Ninth Annual Meeting

1992

USDA/ARS, Beltsville, MD
July 27-28

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INTRODUCTION

Welcome to the ninth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and this meeting is to assemble scientists from the Mid-Atlantic region of the United States for the exchange of information and ideas in plant molecular biology. This meeting is designed to be reasonably priced and easily accessible so as to entice the greatest number of students, post-docs, and other scientists to attend and participate in presentations and discussions. In an attempt to attract even more participants, the mailing list was updated this year to include those individuals from this region who are listed in the current directory for the International Plant Molecular Biology Society.

We are proud to host this year's meeting at the USDA Beltsville Agricultural Research Center in the newly renovated auditorium and conference rooms of the Administration Building. BARC is undergoing exciting renovations. The Plant Molecular Biology Laboratory was one of the first buildings to be renovated. Among the planned improvements for the next three years are the renovation of the greenhouses and the addition of a new building for the Fruit, Vegetable, and Florist and Nursery Crops Laboratories .

Many people have been involved in organizing this meeting and we wish to express our thanks for the time and effort they have invested. It has paid off. We also wish to thank our sponsors and exhibitors whose generous contributions allow us to provide a high quality meeting with an exciting array of speakers at a minimal cost. Last, but not least, we thank you for your participation, and we hope you will continue to support the efforts of this society.

Frank Turano
Susan Koehler
Co-Organizers

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1992 MAPMBS Organizing Committees

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Camelia Smith	504-6478	USDA,SARL
Frank Turano	504-6145	USDA, CSL
Kathy Kamo	504-5350	USDA, FNCL
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Dave Straney	405-1622	UMCP, Botany
Registration		
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Sally Van Wert	436-7612	USDA, APHIS
Funding		
Deborah Loer	504-5258	USDA, PMBL
Karen Lewin	504-5304	USDA, PMBL
Local Arrangements		
Susan Koehler	504-6091	USDA,PMBL
Frank Turano	504-6145	USDA, CSL
Gregg Silk	504-5304	USDA, PMBL
Jian Ping Cheng	504-6478	USDA, SARL
Abstracts		
Greg Wadsworth	504-5304	USDA, PMBL
Gregg Silk	504-5304	USDA, PMBL
Susan Koehler	504-6091	USDA,PMBL
Frank Turano	504-6145	USDA, CSL
Cleo Hughes	504-5304	USDA, PMBL

GENERAL INFORMATION

HOLIDAY INN COLLEGE PARK

If you are staying at the College Park Holiday Inn on Route 1 and you made your reservation by July 15, then you should be receiving the reduced conference rate : $70.15 (single occupancy) or $41.98 (double occupancy)/per person. These prices include taxes, complimentary buffet breakfast at DiRico's restaurant (located at the hotel), one complimentary cocktail per day, free local phone calls, 10% off dinner entree at DiRico's restaurant, free cable TV, use of indoor pool, whirlpool, sauna, and exercise room. The hotel is located only 30 minutes from Washington D.C. The closest metro station is the Silver Springs Station.

SESSIONS

Registration, plenary sessions, poster sessions, exhibits and coffee breaks will be held at the USDA/ARS BARC-West in Building 003 (clock tower building on Circle Dr). Plenary sessions and the keynote address will be held in the auditorium. Poster sessions and exhibits will be held across the hall in the conference room, and the coffee breaks will be set up in the "cafeteria" (not yet occupied) across from the conference room. Park in any unmarked spots. Extra space is usually available to the right or below Bldg 006 behind Bldg 003.

LUNCHES, SOCIAL HOUR/RECEPTION, AND BANQUET

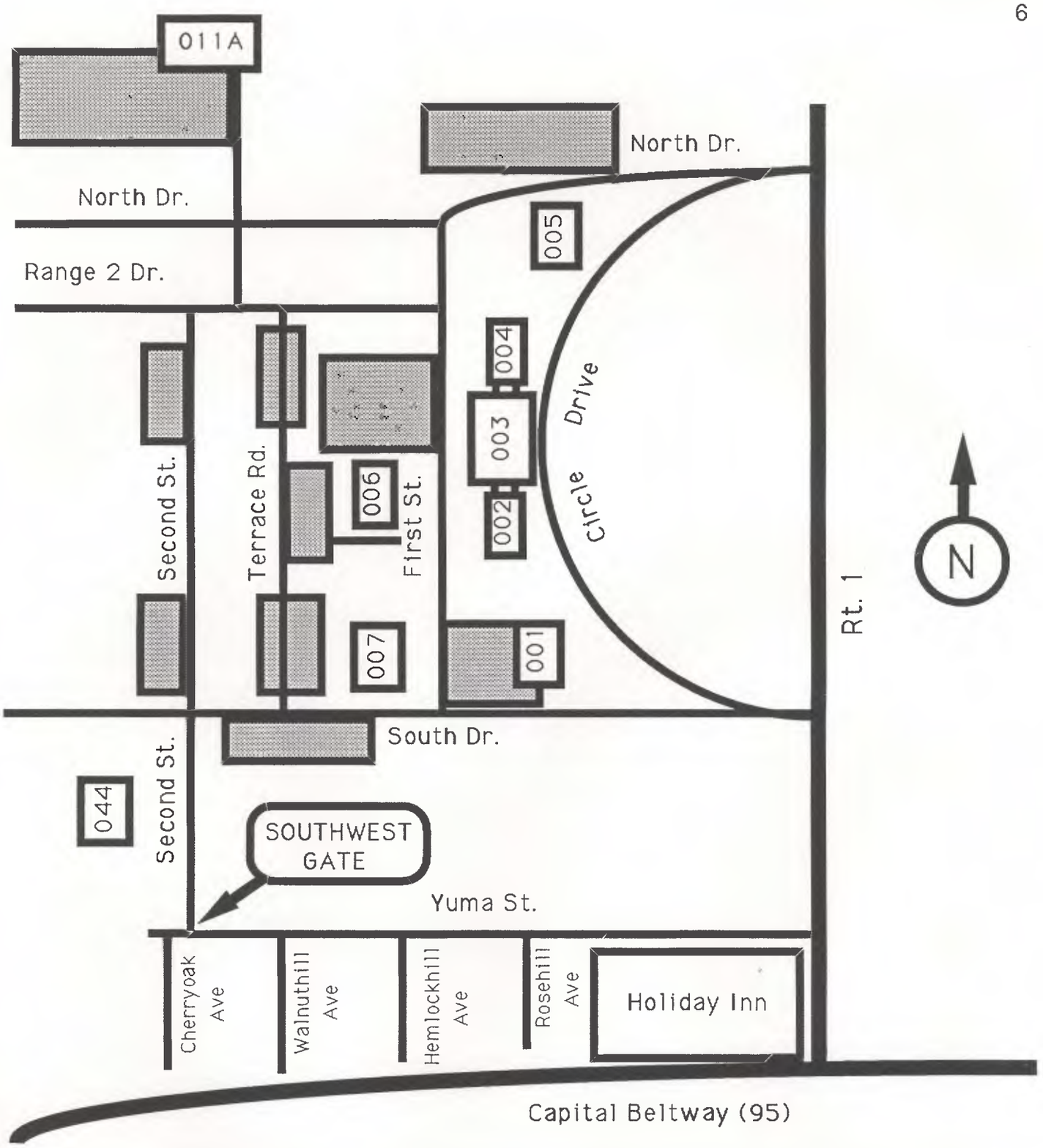
If you pre-registered, your lunches and banquet are included in the registration price. If you did not pre-register, you could not be included in our count for the catering service and so meals are not included, however several restaurants are within walking distance on Route 1 north of the USDA/BARC. The lunches, social hour, and banquet will be held in the Grand Ballroom of the Holiday Inn College Park which is a short walk (or drive) away. See directions below. Vegetarian selections will be available at all meals. The social hour before the banquet will feature hors d'oevres and a cash bar. At the banquet be sure to display the colored card indicating your entree selection which you should have received with your name tag during the meeting registration. This will allow the waiters to serve the meals in a more timely and efficient manner.

