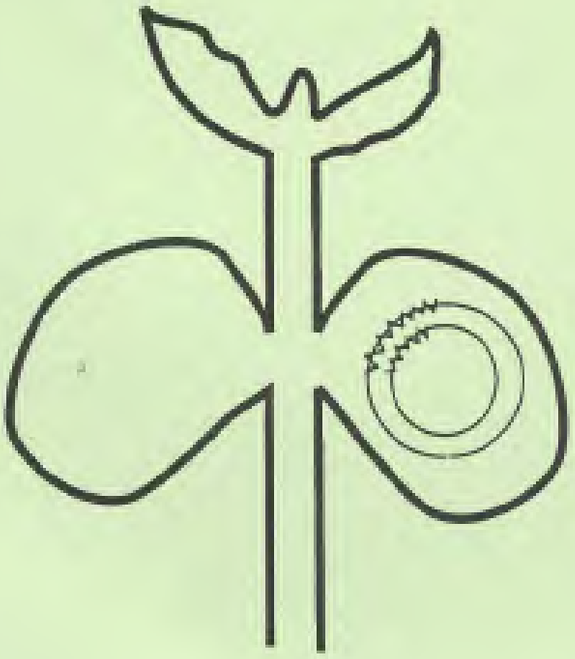
Mid-Atlantic Plant Molecular Biology Society

Sixth Annual Meeting



August 10 & 11, 1989

University of Maryland
Baltimore County

CONTENTS

INTRODUCTION

Welcome to the sixth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. This Society was formed to provide a forum devoted to the exchange of ideas and information concerning plant molecular biology. Hopefully, the Society will ensure scientists in the Mid-Atlantic region of a high quality, accessible and affordable plant molecular biology meeting each year. The Society especially wishes to encourage graduate student and postdoctoral participation in presenting oral reports, posters, and contributing to discussions.

Since its inception six years ago, the MAPMBS has grown steadily in attendance and participation. Each year MAPMBS grows geographically as well. The Society has been strengthened with financial support from industry and with the more formal presentations by certain exhibitors.

On Friday, August 11, at 9:55 am, there will be a brief business meeting which the entire membership is urged to attend. Changes and suggestions for program, format and location of the 1990 meeting are invited.

The MAPMBS meeting this year would not be possible without the efforts of many people. A listing of committee members is on the following page.

Benjamin F. Matthews
Ellen M. Reardon
Co-Chair
Organizing Committee

1989 MAPMBS ORGANIZING COMMITTEE

PROGRAM

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LOCAL ARRANGEMENTS

Frank Turano
Lynn Zimmermann

GENERAL INFORMATION

UMBC The Baltimore County campus is the youngest of the eleven campuses of the University of Maryland system. Since the first students were admitted in 1966, the enrollment has grown to more than 9500 students. The campus is located just 10 minutes south of Baltimore and approximately 45 minutes north of Washington, D.C.

If you have free time after the meeting or would enjoy some night life Thursday evening, you should visit Baltimore's Inner Harbor which contains many excellent restaurants and shops. Other points of interest in the Inner Harbor are the National Aquarium and the Science Center. The Inner Harbor is easily reached by going north on I-95 and following the signs.

SESSIONS All plenary and poster sessions will be held in the ballroom of the University Center of the UMBC Campus. Refreshments will be provided during the breaks in the atrium outside the ballroom. The exhibitors will also be located in the atrium and we hope you will take some time to visit their displays.

MEALS If you pre-registered, your lunches and barbecue have been prepaid. Lunches will be provided in the Faculty/Staff Dining Room (about a 5 min walk from the ballroom).

If you did not pre-register, you are on your own for lunch and dinner Thursday evening. There will be a limited number of lunch and barbecue tickets available at registration. There is a cafeteria on the first floor of the University Center or you may choose to venture off campus.

The barbecue will be held on Thursday at 6:30 pm on the first floor of the University Center (outside).

The sixth annual meeting of the Mid-Atlantic Plant Molecular Biology Society has been made possible by the generous support of the following institutions:

CORPORATE SPONSORS

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We are sincerely grateful for their support.

Ellen M. Reardon
Funding Committee

SPECIAL PRESENTATIONS BY EXHIBITORS

BALTIMORE INSTRUMENT COMPANY. **Jim Hofstetter.** Microinjection and micromanipulation for genetic engineering of plant tissues.

INTERNATIONAL BIOTECHNOLOGIES INCORPORATED. **Richard Vento.** Interactive sequence analysis on a microcomputer.

HOEFER SCIENTIFIC. **Robert Kane,** Beth Miller and Jan Pettitt. Electroporation: recent advances.

HYDRO SERVICES AND SUPPLIES. **Natalie Mason.** Control of organics in ultra-pure water.

SCHLEICHER AND SCHUELL. **John Poisson.** New Schleicher & Schuell products for molecular biology.

DU PONT DE NEMOURS & COMPANY. **Ed Chait,** Lincoln Muir and Melvin Lewis. Advances in biotechnology instrumentation for agriculture.

PHARMACIA-LKB. **Dan Bolling** and Rob Mervis. Cloning and screening in plant vectors.

OTHER EXHIBITORS

AMERICAN TYPE CULTURE COLLECTION. Patrick Burke

BECKMAN INSTRUMENTS. Karen diPianelli and Laurie Nestler.

BIORAD. Mary Ann Ireland.

NATIONAL AGRICULTURAL LIBRARY. Suzanne Nanis.

PROGRAM

Thursday, August 10, 1989

8:00 - 8:45	REGISTRATION	
8:45 - 8:50	WELCOME: Benjamin Matthews	
8:50	SESSION I: GENE REGULATION I	PAGES 11-14
	Convener: Frank Turano	
8:50 - 9:20	DAVID STERN. Function of Plastid 3' Inverted Repeats in Gene Expression.	
9:20 - 9:40	GREGG W. SILK. Regulation of the Steady-State Transcript Levels of the Elongation Factor Gene (tuf) in the Chloroplast of *Chlamydomonas*.	
9:40 -10:00	NADA SIMOVIC. Molecular Cloning, Restriction and Sequence Analysis of 18S Ribosomal RNA Gene in *Fragaria x ananassa* Duch. Cultivated Octoploid Strawberry.	
10:00-10:20	JAMES SAUNDERS. Pollen Transformation in Tobacco Using Electroporation.	
10:20-10:35	SEP*. Robert Kane. HOEFER SCIENTIFIC. Electroporation: Recent Advances.	
10:35-11:05	Break	
11:05	SESSION II: PLANT DEFENSE I	PAGES 15-16
	Convener: John Hammond	
11:05-11:35	SONDRA LAZAROWITZ. Molecular Genetics of the Gemini Viruses Maize Streak and Squash Leaf Curl.	
11:35-12:05	LESLIE DOMIER. Infectious in vitro Transcripts from Cloned cDNA of Tobacco Vein Mottling Virus.	
12:05-12:20	SEP*. John Poisson. SCHLEICHER & SCHUELL. New Schleicher & Schuell Products for Molecular Biology.	
12:20 -1:20	Lunch	
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2:10 - 2:25 SEP*. Jim Hostetter. BALTIMORE INSTRUMENT COMPANY. Microinjection and Micromanipulation for Genetic Engineering of Plant Tissue.

2:25 - 2:55 Break

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Convener: Barbara Wilson

2:55 - 3:25 CAROLE CRAMER. Molecular Cloning and Defense-Related Expression of HMG CoA Reductase.

3:25 - 3:45 MELINDA N. MARTIN. Latex: A Plant Defense System.

3:45 - 4:00 SEP*. Ed Chait. DUPONT. Advances in Biotechnology Instrumentation for Agriculture.

4:00 - 4:30 POSTERS (Authors in Attendance). PAGES 31-45

N.R. APUYA et al., The Heat Shock Response of Carrot Embryos in Culture

L.C. CHILDS et al., Expression of the *Cab II* Multigene Family in Pea

M. GALLO et al., *Cis*-acting Elements Required for Light Regulation of Pea Ferredoxin I Gene Expression are Located Within the Transcription Unit

C.H. HWANG AND J.L.ZIMMERMAN, Differential Expression of the Heat Shock Genes at Different Temperatures in Cultured Carrot Cells: Characterization of the Heat Shock Transcription Factor

A.D. LLOYD et al., Identification and Analysis of Ripening Stage Specific Genes in Tomato

L.A. NEWMAN et al., Synthesis of Two Chromoplast-Specific Proteins During Fruit Development in *Capsicum Annuum*

A. NOUEIRY et al.,Preliminary Studies of Differential Activity and Genetic Nature of Alcohol Dehydrogenase (E.C. 1.1.1.1.) Isozymes in Various Plant Parts of Octoploid Strawberry, Fragaria x ananassa Duch,. Genotype 8343-6

M.A. OSMAN AND M.S. DOBRES, Developmental Regulation of a Bud Specific Gene in Pea

N. OSSANNA AND S. MISCHKE, Transformation of the Filamentous Fungus *Gliocladium virens* to develop Benomyl Resistant Strains for Biocontrol of Plant Diseases

J. PRINCIPE et al., Phytochrome induced Flowering in a Photoperiod Insensitive Barley Mutant: 2-D Gel Electrophoresis Comparison

K. SHAH AND D. OLIVER, Cloning and Sequencing of the cDNA for the P-Protein of the Glycine Decarboxylase Complex From Peas

N. SIMOVIC et al., The Nucleotide Sequence of 5.8S Ribosomal RNA and Two Internal Transcribed Spacers (ITS1 and ITS2) From Octoploid Strawberry (Fragaria x ananassa Duch.)

W.G. START et al., Stable Transformation of Embryonic Maize Cultures by Microprojectile Bombardment

L. WANG AND J.L. ZIMMERMAN, Cloning and Characterization of Carrot Heat Shock Genes

C.A. WOZNIAK AND L.D. OWENS, B-Glucuronidase activity in non-Transformed *Beta vulgaris* L. (Sugarbeet) Tissues

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IAN SUSSEX. New Perspectives on Plant Meristems. Dr. Sussex will be introduced by Kathryn Kamo.

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6:30 Barbecue

Friday, August 11, 1989

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Convener: Mark Tucker

8:30 - 9:00 PAMELA GREEN. Transcriptional and Posttranscriptional Mechanisms for Gene Regulation in Higher Plants.

9:00 - 9:20 GOYKO JELENKOVIC. Structural Features of an Alcohol Dehydrogenase Gene in Cultivated Octoploid Strawberry (*Fragaria x ananassa* Duch.).

9:20 - 9:40 ANN CALLAHAN. Characterization of cDNAs from Developmentally Regulated Genes in Peach Fruit.

9:40- 9:55 SEP*. Dan Bolling. PHARMACIA-LKB. Cloning and Screening in Plant Vectors.

