification. In wind-stimulated plants, there are complex changes in the accumulation pattern of *TCH4*. The identification of *TCH4* as an XET provides insight into the physiological relevance of the regulation of expression of the *TCH* genes by environmental stimuli. The placement, mechanical properties and regulation of expansion - in direction and magnitude - of the cell wall are fundamental in determining plant form. Thus, environmental regulation of expression of genes such as *TCH4* whose protein products likely directly alter cell wall properties may underlie plant developmental responses to environmental stimuli.

ROM1 is a transcriptional repressor protein that modulates temporal expression of seed storage protein and lectin genes during the transition from late cotyledon to maturation stages of embryogeny. Mauricio M. Bustos, Hans G. Eiben, Maw-Shenq Chern and Andrew J. Bobb. Department of Biological Sciences, UMBC, 5401 Wilkens Avenue, Baltimore MD 21228.

The transcription of storage protein (phaseolin) and lectin (PHA) genes is up regulated by factor PvALF during the early stages of bean seed maturation. PvALF is related to the *ABI3* and *VP1* gene products from *Arabidopsis thaliana* and maize. However, like ABI3, PvALF is expressed continuously during embryogenesis, suggesting that temporal regulation of phaseolin and lectin transcription involves additional proteins. Regulator-of-*MAT*-1 (ROM1) is a basic leucine-zipper protein that recognizes common sites on the seed-specific enhancer regions of phaseolin and PHA-L promoters. Binding of ROM1 to these enhancers leads to inhibition of PvALF-mediated activation. Maximal expression of ROM1 mRNA coincides with the end of the cotyledon stage, when phaseolin and lectin genes are inactive, and declines rapidly as both genes are induced at the onset of maturation. A proline-rich domain distinct from the ROM1 DNA-binding, dimerization and nuclear targeting regions is required for transcriptional repression. A different repressor, ROM2, has similar characteristics to ROM1 but is expressed primarily at the beginning of the period of seed desiccation when phaseolin and PHA genes are turned off. A model for the developmental regulation of early maturation genes is proposed based on inhibition of transcription by the repressor proteins ROM1 and ROM2 which oppose activators such as PvALF.

CHARACTERIZATION OF A SOYBEAN cDNA CLONE ENCODING THE CYTOPLASMIC ISOZYME OF ASPARTATE AMINOTRANSFERASE

Joan S. Gebhardt, Gregory J. Wadsworth and Benjamin F. Matthews
U.S. Department of Agriculture, Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD 20705

Aspartate aminotransferase (AAT; EC 2.6.1.1) catalyzes the reversible transfer of the amino group of aspartate to α-ketoglutarate to form oxaloacetate and glutamate. In plants AAT plays an important role in hydrogen shuttles, carbon shuttles and nitrogen distribution. Many plants including soybean have multiple isozymes of AAT. Five isozymic forms of soybean AAT can be distinguished by native agarose or polyacrylamide gel electrophoresis. These forms are designated AAT1 to AAT5 based on their rates of migration from slowest to fastest. These isozymes are differentially expressed in different organs during soybean development. Organelle fractionation studies indicate that AAT1, AAT4, and AAT5 are located in the glyoxysomes, mitochondria and chloroplasts, respectively. A soybean leaf cDNA clone was isolated by hybridization to a carrot AAT cDNA clone at low stringency. The cDNA contained an open reading frame encoding a 45,600 Da protein with the most sequence similarity to several plant cytoplasmic AATs. The coding region of the cDNA was cloned in-frame with the *lacZ* gene in pUC18. *E. coli* transformed with this construct expressed a novel protein with AAT activity that migrated with the soybean AAT2 isozyme on polyacrylamide gels. Antibodies raised against the bacterially-expressed AAT protein reacted with AAT2 of soybean in western blots and immunoprecipitation experiments, providing further evidence that the cDNA clone encodes the soybean cytoplasmic isozyme AAT2. The antibodies also reacted with AAT1 and AAT3 but not with AAT4 and AAT5. Alignment of the cDNA clone with other plant cytoplasmic AATs revealed a 64 amino acid N-terminal extension that contained two additional ATG start codons and a putative glyoxysomal/peroxisomal targeting signal. The N-terminal amino acid sequence of purified AAT1 coincided with the predicted amino acid sequence encoded by the cDNA. This evidence suggests that a single gene may encode both the cytoplasmic and glyoxysomal isozymes of AAT.

GENETIC ANALYSIS OF CIRCADIAN RHYTHMS IN ARABIDOPSIS: David E. Somers, Dept. of Biology, University of Virginia, Charlottesville VA.

The role of a biological clock in regulating processes such as leaf movements, stomatal aperature and flowering time has been well established. Circadian rhythms in mRNA levels have also been demonstrated in a number of plants. However, we have recently developed a novel circadian phenotype in transgenic tobacco and Arabidopsis which is based on *in vivo* luminescence catalyzed by firefly luciferase. Using a low-light video imaging system, we have shown that a *cab2*-luciferase transgene can accurately report the temporal and spatial expression patterns of the endogenous *cab2* gene. With this non-destructive assay we can routinely monitor a large number of seedlings every 2-3 hours for up to a week to obtain accurate and reproducible estimates of the period, phase and amplitude of the circadian rhythm.

We have applied this technique towards the genetic dissection of the components of the circadian clock in Arabidopsis. A simple model of the clock posits an input (entrainment) pathway, a central oscillator and an output pathway. Using photomorphogenic mutants deficient in phytochrome (*phyA*, *phyB*, *phyAphyB*, and *hy1*) and blue light photoreceptors (*cry1*), as well as mutations downstream of these (*det1*, *cop1*, *hy5*) we have established that light input through both the phytochrome and blue light photoreceptor pathways control circadian period (1). As well, the *det1* and *cop1* mutations have now been shown to modify the circadian period in both continuous darkness and continuous light, suggesting that they encode critical components of either the input and output pathways, or of the oscillator itself.