DIRECTIONS FROM THE MEETING SITE TO THE HOLIDAY INN

Exit through the back of Bldg 003 and turn left down First St. Turn right at the stop sign at South Dr. just next to Bldg 007. Turn left at Second St. Go past Bldg. 044 and left through the gate onto Yuma St. The Holiday Inn is located at the end of Yuma St. and Route 1. The gate at Yuma St. should be unlocked by 8:00 am on Monday and Tuesday, July 27-28, and closed by 8:00 pm on Monday and 6:00 pm on Tuesday.

FREE POST-MEETING TOUR OF BARC

Your BARC information packet contains extensive information about this 7200 acre research facility. An air-conditioned bus tour will leave from in front of Bldg 003 at 3:00 pm on Tuesday July 28. The bus will first tour BARC-West, where most of the plant, climate stress and virology research is conducted. The bus will return to Bldg 003 prior to continuing to BARC-East where most of the animal, insect, and human nutrition research is conducted. The bus will stop at the visitors center and then proceed back to Bldg 003. The entire tour will last approximately 1.5 hours.



USDA Buildings are identified by number. The meeting will be held in the Auditorium and Conference Room in Building 003.

The distance from the Holiday Inn to Bldg. 003 is approximately 0.4 miles via Rt.1 and approximately 0.5 miles via Yuma/Second/South/First St.

= Parking Lot

MAPMBS MEETING SCHEDULE

Monday, July 27, 1992

8:00 AM Registration

9:00 AM Opening Remarks - Frank Turano

REGULATION
(Moderator: John Watson, Univ. of Maryland)

9:05 AM Pamela Green, Thomas Newman, Masaru Ohme-Takagi, Crispin Taylor, Pauline Bariola, Christie Howard, Yang Yen, Pedro Gil and Wan-Ling Chiu (Michigan State Univ.) "Determinants of mRNA Stability in Higher Plants"

9:35 AM Benjamin Matthews, Jane Weisemann, Karen Lewin, Greg Wadsworth and Joan Gebhardt (USDA/ARS/PMBL) "Cloning and Analysis of cDNAs Encoding Bifunctional Aspartokinase-Homoserine Dehydrogenase Activies in Carrot and Soybean"

9:55 AM Zhenbio Yang, Carole Cramer and John Watson (Univ. of Maryland, College Park) "Molecular Cloning and Characterization of a cDNA Encoding a Pea Homolog of the Protein Farnesyltransferase β Subunit"

10:15-10:50 AM **COFFEE BREAK, POSTER SETUP & EXHIBITORS**

10:50 AM Teh-hui Kao (Penn. State Univ.) "Molecular Dissection of the Self-Incompatibility System in Solanaceae "

11:20 AM Deborah Loer and Eliot Herman (USDA/ARS/PMBL) "Molecular and Structural Studies of Soybean Oil Body Assembly"

11:40-1:00 PM **LUNCH**

PLANT/MICROBE INTERACTIONS
(Moderator: Dave Straney, Univ. of Maryland)

1:00 PM John Hammond and Kathryn Kamo (USDA/ARS/FNCL) "Potyvirus Resistance Derived from Antisense RNA and Native or Chimeric Coat Protein Genes"

1:30 PM Peter McGarvey, L.M. Geletka and J.M. Kaper (USDA/ARS/MPPL) "Transgenic Tomato Plants Resistant to Cucumber Mosaic Virus Through the Expression of Satellite RNA and Coat Protein"

1:50 PM Gusui Wu and J.M. Kaper (Univ. of Maryland, College Park) "Widely Separated Sequence Elements Within Cucumber Mosaic Virus Satellites Determine Their Ability to Induce Lethal Tomato Necrosis"

2:10 PM Jan Leach (Kansas State Univ.) "Genetics and Physiology of Resistant Interactions Between Bacteria and Rice"

2:40 PM Yingxian Xiao, Y. Lu , S. Heu and S.W. Hutcheson, (Univ. of Maryland, College Park) "Regulation of Gene Expression and Molecular Characterization of *Pseudomonas syringae pv. syringae* HRPIII Gene"

3:00 PM Teresa Synder, Paul Fritz and Douglas Furtek (Penn. State Univ.) "Isolating a Genomic Chitinase Clone from *Theobroma cacao* Using a PCR Generated Probe"

3:20-4:30 PM **COFFEE BREAK, POSTER SESSION & EXHIBITORS**

KEYNOTE ADDRESS
(Introduction: Nichole O'Neill, USDA/ARS)

4:30 PM Richard Dixon, Maria Harrison, Christopher Lamb, Gary Loake, Carl Maxwell, Weiting Ni, Abraham Oommen, Nancy Paiva and Lloyd Yu (The Samuel Roberts Noble Foundation) "Transcriptional Control of Phenylpropanoid Biosynthesis"

5:45-6:45 PM **SOCIAL HOUR**

6:45 PM **DINNER**

Tuesday, July 28, 1992

8:00 AM Open Viewing of Posters

8:50 AM Business meeting

REGULATION
(Moderator: Marvin Edelman, Weizmann Institute, Israel)