9:55-10:30 Business Meeting and Break

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11:30-11:45	SEP*. Richard Vento. INTERNATIONAL BIOTECHNOLOGIES INCORPORATED. Interactive Sequence Analysis on a Microcomputer.	
11:45-12:00	SEP*. Natalie Mason. HYDRO SERVICES AND SUPPLIES. Control of Organics in Ultra Pure Water.	
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2:15 -2:35	FRANK TURANO. Identification of Genes Encoding Aspartate Aminotransferase in Plants.	
2:35-2:55	BENJAMIN MATTHEWS. Regulation of Homoserine Dehydrogenase from Carrot.	

2:55-3:00 SUMMARY AND FAREWELL. Benjamin Matthews

*SEP: Special Exhibitor Presentation.

FUNCTION OF PLASTID 3' INVERTED REPEATS IN GENE EXPRESSION.
Hsu-Ching Chen, Cynthia C. Adams and David B. Stern. Boyce Thompson Institute for Plant Research, Cornell University, Tower Road, Ithaca, NY 14853

Post-transcriptional menchanisms play an important role in the accumulation of mRNAs during plastid development and differentiation in spinach. One important element appears to be an inverted repeat (IR) sequence that can potentially form a stem/loop structure, found in the 3' non-coding region of most plastid transcription units. Using *in vitro* assays, we have shown that although these 3' IRs do not efficiently terminate transcription, they appear to participate in mRNA 3' end formation and stabilization. The function of 3' IRs as processing and stability elements has been investigated in detail by mutagenesis and *in vitro* assays of 3' end stability in chloroplast protein extracts. We have also characterized several proteins that specifically associate with 3' IR-RNAs *in vitro* and may be *trans*-acting factors that influence the efficiency of mRNA processing and/or RNA stability. A model for mRNA maturation and the regulation of plastid stability is discussed.

1. Stern and Gruissem; *Cell* 51:1145 (1987)
2. Stern and Gruissem; *Plant Molec. Biol.* (in press)
3. Stern, Jones and Gruissem; *J.Biol.Chem.* (in press)

Regulation of the Steady-State Transcript Levels of the Elongation Factor Gene (*tuf*) in the Chloroplast of *Chlamydomonas*

Gregg W. Silk, Madeline Wu, Department of Biological Sciences, UMBC, Baltimore, MD 21228

Elongation factor EF-Tu is a protein essential for peptide elongation in prokaryotes and the chloroplast (Cp). Many Cp proteins are translationally regulated, but the regulatory mechanisms are largely unknown. The regulation of EF-Tu expression could be part of the process controlling the expression of many Cp genes.

EF-Tu of the unicellular alga *C. reinhardtii* is encoded by the *tuf* gene of the Cp genome. The *tuf* gene encodes the only light-regulated *Chlamydomonas* Cp transcript identified in our lab. Northern blots showed *tuf* RNA comprises a group of transcript species, some much longer than needed to encode EF-Tu. Slot blots of RNA were used to measure the steady-state levels of chloroplast transcripts.

tuf RNA is light regulated during development, increasing during the greening of dark-grown cells. A large 1,900 base transcript appears to show the greatest increase. This is the first report of a *C. reinhardtii* Cp transcript which accumulates during greening. This increase is strain-specific, and was not observed in a strain which stays green in darkness.

We have not observed similar changes in the steady-state levels of other Cp transcripts. Study of chloroplast RNA decay in rifampicin-treated cells showed that *tuf* RNA has a much shorter half life than previously described Cp transcripts. Northern blots showed the decay of *tuf* RNA is a complex process in which the largest transcript disappears first.

MOLECULAR CLONING, RESTRICTION, AND SEQUENCE ANALYSIS OF 18S RIBOSOMAL RNA GENE IN *Fragaria x ananassa* Duch. - CULTIVATED OCTOPLOID STRAWBERRY

Nada Simovic, David Wolyn, Gojko Jelenkovic
Dept. of Horticulture and Forestry,
Rutgers University, New Brunswick, NJ 08903

Considerable information concerning ribosomal RNAs and encoding family of repeated genes (rDNA) has accumulated. Nonetheless, questions on primary structure and regulation of eucaryotic rRNA genes, secondary and tertiary structure of rRNAs, assembly and function of eucaryotic ribosomes (especially in plants) remain to be answered. The significance of high variability in the rRNA gene number among and within a species, and the mechanism by which an inter and intrachromosomal gene homogeneity is maintained presents another intriguing problem. Considering information about the primary structure fundamental for resolution of some postulated problems, we have identified and isolated five ribosomal DNA (rDNA) clones from lambda genomic library of octoploid strawberry - *Fragaria x ananassa* Duch.

The clone λ Frl was restricted with Bam HI, and subcloned into pGEM-3. Subclones bearing 18S rDNA were identified and sequenced. The strawberry 18S rRNA gene is 1804bp long and has 49% GC content. The comparison to corresponding genes from soybean, maize, rice, frog, rat and yeast has demonstrated a high level of sequence conservation, especially among the plants (over 90%). The strawberry 18S rDNA exerts higher homology to the gene sequence from lower eucaryote yeast (81.5%), than to amphibian (78.6%) or mammalian (77.3%) sequences.

When aligned for maximal homology, eucaryotic 18S rRNA genes exhibit a pattern of defined homologous regions interrupted by variable sequences. Five regions of extensive homology are located at similar positions in all analyzed genes. The strawberry 18S rRNA gene shares low sequence homology with *E. coli* 16S rDNA. However, within the conserved regions, short stretches are found to be identical in sequence and relative position to bacterial 16S rDNA. Six highly variable regions, share less than 50% overall homology among eucaryotes. Due to compensating base substitutions the variable regions from different species fold in homologous secondary structures. Sequence homologies and secondary structure similarities with *E. coli* 16S rRNA were used to speculate about functionally important regions in strawberry 18S rRNA.

POLLEN TRANSFORMATION IN TOBACCO USING ELECTROPORATION

James A. Saunders, Benjamin F. Matthews, and Aref Abdul-Baki, Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705

Gene transfer in plants has been restricted to the use of protoplast or tissue culture systems or it has been confined to plants which can function as a host for Agrobacterium tumefaciens. We are developing a procedure which utilizes electroporation techniques on germinating pollen to effect a functional pollen transformation. The subsequent progeny that result from the pollination of flowers with this pollen represent paternally inherited transformed plants. Our initial results have shown that the conditions for the optimization of pollen electroporation differ substantially from protoplast electroporation parameters. Expression of GUS activity which was electroporated into the pollen was seen both in the pollen as well as the plants produced from the pollen. Southern hybridizations of the GUS DNA in the pollen indicates that the electroporated DNA is incorporated into the pollen genomic DNA within 24 hrs of the electroporation treatment. Further analysis of the transformed plants are underway to determine stability and expression of the GUS activity.

ROLE OF MOVEMENT AND REPLICATION FUNCTIONS IN SYMPTOM DEVELOPMENT AND HOST RANGE PROPERTIES OF THE GEMINIVIRUSES MAIZE STREAK AND SQUASH LEAF CURL. S.G. Lazarowitz[1], A.J. Pinder[1], V.D. Damsteegt[2] and S.G. Rogers[3]. [1]Department of Embryology, The Carnegie Institution of Washington, Baltimore, MD 21210; [2]Virology Lab, USDA-ARS, Frederick, MD 21701; [3]Biological Sciences, Monsanto Company, St. Louis, MO 63198.

Maize streak (MSV) and squash leaf curl (SqLCV) viruses are representative of the leafhopper-transmitted single-component and whitefly-transmitted bipartite geminiviruses, respectively. The genome of MSV is an ~2.7 kb single-stranded circular DNA with sufficient coding capacity for only three average sized proteins, yet the virus is capable of causing severe disease of many monocots with symptoms of chlorosis and stunting. Through the analysis of deletion and gene replacement mutants, we have identified MSV gene functions essential for systemic spread and symptom developement. Using *Agrobacterium*-mediated inoculation of maize seedlings and a unique scheme whereby gene replacement mutants were generated in the plant following inoculation, we have found that unlike the bipartite geminiviruses, the capsid gene of MSV is essential for systemic spread and subsequent disease development. Our analyses have also identified a small "+" strand open reading frame (ORF) predicted to encode an ~11 kd protein as functioning and being essential for movement, and have localized the viral sequences supporting autonomous replication to a 1.7 kb segment containing the two viral intergenic regions and 2 "-" strand overlapping ORFs.

SqLCV contains two ~2.7 kb single-stranded DNA genomic components (A and B). The genomic organization of SqLCV is identical to that of other bipartite geminiviruses such as tomato golden mosaic virus and bean golden mosaic virus. There are 6 ORFs, 4 on the A component and 2 on the B component, diverging either side of an ~200 base intergenic common region. The sequence of the common region is identical in the two genomic components and contains a ~30 base sequence element which has the potential to form a hairpin structure and is conserved in all geminiviruses. By molecular analysis we have identified two highly homologous SqLCVs, SqLCV-E (extended) and SqLCV-R (restricted), which can be distinguished by their genomic maps and host range properties. While SqLCV-E independently segregates, virus transmission studies and the analysis of field samples demonstrate that SqLCV-R has an unorthodox pattern of transmission with its genomic components often co-segregating with those of SqLCV-E. Using *Agrobacterium*-mediated inoculation of the cloned viral genomic components, the permissive host range of SqLCV-R was found to be a subset of that of SqLCV-E. In restrictive hosts, SqLCV-R has a cis-acting defect in replication and is found to replicate inefficiently. The replication of SqLCV-R in these restrictive hosts can be transactivated by the A component of SqLCV-E. Evidence suggests that this host-dependent replication defect in SqLCV-R may result from a defect in transcription of AL1, the one viral gene essential for replication. A mutant genomic B component of SqLCV-R (B_R*) has also been identified which is defective in tobacco, but functions in other host plants. Leaf disc analyses demonstrate that while B_R* is not infectious in tobacco, it replicates normally in this host. Thus, this B_R* component has a host dependent defect in expressing a function essential for systemic movement.

INFECTIOUS *IN VITRO* TRANSCRIPTS FROM CLONED cDNA OF THE POTYVIRUS, TOBACCO VEIN MOTTLING VIRUS Leslie L. Domier, USDA-ARS, Dept. of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

Full-length cDNA copies of tobacco vein mottling virus (TVMV) RNA were constructed downstream from either bacteriophage T7 or T3 RNA polymerase promoters. The plasmids were designed to produce *in vitro* transcripts containing, respectively, one or two G residues at the 5' terminus not derived from the TVMV sequence and a single additional C residue at the 3' terminus following the poly(A) tail. Introduction of transcripts from either plasmid into tobacco mesophyll protoplasts resulted in the accumulation of TVMV coat protein and RNA. Neither coat protein nor viral RNA accumulated in protoplasts inoculated with linearized cDNA or with *in vitro* transcripts synthesized in the absence of m^7GpppG. Tobacco seedlings inoculated with *in vitro* transcripts developed characteristic symptoms after seven days, but the symptoms were initially not as severe as those induced by native TVMV RNA. Symptoms produced by native viral RNA and *in vitro* transcripts were, however, indistinguishable three weeks after inoculation.