A second application of this assay has been in the development of a novel screen for circadian rhythm mutants. The initial selection was based on the identification of mutagenized luminescent individuals which free-run out of synchrony with wild-type seedlings. Subsequent rescreening identified 11 late-cycling (long period), 7 early cycling (short period), 1 reduced amplitude and 2 late phase mutants (2). In addition to a 3 h shorter period of *cab*-luciferase cycling, one semi-dominant short-period mutant (*toc 1*) also showed a comparable reduction in the period length of the leaf movement rhythm. These results are consistent with the possibility of TOC1 encoding a clock component. Mapping of the remaining period mutants is underway to determine if other alleles of TOC1 were recovered. The discovery of arrhythmic, long-period and short-period alleles at the same locus would strongly implicate TOC1 as a component of the central oscillator.

A third approach has been to cross the reporter construct into previously identified mutants whose phenotypes are likely to have links to the clock. Our focus has been on early and late flowering mutants. Preliminary results have identified lines in which both the period and amplitude of the *cab*-luciferase rhythm is modified. We are currently testing the strength of this potential link between *cab* circadian rhythm and flowering time.

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(1) A.J. Millar, M. Straume, J.Chory, N.-H. Chua, S.A. Kay, *Science* **267**, 1163 (1995).
(2) A.J. Millar, I.A. Carre, C.A. Strayer, N.-H. Chua, S.A. Kay, *Science* **267**, 1161 (1995).

THE ETHYLENE RESPONSE IN *ARABIDOPSIS*: A TWO-COMPONENT GENE FAMILY Caren Chang[1], Jian Hua[2], Hajime Sakai[2], and Elliot Meyerowitz[2]; [1] Department of Plant Biology, University of Maryland, College Park, MD 20742; [2] Biology Division, California Institute of Technology, Pasadena, CA 91125

We are interested in the molecular basis for the perception and signal transduction of the gaseous plant hormone ethylene. Mutants of the *Arabidopsis ETR1* locus display pleiotropic insensitivity to ethylene, indicating that the *ETR1* gene plays a central role in ethylene responses. The *ETR1* gene was cloned, and the four known *ETR1* mutant alleles, which are all dominant to the wild type, were found to contain missense mutations within the amino-terminal portion of the deduced ETR1 protein. This amino-terminal domain has no clear homologs in the current sequence databases, however the predicted ETR1 carboxyl-terminal portion shows a high degree of sequence similarity to both components of a large family of bacterial signal transducers known as the two-component regulators. The two-component regulators have not been reported previously in higher eukaryotes. This finding suggests that an early step in ethylene signal transduction might involve a histidine/ aspartate phosphorylation mechanism as described for the bacterial two-component regulators.

Recently, we isolated two additional two-component homologs in *Arabidopsis* called *ETR2* and *ERS*. Both appear to be highly related to *ETR1*; as with ETR1, missense mutations in the amino-terminal domains of ETR2 and ERS confer dominant ethylene insensitivity. The similarities between ETR1, ETR2 and ERS suggest that the perception and signal transduction of the ethylene hormone might involve a family of proteins having redundant functions, which could explain our lack of recessive mutations in these genes.

In the emerging picture of the ethylene-response pathway, *ETR1*, *ETR2* and *ERS* act upstream of *CTR1*, which encodes a protein kinase resembling the Raf protein kinase family known in animals. Raf is a kinase in the conserved mitogen-activated protein (MAP) kinase cascades involved in animal cell differentiation and growth. These animal signaling pathways generally begin with transmembrane tyrosine protein kinase receptors. However, in the ethylene-response pathway in plants, a Raf-like protein kinase appears to be regulated through several two-component regulators, namely ETR1, ETR2 and ERS.

IDENTIFYING INTERACTING PROTEINS: THE YEAST TWO-HYBRID SYSTEM.

Karen L. Clark and Caren Chang; Dept. of Plant Biology, University of Maryland, College Park, MD 20742

Identifying interacting proteins either in signal transduction pathways, multi-protein complexes, metabolic pathways, or other processes is a goal sought by many researchers in different fields. A new technique to address this question, the two-hybrid system, is a protocol that takes advantage of the properties of transcriptional activators (Fields and Song 1989). The *Saccharomyces cerevisiae* Gal4 transcriptional activator can be considered to consist of two separable domains: an N-terminal DNA binding domain and a C-terminal transcriptional activation domain. The DNA binding domain recognizes and binds to the GAL-UAS, a palindromic 17-mer sequence, thus bringing the transcriptional activation domain to an appropriate location to interact with the transcriptional machinery and, hence, activate transcription of the gene. The exploitable feature of these domains is that they need not be on the same molecule to activate transcription. Indeed, if they are present on separate proteins and those two proteins interact, then transcriptional activation will occur. This property is the basis of the two-hybrid system. Since the original presentation by Dr. Stan Fields (Fields and Song 1989), many researchers in many areas of study have used this system to identify interacting proteins, including Ras-Raf (Vojtek et al. 1993; Zhang et al. 1993), Cdk2-Cip1 (Harper et al. 1993), and Max-Mxi1 (Zervos et al. 1993).

We are using the two-hybrid system to identify proteins that interact with the *Arabidopsis thaliana* protein ETR1. Since ETR1 is an upstream component of the ethylene response pathway and is probably an ethylene receptor, we want to identify downstream components with which it associates. We have isolated 15 candidate clones to date and are in the process of retesting and confirming their interaction.

Fields and Song. (1989). Nature **340**:245.
Vojtek et al. (1993). Cell **74**:205.
Zhang et al. (1993). Nature **364**:308.
Harper et al. (1993). Cell **75**:805.
Zervos et al. (1993). Cell **72**:223.

The Development of a Genetic Transformation System for Citrus

Citrus is a major fruit crop and makes up 41% and 21% respectively of the total tonnage and value of fruits grown in the U.S. Variety development by hybridization has been difficult because of Citrus' apomictic sexuality; therefore, most Citrus varieties, particularly sweet orange and grapefruit, are bud sport selections. Also, no sweet orange, grapefruit, lemon, or mandarin genetic transformants have been reported, partly due to difficulties in identifying an efficient method of selection. To develop a visual selection scheme, protoplasts were isolated from embryogenic sweet orange (*Citrus sinensis* (L.) Osbeck cv. 'Hamlin') suspension cultures derived from ovule nucellar tissue. An electroporation protocol was optimized for a number of variables including field strength, buffer, and DNA concentration. Various plasmid constructs were tested for their utility in visual selection. A plasmid (p35S-GFP) carrying the gene for the green fluorescent protein (GFP) from the bioluminescent jellyfish *Aequorea victoria* was constructed by replacing the GUS coding sequence of pBI221 with a functional GFP gene, thereby placing the GFP gene under the control of the CaMV 35S promoter. Protoplasts were viewed by incident-light fluorescence microscopy 24 h after electroporation, and 20-60% of the protoplasts emitted an intense green light when illuminated with blue (450-490 nm) light.