9:00 AM Eric Lam, Olga Perisic and Luis Romero (Rutgers State Univ.) "Molecular Analysis of Light Regulated Gene Expression"

9:30 AM Mauricio Bustos, Helen Murphy and Maw-Shenq Chern (Univ. of Maryland, Baltimore County) "Two Conserved Regions of the Phaseolin Seed-Specific Enhancer UAS1 Interact *In-vitro* with Nuclear Proteins from Developing Bean Embryos"

9:50-11:00 AM **COFFEE BREAK, POSTER SESSION & EXHIBITORS**

11:00 AM Howard Hershey, Mark Chapman, Hugo Dooner, James English, Edward Ralston, Sonja Schmitz, Timothy Stoner and James Wong (Du Pont) "Development of a Chemically-Inducible Gene Expression System for Transgenic Plants"

11:30 AM Hortense Dodo, Paul Fritz and Douglas Furtek (Penn. State Univ.) "Cloning and Sequencing of a Gene Encoding a 21 kDa Trypsin Inhibitor from *Theobroma cacao*"

11:50-1:10 PM **LUNCH**

REGULATION
(Moderator: Sally Van Wert, USDA/APHIS)

1:10 PM Elizabeth Kemmerer, Susan Baird, Panos Kalaitzis, Susan Koehler, Gail Matters, Roy Sexton and Mark Tucker (USDA/ARS/PMBL) "Hormonal and Tissue-Specific Regulation of Cellulases"

1:30 PM Lauren McHenry and Mark Guiltinan (Penn. State Univ.) "Transgenic Tobacco Plants Expressing an Epitope-Tagged DNA Binding Protein"

TRANSFORMATION

(Moderator: Sally Van Wert, USDA/APHIS)

1:50 PM	Rob Griesbach and John Hammond (USDA/ARS/FNCL) "Transformation of Orchid Embryos via Electrophoresis"
2:20 PM	Maud Hinchee, Fred Perlak, Yvonne Muskopf, Dave Stark, Toni Armstrong, Mike Fromm, Chuck Armstrong, Harry Klee, Jeanne Layton, Steve Padgette, Renee Rozman, Xavier Delannay, Rob Horsch and Rob Fraley (Monsanto) "Plant Transformation and Crop Improvement"
2:50 PM	Closing Remarks - Susan Koehler
3:00 PM	Tour of BARC

POSTER SESSIONS

(Monday 3:20-4:30 PM & Tuesday 9:50-11:00 AM)

Poster (page)

1 (32) "Recombinant Fab Antibody Fragments Which Bind Bean Yellow Mosaic Potyvirus Coat Protein" Leslie M. Palmer and Ramon L. Jordan (USDA, Beltsville)

2 (33) "The Nucleotide Sequence of a Cucumber Mosaic Virus Strain Deficient in Satellite RNA Replication" P.B. McGarvey, F. Cellini, M. Tousignant and J.M. Kaper (USDA, Beltsville/Metopontum Agrobios, Italy)

3 (34) "Agrobacterium-Mediated Cotransformation and Inheritance of Multiple Genetic Markers" Hema Bandaranayake and Paul J. Bottino (Univ. of Maryland, College Park)

4 (35) "Akinete Differentiation and Germination" Shane Hardin and Bob Fisher (Virginia Commonwealth Univ.)

5 (36) "Microsurgical and Hormonal Analyses of an Arabidopsis Mutant Altered in Organ Elongation" Roxanne H. Fisher, Kathy Barton, Jerry D. Cohen, R. Scott Poethig and Todd J. Cooke (USDA, Beltsville/Univ. Pennsylvania/Univ. of Maryland, College Park)

6 (37) "Effects of Genomic Methylation on RAPD Patterns in *Brassica oleracea*" Steven J. Gagliardi, C. Lan and Fang-Sheng Wu (Virginia Commonwealth Univ.)

7 (38) "Use of Polymerase Chain Reaction and Serology for Detecting Heat Shock Proteins in Heat-Sensitive and Tolerant Tomato Genotypes" Magdy S. Montasser, Aref A. Abdul-Baki and Benjamin F. Matthews (USDA, Beltsville)

8 (39) "Application of the Diffusible Factor Hypothesis in the Induction of *a*-amylase During Seed Germination in Two Cultivars of Rice" Mary A. Smith and Angela Mosley (Univ. of Richmond)

9 (40) "Photosystem I Protein PsaL cDNA Clone from Maizé" Alan H. Christensen, Jennifer Stevens and Peter H. Quail (George Mason Univ./Univ. of CA Berkeley/USDA Plant Gene Expression Center)

10 (41) "Deletion Analysis of a Polyubiquitin Gene (UBI-l) Promoter from Maize" Donna M. Fox and Alan H. Christensen (George Mason Univ.)

11 (42) "Gene Structure of APRT in *Arabidopsis thaliana*" Sunita K. Agarwal, Barbara A. Moffatt and Dennis A. Schaff (Univ. of Delaware/ Univ. of Waterloo)

12 (43) "Cloning of Soybean Asparagine Synthetase Gene" Cleo A. Hughes and Benjamin F. Matthews (USDA, Beltsville)

Poster (page)

13 (44) "Use of PCR in the Molecular Cloning of the Soybean Gene Encoding Dihydrodipicolinate Synthase" Gregg W. Silk and Benjamin F. Matthews (USDA, Beltsville)

14 (45) "Identification of Transcripts Expressed in the Corn Root Primary Response to Environmental Nitrate" Margaret Redinbaugh, Jacquline M. Vrba-Kohler and Wilbur H. Campbell (USDA/NC State Univ./Michigan Technological Univ.)