DEFINITION AND MAPPING OF AN ETHYLENE PROMOTER FROM *SOLANUM TUBEROSUM*.

John J. Gaynor[1] and Dominique Roby[2]. [1]Department of Biological Sciences, Rutgers University, Newark, NJ 07102, and [2]CNRS, Universite Paul Sabatier, Toulouse, FRANCE.

The phytohormone ethylene exerts a powerful influence on the growth and development of higher plants. In an effort to understand how vanishingly small amounts of this gaseous hormone can have such profound effects, we have studied the expression of endochitinase, a putative defense protein which is tightly regulated by ethylene. This enzyme is also induced by pathogens, abiotic elicitors, and environmental insults. In potato, chitinase is encoded by a small multigene family, and is regulated at the transcriptional level. In order to investigate the nature of this control, the transient expression of a chimeric gene containing 1.2 kb of the 5' flanking sequences of a potato endochitinase gene (pRU8713), fused to the reporter gene GUS, was studied in PEG-treated potato protoplasts. 1-Aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ethylene, and a fungal elicitor isolated from *Colletotrichum lagenarium*, caused a high and transient expression of the reporter gene. A five-fold induction of GUS activity was observed within 2 hours of incubation with ACC, and a 40-fold induction was reached after 48 hours. Analysis of a nested set of deletion mutants for this putative ethylene promoter suggests that the information specifying ethylene sensitivity is limited to a 238 bp fragment found *ca.* 150 nucleotides upstream from the transcription start site. Further analysis of these deletion mutants, in both transgenic plants and transient expression assays, should permit us to further define the *cis*-acting elements required for ethylene-induced transcription as well as the *trans*-acting factors which may bind to such elements.

Supported by a grant from the National Science Foundation (DMB 8704677).

HORMONAL AND TISSUE-SPECIFIC REGULATION OF CELLULASE GENE EXPRESSION: Mark Tucker, Roy Sexton, Elena del Campillo, Lowell Lewis, Stephen Milligan; USDA, ARS, PSI, PMBL, Beltsville, MD and MPB, Univ. of Calif., Berkeley, CA

The physiology and anatomy of abscission has been studied in considerable detail. Abscission, therefor, has the potential to be a good system to study developmental, hormonal, and cell-specific regulation of gene expression. An increase in cellulase activity has been documented for abscission in many different plants. We have identified a cDNA clone for a bean abscission cellulase mRNA. The cellulase mRNA accumulates in abscising tissue in response to ethylene. Although cellulase mRNA accumulation is greatest in the separation layer, cellulase mRNA also increase in response to ethylene in adjacent stem and petiole tissues. Experiments with 2,5-norbornadiene, a competitive inhibitor of ethylene action, shows that ethylene is required not only to initiate cellulase gene expression in abscission but also to maintain its expression. Auxin applied prior to an ethylene treatment blocks abscission and cellulase mRNA accumulation. Application of the auxin analogs, aNAA and 2,4-D, 48 hrs after ethylene treatment of bean explants began inhibited further accumulation of cellulase mRNA. The results support an earlier observation that high concentrations of auxin can overcome ethylene induced cellulase gene expression in abscission.

MOLECULAR CLONING AND DEFENSE-RELATED EXPRESSION OF HMG CoA REDUCTASE. C.L. Cramer, H.S. Park, C.J. Denbow, Z. Yang, and G.H. Lacy, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) mediates a major rate-limiting step in the isoprenoid pathway. In higher plants, HMGR is involved in synthesis of sterols, carotenoids, electron transport components, rubber, gibberellins, and abscisic acid. In the Solanaceae, HMGR also directs the synthesis of sesquiterpenoid phytoalexins associated with disease resistance. Gene sequences encoding HMGR were cloned from a tomato genomic library based on cross-hybridization with a yeast HMG1 [Basson et al. (1986) PNAS 83:5563] probe. Partial sequence analysis reveals regions exceeding 65% nucleic acid and 70% derived amino acid identity with yeast and human HMGR. Tomato HMGR sequences hybridize to tomato and potato mRNA species of about 2.5 kb suggesting that the encoded plant HMGR (estimated at 65-80 kD) is substantially smaller than yeast or mammalian HMGR (97-100 kD). In tomato, HMGR is encoded by a small multigene family of at least three divergent members which contrasts with Arabidopsis, Drosophila, and mammalian systems where a single gene per haploid equivalent is found.

We have analyzed the defense-related induction of HMGR mRNA levels in tomato cells and potato tubers. In tomato suspension-cultured cells, HMGR mRNA levels are highly elevated after treatment with fungal elicitors isolated from the pathogens, Fusarium oxysporum or Verticillium albo-atrum. The induction kinetics are not as rapid as those for phenylalanine ammonia-lyase mRNA, a molecular marker for a distinct defense-related pathway. In potato tubers, HMGR mRNAs show a very rapid induction in response to wounding with mRNA levels peaking at 30-60 min. Tuber slices inoculated with the soft rot bacterium, Erwinia carotovora, show a second peak of HMGR induction with an RNA maximum at 12-14 hr. Analyses utilizing a gene specific probe suggest that distinct isogenes are activated in response to wounding versus infection. Thus, plants may utilize differential expression of specific HMGR isogenes to cope with the varied developmental and stress-related requirements for distinct isoprenoid products.

LATEX: A PLANT DEFENSE SYSTEM

Melinda N. Martin* and John J. Gaynor, Department of Biological Sciences, Rutgers University, Newark, NJ 07102

Over 12,500 species of plants representing approximately thirty families contain cells of unknown function called laticifers. The cytoplasm or latex of these cells is often filled with large amounts of relatively few proteins and secondary metabolites, indicating a highly specialized function. We have obtained data which suggests that the function of laticifers may be defensive. In many cases, the putative defense protein chitinase/lysozyme, which is capable of hydrolyzing the chitin component of fungal cell walls and the peptidoglycan component of bacterial cell walls, has been identified immunologically and enzymatically as one of the major latex proteins, often representing as much as twenty-five percent of the soluble protein. In most other tissues of dicotyledonous plants , the level of chitinase/lysozyme activity is very low but is inducible by ethylene as well as by physical and biological stresses. In the latex of *Hevea brasiliensis*, the commercial rubber tree, we have established that the "chitinase/lysozyme" actually consists of small families of both acidic and basic chitinases. The acidic proteins have chitinase activity but lack lysozyme activity. In contrast, the basic proteins have a high lysozyme specific activity and a very low chitinase specific activity suggesting that in the latex of this plant distinct proteins exist for defense against bacterial and fungal pathogens. Several techniques have been employed to investigate both the developmental and stress induced expression of the chitinase/lysozymes as well as other latex proteins.

NEW PERSPECTIVES ON PLANT MERISTEMS. Ian M. Sussex, Department of Biology, Yale University, P.O. Box 6666, New Haven CT 06511.

Apical meristems are located at the tip of each shoot and root and produce the cells that will become the differentiated tissues and organs of the plant. There is extensive information about the structural organization of meristems of a wide variety of vascular plants throughout the whole life cycle, but we still do not have an adequate understanding of how meristem function is orgnaized and regulated.

Classical experiments of meristem surgery and in vitro culture have provided some insight into these questions. From these experiments we know that the apical meristems of flowering plants are stably determined for their specific functions, and that most, but not all, of the signals that regulate meristem function originate within the meristem itself. Meristems are, therefore largely self-organizing and self-regulating.

In our lab we are using molecular and genetic approaches to analyze the following aspects of meristem functional organization and regulation: 1) What is the pattern of gene expression in cells that are becoming organized as a new meristem? 2) How are the cells partitioned from the meristem into the plant organs? 3) What are the changes in gene expression as dormant meristems begin to grow? 4) How do cells within the meristem interact with each other? I will describe the results of these investigations.

TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL MECHANISMS FOR GENE REGULATION IN HIGHER PLANTS. Pamela J. Green[1], Cris Kuhlemeier[2], Thomas C. Newman[1], Maria Cuozzo[3], Mun-Heng Yong[4], Crispin Taylor[1] and Nam-Hai Chua[5]. [1]MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824. [2]Botanische Institut, Universitat Bern, CH-3013 Bern, Altenbergrain 21, Switzerland. [3]Biochemistry Dept., University of Maine, Orono, ME 04469. [4]Institute of Molecular and Cell Biology, National University of Singapore, Republic of Singapore. [5]Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021.

The pea rbcS-3A gene, which encodes the small subunit of ribulose 1,5 bis-phosphate carboxylase-oxygenase, is regulated by light at the transcriptional level. Expression of mutant derivatives of rbcS-3A in transgenic tobacco has shown that light-responsive transcription is mediated by a complex set of elements located 5′ of the transcription start site. A nuclear protein factor that binds to several homologous sequences in this region has been identified from in vitro experiments and designated GT-1. We have observed a strong correlation between the sequences required for GT-1 binding in vitro and activation of transcription in the light in vivo. This indicates that GT-1 is an activator of rbcS-3A transcription. In mature plants, 170 bp of 5′ flanking sequences are sufficient to direct light-dependent transcription of rbcS-3A. In this context, two GT-1 binding sites are required for expression and in vitro experiments indicate that there is an interaction between the factors bound at these sites. Sequences located more than 170 bp 5′ of the transcription start are only dispensable in mature leaves of transgenic tobacco. In young leaves and seedlings, additional 5′ flanking sequences are required for full expression.

In contrast to transcription, very little is known about post-transcriptional processes such as mRNA degradation. We have begun to study the molecular mechanisms that control mRNA stability because mRNA degradation rates can vary over a wide range in plants and therefore can play a major role in gene expression. Degradation rates for individual nuclear encoded transcripts have not been measured directly in plants, but indirect measurements indicate that some mRNAs (such as auxin inducible transcripts) are very unstable. Our efforts have focused on the development of systems to measure mRNA half-lives of specific transcripts in vivo, the identification of the mRNA sequences that control degradation rates, and the identification of the trans-acting factors that recognize such sequences. Progress in these areas using Arabidopsis and tobacco will be discussed.

Structural features of an alcohol dehydrogenase gene in cultivated octoploid strawberry (*fragaria* x *ananassa* Duch)

David Wolyn, Gojko Jelenkovic
Department of Horticulture & Forestry
Rutgers University, New Brunswick NJ 08903

An alcohol dehydrogenase gene (Adh) was isolated from octoploid strawberry (*Fragaria* x *ananassa* Duch.) and sequenced. Ten exons, separated by nine introns, were identified based on homology with maize Adh cDNA. The nine introns each begins with GT, terminate with AG and are AT rich (65-72%). The 5^1 and 3^1 consensus splice signals (A/G) AGGGTA (T/A) and (C/T) AG (C/G), respectively, are identical to those of other plant genes. The coding sequence of strawberry Adh was 74-76% homologous with genes from maize, pea, Arabidopsis and barley, while comparisons with the predicted 380 amino acid protein sequence for strawberry showed 81-85% homology. Like in other dicotes the second amino acid in the first exon of strawberry Adh gene is serine while in the monocots at the same position is an alanine residue. Nucleotide sequence similar to the 5^1 regulatory region of maize Adh, thought to regulate anaerobic induction and translation were identified in the strawberry gene, in addition to four tandem repeats of TGGTTT, a putative core sequence for anaerobic induction.