RECOVERY OF PLASMID DNA FROM TRANSFORMANTS OF THE MOSS, *Physcomitrella patens*: John C. Wallace, Dept. of Biology, Bucknell University, Lewisburg, PA 17837.

The ability to create transgenic plants has revolutionized many aspects of both basic plant science and agricultural biotechnology. Many of the fundamental events that take place during the processes of transformation remain largely unknown, however. The moss *Physcomitrella patens* is quite easily transformed via particle bombardment, from which three classes of antibiotic-resistant transformants appear: those that express the transgene for only up to a few weeks time (transients); those that will express the gene permanently only if maintained on selective medium (unstables); and those that continue expression regardless of culture conditions (stables). Southern analysis of genomic DNA from the unstables has suggested that they maintain transforming DNA as extrachromosomal plasmids. Rescue of those plasmids by electroporation into *E. coli* has been performed. Analysis of these rescued plasmids will be presented, as well as the potential utility of this system in the isolation and cloning of plant genes of unknown function.

MECHANISM AND FUNCTION OF THE OXIDATIVE BURST IN PLANT DISEASE RESISTANCE.

Chris Lamb, Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 NorthTorrey Pines Road, La Jolla, California 92037.

Treatment of bean or soybean cells with fungal elicitor causes a rapid insolubilization of pre-existing (hydroxy)proline-rich structural proteins in the plant cell wall. This insolubilization, which involves H_2O_2-mediated oxidative cross-linking, is initiated within 2 to 5 min, and is complete within 10-20 min, and hence precedes the expression of transcription-dependent defenses (1). Oxidative cross-linking makes the cell wall refractory to digestion by microbial protoplasting enzymes and is strictly dependent on gene-for-gene mediated incompatibility in an isogenic setting (2). Thus stimulus-dependent oxidative cross-linking of wall proteins likely has an important function in the initial stages of plant defense. Cross-linking is substrate controlled by the rapid generation of H_2O_2 at the cell surface. In addition, we have recently shown that H_2O_2 from this oxidative burst also functions as a local trigger of programmed death in challenged cells and as a diffusible signal for the induction in adjacent cells of genes encoding cellular protectants such as glutathione S-transferase and glutathione peroxidase (3). Thus, H_2O_2 from the oxidative burst plays a key role in the orchestration of a localized hypersensitive response during the expression of plant disease resistance (4). Emerging evidence will be presented that H_2O_2 triggers a sustained Ca^{2+}-influx to induce apoptotic cell death and that salicylic acid potentiates the hypersensitive response by positive feedback modulation of an early step in the signal pathway.

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USING MYCOVIRUSES TO UNDERSTAND AND CONTROL FUNGAL PATHOGENESIS: Donald L. Nuss, Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, MD 20742-3351

Cytoplasmically-transmissible RNA viruses of the genus *Hypovirus* cause reduced virulence (hypovirulence) in the chestnut blight fungus *Cryphonectria parasitica*, providing a paradigm for the general use of viruses to understand and control fungal pathogenesis. The development of a full-length infectious hypovirus infectious cDNA has made it possible to engineer hypovirulent *C. parasitica* strains with improved biological control potential and to extend virus-mediated virulence-attenuation to new fungal species not previously reported to harbor viruses. Several lines of evidence indicate that hypovirus infection alters signal transduction pathways involved in normal fungal gene expression including pathways that govern response to nutrient availability. In the most resent extension of these studies, we have found that the accumulation of a heterotrimeric GTP-binding protein α subunit of the G_i class, designated CPC-1, is reduced in hypovirus-infected *C. parasitica* strains. Transgenic co-suppression, a phenomenon frequently observed in transgenic plants, reduced CPC-1 accumulation in virus-free fungal strains. Significantly, the resulting transgenic fungal strains were also hypovirulent. Subsequent differential mRNA display analysis revealed a constellation of potential reporter genes that are identically regulated in hypovirus infected and in the transgenic CPC-1 suppressed strains. These results provide the first evidence for the involvement of G protein-linked signal transduction in fungal pathogenesis and suggest a molecular basis for virus-mediated hypovirulence. They also reinforce the emerging view that the role of putative fungal virulence determinants must be considered within the broader context of the regulatory pathways that govern their elaboration at the host-pathogen interface.

GENETIC RELATEDNESS OF PRUNUS NECROTIC RINGSPOT VIRUS ISOLATES CORRELATES WITH DISEASE SEVERITY

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Prunus necrotic ringspot virus (PNRSV) occurs worldwide and is a serious pathogen of *Prunus* species, including peach and sweet cherry. PNRSV is a member of a serologically interrelated group of ilarvirus subgroup III. Its genome is characterized by a tripartite, message-sense, single-stranded RNA genome. RNA3 encodes two polypeptides, a polypeptide with homology to proteins required for cell-to-cell movement of plant viruses, and the capsid or coat protein. We have determined the complete nucleotide sequence of RNA3 of the peach PE-5 isolate of PNRSV and have made DNA constructs to introduce the coat protein gene into peach and cherry for coat protein mediated resistance to PNRSV. Several biological isolates of PNRSV have been isolated from perennial fruit crops. Although some of the isolates differ greatly in pathology, they do not exhibit extensive differences in particle characteristics. Sequence analysis of PCR products obtained from the RNA3 of these isolates reveal that the primary sequence shows strong correlation to symptom and serological phenotypes; single amino acid residues in the predicted translation products also appear to be conserved between the symptom types.

Approach to Taxonomy for Unculturable Mycoplasmalike Organisms.