15 (46) "Nucleotide Sequence and Organization of Soybean Chalcone Synthase Multi-Gene Family" Shinji Akada, Shain-Dow Kung and Shyam K. Dube (Univ. of Maryland, College Park)

16 (47) "Organization and Expression of Phenylalanine Ammonia-Lyase Genes in Tobacco *(Nicotiana tabacum)*" Tomoko Fukasawa-Akada, Shyam K.Dube and Shain-Dow Kung (Univ. of Maryland, College Park)

17 (48) "Primary Structure and Expression of S-RNase Homologs in *Arabidopsis thaliana*" Pauline A. Bariola, Crispin B. Taylor, Michael T. Verburg and Pamela J. Green (MSU-DOE)

18 (49) "Cloning and Characterization of Genes Expressed During Carrot Somatic Embryogenesis" Xiaoying Lin, Gwo-Jiunn H. Hwang and J. Lynn Zimmerman (University of Maryland, Baltimore County)

19 (50) "Photoregulated Expression and Sequence Analysis of PsPK5, a Protein Kinase Homolog from *Pisum sativum* L." Xia Lin and John C. Watson (Univ. of Maryland, College Park)

20 (51) "Changes in Gene Expression Associated with Chilling-Unit Accumulation in Different Blueberry Varieties" Muthalif M. Mubarack and Lisa J. Rowland (USDA, Beltsville/Univ. of Maryland, College Park)

21 (52) "Induction of l-Aminocyclopropane-l-Carboxylate Synthase in Tobacco Treated with an Ethylene Biosynthesis-Inducing Xylanase" Adi Avni, Bryan A. Bailey, Autar K. Mattoo and James D. Anderson (USDA, Beltsville)

22 (53) "A Wound-Repressed Tomato cDNA Clone Encodes a Glycine-Rich Protein Transcript that is Expressed in Vascular Tissue" Barbara L. Parsons and Autar K. Mattoo (USDA, Beltsville)

DETERMINANTS OF mRNA STABILITY IN HIGHER PLANTS

Pamela J. Green*, Thomas C. Newman, Masaru Ohme-Takagi, Crispin B. Taylor, Pauline A. Bariola*, Christie Howard*, Yang Yen, Pedro Gil, and Wan-Ling Chiu. MSU-DOE Plant Research Laboratory and *Department of Biochemistry, Michigan State University, E. Lansing, MI 48824-1312.

As a first step towards elucidating the fundamental principles that govern mRNA stability in higher plants, we have begun to identify mRNA sequences and cellular factors that facilitate general and selective mRNA decay. Certain transcripts are known to be very unstable in plants and we have been particularly interested in the identification of the mRNA sequences that mediate this effect. To this end we have developed a system to measure directly mRNA decay rates in stably transformed tobacco cells and have used this system to measure the stability of a variety of endogenous and genetically engineered transcripts. These studies have identified two different kinds of sequences that cause mRNA instability when inserted into the 3' untranslated region of reporter genes. The first instability sequence, DST, derives from the Small Auxin Up RNA (SAUR) genes of soybean (McClure et al., 1989, Plant Cell, 1:229-239) which are known to encode unstable transcripts. The second element is a synthetic sequence consisting of multiple copies of the AUUUA motif, considered to be an mRNA instability determinant in mammalian cells. Our experiments have shown that multiple copies of the AUUUA motif or the DST sequence are sufficient to markedly destabilize GUS and globin reporter transcripts in stably transformed tobacco cells. These sequences also decrease transcript accumulation in regenerated plants, indicating that repeats of the AUUUA motif and the DST sequence can destabilize transcripts in transgenic tobacco. Among the cellular factors that participate in mRNA degradation, ribonucleases (RNases) undoubtedly play a major role. Using a substrate-based gel assay we have identified the major RNases of *Arabidopsis* and have studied their biochemical properties. Our long term goal is to use RNase mutants to determine which activities are important for mRNA decay, and a screen for such mutants is in progress. In addition, we have identified three RNase genes (RNS1, RNS2, and RNS3) from *Arabidopsis* that are homologous to the S-RNases associated with self-incompatibility. The expression of the RNS genes in *Arabidopsis*, which is self-compatible, suggests that these RNases have a more general function in RNA metabolism. Our data indicate that the RNS gene products and related "S-like" RNases from self-compatible plants, diverged from the S-RNases before speciation. Expression studies support a role for RNS2 during senescence in *Arabidopsis*.

Cloning and analysis of cDNAs encoding bifunctional aspartokinase-homoserine dehydrogenase activities in carrot and soybean.

Benjamin F. Matthews, Jane M. Weisemann, Karen M. Lewin, Greg J. Wadsworth and Joan S. Gebhardt. USDA ARS, Plant Molecular Biology Laboratory, Beltsville, MD 20705.

The essential amino acids, lysine, threonine, methionine and isoleucine are derived from aspartate. Aspartokinase, the first enzyme committed to the synthesis of these amino acids, converts aspartate to β-aspartyl phosphate. In many plants aspartokinase activity is inhibited by the pathway end products, lysine and threonine. The third enzyme in the pathway, homoserine dehydrogenase, is the branch-point enzyme leading to threonine and methionine production and is feedback inhibited by threonine.

Aspartokinase is encoded by one or more genes in most bacteria as is homoserine dehydrogenase. In contrast, E. coli possesses two isozymes, AKI-HSDHI and AKII-HSDHII, which contain aspartokinase and homoserine dehydrogenase activities on the same bifunctional protein. E. coli also has a third aspartokinase activity, AKIII, which isn't bifunctional.

Our laboratory has cloned cDNAs encoding bifunctional aspartokinase-homoserine dehydrogenase (AK-HSDH) activities from carrot and soybean. A bifunctional AK-HSDH was purified from carrot cell suspension cultures and oligonucleotide primers based on amino acid sequences of this enzyme were used to amplify a portion of the gene from carrot cDNA. The amplified fragment was used to screen a carrot cDNA library to identify homologous clones. Two overlapping clones were isolated. The complete cDNA is >3000 bp containing a single open reading frame. Soybean cDNA clones were isolated using the carrot cDNA probe. A comparison of the carrot and soybean DNA sequences indicates >85% homology between the AK-HSDH cDNAs in the region encoding the mature protein. The carrot and soybean cDNAs are more highly homologous to the E. coli thrA gene (AKI-HSDHI) than to the metL gene (AKII-HSDHII). A portion of the carrot and soybean cDNAs encode for chloroplast transit peptides.

Enzyme-specific staining of electrophoretic gels demonstrates the presence of four HSDH isozymes in soybean. Preliminary molecular analysis suggests that there may be two or more genes encoding these enzyme activities in soybean. This is suggested by results of differential screening of genomic libraries and Southern blots.