CHARACTERIZATION OF CDNAS FROM DEVELOPMENTALLY REGULATED GENES IN PEACH FRUIT

Ann Callahan, Peter Morgens, Reuben Cohen, and Paul Wright USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

We are studying peach fruit development at the gene expression level to identify genes that will be potentially useful in modifying fruit characteristics such as softening. These genes could then be modified and introduced into desirable peach cultivars by means of transformation and regeneration. We would also like to determine if these genes can be used as markers in breeding programs. To these ends, cDNA libraries have been constructed from both young fruit and ripe fruit. Nine clones of potential interest have been identified by 1) differential hybridization and 2) hybridization with probes for known enzymes derived from other species. Most of these clones are homologous to RNAs that increase during fruit development. The expression patterns of several clones correlate strongly with the degree of softening during the final stages of maturation. Southern analyses indicate that most of these cDNA clones are derived from low copy number genes. The cDNAs are being sequenced to determine if they are related to known genes.

Since fruit characteristics such as size, color, softening rates, and flavor vary considerably in peach cultivars, we hypothesized that genes associated with fruit development might be used to detect genetic variability through northern and Southern analyses. The expression and structure of these genes was examined in 10 cultivars whose fruit phenotypes range from small, white flesh, freestone and bitter flavor to large, yellow flesh, clingstone with commercial quality flavor. The same general patterns of expression throughout development were seen with the exception of a peach-almond hybrid (with almond-like fruit) which had very low amounts of detectable homologous RNA. Gene structures were compared by hybridizing labeled cDNA inserts to EcoRI digested total DNA. Several of the cDNAs detected restriction fragment length polymorphisms (RFLPs) in the peach cultivars while most of the clones detected polymorphisms in an almond and a peach-almond hybrid. The cDNAs that detected RFLPs are being used to analyze crosses to determine their usefulness as markers in constructing genetic linkage maps for peach.

SINGLE-CHAIN ANTIBODY™ PROTEINS Robert E. Bird, Genex Corporation, 16020 Industrial Drive, Gaithersburg, Maryland 20877.

Single-chain antibody™ (SCA™) proteins are novel recombinant polypeptides, composed of an antibody variable light-chain amino acid sequence (V_L) tethered to a variable heavy-chain sequence (V_H) by a designed peptide that links the carboxyl terminus of the V_L sequence to the amino terminus of the V_H sequence. These proteins have the same specificities and affinities for their antigens as the monoclonal antibodies whose V_L and V_H sequences were used to construct the recombinant genes. Three of these proteins, one derived from the sequence for a monoclonal antibody to growth hormone and two derived from the sequences of two different monoclonal antibodies to fluorescein, were designed, constructed, synthesized in Escherichia coli, purified and assayed. These proteins are expected to have significant advantages over monoclonal antibodies in a number of applications. The activity of these proteins will be discussed.

MAPPING ANTIGENIC SITES IN ZEINS BY SYNTHETIC PEPTIDES:
Asim Esen. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

In recent years, the use of synthetic peptides has become one of the powerful tools for isolating and characterizing genes and gene products. They are commonly used in identifying and mapping sequential antigenic sites (epitopes) on proteins and relating sequenced genes to their corresponding polypeptide products. Zein, a group of highly hydrophobic proteins that constitutes 50-60% of the total protein in corn endosperm, presents a special challenge in this respect. There are four classes of zein: α, β, γ, δ. Of these, α-zein is the most abundant (80% of the total) and polymorphic, containing 25-30 polypeptides (23.8 to 26.7 kD) encoded by members of a multigene family. Complete primary structures of more than 15 α-zein polypeptides have been deduced from cloned cDNA and genomic DNA sequences. However, it is not known which *in vivo* polypeptides correspond to which of the primary structures deduced from nucleic acid sequences. γ-Zein (10-15% of the total) is second in abundance and represented by two polypeptides (18 and 27 kD). Twenty-three (17-mer) oligopeptides corresponding to the predicted antigenic epitopes and to other selected regions of α-zein (23.8 and 26.7 kD) and three corresponding to those of γ-zein$_1$ (27 kD) were synthesized. The peptides were used to identify sequential antigenic epitopes and also to define the regions of zein primary structure that reside on the surface of the folded protein. The peptides corresponding to α-zein sequences were reacted, both in the free and BSA-conjugated forms, with seven different antisera to intact α-zein polypeptides using an enzyme-linked immunosorbent assay (ELISA). Sixteen of the twenty-three oligopeptides yielded positive reactions with one or more of the seven different antisera. The results indicated that the reactive oligopeptides contain sequential epitopes corresponding to sequences that reside partly or entirely on the surface of the folded protein and that most of the immune response to α-zeins is directed to shared epitopes. The seven nonreactive peptides are thought to be not antigenic because either the regions of the protein corresponding to them are not immunogenic or the peptides have a conformation different from that in the intact protein. In addition, only a few of the peptides corresponding to variants of the 20-mer α-zein repeating unit reacted with antisera to the native protein. These results are not entirely consistent with the structural model proposed for α-zeins by Argos et al., (1982). Similar studies with three different antisera to γ-zein$_1$ indicate that the region of the protein containing 8 tandem repeats of the sequence PPPVHL is the site of one or more immunodominant epitopes. In one case the entire antibody repertoire of an antiserum recognizes epitopes within this region. These antisera yielded positive reactions with the 17-mer peptide (peptide 37) derived from the repeat region and little or no reaction with the two other 17-mer peptides. In addition, antiserum raised to peptide 37 was found to react with both the homologous antigen (peptide 37) and the intact γ-zein$_1$. Peptide 37 was also shown to block the binding of antibodies to γ-zein$_1$ in competition assays. Subsequent to these results, the shorter 6-mer (peptide 82), 9-mer (peptide 81) and 12-mer (peptide 80) versions of peptide 37 were synthesized and reacted with anti-peptide 37 serum and also with each of the three anti-γ-zein$_1$ sera. In these reactions the reactivity and the blocking ability in competition assays increased in proportion to the length of the peptide. Based on these data, it was concluded that the repeat region of γ-zein$_1$ is the site of one or more epitopes varying in length from 6 to more than 12 residues. The data also suggest that the repeat region is exposed on the surface of the folded protein and probably occurs as a mobile, random coil.

Tryptophan Biosynthesis in Arabidopsis: Genetic Redundancy and Conditional Auxotrophic Mutations. Robert L. Last, Boyce Thompson Institute at Cornell University, Ithaca, NY 14853-1801.

Although a great deal is known about the regulation of amino acid biosynthesis in microbes, little is known about the structure and regulation of amino acid biosynthetic genes in plants. The tryptophan pathway is of special interest in plants because it is the precursor to the hormone indole 3-acetic acid (IAA). We are studying tryptophan biosynthesis in *Arabidopsis thaliana* by isolating mutants defective in trp biosynthesis and cloning genes that encode the biosynthetic enzymes. Recessive auxotrophic mutations that define three complementation groups (*TRP1-TRP3*) have been identified by selection for plants that are resistant to 5-methylanthranilate+tryptophan. *trp1-1* plants are deficient in anthranilate phosporibosyltransferase activity while *trp2-1* plants appear to be deficient in tryptophan synthase B activity.

Unexpectedly, plants that are homozygous for any of the auxotrophic alleles exhibit a conditional Trp^- requirement: they are Trp^+ under standard growth conditions (150μEinsteins of light) and Trp^- under very low light intensities (25μEinsteins). Our working hypothesis is that the conditional phenotype results from differential expression of duplicated genes. Consistent with this hypothesis, two unlinked Arabidopsis tryptophan synthase B genes have been isolated. The inferred amino acid sequences of these genes show great similarity: they are 95% identical and both encode potential NH_2-terminal chloroplast targeting sequences. However, in mature leaf tissue the *TSB1* mRNA has an approximately 10 fold higher steady-state level than that of the *TSB2* gene. This result suggests that the *trp2-1* mutation is in the *TSB1* gene, and that expression of the less active gene is insufficient to allow plants to grow under optimal culture conditions (higher light levels). Questions currently under study include: 1. Does *trp2-1* map to the *TSB1* locus? 2. Is the *TSB2* gene expressed at higher levels in other tissues or different stages of development?

THE USE OF AUXIN MUTANTS TO STUDY IAA METABOLISM IN HIGHER PLANTS

Janet P. Slovin[1], Bruce G. Baldi[2], and Jerry D. Cohen[2], [1]Dept. of Botany, University of Maryland, College Park, MD 20742 and [2]USDA/ARS Plant Hormone Lab., Beltsville Agricultural Research Center, Beltsville, MD 20705.

The primary interest of our laboratory is the regulatory metabolism of the plant hormone indole-3-acetic acid (IAA). We want to understand how a plant regulates *how, how much, where, and when* to increase the levels of free and conjugated IAA in specific plant tissues. The study of IAA biochemistry is not straight forward: the compound is light sensitive, labile, easily obtained by non-enzymatic mechanisms from tryptophan and other indolic compounds, is present in very low amounts, etc. Even the routine quantitative analysis of IAA from plants has, until recently, been laborious and subject to analytical errors. These analytical problems have been largely overcome by use of quantitative gas chromatography-mass spectrometry. We are continuing the development of a new system, using the small aquatic monocot *Lemna gibba* G-3, which has also allowed us to overcome many of the biological difficulties of studying the metabolism of IAA.

Using tissue culture, or chemical mutagenesis of intact plants, we have been able to generate various types of somaclonal variants or mutants of *Lemna*. One such variant, jsR_1 is approximately 1.5 times the size of the parent line (PL) and is especially useful to us because it contains up to 100 times the levels of free IAA as PL and, unlike PL, it contains no detectable IAA conjugates. We are using stable isotope techniques to measure the kinetics of IAA metabolism in jsR_1 and to determine in detail the pathway of isotope incorporation in the indolic pools in these plants. These studies should lay the groundwork and provide the model for the techniques required for the efficient use of mutants to understand auxin metabolism in higher plants.

Additional mutants are being produced and selected for specific metabolic abnormalities of interest as described in the following table:

Methods for the selection of metabolic mutants with potential utility for studies of auxin metabolism.