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Mycoplasmalike organisms (MLOs) (now called phytoplasmas) are unculturable, wall-less prokaryotes that cause disease in a wide variety of economically important plant crops. Inability to isolate MLOs in pure culture has made it difficult to characterize these pathogens and determine their taxonomy by traditional methods. To provide a basis for eventual establishment of their taxonomy, the phylogenetic relationships of MLOs to *Mollicutes* and culturable bacteria and the relatedness among a wide range of MLOs were investigated by parsimony analyses of PCR-amplified conserved 16S rRNA and ribosomal protein (rp) gene sequences. These analyses revealed that MLOs form a monophyletic clade within the larger *Acholeplasma* clade within the *Mollicutes* and delineated 11 distinct subclades within the MLO clade. The inferred phylogenies suggested that MLOs be represented taxonomically at the minimal level of genus, and that each distinct sub-clade should represent at least a distinct species under this genus. Further categorization of MLO strains comprising the two largest putative MLO species could be obtained by restriction fragment length polymorphism (RFLP) analyses of PCR-amplified 16S rRNA, rp, and chromosomal gene sequences.

ANTIBODY EXPRESSION IN TRANSGENIC PLANTS: FROM PRODUCTION TO PROTECTION. Ramon Jordan, USDA, Agricultural Research Service, US National Arboretum, Floral and Nursery Plants Research Unit, Beltsville, MD

Recent attempts to confer resistance to plant viruses by transformation and genetic engineering with viral and antiviral genes have provided promising new strategies for virus control. The major approaches that have been developed to generate transgenic resistance include expression of viral coat protein coding sequences, transformation of plants with other viral gene products, such as viral replicase, and expression of untranslatable sense or anti-sense viral transcripts.

One of our research goals is to develop a novel method for establishing virus resistance in plants by producing transgenic plants expressing monoclonal antibody proteins (as Fabs and/or single-chain antibodies) capable of binding and inhibiting, or interfering with, the mode(s) of action of specific viral structural and non-structural gene products. These target proteins include viral coat protein, helper component and replicase. Expression of antibodies in plants that bind antigens essential for pathogenesis could ameliorate the symptoms of infection by binding to or affecting 'functional' epitopes, or simply through a reduction in the effective titer of the target antigen. The effective "neutralization" of one or more of these viral proteins should interfere with the efficiency of virus assembly, movement of virus within the host, symptomotology, aphid transmission, and/or virus replication and thereby form the basis of a mechanism for conferring disease tolerance or resistance to the engineered host plant. One of the advantages of this type of 'anti-viral antibody-mediated protection' would be that no viral genes need be introduced into the environment as part of the transgenic plant.

Several approaches have been employed to express antibodies in plants. Hiatt et al (1989) demonstrated that plants transformed with antibody genes were able to produce serologically active antibodies and even suggested that this technology would be applicable to studying plant-pathogen interactions. At least six reports of 'plantibody' or 'phytoantibody' production in plants have since been published. In most cases the plant-produced antibody, when purified, was found to possess the antigen binding properties of the original monoclonal antibody (During et al., 1990; Hein et al., 1991; Hiatt and Ma, 1992); however, a plant-produced variable heavy domain antibody did not (Benvenuto et al., 1991). More recently a functional (in planta) anti-phytochrome single-chain antibody (scFv) was successfully produced in transgenic tobacco (Owen et al., 1992). Functional antibody could be purified from transgenic plants and seeds from transgenic R1 progeny displayed abberrant phytochrome-dependent germination. This was the first demonstration that a functional antibody fragment that binds a plant cell antigen could be synthesized in plant cells. Taviadoraki et al (1993) presented evidence that constituitive expression in transgenic plants of a scFv directed against a plant tombusvirus caused reduction of infection incidence and delay in symptom development.

MOLECULAR DETECTION OF APPLE CHLOROTIC LEAFSPOT AND APPLE STEM GROOVING VIRUSES

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Apple chlorotic leafspot trichovirus (ACLSV) and apple stem grooving capillovirus (ASGV) infect rosaceous fruit trees. Low virus concentrations in woody hosts are an obstacle to reliable detection of the viruses. Thus, reverse transcriptase polymerase chain reaction (RT-PCR) procedures have been developed that detect virus-specific fragments of the RNA genomes. The rapidity with which samples can be tested for ACLSV and ASGV, combined with a limit of detection estimated to be at the picogram levels, provides significant advantages for routine detection. These systems were validated by sequencing fragments amplified from leaves of apple, cherry, and Asian pear trees growing in South Carolina. Comparisons with published sequences indicated that the three fragments from the ACLSV isolates have very high (>97.8%) percent identity values when compared to the corresponding region of a plum isolate from France. The fragments amplified from the ASGV isolates were similar (>89.8% identities) to the corresponding region of an apple isolate from Japan.

These detection systems have been found to be applicable to an extensive array of isolates of the viruses, including ASGV isolates of worldwide origin. The systems were used to investigate the epidemiological properties of the viruses. Both viruses were detected in apple bark, blooms, fruits, leaves, and roots, indicating that the viruses are located throughout infected, actively growing apple trees. ACLSV and ASGV were detected in both the scions and rootstocks of established apple trees that displayed no symptoms of infection, suggesting that latent infections are not a result of confinement of the viruses to either scion or rootstock. Both viruses are evenly distributed throughout the leaves of actively growing trees. Thus, composite samples of only a few leaves are sufficient to conduct accurate diagnoses. Finding both viruses in root tissue validates previous suppositions that spread in the field may occur by underground contact among roots.

TRANSCAPSIDATION IN POTYVIRUS-INOCULATED TRANSGENIC PLANTS EXPRESSING VARIOUS FORMS OF POTYVIRUS COAT PROTEIN.

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Transgenic plants expressing potyviral coat proteins have been shown to exhibit varying degrees of resistance to the virus from which the gene was derived, and in some cases to related viruses. One perceived risk of growing such plants in the field is the possibility for transcapsidation (the incorporation of the transgene coat protein into particles of a virus able to infect the transgenic plants). Transcapsidation could alter aphid transmission characteristics, as different potyviruses are transmitted by different species of aphids. However, transcapsidation could only influence a single passage from a transgenic plant to a non-transgenic plant, as only the coat protein encoded by the viral genome would be present in cells of the non-transgenic plant.