MOLECULAR CLONING AND CHARACTERIZATION OF A cDNA ENCODING A PEA HOMOLOG OF THE PROTEIN FARNESYLTRANSFERASE β SUBUNIT

Zhenbiao Yang*, Carole L. Cramer†, and John C. Watson*§‡
*Department of Botany, §Maryland Agricultural Experiment Station, and ‡Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742-5815
†Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Protein farnesyltransferase (FTase) is a heterodimeric enzyme that post-translationally modifies proteins by attaching a farnesyl moiety to a C-terminal cysteine residue. The isoprenylation is a prerequisite for membrane association and *in vivo* activity of many regulatory proteins such as a family of *ras* proteins. Genes encoding both the α and β subunits of the FTase have recently been cloned and characterized from yeast and mammalian cells. To isolate a plant homolog, degenerate oligonucleotides, corresponding to the conserved regions of the β subunit, were used as primers for polymerase chain reactions to amplify pea cDNA. Amplification of cDNA synthesized from total celluar RNA isolated from pea buds generated a 171 bp fragment with an open reading frame of 57 amino acids that shows 65% identity to the rat β subunit. Using the 171 bp fragment as hybridization probe to screen a pea cDNA library, one full-length cDNA clone was obtained that contains an open reading frame encoding a polypeptide of 419 amino acids. The predicted amino acid sequence exhibits 48% and 40% identity to the rat and yeast β subunits, respectively, indicating that the cloned cDNA encodes a pea homolog of the FTase β subunit. Genomic DNA gel blot analysis suggests that the pea homolog is encoded by a single gene. RNA gel blot analysis shows that the pea FTase β subunit is expressed as a single transcript of approximately 1.7 kb in the buds of pea seedlings. Preliminary results suggest that levels of the β subunit transcript increase rapidly and transiently just at the onset of active cell division both in the apical buds of pea seedlings and in tomato cell cultures.

MOLECULAR DISSECTION OF THE SELF-INCOMPATIBILITY SYSTEM IN SOLANACEAE: Teh-hui Kao, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802.

Self-incompatibility is an intraspecific reproductive barrier which prevents crosses between genetically related individuals of the same species. Gametophytic self-incompatibility is a major type of self-incompatibility, and has been best studied in solanaceous species. In the simplest case, it is controlled by a single multi-allelic locus, the S-locus. Pollen bearing an S-allele identical to one of the two alleles carried by the pistil suffers growth arrest in the style. To address the questions of the mechanism of self-recognition and self-rejection, the S-allele product (S-protein) in the pistil has been identified in a number of solanaceous species. To date, cDNAs for 21 S-proteins from 6 solanaceous species have been reported, and comparison of the deduced amino acid sequences has revealed primary structural features of the S-protein and the evolution of S-allele polymorphism. One surprising finding is the sequence similarity between S-proteins and RNase T1 and RNase Rh of fungi. A number of S-proteins have now been shown to be ribonucleases; the enzymatic properties of three Petunia inflata S-proteins have been characterized. P. inflata pistils appear to contain a large number of ribonucleases, in contrast to leaf and petal which contain relatively few. One of these ribonucleases, designated RNase X2, has been characterized, and cDNA and genomic clones encoding it have been isolated and sequenced. RNase X2 shares similar properties and sequences with S-proteins, yet it is distinct from S-proteins, and is most likely not associated with self-incompatibility. Comparison of two alleles of P. inflata S-gene revealed that the flanking regions are extremely heterogeneous and rich in repetitive sequences. These results coupled with statistical analysis of nucleotide sequences suggest that recombination is suppressed at the S-locus to allow S-alleles to be stably maintained in a population. The cause of the breakdown of self-incompatibility in a cultivar of P. hybrida is being studied by examining the inheritance of self-incompatibility in interspecific hybrids between it and self-incompatible P. inflata. Results obtained so far have led to the formulation of a model which invokes a modifier locus, in addition to the S-locus, for the genetic control of self-incompatibility. This model postulates that two independent mutations, one at the S-locus and the other at the modifier locus, are responsible for the breakdown of self-incompatibility in this cultivar of P. hybrida.

MOLECULAR AND STRUCTURAL STUDIES OF SOYBEAN OIL BODY ASSEMBLY. Deborah S. Loer and Eliot M. Herman. USDA/ARS, Plant Molecular Biology Laboratory, Beltsville, MD 20705

Soybean seed oil accumulates as oil bodies, cytoplasmic droplets of triglycerides bounded by a monolayer of phospholipids in which a few distinct proteins (oleosins) are embedded. We are interested in studying the ontogeny of oil bodies and the mechanism of their assembly because of their importance to triglyceride accumulation and storage. We have focused on investigating the role of the endoplasmic reticulum (ER) in oil body assembly. Our laboratory has isolated and characterized a cDNA clone for the 24 kDa soybean oleosin (Kalinski, et al. PMB 17: 1095-1098, 1991). The oleosin cDNA clone was used to synthesize mRNA *in vitro*, which was then translated in a reticulocyte system in the presence of canine microsomes. After translation was completed, microsomes were separated from soluble components of the lysate by centrifugation through a sucrose gradient and gradient fractions were analyzed by SDS-PAGE fluorography. Our results show that oleosin is specifically associated with the microsomal fraction and that oleosin remains associated even after extraction with sodium carbonate, indicating that oleosin can integrate into a bilayer membrane. We found that there is a cotranslational requirement for microsomes in order for membrane association to occur, indicating the 24 kDa soybean oleosin is synthesized on the ER. A new model of oil body assembly involving conformational changes of the oleosin protein through transient association with the ER membrane is proposed.

POTYVIRUS RESISTANCE DERIVED FROM ANTISENSE RNA AND NATIVE OR CHIMERIC COAT PROTEIN GENES. John Hammond and Kathryn K. Kamo, USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705.

An antisense (AS) RNA construct consisting of the C-terminal portion of the coat protein (CP) gene and complete 3' non-coding sequence of bean yellow mosaic virus (BYMV), and driven by the cauliflower mosaic virus (CaMV) 35S promoter, was used to obtain transgenic Nicotiana benthamiana plants by Agrobacterium-mediated transformation. Other plants were transformed with constructs designed to express the BYMV CP gene or chimeric CP genes. The original transformants from each construct were allowed to self. R1 plants carrying the introduced gene were selected on the basis of polymerase chain reaction (PCR) and/or ELISA (for CP-expressing plants) with monoclonal antibodies. Homozygous R2 populations were selected similarly, and then challenged with various potyviruses to determine the levels and types of resistance conferred by each construct.

Plants expressing AS RNA displayed a range of resistance, from minimal to apparent immunity from infection by BYMV; no resistance was observed to other potyviruses. Differences between individual transformants possibly reflect variation in the level of RNA expression, or tissue specificity of expression.

Plants expressing native BYMV CP also showed a range of resistance, with a minimal degree of resistance to other potyviruses. Chimeric CPs, with the N-terminal domains of BYMV fused to the C-terminal domains of pepper mottle potyvirus or zucchini yellow mosaic potyvirus, differed in their response to challenge with several potyviruses. At least one transformant of each chimeric CP showed milder symptoms than non-transgenic controls when inoculated with BYMV, and some resistance to potato virus Y. Differences between individual transformants may reflect variations in levels and sites of CP expression.