Selection technique or agent	Mechanism	Possible Phenotypes
IAA general metabolism (Products formed from exogenous feeds of ^{3}H-IAA)	Product screening by TLC/autoradiography or HPLC/radiochromatography ("brute force")	Plants which cannot form certain metabolic products of IAA (altered conjugation or catabolism)
2,4-D, IAA, NAA, 4-Cl-IAA	Positive ability to grow at high levels of selecting agent	Increased conjugation or degradation; decreased receptor sensitivity
5-Br-IAA-alanine and other toxic auxin analog conjugates	Positive selection for ability to grow in presence of selecting agent	Reduced or no capacity for conjugate hydrolysis or resistance to toxic auxin analog
5-methyl-anthranilate in presence of added L-tryptophan	Tryptophan auxotrophs selected	See [RL Last and GR Fink, 1988, Science 240:305-310] Phosphoribosyl-transferase, tryptophan synthase A, etc reduced
Tryptophan analogs:		Altered form of:
5-methyl-tryptophan alpha-methyl-tryptophan 7-aza-tryptophan 5-Br-tryptophan	Positive selections for growth in presence of selecting agent	Anthranilate synthetase, tryptophan synthase, etc. Reduced production of 5-Br-IAA
Gross morphology	Selections based on size, frond shape, root growth	Altered level or rate of IAA production

IDENTIFICATION OF GENES ENCODING ASPARTATE AMINOTRANSFERASE IN PLANTS

Frank J. Turano, Barbara J. Wilson, Dennis A. Schaff and Benjamin F. Matthews, Plant Molecular Biology Laboratory, U. S. Department of Agriculture, Beltsville, MD 20705

The molecular mechanisms that regulate and orchestrate the expression of genes encoding isoenzymes in various plant organelles are not fully understood. To gain a better understanding of these mechanisms, we are studying the differential expression of aspartate aminotransferase (AAT) isoenzymes in plants. There are as many as five AAT isoenzymes which have been localized to different parts of the plant cell. AAT plays a key role in nitrogen metabolism, hydrogen shuttles (malate-aspartate), and carbon shuttles. We have approached the problem from two levels. First, we decided to identify the different isoenzymes in carrot cell suspension cultures. Once the isoenzymes have been identified, the isoenzymes will be purified and characterized. Second, we wanted to identify the gene(s) that code the different isoenzymes. This approach will enable us to 1) gain a better understanding of the function and regulation of the AAT isoenzymes in plants, 2) study and understand the complexity of the AAT gene family and 3) identify and characterize the gene(s) encoding the different isoenzymic forms of AAT.

Three aspartate aminotransferase isoenzymes were identified from extracts of carrot cell suspension cultures. These isoenzymes were separated by DEAE chromatography. When the isoenzymes were analyzed on native gradient polyacrylamide gels, the relative molecular weights of the isoenzymes were 111,000$\pm$5000, 105,000$\pm$5000, and 94,000$\pm$4000 daltons. The largest molecular weight form (Form I) has been purified to apparent homogeneity (>300 fold) using immunoaffinity chromatography with rabbit anti-pig AAT antibodies. Form I has a subunit size of 43,000 M_r, as determined on SDS-PAGE. IEF-PAGE has resolved three bands at pI of approximately 5.2. There are indications that Form I may not be composed of identical subunits. Kinetic parameters for Form I were determined; K_m values of 23.6, 2.8, 0.05 and 0.22 mM were obtained for glutamate, aspartate, oxaloacetate and α-ketoglutarate, respectively. Form I has a pH optimum from 7.5 to 10.0. The kinetic mechanism is a ping-pong-bi-bi mechanism.

We have taken several approaches to identify the gene(s) that encode the different AAT isoenzymes. First, we have purified an AAT isoenzyme to apparent homogeneity (>300 fold). The pure protein was used for amino acid composition analysis, amino acid sequencing and antibody production. Secondly, we have screened cDNA libraries with animal AAT cDNA clones. A 620 bp clone has been sequenced. A 226 bp region has a 50% identity with the 3' end of a known mammalian AAT gene.

REGULATION OF HOMOSERINE DEHYDROGENASE FROM CARROT.

Benjamin F. Matthews, Frank J. Turano, Ann C. Gray, Margaret J. Farrar and Ramon L. Jordan. USDA-ARS Plant Molecular Biology and Florist and Nursery Crops Laboratories, Beltsville, MD 20705

Homoserine dehydrogenase (HSDH) from cell suspension cultures of carrot has been purified to apparent homogeneity. The enzyme is composed of subunits of equal molecular weight (M.=85,000 +/- 5,000). The enzyme can be reversibly converted into a 180 Kd K-form or a 240 Kd T-form. When the enzyme is dialyzed in the presence of 5 mM threonine, the enzyme is converted into the 240 Kd T-form which is completely inhibited by 5 mM threonine. When the enzyme is dialyzed in the presence of 100 mM K^+, the enzyme is converted into the 180 Kd K-form which is not inhibited by threonine. Polyacrylamide electrophoretic gels stained for HSDH-specific enzyme activity or stained for protein were compared with western blots to verify the interconversion of the T and the K forms. The enzyme also can polymerize to form catalytically active higher molecular weight aggregates ranging in size up to 720 Kd under certain conditions. Polyclonal antibody from mouse was raised against the T-form (240 Kd) of carrot HSDH. Specificity of the mouse antisera was verified by immunoprecipitation of HSDH activity and by western blot analysis. The antisera also cross-reacted with soybean HSDH, but did not cross-react with either of the two forms of E. coli HSDH. Efforts are underway to identify clones encoding carrot HSDH. A model for the in vivo regulation of threonine biosynthesis in the chloroplast based on the interconversion of HSDH forms will be presented.

THE HEAT SHOCK RESPONSE OF CARROT EMBRYOS IN CULTURE

Nestor R. Apuya, Cynthia O'Carroll, J. Lynn Zimmerman
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Md 21228

Heat shock treatment (3 hr at 38°C) of somatic embryos of carrot (Daucus carota) at different stages of early embryogenesis produced a family of small heat shock (HS) proteins including the 17.5 kD protein. Based on 2-D PAGE analysis, heat-shocked callus cells and globular, heart, torpedo, and plantlet embryos were found to contain similar pattern of small HS proteins and similar quantities of 17.5 kD protein. However, based on run-on transcription analysis of nuclei from the tissues mentioned, it was found that HS induction of transcription of HS17.5b gene (corresponding to the 17.5 kD protein) was significantly reduced in globular embryos. The induction was highest in callus tissues (65-fold, relative to globular) and was relatively lower in heart/torpedo (5-fold) and plantlet stage (11-fold). The accumulation of HS17.5b mRNA, as shown by Northern analysis, followed a trend parallel to the transcription rate i.e., the steady state level of HS17.5b transcripts was highest in callus, was lowest in globular stage and began to increase at the heart/torpedo stage. These data strongly suggest that at globular stage a mechanism is operating at the translational level such that substantial production of 17.5 kD protein is ensured. Somehow, this mechanism compensates for the low level of HS17.5b transcription. Preliminary studies of HS protein synthesis rates during embryogenesis indicate that this might be true. Specific incorporation of ^{35}S-Met during HS (cpm/ug protein) was found to be significantly higher at the globular stage.

The effect of HS treatment on the developmental progress of somatic embryogenesis has also been investigated. We have found that globular embryos are specifically and irreversibly arrested in their development by exposure to a brief HS. The characteristics of this phenomenon are quite similar to the production of "phenocopies" in Drosophila. This indicates that the production of a critical determinant necessary for the transition from globular to heart stages is being "blocked" by HS treatment. The relationship of this block to the translational compensatory mechanism for the HS17.5b gene (and possibly other HS genes) at globular stage is under investigation.

EXPRESSION OF THE *CabII* MULTIGENE FAMILY IN PEA

L.C.Childs, M.J. White, B. Fristensky, D. Falconet, and W.F. Thompson. Depts. of Genetics and Botany, North Carolina State University, Raleigh, NC 27695-7614.

The chlorophyll a/b (*CabII*) binding polypeptides of the light-harvesting antenna complex of photosystem II (LHC II) are encoded by at least six closely related nuclear genes in pea. Sequences for AB66, AB80, and AB96 have been published (1,2). In collaboration with J.Watson and B.Roe, our lab has sequenced three additional *cab* genes(AB8, AB9, and pEA215). Another cDNA (pEA315) has been identified as a *cab* gene candidate. Some evidence that the genes are regulated differentially comes from earlier fluence response and escape from far-red reversibility data on pEA215 and pEA315 as compared with total *cab* mRNA behavior (3,4). With the above sequence information we have made gene-specific probes and checked their specificity. We now plan to use these to study differential expression and to analyze chromatin structure.

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Cis-ACTING ELEMENTS REQUIRED FOR LIGHT REGULATION OF PEA FERREDOXIN I GENE EXPRESSION ARE LOCATED WITHIN THE TRANSCRIPTION UNIT.

M. Gallo, R. C. Elliott, L. F. Dickey, B. R. Fristensky, T. J. Pedersen, and M. J. White, and W. F. Thompson. Departments of Genetics and Botany, North Carolina State University, Raleigh, NC 27695, USA

Light responses of the pea ferredoxin I gene (*Fed-1*) differ from those of other light responsive genes such as *RbcS* and *Cab* (Thompson, Plt Cell Env 11, 319, 1988.). Our recent results indicate that *Fed-1* also has several features appropriate to a major model system. Notably, analysis of its regulation will be greatly simplified by the fact that it is a single copy gene, and the absence of introns removes the need to consider complex RNA processing pathways. We have started an experimental analysis of *Fed-1* regulatory mechanisms by introducing an intact gene and several chimaeric constructs into tobacco plants. The intact gene is correctly transcribed and translated, producing a protein which is imported into host cell chloroplasts and processed to its normal mature size. *Fed-1* mRNA accumulation in these plants is strongly light dependent, as it is in pea leaves. In experiments with chimaeric gene constructs, however, the promoter fails to respond to light even though it is transcriptionally active, and sequences downstream of the gene also fail to confer light sensitivity. Only the transcription unit conveys normal light responsiveness. This unusual result is most consistent with the hypothesis that light affects *Fed-1* mRNA stability rather than transcription of the gene.

DIFFERENTIAL EXPRESSION OF THE HEAT SHOCK GENES AT DIFFERENT TEMPERATURES IN CULTURED CARROT CELLS: CHARACTERIZATION OF THE HEAT SHOCK TRANSCRIPTION FACTOR

CHEOL HO HWANG AND J. LYNN ZIMMERMAN
DEPT. OF BIOL. SCI., UMBC, CATONSVILLE, MD 21228

In response to heat shock plants produce a new set of proteins ,the Heat Shock Proteins, including a complex set of low molecular weight HSPs. Some of the low molecular weight HSPs are believed to be involved in conferring thermotolerance to plants by localizing into the organneles and by forming HS granules in the cytoplasm. It appeared that each low molecular weight HSP may have a specific role even though they are closely related each other in terms of DNA sequence homology.