We have challenged transgenic plants expressing either wildtype or chimeric potyvirus coat protein constructs, and purified virus particles from these plant. Most of the transgene coat proteins are uniquely identifiable using a monoclonal antibody specific for an epitope in the amino terminus of the strain of bean yellow mosaic virus from which the amino-terminal domain of these constructs was derived. Using Western blots and indirect ELISA, we estimated that between 0.5% and 25% of the coat protein was derived from the transgene, depending upon the virus/transgene combination. The degree of transgene coat protein incorporation was not apparently correlated with the extent of virus resistance, suggesting that re-encapsidation of incoming viral RNA (potentially inhibiting translation and therefore replication) is not a major factor in potyvirus coat protein-mediated resistance.

AFLP: a novel PCR-based assay for plant and bacterial DNA polymorphism. Jhy-Jhu Lin. Agricultural Biotechnology and Molecular Biology R&D, Life Technologies, Inc., 8717 Grovemont circle, Gaithersburg, MD 20878

Amplifed Restriction Fragment Polymorphism (AFLP) is a powerful technique for the demonstration of the DNA polymorphism in plants and microorganisms. The resolution of the DNA polymorphism by AFLP depends on the number of selective nucleotides in the EcoR I and Mse I primers as well as the complexity of the genomic DNA. Although the concentration of genomic DNA has little effect on AFLP, the quality of genomic DNA is critical. Significantly, different patterns between octopine strains and nopaline strains of *Agrobacterium tumefaciens* have been demonstrated based on AFLP. This suggested that analysis of genomic DNA as well as the functional assay of the Ti plasmid is required for the nomeclature of *A. tumefaciens*. Furthermore, direct comparison between AFLP, RAPD, and RFLP in soybean indicates that AFLP has several advantages: (1) superior performance in the demonstration of DNA polymorphism, (2) faster time for screening molecular markers, and (3) reproducibility.

OVEREXPRESSION OF A FUNGAL PHYTASE GENE IN SOYBEAN SUSPENSION CULTURE CELLS TRANSFORMED BY PARTICLE BOMBARDMENT

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Phytate (*myo*-inositol hexaphosphate) is a major storage form of phosphorus found in plant seeds and comprises 61% of phosphorus reserves in soybean. Phytate in soybean meal-based animal feed cannot be utilized efficiently by non-ruminant animals and is excreted in manure. This contributes to phosphorus pollution associated with animal production. Supplementation of animal feed with a preparation of fungal phytase (E.C. 3.1.3.8) from *Aspergillus niger* is effective at reducing phosphorus levels in manure. Our longterm goal is to reduce excreted phosphorus levels by overexpressing a fungal phytase gene (*phyA;* 1) in soybean seeds. We have transformed soybean suspension culture cells (cv. Williams 82) with several different phytase constructs via particle bombardment and have confirmed stable integration by Southern analysis. Transgenic cell lines containing a phytase vector with dual-enhanced CaMV 35S promoter and a patatin endoplasmic reticulum (ER) signal peptide secreted active phytase into the culture medium. The *phyA* gene under control of a seed specific promoter is not expressed in suspension culture cells and constructs lacking an ER signal sequence failed to express phytase activity in the medium or within the cells. It appears that fungal phytase requires processing in the endomembrane system of soybean for activity. Preliminary western blot results indicated that the secreted fungal phytase in the transgenic soybean cell lines is about 85 kD, similar to the size of *phyA* product from *A. niger* (2). The optimal temperature for the secreted phytase is pH dependent (66 ^{0}C at pH 4.5, 63 ^{0}C at pH 5.0 and 58 ^{0}C at pH 5.5) and at 55 ^{0}C there are two peaks of activity (at pH 3.0 and 5.5), similar to the phytase enzyme from *A. niger* (2). We have also bombarded regenerable soybean somatic embryos in an effort to obtain transgenic plants. Bombarded embryos were selected on hygromycin for 6 weeks prior to transfer to regeneration medium. Selected somatic embryos have progressed to the torpedo embryo stage.

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BIOLISTIC-MEDIATED TRANSFORMATION OF SUGARBEET WITH NOVEL PLANT PATHOGEN DEFENSE GENES. Gordon W. Snyder, John C. Ingersoll, and Lowell D. Owens. USDA/ARS, PMBL, Beltsville, MD 20705.

The goal of our research is to use genes encoding pathogen-defense related proteins under transcriptional control of stress or wound inducible promoters to produce plants with enhanced disease resistance. A sugarbeet transformation method has been developed using callus generated from seedling hypocotyls. Seeds were germinated for 3 weeks in the dark at 27°C on MS media supplemented with 1.0 mg/l 6-benzylaminopurine (BA), and 0.5 mg/l 2,3,5-triiodobenzoic acid (TIBA). The shoot and root were removed from the seedlings and the hypocotyls cut into 1-2 cm sections and placed on MS media containing 1.0 mg/l BA. Hypocotyls were incubated in the dark at 30°C for 4-8 weeks. The embryogenic callus was harvested, spread on a sterile filter paper circle on top of MS media containing 0.3 mg/l BA, 0.1 mg/l α-naphthaleneacetic acid (NAA), and osmoticum. Following a 4 hour incubation the callus was subjected to particle bombardment using gold particles coated with plasmid DNA containing a promoter-cDNA fusion. After 3 d, the callus was transferred to MS media containing 1.0 mg/l BA, 200 mg/l cefotaxime, and 200 mg/l kanamycin and incubated in the light at 24°C. Promoters used in this study included the CaMV 35S, and those derived from genes encoding osmotin and pathogenesis related protein-S (PR-S) from tobacco, and proteinase inhibitor II (Pin II) from potato. The promoters were cloned 5' to cDNA's encoding either β-glucuronidase (GUS), osmotin, PR-S, barley leaf thionin, and cecropin. Preliminary results indicate that at least one transgenic plant can be obtained from 3-5 g of callus tissue following particle bombardment and selection.