Resistances to initial infection and to replication and/or systemic spread were each observed in both AS and CP plants. Deleted constructs are being prepared with the aims of separating the two types of resistance, determining the mechanisms of resistance, and which domains confer viral specificity.

TRANSGENIC TOMATO PLANTS RESISTANT TO CUCUMBER MOSAIC VIRUS (CMV) THROUGH THE EXPRESSION OF SATELLITE RNA AND COAT PROTEIN

P.B. McGarvey, L.M. Geletka, and J.M. Kaper
Microbiology and Plant Pathology Laboratory, PSI, ARS, USDA
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Cucumber mosaic virus (CMV) is one of the most economically damaging virus to field grown vegetables in the world. Recent outbreaks have occurred in China, Italy, and Spain, resulting in large crop losses. The United States has also suffered from CMV outbreaks on a smaller scale. The goal of our research is to produce transgenic tomato plants resistant to CMV.

Satellite RNAs are small RNA molecules found in association with many plant viruses and can characteristicly modify the diseases induced by the virus. In most cases the satellite RNA will reduce the severity of viral infections either when coinoculated with the virus or when satellite sequences are expressed in transgenic plants. The expression of coat protein genes in transgenic plants has also been shown to prevent or delay viral infection in a number of plant viral systems.

We have used Agrobacterium mediated plant transformation to produce transgenic tomato plants that express CMV satellite RNA and plants that express CMV coat protein. F1 generation plants were challenge inoculated with purified virus and purified viral RNA and their resistance evaluated biologically via symptomatology and molecularly via virus replication. The results of these experiments will be presented.

Widely separated sequence elements within cucumber mosaic virus satellites determine their ability to induce lethal tomato necrosis

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To determine the structural requirements for cucumber mosaic virus (CMV) satellites (designated CARNA 5 for CMV-Associated RNA 5) to elicit lethal tomato necrosis, three variants D-, S- and Y-CARNA 5 were used in the construction and cloning of chimeric cDNAs. D- and S-CARNA 5 are necrogenic and nonnecrogenic prototypes, respectively, and Y-CARNA 5 possesses the 3' conserved necrosis determining region but does not cause lethal tomato necrosis. Its 5' half harbors a insertion/deletion region that results in a molecule about 30 nucleotides longer than other CARNA 5 variants. Tomato bioassays were conducted with RNA transcripts of all six chimeric combinations of the 5' and 3' halves of Y-, D- and S-CARNA 5 divided by a common restriction site, as well as with a mutated CARNA 5 chimera. None of the chimera containing the 5' half of Y-CARNA 5 induced lethal necrosis in tomato even when their 3' halves possessed the conserved necrogenic element. Chimera with the 3' half of Y-CARNA 5 elicited only partial or restricted necrosis which was much less severe than that induced by prototype necrogenic D-CARNA 5, and often was not lethal. Site-directed mutation of a single nucleotide in proximity to the necrogenic element of such a chimera containing the 5' half of Y-CARNA 5, restored much lethal necrogenicity. The results reveal the presence of structural elements in Y-CARNA 5 that modulate or even suppress the expression of the 3' conserved necrosis determining element. They indicate that in CMV satellites widely separated sequence elements constituting a three dimensional requirement are responsible for eliciting lethal necrosis in tomato.

GENETICS AND PHYSIOLOGY OF RESISTANT INTERACTIONS BETWEEN BACTERIA AND RICE. Jan E. Leach, Kansas State University, Department of Plant Pathology, Manhattan, KS 66506-5502.

Xanthomonas oryzae pv. *oryzae*, the bacterial blight pathogen of rice, contains three avirulence genes (*avrxa5*, *avrXa7* and *avrXa10*) that are related to *avrBs3*, an avirulence gene from the pepper pathogen *X. campestris* pv. *vesicatoria*. The gene *avrXa10*, which specifies the resistant phenotype on rice cultivars with the bacterial blight resistance gene *Xa-10*, like *avrBs3*, contains a 102 bp repeat sequence, although *avrXa10* contains fewer copies than *avrBs3* (15.5 vs. 17.5). Each 102 bp repeat of *avrXa10* contains a six nucleotide variable region, and the order of the repeat units within *avrXa10* is distinct from *avrBs3*. The number of repeat copies in *avrXa7* was estimated at 25. Experiments are in progress to determine the location of the avirulence genes within the bacteria and the portions of the genes responsible for avirulence activity. The physiological events associated with resistant interactions were investigated using the cloned avirulence genes and near-isogenic rice cultivars with single bacterial blight resistance genes. Resistance in rice to *X. oryzae* pv. *oryzae* is characterized by an increase in the extracellular activity of three peroxidases, lignin deposition, host cell death, and a decrease in the rate of bacterial multiplication. The timing of these events is dependent on the specific avirulence gene - resistance gene interaction, that is, the responses associated with resistance occur later in interactions involving *avrxa5-xa-5* and *avrXa7-Xa-7* than those between *avrXa10* and *Xa-10*.

REGULATION OF GENE EXPRESSION AND MOLECULAR CHARACTERIZATION OF PSEUDOMONAS SYRINGAE PV. SYRINGAE HRPIII GENE.

Xiao, Y., Y. Lu*, S. Heu and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742. *Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

We have previously reported the isolation of a 31 kb hrp (hypersensitive response and pathogenicity) gene cluster from Pseudomonas syringae pv. syringae 61 that enables nonpathogenic bacteria to elicit the hypersensitive response in tobacco leaves. TnphoA and Tn5-gusA1 mutageneses have shown that this gene cluster contains at least 13 hrp genes and one hrm gene. These loci are organized into at least 9 apparent transcriptional units. Enhanced expression of hrp-uidA fusions were detected in the seven of these transcriptional units after bacteria were inoculated into tobacco leaves. To understand the mechanism of gene regulation, we constructed a hrpIII-uidA transcriptional fusion . A plasmid, pYX5112-3-S, which carries the fusion, was conjugated into 13 different Pseudomonas syringae pv. syringae 61 hrp/hrm::TnphoA mutants. The expression of the hrpIII-uidA transcriptional fusion decreased in six of the 13 mutants. The mutated hrp/hrm loci within the six mutants appear to be required for activation of hrpIII expression.