An analysis of the HSPs induced at various temperatures in carrot cells revealed a non-coordinated regulation of the induction among low molecular weight HSPs. Some were induced to a maximal level in response to very mild heat shock (30°C) while others were induced maximally by much higher temperatures (i.e.38°C). This is particularly interesting in the light of the facts that all heat shock genes are basically regulated in the same way through the interaction between a small,highly conserved DNA sequence,the heat shock element (HSE) and a single transcription factor, the Heat Shock Transcription Factor (HSTF). In order to achieve subtle levels of heat shock regulation (such as those observed in carrot cells), there must exist the potential to modify one or both of these regulatory units. In fact, in yeast, there is some evidence that different forms of active HSTF exist at different temperatures. These different forms might recognize subtle differences among the HSEs of different heat shock genes and allow differential induction.

As a first step toward determining if this type of control might operate in plants, we have begun to characterize HSTF in our system, carrot cultured cells by using Southwestern analysis and have identified a protein which may be the carrot HSTF.

IDENTIFICATION AND ANALYSIS OF RIPENING STAGE SPECIFIC GENES IN TOMATO

Andrew D. Lloyd, Ann Callahan and Peter Morgens, USDA, ARS, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430

cDNA clones of mRNAs that are expressed during different stages in the ripening of tomato fruit (Lycopersicon esculentum Mill. var. Pixie) have been isolated by differential hybridization. Corresponding genomic clones from an EMBL3 tomato genomic library were isolated using these cDNA clones. Expression of clone 102 increased during ripening, while clone 204 showed peak expression in green fruit, with mRNA levels decreasing as ripening progressed. Insert DNA from genomic clones 102 and 204 was transferred into a Bluescript plasmid vector and analyzed by restriction enzyme mapping. Southern blot analysis of the genomic clone of 204, pAL204, using the 204 cDNA as a probe, indicated that all genomic sequences complimentary to the cDNA were present in a 2.0 kb Eco R1 fragment. The DNA sequences of this genomic fragment, as well as that of the cDNA clones for both 102 and 204 is being determined.

ABSTRACT

SYNTHESIS OF TWO CHROMOPLAST-SPECIFIC PROTEINS DURING FRUIT DEVELOPMENT IN *CAPSICUM ANNUUM*. Lee A. Newman, Noureddine Hadjeb, and C. A. Price. Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759.

The time-course of accumulation of two membrane proteins during fruit ripening was examined by SDS-polyacrylamide gel electrophoresis and western blots in tissue extracts of fruits of *Capsicum annuum* L., vars. Emerald Giant, Albino, and DNAP VS-12. The proteins, named ChrA and ChrB, were previously shown to occur specifically in chromoplasts. Fruit development was divided into five stages arbitrarily based on the accumulation of carotenoids. ChrA was not detectable in immature fruits, but accumulated to a high level in the fully mature, red fruit. ChrB was not detectable in the first stage of fruit maturation, but appeared in the second stage of maturation when carotenoids were first seen, and in all later stages. The patterns of accumulation were similar in chromoplasts that arose from chloroplasts or leucoplasts. We conclude that ChrA and ChrB are synthesized *de novo* during chromoplast development.

PRELIMINARY STUDIES OF DIFFERENTIAL ACTIVITY AND GENETIC NATURE OF ALCOHOL DEHYDROGENASE (E.C. 1.1.1.1.) ISOZYMES IN VARIOUS PLANT PARTS OF OCTOPLOID STRAWBERRY, Fragaria x ananassa Duch., GENOTYPE 8343-6.

Amin Noueiry, David Wolyn, Gojko Jelenkovic
Department of horticulture
Rutgers University
New Brunswick, N.J. 08903

The alcohol dehydrogenase (ADH) genes in plants are involved in a response mechanism where the plant reverts to alcoholic fermentation to survive and continue respiration under conditions of transient hypoxia. In fruits ADH interconverts alcohols and aldehydes, which are major flavor compounds, therefore contributing to the quality of the fruit.

Roots, leaves, runners, seeds, and fruits of the 8343-6 genotype of cultivated octoploid strawberry were assayed for ADH activity. In all investigated strawberry organs, a low basal activity, although not at the same level, has been found. The highest total activity(Units/gm fresh wt) was observed in seeds and the lowest one in leaves.

Fruits were assayed at four different developmental stages: green, white, orange and red stages. The activity of ADH increases with development of the fruit reaching its maximum at the third stage. This increase in the activity, however, is not parallel to the continuous increase in total protein content of the fruit. Seeds collected from fruits at the same developmental stages had an overall increasing total protein content, with red seeds having the highest concentration. On the other hand total NAD-dependent ADH activity was highest in white seeds, significantly lower in orange seeds, and not significantly different in red seeds.

Under anaerobic conditions, the level of activity in roots, leaves and runners did not increase. ADH activity almost doubled when fruits in the orange stage were induced with acetaldehyde over a period of twenty four hours.

ADH isozymes could not be separated on starch, agarose or native polyacrylamide gel electrophoreses. On agarose gels, a single diffuse band is observed from the fruit samples. However, isoelectric focusing on polyacrylamide gels (pH gradient 4-6.5) revealed four and six bands in fruit and seed samples respectively. Genetic analysis showed the existance of three differentially expressed genes in the genotype studied: Adh1, expressed in the seeds and fruits; Adh2, found only in the seeds; Adh3, expressed only in fruits.

ABSTRACT

Developmental Regulation of a Bud Specific Gene in Pea

Mahasin A. Osman and Michael S. Dobres, Department of Bioscience and Biotechnology Drexel University, Philadelphia, PA 19104.

A striking example of organ-and stage -specific gene expression in pea (*Pisum sativum* L.) was recently reported (Dobres & Thompson,1989). Transcripts for the cDNA clone pEA207 (Thompson *et al*, 1983) accumulate in the terminal bud of the pea plant and are either absent or present at very low levels in the expanded leaves below the bud. The deduced amino acid sequence of this transcript shows 49% similarity to the phytohemagglutinin lectin sequence of kidney bean and 37% to the pea seed lectin. All the essential residues involved in metal binding by lectins are present in the sequence (Dobres & Thompson, 1989).

We have examined the relationship between the accumulation of pEA207 and cell division. pEA207 mRNA is present at low level in root tips that, like buds, contain high levels of dividing cells; This indicates that pEA207 accumulation is not purely a function of cell division, rather, it might relate to the position of the expressing cells in the shoot apex. This hypothesis was tested by measuring the relative accumulation of pEA207 RNA in dormant and growing lateral buds. We found that pEA207 mRNA accumulates to equal levels in dormant and growing lateral buds. It appears that pEA207 RNA accumulates as a function of position rather than growth rate.

The PEA207 accumulation was measured in embryonic shoot (plumule) and embryonic root (radicle) and found to display shoot specificity of expression as early as 24 hours after imbibition. A comparison of pEA207 mRNA accumulation in young (8 DAF) and mature seeds (20 DAF) revealed the presence of significant amounts of the transcript in the immature seeds (8 DAF) and none in 20 DAF ones. We intend to use pEA207 as a tool to further investigate the relationship between gene expressin and shoot development.

TRANSFORMATION OF THE FILAMENTOUS FUNGUS *GLIOCLADIUM VIRENS* TO DEVELOP BENOMYL RESISTANT STRAINS FOR BIOCONTROL OF PLANT DISEASES. Nina Ossanna and Sue Mischke, Biocontrol of Plant Disease Laboratory, USDA-ARS, Beltsville, MD 20705

Biocontrol agents are more useful for programs of integrated pest management if they are not sensitive to agricultural chemicals such as benomyl, a methyl benzimidazole carbamate (MBC) fungicide which acts as an antimitotic by binding to β-tubulin and preventing microtubule formation. *Gliocladium virens* is an effective antagonist of several important plant pathogens and a genetic engineering program has been undertaken to develop strains with improved characteristics, including tolerance to benomyl. Methodology was developed to isolate and regenerate protoplasts of this filamentous fungus, as well as to transform them with exogenous DNA. Transgenic strains of benomyl-resistant *G. virens* were obtained by transformation with a benomyl-resistant β-tubulin gene from *Neurospora crassa*. Southern blot analysis shows that the *N. crassa* DNA integrated randomly into the chromosome and was present in multiple copies.

PHYTOCHROME INDUCED FLOWERING IN A PHOTOPERIOD INSENSITIVE BARLEY MUTANT: 2-D GEL ELECTROPHORESIS COMPARISON

Jan Principe, G.F. Deitzer Dept. Hort., Univ. Maryland, College Park, MD. 20742 and W.R. Hruschka, Instrumentation Research Laboratory, Bldg 002, USDA/ARS, BARC-West, Beltsville, MD 20705.

Flowering in barley (*Hordeum vulgare* L.) is promoted by FR light that is absorbed by phytochrome. Floral induction is saturated by 72h of continuous light containing FR. A single gene mutant (BMDR-1) was isolated that is insensitive to both FR and photoperiod. Floral induction was compared to an isogenic, sensitive cv. (BMDR-8) by addition of FR to 24h photoperiods. Floral induction of BMDR-1 takes place after 5 days under both conditions. BMDR-8 is the same as BMDR-1 under 24h + FR but is significantly delayed under 24h - FR. The results suggest that BMDR-1 lacks a gene for the production of a floral inhibitor in leaves the expression of which is repressed by FR in BMDR-8. To test this, total phenol extracted proteins were resolved during the first 5 d by 2-D gel electrophoresis and compared in both isotypes under light with or without FR. Specific differences in protein patterns were detected by video computer digital analysis between BMDR-1 and BMDR-8. Their regulation by FR and relation to flowering will be discussed.

THE NUCLEOTIDE SEQUENCE OF 5.8S RIBOSOMAL RNA AND TWO INTERNAL TRANSCRIBED SPACERS (ITS1 AND ITS2) FROM OCTOPLOID STRAWBERRY (Fragaria x ananassa Duch.)

Nada Simovic, David Wolyn, Gojko Jelenkovic
Dept. of Horticulture and Forestry,
Rutgers University, New Brunswick, NJ 08903

Nucleotide sequences of ribosomal RNAs (rRNA), their secondary and tertiary structures, and interaction with ribosomal proteins are fundamentally similar in procaryotes and eucaryotes. However, in the large ribosomal subunit of eucaryotes (apart from 26-28S and 5S rRNAs, which have functional counterparts in procaryotes) an additional small ribosomal RNA is present-5.8S rRNA. The relatively small size of this molecule and its uniqueness in eucaryotes encouraged early sequencing. Currently, a number of 5.8S rRNA sequences from different species are available. Nonetheless, information on plant 5.8S rRNAs, encoding genes (rDNA), and internal transcribed spacers are scarce. Bearing this in mind, we have cloned and sequenced the 5.8S rRNA gene and internal transcribed spacers (ITS1 and ITS2) from cultivated octoploid strawberry (Fragaria x ananassa Duch).