Post-transcriptional Regulation of Gene Expression During Carrot Somatic Embryogenesis

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It is generally believed that transcriptional activation is the major mechanism for regulating gene expression during plant embryogenesis. This thought is partially fostered by the lack of well-documented examples of developmentally regulated genes that are primarily controlled through post-transcriptional processes. Northern analyses on more than 50 developmentally regulated genes isolated from carrot somatic embryos suggested the presence of a class of genes that are regulated, at least in part, at the post-transcriptional level, based on their relative abundance in total versus polysomal RNA. Further characterization indicates that the preferential accumulation of mRNAs from this class of genes in the polysomal fraction is mainly due to a shift of these RNAs from the monosome fraction to the polysome fraction. Interestingly, however, we have determined that not all of these polysome-associated mRNAs are EDTA-sensitive (i.e. a substantial portion of the polysomal RNA cannot be "EDTA-released), which is in contrast to the general belief that polysome-associated mRNAs are translationally active and EDTA-releasable. In addition, the percentage of EDTA-insensitive polysomal RNA changes at different developmental stages and is greatest at the globular embryo stage. We are focusing our current analysis on understanding the nature and significance of these EDTA-insensitive complexes in the cytoplasm of developing embryos.

In investigating the mechanisms responsible for the developmental shift of these mRNAs from the monosome to polysome pools, we have determined that modulation of the length of the poly(A) tail does not seem to be a factor. We are now analyzing the *cis* - elements responsible for this regulatory pattern by both *in vitro* and transgenic plant assays in an attempt to dissect the important elements for post-transcriptional regulation of genes in developing embryos.

Glutathione Reductase Isoforms In Isolated Chloroplasts From Pea (*Pisum sativum* L., cv. Nugget) Protoplasts Change In Response To Oxidative Stress. Camellia Moses Okpodu and Ruth G. Alscher. Dept. of Plant Pathology, Physiology and Weed Science, Blacksburg, Virginia 24060-0330

Considerable physiological and biochemical evidence has accumulated to support the hypothesis that the deleterious action of sulfur dioxide on foliar tissue depends on light and a functioning photosynthetic machinery. Although the stomatal apparatus has also been implicated, a major site of action appears to be the chloroplast. Previously we showed that protoplasts isolated from pea cv. Nugget were differentially sensitive to oxidative stress as examined by the relative responses of CO_2-dependent oxygen evolution and glutathione reductase (GR) activities in the presence of 10 mM sulfite when compared to cv. Progress. In Nugget, protoplasts exposed to sulfite for 1 hour resulted in a 60% decrease in CO_2-dependent oxygen evolution after which they returned to control levels. GR activity increased 30% at the end of 1 hour and by the 2 hour time point it had returned to control levels. In one-dimensional native gels, stained for GR activity, there was an increase at 30 minutes after which the GR activity returned to control levels. Protein extracts of chloroplasts isolated from protoplasts following a 30 minute treatment with sulfite were further resolved on native analytical IEF. Distinct isoforms of GR were resolved in the treated chloroplasts on IEF using an activity stain for GR activity when compared to the control treatment. The isoform profile in the sulfite treated chloroplasts differed from that detected in the control chloroplasts. Future work is underway to further characterize the isoforms.

CONSTITUTIVE EXPRESSION OF AN ETR1 HOMOLOG FROM TOMATO

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The *Arabidopsis etr1* mutant is a dominant mutation affecting ethylene signal transduction. Recently, Chang et al. (Science 262: 539-544) identified and sequenced the *ETR1* gene and cDNA responsible for this mutant phenotype. Using the ETR1 cDNA as a probe, we identified a cDNA homolog (eTAE1) from tomato. eTAE1 contains an open reading frame encoding a polypeptide of 754 amino acid residues. Its nucleic acid sequence is 67% identical to the *Arabidopsis* ETR1 cDNA and the deduced amino acid sequence for eTAE1 is 81% identical and 90% similar to the *Arabidopsis* ETR1 sequence. Southern blot analysis of restriction enzyme digested tomato genomic DNA suggests that 3 or more *ETR1* homologs exist in tomato. RNA blots show that eTAE1 mRNA is constitutively expressed in all the tissues examined. The expression pattern of eTAE1 was not affected by ethylene, silver (an inhibitor of ethylene action) or auxin.

ASSEMBLY OF WHEAT STORAGE PROTEINS INTO PROTEIN BODIES

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Wheat storage proteins are co-translationally sequestered into the endoplasmic reticulum (ER) and then assemble into large complexes termed protein bodies. The major wheat storage proteins are the sulfur-rich gliadins, which are structurally divided into two discrete domains, an N-terminal extended 'repetitive' domain containing alternating nonpolar and uncharged polar residues and a C-terminal globular domain containing 6 to 8 cysteine residues which are mostly intramolecular bound.

Gliadins, extracted from developing wheat grains, were unable to assemble spontaneously *in vitro*. However, assembly did occur following reduction of the intramolecular disulfide bonds at their C-terminal region. This suggested that assembly of the gliadins *in vivo* occurs before the complete formation of disulfide bonds at the C-terminal region. Indeed, we found that this assembly process occurs in vivo within a few minutes following protein synthesis. Moreover, after a few minutes, the vast majority of these newly synthesized proteins are already oxidized to an extent where free thiol groups are not exposed. From these results, we hypothesize, that gliadin assembly occurs rapidly following (or during) protein translation and this process is also accompanied with formation of intramolecular disulfide bonds by a mechanism, which is apparently regulated by the redox potential within the ER.

DEVELOPMENTAL REGULATION OF EXPRESSION AND ACCUMULATION OF SOYBEAN VEGETATIVE-CELL THIOL PROTEASE

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Plants possess a salvage process in which proteins accumulated during prior developmental stages are catabolized and recycled as building blocks for new rounds of synthesis. Soybean leaves and pods accumulate vegetative storage proteins prior to and during reproductive stage that function as transient nitrogen resources. These proteins are mobilized to provide carbon and nitrogen for the synthesis of seed storage proteins during seed maturation. We have identified thiol protease cDNAs that correspond to mRNAs that are expressed in soybean leaves and pods. Two distinct cDNA isoforms were identified. Southern blots indicate that soybean thiol proteases are encoded by a small gene family. The developmental and organ specific distribution was examined with a specific antibody. The expression of the vegetative-cell thiol protease mRNA and accumulation of thiol protease protein in soybean leaves occur during leaf expansion, but only after the leaves are sufficiently mature for the sink to source transition. The expression of leaf thiol protease mRNA is diurnally regulated. The leaves of podded and depodded soybean plants both express the thiol protease mRNAs, however depodding soybean plants partially represses thiol protease gene expression. The vegetative-cell thiol protease is expressed and accumulated in pods throughout seed maturation. Chemical stress and insect wounding does not induce the vegetative thiol protease. The accumulation of the vegetative-cell thiol proteases is likely to be responsible for amino acid salvage from soybean proteins. Supported in part by a USDA/NRI grant to EH.