Nine kb DNA fragment from the left part of the cluster has been sequenced. In the region corresponding to hrmA, hrpI, hrpII, hrpIII and hrpIV, there are 5 open reading frames which encode 5 putative proteins with the predicted size 41kD, 68kD, 42kD, 40kD and 67kD, respectively. The results from sequence analysis of hrpIII and related region will be discussed.

ISOLATING A GENOMIC CHITINASE CLONE FROM THEOBROMA CACAO USING A PCR GENERATED PROBE: Teresa Snyder, Paul Fritz and Douglas Furtek, ACRI Cocoa Molec.Biol. Lab, Penn State University, University Park, PA 16802.

In approximately 40 tropical countries, over five million people grow T. cacao, the only source of chocolate and cocoa butter. Crop loss from fungal and viral diseases greatly reduces income and completely decimates the crop during severe outbreaks. Thus, a major goal of breeders is to develope disease resistant varieties. Chitinases have hydrolytic activity that degrades fungal cell walls and releases oligosaccharides that elicit defense responses in many plants.

To isolate a chitinase clone from T. cacao, we obtained a 115 bp probe using polymerase chain reaction (PCR). Primers were designed from highly conserved chitinase sequences from other plants. First strand cDNA from pod and seeds was used as the template. The probe is 100% identical to a tobacco sequence at the amino acid level and highly homologous to bean, potato and poplar chitinases. Initial screening of a genomic library yielded seven positive clones. One of these clones has been subcloned into a Bluescript vector for sequencing.

Initial data indicates the genomic clone sequence is most highly homologous to tobacco, potato, and rice chitinases, and a poplar wound induced protein. This is the first genomic clone isolated from T. cacao that may have an impact on disease resistance. Future characterization and use of the clone will be discussed.

TRANSCRIPTIONAL CONTROL OF PHENYLPROPANOID BIOSYNTHESIS. Richard A. Dixon[1], Maria J. Harrison[1], Christopher J. Lamb[2], Gary Loake[1,2], Carl A. Maxwell[1], Weiting Ni[1], Abraham Oommen[1], Nancy L. Paiva[1] and Lloyd M. Yu[1,2].
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Phenylpropanoid biosynthesis in legumes represents an excellent model system for understanding the complex control mechanisms which underlie the developmental and environmental regulation of plant secondary metabolism. The central phenylpropanoid pathway leads to the synthesis of a range of functionally distinct plant phenolic compounds from the amino acid L-phenylalanine. In legumes, these include antimicrobial isoflavonoid phytoalexins induced in response to pathogenic fungi and bacteria, chalcones and flavones which induce the nodulation genes of *Rhizobium meliloti*, UV-protective flavonoids, soluble and cell-wall-bound hydroxycinnamic acids and the phenylpropane polymer lignin. Many of the biosynthetic enzymes involved in the elaboration of these important secondary metabolites have been characterized.

We have cloned genes encoding L-phenylalanine ammonia-lyase (first committed step in phenylpropanoid synthesis), chalcone synthase (first enzyme of the flavonoid branch pathway), chalcone isomerase (flavonoid branch), chalcone 2'-O-methyltransferase (synthesis of a *nod* gene inducer), isoflavone reductase (phytoalexin pathway) and caffeic acid O-methyltransferase (lignin pathway) from bean or alfalfa. Their induction in response to developmental and environmental cues is the result of increased transcriptional activation. We review the tissue-specific expression patterns, induction kinetics and genomic organization of these genes. Deletion and mutational analyses utilizing stable transformation and transient assay systems have defined *cis*-elements required for quantitative expression level, developmental expression, inducibility by microbial elicitors and modulation by phenylpropanoid pathway intermediates. These elements include GT-1-like enhancer/silencer regions, the G-box (core consensus CACGTG) and the H-box (CCTACC(N_7)CT(N_4)A). Three *trans*-acting factors which specifically recognize some of these elements have been purified to near homogeneity. The role of such factors in the integration of developmentally and environmentally modulated signal transduction pathways will be discussed.

MOLECULAR ANALYSIS OF LIGHT REGULATED GENE EXPRESSION: Eric Lam, Olga Perisic and Luis Romero; AgBiotech Center, Box 759, Waksman Institute, Rutgers State University, Piscataway, NJ 08854.

Light plays an indispensible role in the life-cycle of plants. In addition to providing the energy for photosynthesis, it is also a critical environmental signal that regulates processes as diverse as seed germination and flowering. Although the photoreceptor phytochrome has been studied extensively since its discovery in the 1950's, the mechanism of its action remains largely unknown. One level of phytochrome control that has been well-studied in recent years is that of gene expression. In our laboratory, we are interested to elucidate the signal transduction pathway by which activated phytochrome (Pfr) modulate transcription. We hope this approach may serve as a paradigm for plant receptor function and transcriptional control.

Cis- and Trans-acting Elements for Light-Responsive Transcription. To regulate gene expression at the transcription level, light must somehow modulate the activity of certain cis- and trans-acting elements. One of the most well-studied cis-element that is involved in light-responsive transcription is the so-called Box II element, originally defined in the promoter of pea rbcS (small subunit of ribulose-1,5-bisphosphate carboxylase) genes. From mutational and chimeric promoter studies in transgenic tobacco, Box II is likely a positive element that is critical for light-regulated transcription. To elucidate the mechanism of action for Box II, we are interested to characterize the nuclear factors that interact specifically with this sequence. To this end, we have now isolated a tobacco cDNA clone that encodes a protein which interacts with Box II *in vitro*. Properties of this cDNA clone will be reported.

Signal Transduction Intermediates Between Pfr and Light-Responsive Promoters. The study of signal transduction events in plants has been hampered by the complexity of *in Planta* assays. The use of homogeneous cell suspensions will avoid problems such as heterogeneity of cell-types and accessibility of exogenously-applied modulators of enzyme action. We have found previously that a photoautotrophic soybean cell suspension, SB-P, retains characteristics that are quantitatively and qualitatively similar to that of soybean leaves. Using this cell suspension, we now demonstrated that well-characterized G-protein modifiers can uncouple light-regulated gene expression from the control of phytochrome. From a combination of activator and inhibitor studies, we propose that Pfr may act through a Gs-type heterotrimeric G-protein to activate a calmodulin-dependent enzyme. A working model of light-responsive gene expression that incorporate the present findings and recent results from other laboratories will be discussed.

Two conserved regions of the phaseolin seed-specific enhancer UAS1 interact *in-vitro* with nuclear proteins from developing bean embryos. Mauricio M. Bustos, Helen Murphy and Maw-Shenq Chern, Dept. of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, 21228.