The strawberry 5.8S rRNA gene is 164 nucleotides long, and its GC content is 55%. Alignment with corresponding plant sequences shows homology over 90%. The homology to lower eucaryotic sequence from yeast is 82%, and the homology to amphibian (Xenopus) and mammalian (mouse) genes is 70%. Conservation of short sequences, and two highly variable regions were observed within analyzed genes. The secondary structure model for the strawberry 5.8S rRNA was constructed based on the gene sequence, and it features five secondary structure helices, two of which have characteristic nucleotide composition: one is A+U rich, the other is G+C rich.

The 5.8S rRNA gene in strawberry is separated from the 18S rDNA by 253bp long spacer (ITS1), and from 26S rDNA by 207bp long spacer (ITS2). The comparison to available spacer sequences from yellow lupine demonstrates considerable sequence variation. However, two regions in ITS1 and one in ITS2 appear conserved. Whether these regions play a role in ribosomal precursor processing remains to be answered.

CLONING AND SEQUENCING OF THE cDNA FOR THE P-PROTEIN OF THE GLYCINE DECARBOXYLASE COMPLEX FROM PEAS

Ketan Shah & David Oliver Department of Biochemistry, University of Idaho, Moscow, ID 83843

The glycine decarboxylase complex of pea leaf mitochondria is a key reaction of photorespiration. It is composed of four (P, T, L & H) nuclear-coded proteins. A cDNA for the P-protein, P-5-1, was selected from lambda gt11. A o.7 kb EcoR1 cDNA insert from P-5-1 was subcloned into pBSKS. The recombinant single-stranded (ss) pBSKS phagemid DNAs from different E. coli JM101 clones were rescued with helper phage VCSM13. DNA sequencing was performed by the dideoxy (Sanger) method using both ss and double-stranded template DNAs. Sequence analysis showed a poly A of atleast 70 nucleotides at 5'end of cDNA which represents the polyadenylate tail of the mRNA. Since the cDNA sequenced is little more than 1/5 of the length of the complete 3.2 kb mRNA for the 114 kd P-protein, larger cDNA inserts are being screened from the cDNA library using the 0.7 kb cDNA probe as well as using a polyclonal antibody raised against the purified P-protein. Identification of genes for P-protein from genomic clones in pea nuclear DNA library constructed in lambda EMBL vector will be presented.

STABLE TRANSFORMATION OF EMBRYOGENIC MAIZE CULTURES BY MICROPROJECTILE BOMBARDMENT

Start, W.G., T.R. Adams, W.R. Adams, S.A. Chambers, V.C. Courreges, R.J. Daines, M.L. Mangano, J.V. O'Brien, T.M. Spencer, N.G. Willetts, W. Gordon-Kamm, A.P. Kausch, R.W. Krueger, P.G. Lemaux, and C.J. Mackey.
DeKalb-Pfizer Genetics, Pfizer Central Research, Groton, CT 06340.

Stable genetic transformation of embryogenic maize cultures was accomplished by microprojectile bombardment. Type II callus from A188xB84 was used to initiate embryogenic suspension cultures, which were bombarded with a mixture of two plasmids, one encoding neomycin phosphotransferase, APH(3')-II, and one encoding β-glucuronidase, GUS. To optimize bombardment parameters, a histochemical assay for transient GUS expression was used to assess the numbers of cells expressing the introduced DNA two days post-bombardment. Plates of bombarded suspension cells were placed under selection of the aminoglycoside, G418. Callus which stably expresses APH(3')-II was recovered using two different selection protocols. Presence of the *neo* gene was confirmed by Southern analysis and the polymerase chain reaction. Efforts to regenerate this material have been suspended because the age of this particular suspension culture probably would preclude success in these efforts. Nonembryogenic maize suspension cultures were bombarded with a mixture of plasmids encoding APH(3')-II, GUS and the crystal toxin protein from *Bacillus thuringiensis* var. *kurstaki*. Cells were selected on kanamycin and then analyzed for the presence of the nonselected genes. Cotransformation frequencies were 100% for the nonselected genes; coexpression frequencies with the GUS gene were approximately 50%.

CLONING AND CHARACTERIZATION OF CARROT HEAT SHOCK GENES

Liqun Wang, Nestor Apuya and J.Lynn Zimmerman
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, MD 21228

The heat shock phenomenon is ubiqutious and has long been considered as a model system for the study of gene regulation. Our lab has focused on understanding what is necessary and sufficient for heat shock induction to occur in cultured carrot cells.

Two related but non-identical clones whose expression is heat shock-dependent have been isolated from our carrot genomic library and completely sequenced. Comparison of both genes reveals 90% DNA homology evenly distributed throughout their coding regions and a high degree of homology to similar size heat shock genes documented from other plant species. The putative promoter regions of both genes contain multiple heat shock consensus elements, and in one gene analyzed, this region is proceded by a long stretch of repetitive AT rich sequence (with A+T content of 89%). A relatively long untraslated leader sequence also exists at the 5' end of the coding sequence. Since both genes display the characteristic features of typical heat shock genes, the functions of each component within the genes now can be studied to learn the mechanism of the heat shock induction and to produce a minimal size heat-inducible plant promoter.

B-GLUCURONIDASE ACTIVITY IN NON-TRANSFORMED *Beta vulgaris* L. (SUGARBEET) TISSUES.

Chris A. Wozniak and Lowell D. Owens, USDA/ARS, Plant Molecular Biology Laboratory, BARC-West, Beltsville, Maryland 20705.

As a readily assayable marker of plant cell transformation, B-glucuronidase (GUS) has proven to be invaluable in the analysis of foreign DNA introduction techniques with several plant species. Histochemical, colorimetric, fluorometric and immunochemical methods exist for documenting the presence of GUS in transformed plant tissues. During an analysis of *Agrobacterium*-mediated transformation of sugarbeet cells, we have found the histochemical assay useful in identifying transformed (blue) cells, whereas no hydrolysis of the substrate (X-gluc) occurred in non-transformed (white) cells. Fluorometric analysis of axenically grown shoots and calli of cultivar 'REL-1', however, yielded significant levels of enzyme activity capable of hydrolyzing the fluorogenic substrate 4-methylumbelliferyl-B-D-glucuronide (MUG), as well as several other related glycosides. The enzyme(s) responsible is resistant to some common proteases, relatively heat stable and has been detected in three cultivars tested to date. Western blot analysis has revealed the presence of four peptides which are immunoreactive to polyclonal antiserum raised against GUS of *E. coli* origin. Attempts to culture microbial contaminants/endophytes on common bacteriological media has proven negative for these tissues. Current aims focus on Southern analysis of transformed and non-transformed tissues to assess the utility of GUS as a marker in the genetic engineering of sugarbeet.

NESTOR R. APUYA
DEPARTMENT OF BIOLOGICAL SCIENCES
UMBC
CATONSVILLE MD 21228
301-455-2263

NEAL BARNETT
BOTANY DEPARTMENT
UNIVERSITY OF MARYLAND
COLLEGE PARK MD 20742
301-454-0248

SHARON BILLINGS
BLAKE HALL, COOK COLLEGE
RUTGERS UNIV.
NEW BRUNSWICK NJ 08903

ROBERT BIRD
GENEX CORPORATION

GAITHERSBURG MD 20877
301-258-0552

BARRY BUCHBINDER
LIFE TECH, INC.
PO BOX 6009
GAITHERSBURG MD 20850
301-670-8367

ANN CALLAHAN
USDA-ARS, APPALACHIAN FRUIT RES STATION
45 WILTSHIRE RD.
KEARNEYSVILLE WV 25430
304-728-2356

PAMELA J. CASCONE
PROGRAM IN MOLEC & CELL BIOL
UNIV OF MASSACHUSETTS, GRAD RESRCH TOWER
AMHERST MA 01003
413-545-0277

DONNA CATTELL
PENN STATE UNIVERSITY
BOX 157
PINE GROVE MILLS PA 16868
814-238-8130

MIGUEL C. CERVANTES
WAKSMAN INSTITUTE
RUTGERS UNIVERSITY
PISCATAWAY NJ 08855-0759
201-932-2121

L. C. CHILDS
DEPARTMENT OF GENETICS
NORTH CAROLINA STATE UNIVERSTIY
RALEIGH NC 27695-7614
919-737-7166

ALAN H. CHRISTENSEN
DEPARTMENT OF BIOLOGY
GEORGE MASON UNIVERSITY
FAIRFAX VA 22030-4444

JOSEPH A. COZZITORTO JR.
ENICHEM AMERICAS INC.
2000 PRINCETON PK. CORP. CTR.
MONMOUTH JCT. NJ 08852
201-422-3660

CAROLE CRAMER
DEPT PLANT PATH, PHYSIOL & WEED SCIENCE
VA POLYTECH INSTITUTE & STATE UNIVERSITY
BLACKSBURG VA 24061
703-961-6757