SENSITIVITY TO PARAQUAT IN PEA (PISUM SATIVUM L.) IS RELATED TO DEVELOPMENT AND CORRELATES WITH THE BEHAVIOR OF SCAVENGING ENZYMES: Janet L. Donahue, Camellia Moses Okpodu, Carole L. Cramer, Elizabeth A. Grabau and Ruth Grene Alscher, Virginia Tech, Blacksburg, VA 24061

The youngest (tertiary) leaves of 14-day-old peas were 6.5 times more resistant to paraquat stress (10^{-4} M) than were older (primary or secondary) leaves, as evidenced by degree of leaf injury over a 48 hour post-application period. Activities of the scavenging enzymes ascorbate peroxidase (AP), superoxide dismutase (SOD) and glutathione reductase (GR) in untreated tertiary leaves were 3-4 times higher in GR activity, 2.5-3.5 times higher in SOD activity, and 1.9-2.3 times higher in AP activity than primary leaves, where tertiary leaves were 1.9, 2.3 and 1.6 times higher in these enzyme activities (respectively) than secondary leaves. In addition, the paraquat-treated tertiary leaves showed a steady, significant increase in GR and AP activities over the course of 48 hours post-exposure, reaching a 20% increase in AP above controls, and a 25% increase in GR. Differences in activities of treated and control leaves for primary and secondary leaves were not significant. The steady state levels of SOD and GR mRNAs in the control plants were 5 times and 2 times higher (respectively) in tertiary leaves than in secondary, and 5 times higher in tertiary than in primary leaves. There was no significant increase in the abundance of GR, SOD, and AP mRNAs during the 48 hours following the insult. We infer that the developmental regulation evident in the varying activity levels and mRNA amounts of the antioxidant enzymes in the different ages of leaves plays a large part in the resistance to paraquat. This research was supported by the Binational Agricultural Research and Development Fund, #US2043-91.

Polymorphic Analysis Of *Arabidopsis thalianas* and *E. coli* Strains Using Amplified Fragment Length Polymorphism(AFLP)

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ABSTRACT

The DNA polymorphism of different ecotypes of *Arabidopsis thalianas* and different strains of *E.coli* were demonstrated using a novel PCR based assay - Amplified Fragment Length polymorphism (AFLP). In AFLP, the number of selective nucleotides in the EcoR I and Mse I primers for the selective amplification as well as the complexity of the genomic DNA determine the number of amplified DNA fragments. No difference in the AFLP patterns was observed using different amounts(100 ng to 5 ug) of genomic DNA from both A. *thalianas* and *E.coli*. The effect of partially digested genomic DNA was shown to increase numbers of amplified DNA fragments.

ISOLATION AND CHARACTERIZATION OF cyc*Zm*W, A FUNCTIONAL CYCLIN HOMOLOGUE IN *Zea mays*

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The cell cycle-dependent protein kinase $p34^{cdc2}$ functions with its regulatory cyclin subunits to regulate entry into and progression through the cell cycle in virtually all eukaryotes. In order to investigate the regulatory role of this protein kinase in plant root development, we have focused on cyclins in maize. Initially, we used a clam cyclin A cDNA as a heterologous probe to screen a corn root tip cDNA library. We obtained a cDNA clone, cyc*Zm*W, that contains an open reading frame capable of encoding a polypeptide of 502 amino acids with a predicted molecular mass of 55kDa. The deduced amino acid sequence of the cyc*Zm*W polypeptide possesses a domain homologous to the cyclin box of mitotic cyclins and a conserved "destruction box" at the amino-terminal. Sequence comparison shows that cyc*Zm*W is highly similar to the "A-like" group II cyclins found in a variety of plants. Southern blot analysis indicates that the cyc*Zm*W is a member of a multigene family. In order to determine whether the cyc*Zm*W is functional as a cyclin gene, we introduced the cDNA into a *CLN*$^-$ mutant of budding yeast (BF305-15d#21) and tested for its ability to complement the mutant phenotype. This *CLN*$^-$ mutant has insertional mutations in *CLN1* and *CLN2*, and conditional expression (under the *GAL* promoter) of *CLN3*. Only truncated mitotic cyclins and G1 cyclins can restore the *CLN* function in the presence of glucose. Complementation studies revealed that cyc*Zm*W was able to rescue a cyclin-deficient mutant. Northern analysis showed that the cyc*Zm*W is expressed differentially in different organs of the maize plant. Transcript levels were highest in root tips and moderate in young roots and 5 day-old shoots, but were not detected in tassels or in mature leaves. Our results suggest that cyc*Zm*W encodes a functional maize cyclin gene that is expressed in or near the apices of roots and young shoots.

Degradation of a Secretory Protein With a Destabilizing Epitope Occurs in the Vacuole

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To understand how plant cells exert quality control over the proteins that pass through the secretory system we examined the transport and accumulation of the bean vacuolar storage protein phaseolin, structurally modified to contain a helix-breaking epitope and carboxyterminal HDEL, an endoplasmic reticulum (ER) retention signal. The constructs were expressed in tobacco with a seed specific promoter. The results show that phaseolin-HDEL accumulates in the protein storage vacuoles, indicating that HDEL does not contain sufficient information for retention in the ER. However, the ER of seeds expressing the phaseolin-HDEL construct contains relatively more phaseolin-HDEL compared to phaseolin in the ER of seeds expressing the phaseolin construct. This result indicates that the flow out of the ER is retarded but not arrested by the presence of HDEL. Introduction into phaseolin of the epitope "himet" (Hoffman et al.., 1988 Plant Molec. Biol. 11:717) greatly reduces the accumulation of HiMet phaseolin compared to normal phaseolin. However, the increased abundance within the ER is similar for both phaseolin-HDEL and HiMet phaseolin-HDEL. Using immunocytochemistry with specific antibodies, HiMet phaseolin was found in the ER, the Golgi stack, and in transport vesicles indicating that it was transport competent. It was also present at an early stage of seed development in the protein storage vacuoles, but was not found there at later stages of seed development. Together these results support the conclusion that the HiMet epitope did not alter the structure of the protein sufficiently to make it transport incompetent. However, the protein was sufficiently destabilized to be degraded by vacuolar proteases.