DEAN CRESS
BIOTECHNOLOGY
727 NORRISTOWN RD
SPRING HOUSE PA 19477

JEANNE CURRY
UNIVERSITY OF MASSACHUSETTS

AMHERST MA 01003
413-545-4380

GERALD F. DEITZER
DEPT OF HORTICULTURE
UNIVERSITY OF MARYLAND
COLLEGE PARK MD 20742
301-454-6322

CYNTHIA DENBOW
PLANT MOLECULAR BIOLOGY
VIRGINIA TECH
BLACKSBURG VA 24061
703-961-4145

HORTENSE W. DODO
PENN STATE UNIV.
433 WARTIK LAB
UNIVERSITY PARK PA 16802
814-865-3680

LESLIE DOMIER
USDA-ARS
DEPT PLANT PATHOLOGY, UNIV OF ILLINOIS
URBANA IL 61801
217-333-0510

ASIM ESEN
DEPARTMENT OF BIOLOGY
VA POLYTECH INSTITUTE & STATE UNIVERSITY
BLACKSBURG VA 24061
703-231-5894

WILLIAM P. FLYNN
PENN STATE PLANT PHYSIOL
111 BORLAND LAB
UNIV. PARK PA 16802
814-865-0269

CLARISSE FRIAS
BLAKE HALL, COOK COLLEGE
RUTGERS UNIV
NEW BRUNSWICK NJ 08903

HUGH FRICK
PLANT SCIENCE DEPARTMENT
UNIVERSITY OF DELAWARE
NEWARK DE 19717-1303
302-451-2534

MARIA GALLO
DEPARTMENT OF GENETICS
NORTH CAROLINA STATE UNIV, BOX 7614
RALEIGH NC 27695
919-737-7166

JOHN GAYNOR
DEPARTMENT OF BIOLOGICAL SCIENCES
RUTGERS UNIVERSITY
NEWARK NJ 07102
201-648-1007

JOE GOFFREDIA
BLAKE HALL, COOK COLLEGE
RUTGERS UNIV
NEW BRUNSWICK NJ 08903

ANN GRAY
USDA, PMBL
B-006, RM 114, BARC W
BELTSVILLE MD 20705
301-344-4376

PAMELA GREEN
MSU-DOE PLANT RESEARCH LABORATORY
MICHIGAN STATE UNIVERSITY
EAST LANSING MI 48824
517-353-4838

ROB GRIESBACH
USDA
BLDG 004, RM 18
BELTSVILLE MD 20705
301-344-3574

SAM B. HA
DEPT CROP & SOIL ENVIRONMENTAL SCIENCE
VA POLYTECH INSTITUTE & STATE UNIVERSITY
BLACKSBURG VA 24061

NOUREDDINE HADJEB
WAKSMAN INSTITUTE
RUTGERS UNIVERSITY
PISCATAWAY NJ 08855-0759
201-932-2121

JOHN HAMMOND
USDA, ARS, FNCL
RM 208, B-004, BARC WEST
BELTSVILLE MD 20705
301-344-3313

ROSEMARIE W. HAMMOND
USDA-ARS, MICROBIAL PLANT PATH LAB
RM 252, B-011A, BARC W
BELTSVILLE MD 20705
301-344-3203

ELIOT HERMAN
USDA-ARS, PMBL
B-006, BARC W
BELTSVILLE MD 20705
301-344-3258

VICTORIA HOLLOWELL
UNIVERSITY OF SOUTH CAROLINA
COKER LIFE SCIENCES BUILDING
COLUMBIA SC 29208
803-777-3179

HEI-TI HSU
USDA-ARS
RM 208, B 004, BARC WEST
BELTVILLE MD 20705
301-344-3570

CHEOL HO HWANG
BIOLOGICAL SCIENCES
UMBC
CATONSVILLE MD 21228
301-455-2263

TARI JAVAD
UNIVERSITY OF SOUTH CAROLINA
COKER LIFE SCIENCES BUILDING
COLUMBIA SC 29208
803-777-3179

GOJKO JELENKOVIC
DEPT OF HORTICULTURE & FORESTRY
RUTGERS UNIV
NEW BRUNSWICK NJ 08903
201-932-8976

RAMON JORDAN
USDA-ARS
BARC W, B-004, R-208
BELTSVILLE MD 20705
301-344-1646

ANDY KALINSKI
USDA-ARS, PMBL
PSI
BELTSVILLE MD 20705
301-344-3258

KATHY KAMO
USDA-ARS, FLORIST & NURSERY CROPS LAB
B-004, RM 208, BARC W
BELTSVILLE MD 20705
301-344-3350

KELLI KOS
USDA-ARS
RM 275, B-011A
BELTSVILLE MD 20705
301-344-4003

ROBERT LAST
BOYCE THOMPSON INSTITUTE
CORNELL UNIVERSITY
ITHACA NY 14853
607-254-1234

SONDRA LAZAROWITZ
DEPARTMENT OF EMBRYOLOGY
CARNEGIE INSTITUTE OF WASHINGTON
BALTIMORE MD 21210
301-467-1414

KAREN LEWIN
USDA-ARS
BARC WEST BLDG 006
BELTVILLE MD 20705
301-344-4376

XIA LIN
BOTANY DEPARTMENT
UNIVERSITY OF MARYLAND
COLLEGE PARK MD 20742
301-454-0243

ANDREW D. LLOYD
USDA-ARS, APPALACHIAN FRUIT RES STATION
45 WILTSHIRE RD.
KEARNEYSVILLE WV 25430
304-725-3451

NAGESWARA RAO MADAMANCHI
DEPT PLANT PATH, PHYSIOL & WEED SCIENCE
VA POLYTECH INSTITUTE & STATE UNIVERSITY
BLACKSBURG VA 24061
703-231-9467

MAURICE MARGULIES
USDA-ARS, PLANT PHOTOBIOLOGY LAB
BARC W
BELTSVILLE MD 20705
301-344-3607

BARBA MARINA
INSTITUTO PATHOLOGIA VEGETALE
VIA C. G. BERTERO 22
ROME, ITALY 00152
06-827-4222

MELINDA N. MARTIN
DEPT OF BIOLOGICAL SCIENCES
RUTGERS UNIVERSITY
NEWARK NJ 07102
201-648-5385

BENJAMIN F. MATTHEWS
USDA-ARS, PMBL
B-006
BELTSVILLE MD 20705
301-344-2730

SUNITA MIDHA
ENICHEM AMERICAS INC.
2000 PRINCETON PARK CORP CENT.
MOHMOUTH JCT NJ 08852
210-422-3771

SUE MISCHKE
USDA-ARS
BIOCONTROL OF PLANT DISEASE LAB
BELTSVILLE MD 20705
301-344-4003

WILLIAM C. MITCHELL
DEPARTMENT OF BIOLOGICAL SCIENCES
RUTGERS UNIVERSITY
NEWARK NJ 07102
201-648-5385

DELORES MOORE
UNIVERSITY OF SOUTH CAROLINA
COKER LIFE SCIENCES BUILDING
COLUMBIA SC 29208
803-777-3179

PETER MORGENS
USDA-ARS, APPALACHIAN FRUIT RES STATION
45 WILTSHIRE RD.
KEARNEYSVILLE WV 25430
304-728-2346

SUZANNE NANIS
USDA-NAL
10301 BALT. BLVD., RM 301
BELTSVILLE MD 20705
301-344-3218

LEE NEWMAN
WAKSMAN INSTITUTE
RUTGERS UNIVERSITY
PISCATAWAY NJ 08855-0759
201-932-2121

AMIN NOUEIRY
DEPT OF HORTICULTURE
RUTGERS UNIVERSITY
NEW BRUNSWICK NJ 08903
201-932-8976

LEE OLSON
CHRISTOPHER NEWPORT COLLEGE

NEWPORT NEWS VA 23606

MAHASIN A. OSMAN
DREXEL UNIVERSITY
269 S 44TH ST
PHILADELPHIA PA 19104
215-382125

LOWELL OWENS
USDA-ARS
BLDG 006
BELTSVILLE MD 20705
301-344-4072

JHANG HO PAK
DREXEL UNIVERSITY
311 N 33RD ST APT. B6
PHILADELPHIA PA 19104
215-222-3454

HEESUNG PARK
DEPT PLANT PATHOL,PHYSIOL & WEED SCIENCE
VA POLYTECH INST & STATE UNIVERSITY
BLACKSBURG VA 24061
703-961-4145

JUDITH B. PHILBRICK
USDA-ARS
RM 118, BLDG 006, BARC WEST
BELTSVILLE MD 20705
301-344-1267

DEBORAH POLAYES
LTI, BRL
8718 GROVEMONT CIRCLE
GAITHERSBURG MD 20877
301-670-8344

CARL A. PRICE
WAKSMAN INSTITUTE
RUTGERS UNIVERSITY
PISCATAWAY NJ 08855-0759
201-932-2920

JAN PRINCIPE
DEPT OF HORTICULTURE
UNIVERSITY OF MARYLAND
COLLEGE PARK MD 20742
301-454-6322

ELLEN M. REARDON
USDA-ARS, PMBL
BARC WEST, B-006
BELTSVILLE MD 20705
301-344-3304

CATHY RONNING
DEPT OF HORTICULTURE
UNIVERSITY OF MARYLAND
COLLEGE PARK MD 20742
301-454-6322

GIDEON W. SCHAEFFER
USDA-ARS, PLANT MOLECULAR BIOLOGY LAB
BARC WEST
BELTSVILLE MD 20705
301-344-4342

HOLLY SCHAUER
USDA CRGO
901 D ST SW
WASHINGTON DC 20251-2200
202-447-7176

KETAN SHAH
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF IDAHO
MOSCOW ID 83843
208-885-7631

GREGG W. SILK
DEPARTMENT OF BIOLOGICAL SCIENCES
UMBC
CATONSVILLE MD 21228
301-455-3482

NADA SIMOVIC
DEPT OF HORTICULTURE & FOREST
RUTGERS UNIV
NEW BRUNSWICK NJ 08903
201-932-8976

MARCIA SLOGER
DEPARTMENT OF BIOLOGICAL SCIENCES
UMBC
CATONSVILLE MD 21228
301-455-3474

JANET P. SLOVIN
USDA-ARS, PLANT HORMONE LAB
BARC WEST B050
BELTVILLE MD 20705
301-344-3632

TERESA SNYDER
PENN STATE UNIVERSITY
111 BORLAND LAB
UNIVERSITY PARK PA 16802
814-865-0269

BILL START
DEKALB-PFIZER GENETICS
PFIZER CENTRAL RESEARCH
GROTON CT 06340
203-441-4766

DAVID B. STERN
BOYCE THOMPSON INSTITUTE
CORNELL UNIVERSITY
ITHACA NY 14853
607-254-1234

IAN SUSSEX
DEPARTMENT OF BIOLOGY
YALE UNIVERSITY
NEW HAVEN CT 06511
203-432-3460

MARK TUCKER
USDA-ARS, PMBL
BLDG 006, RM 207, BARC WEST
BELTSVILLE MD 20705
301-344-1091

FRANK J. TURANO
USDA-ARS, PMBL
BARC WEST, PSI, B-006
BELTSVILLE MD 20705
301-344-3304

PETER P. UENG
USDA-ARS
BARC WEST , BLDG 006
BELTSVILLE MD 20705
301-344-3035

ANGELA E. C. VALINSKI
PROGRAM IN MOLEC & CELL BIOL
UNIV OF MASSACHUSETTS, GRAD RESRCH TOWER
AMHERST MA 01003
413-545-0277

VICKI VANCE
UNIVERSITY OF SOUTH CAROLINA
COKER LIFE SCIENCES BUILDING
COLUMBIA SC 29208
803-777-3179

LIQUN WANG
UMBC
5401 WILKINS AVE
CATONSVILLE MD 21228
301-455-2263

JOHN C. WATSON
UNIV OF MD
DEPT OF BOTANY
COLLEGE PARK MD 20742
301-454-0242

JENNIFER WEBB
USDA-ARS
RM 275, B-011A
BELTSVILLE MD 20705
301-344-4003

JANE WEISEMANN
USDA-ARS, PMBL
BLDG 006 BARC WEST
BELTSVILLE MD 20705
301-344-3304

JOHN G. K. WILLIAMS
DUPONT EXPERIMENTAL STATION
E173/108
WILMINGTON DE 19880-0173
302-695-3674

BARBARA WILSON
USDA-ARS
PMBL
BELTSVILLE MD 20705
301-344-4376

CHRIS A. WOZNIAK
USDA, ARS
B-006, RM 118, BARC WEST
BELTSVILLE MD 20705
301-344-1267

DEOK-CHUN YANG
KOREA GINSENG & TOBACCO RESEARCH INST
302 SHINSEONG-DONG,JOONG-GU
TAEJEON, SOUTH KOREA
703-961-4145