MOLECULAR CLONING OF AN ARABIDOPSIS HOMOLOG OF THE PSPK3 PROTEIN KINASE FROM PISUM SATIVUM L.

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In order to understand the role protein kinases play in photoregulated leaf development, we are investigating a set of genes encoding putative protein kinases in the garden pea. These genes, called PsPK1 through PsPK5, correspond to rare class mRNAs that are differentially expressed during de-etiolation. Exposure of etiolated seedlings to continuous light causes PsPK3 and PsPK5 mRNA levels to decline rapidly. The spectral dependence of the response is consistent with a high irradiance response regulated by type II phytochrome. If the PsPK3 and PsPK5 homologs from Arabidopsis exhibit similarly photoresponsive behavior, then the phytochrome mutants of Arabidopsis should be useful in identifying the molecular species of phytochrome that is responsible. Therefore, the PsPK3 cDNA clone was used in gel blot analyses of Arabidopsis genomic DNA and to screen an Arabidopsis genomic library at moderate stringencies. Restriction mapping and DNA gel blot analyses of the clones isolated suggest that Arabidopsis contains a small multigene family that hybridizes with the PsPK3 cDNA. We sequenced the entire polypeptide coding region from the clone that hybridizes most intensely with the PsPK3 probe. The predicted polypeptide of 477 amino acids contains a putative catalytic domain that is closely related to second messenger-dependent protein kinases and possesses all the invariant amino acids charactistic of protein serine/threonine kinases. PsPK3 and the Arabidopsis kinase are more similar to each other than to any other known protein kinase. Like PsPK3, the Arabidopsis polypeptide has interesting features in that the N-terminal domain is very rich in serines and threonines, whereas the C-terminal domain is rich in basic amino acids (as in many nucleic acid binding proteins). This clone will be used to ask if light regulates protein kinase gene expression in Arabidopsis. (This work was supported by NSF grant IBN-9496325.)

Improved Regeneration Of Plant Tissues : A Novel Medium Format And Membrane-Based Liquid Culture System.

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A convenient concentrate-prepared MS complete liquid medium has been formulated. Preparation of MS medium is achieved by simply adding three components of concentrate-prepared MS liquid medium and the sterilized water together. The requirements of sterilization by autoclave and the ajustment of pH are eliminated. The fresh weight of tobacco suspension cells and calli obtained from the cells grown in the medium prepared from the concentrate-prepared MS complete liquid medium increases 20-30% over those grown in the medium prepared from the MS complete powder medium. The regeneration of shoots from tobacco leaf discs grown in the membrane based containers with the liquid medium made from MS complete concentrate-prepared liquid medium increases 60-70% in the fresh weight and 50% in total number of shoots over those grown in the petri dish with the solid medium prepared from MS complete powder medium. The transgenic shoots containing *gus* gene were regenerated from *Agrobacterium tumefaciens* infected tobacco leaf discs using the membrane based container with the liquid medium made from MS concentrate-prepared liquid medium. In addition, the increase of regeneration of shoots in both fresh weight and the total number of shoots using MS complete concentate-prepared medium were also observed. The application of MS complete concentrate-prepared medium in different crops such as tomato *Arabidopsis* and potato will be discussed.

EFFECT OF PROMOTER-LEADER SEQUENCES ON TRANSIENT EXPRESSION OF REPORTER GENE CHIMERAS BIOLISTICALLY TRANSFERRED INTO SUGARBEET SUSPENSION CELLS

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Conditions for delivery of DNA-coated particles into sugarbeet (*Beta vulgaris* L.) suspension cells were optimized for transient expression analysis of the β-glucuronidase reporter gene (*gus*) fused to various promoter-leader sequences. The optimized protocol consisted of layering 150 mg (FW) of suspension cells onto a 47 mm nylon filter, preincubating 4 h on an osmotica-supplemented (0.25 M) medium, and culturing on the same medium following bombardment. Chimeric constructs consisting of the *gus* coding region fused downstream of promoter-untranslated leader sequences from the tobacco osmotin and PR-S genes, the potato proteinase inhibitor 2 gene (*pin2*) , and the cauliflower mosaic virus (CaMV) 35S promoter were biolistically transferred into the suspension cells. Each construct was expressed in recipient cells at 6 h after bombardment. The osmotin-promoted construct was constitutively expressed and reached expression levels approximately 2.5-fold higher than that of the 35S construct. The PR-S chimera mimicked expression of the constitutively expressed 35S construct but achieved levels almost 50% higher. The *pin2*-promoted chimera displayed wound induction with an ultimate expression level similar to that of PR-S.

CONSTRUCTS FOR IDENTIFYING PROMOTER ACTIVITY IN THE POTATO SPINDLE TUBER VIROID (PSTVd) RNA. C.J.M. Maroon[1,2], P.A. Feldstein[1], R.W. Hammond[3], & T.O. Diener[1,2]. [1] Department of Botany, and [2] Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20740; [3] Molecular Plant Pathology Laboratory, ARS, USDA, Beltsville MD 20705.

The ability of viroids to replicate autonomously in planta implies the presence of a promoter in the RNA. However there is no significant sequence homology between the PSTVd RNA and reported eukaryotic promoters. Instead, they may have structural similarities which allow PSTVd to be recognized by a host-encoded polymerase. To identify the RNA promoter, we made four sets of constructs with permuted PSTVd sequences linked to a reporter RNA, the dm strain of the satellite RNA of tobacco ringspot virus (sTRSV). Circular transcripts derived in vitro from these constructs consist of PSTVd RNA in either orientation upstream of a greater-than-unit length non-infectious negative strand of the dm sTRSV. The primary transcripts process and circularize accurately and efficiently in vitro. In vivo, the promoter within the PSTVd portion of these transcripts will drive production of positive dm sTRSV which will be amplified by TRSV. The bioassay involving these constructs is currently underway. We hope to identify the promoter region(s) using this system.

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