Our laboratory is investigating *cis*- and *trans*-acting factors involved in seed-specific regulation of storage protein genes from the common bean (*P. vulgaris*). An enhancer-like element (UAS1, nucleotides -295 to -109) from a β-phaseolin gene is necessary and sufficient for spatially and temporally regulated transcription in tobacco [1]. This element contains two sequences, Vb1 and Vb2, homologous to the "vicilin-box" conserved in vicilin-like storage globulin genes from pea, bean and soybean [2]. We have found that multiple proteins present in nuclear protein fractions from immature (10-14 mm) bean cotyledons bind to both Vb elements in a sequence-specific manner, and that Vb2-containing DNA fragments partially compete for protein binding to the Vb1 sequence. At least two DNA binding activities were distinguished by their behavior on a Macro-Prep 50 Q anion-exchange chromatographic support. Methylation interference experiments indicate close major groove contacts between some of the proteins and guanines of the Vb2 sequence. Similar contacts were not observed with Vb1 suggesting that the proteins bind to each sequence in a different fashion. We are using PCR and oligonucleotide-directed mutagenesis to ascertain the contribution of individual motifs to overall binding affinity. We will report on the progress made to purify vicilin-box binding proteins (Vb-BPs) from staged bean embryos by standard and oligo-affinity chromatography and on our efforts to clone complementary DNA sequences for Vb-BPs by screening two different expression cDNA libraries from bean embryo polyA$^+$RNA using Vb1 and Vb2 DNA probes.

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DEVELOPMENT OF A CHEMICALLY-INDUCIBLE GENE EXPRESSION SYSTEM FOR TRANSGENIC PLANTS

Howard P. Hershey, Mark Chapman*, Hugo Dooner*, James English*, Edward Ralston*, Sonja Schmitz, Timothy Stoner and James Wong. Du Pont Agricultural Products, Wilmington, DE 19880 and *DNA Plant Technology Corp., Oakland, CA 94608

Several cDNA clones representing maize mRNA species which are induced by substituted benzenesulfonamides have been isolated. Studies examining the induction of these maize sequences showed good specificity of induction in a wide variety of organ systems following chemical treatment and a rapid appearance and disappearance of induced mRNAs. Genomic clones for two of these cDNAs, designated In2-1 and In2-2, have been isolated and characterized. The promoter sequences from these two genes were isolated and fused to several reporter genes. The expression of the resulting recombinant genes was analyzed by transient expression in a monocot protoplast transformation system. In this manner, the locations of cis-acting elements within the inducible promoters that are necessary for chemical responsiveness were identified. These inducible recombinant genes were also stably introduced into a number of plants species. The results of these studies indicate that the regulatory elements derived from the In2-1 and In2-2 promoters may be useful in developing a novel inducible gene expression system for plants that may have both research and commercial applications.

CLONING AND SEQUENCING OF A GENE ENCODING A 21KDA TRYPSIN INHIBITOR FROM *Theobroma cacao*.

H.W. DODO & P.J. Fritz & D.B. Furtek

The Department of Food Science, The Biotechnology Institute, and the American Cocoa Research Institute Molecular Biology Laboratory. The Pennsylvania State University, University Park PA 16802.

KEY WORDS: *Theobroma cacao*, trypsin inhibitor, gene sequence.

The cocoa plant, *Theobroma cacao* is of great commercial value to the food and pharmaceutical industries, and to cocoa producing countries. Its seeds are the sole source of cocoa butter and cocoa powder. Each year up to 30% loss in cocoa bean production is caused by insect infestations. It has been reported that transgenic tobacco plants expressing high levels of a cowpea trypsin inhibitor are more resistant to insect attacks. Thus, increased expression of a trypsin inhibitor gene in the cocoa plant by genetic engineering could enhance the plant's resistance to insects.

The objectives of this study were to establish the function of the abundantly-expressed cocoa seed 21 kDa protein, with high amino acid similarity to Kunitz type trypsin inhibitors, and to clone and analyze the gene.

A cocoa genomic library was constructed in a bacteriophage lambda *gem* 11 vector and screened with a cDNA clone obtained from cocoa seeds. A full-length clone was isolated and sequenced. Analysis of the sequence revealed a single open reading frame with no introns, starting with an initiation codon, ATG at designated position 1 and ending with a termination codon, TAA at position 666. The promoter region contains putative TATA, CAAT, and G boxes. Southern blot analysis and gene-copy number reconstruction experiment indicates that the gene is a member of a multigene family with at least 10-12 copies per haploid genome. Serine proteinase inhibitor assays confirmed that the 21 kDa protein is a trypsin inhibitor.

HORMONAL AND TISSUE-SPECIFIC REGULATION OF CELLULASES: Elizabeth C. Kemmerer, Susan L. Baird, Panos Kalaitzis, Susan M. Koehler, Gail L. Matters, Roy Sexton and Mark L. Tucker; USDA/ARS Plant Molecular Biology Laboratory, Bldg. 006, BARC-West, Beltsville, MD 20705.

Cellulase (endo-1,4-β-D-glucanase) is one of several cell wall hydrolases playing a critical role in many plant developmental processes. We are studying the hormonal and tissue-specific regulation of cellulases during abscission, adventitious rooting and cell division.

We have identified cDNA and genomic clones for bean and soybean abscission cellulase. Using *in situ* hybridization, we showed that abscission cellulase mRNA accumulates in ethylene-induced bean leaf abscission zones and the adjacent vascular tissue. The promoter region of the bean abscission cellulase gene shows three regions of high sequence identity with bean chitinase, another ethylene-induced gene.

We are also studying cellulase expression during adventitious rooting. Exogenously applied auxin induces adventitious rooting in explants. Cellulase facilitates rooting by breaking down cell wall components in the hypocotyls, allowing the new roots to emerge. Cellulase activity increases ten-fold in hypocotyls by the third day after auxin treatment (the day of maximum hypocotyl swelling and root emergence) after which the cellulase activity decreases as the roots elongate. Epicotyl and leaf tissues, which do not swell or form roots, show no distinct pattern of cellulase activity. Experiments with anti-auxin and anti-ethylene agents reveal that it is the ethylene produced by the explants in response to the exogenously applied auxin that induces cellulase activity, however, both auxin and ethylene are required for the development of adventitious roots.

In order to determine whether the same cellulase is active in these and other developmental processes, we used antibody raised against bean abscission cellulase to distinguish between abscission-specific cellulase activities and other cellulase activities. Abscission-specific cellulase antiserum immunoprecipitates most of the cellulase activity in bean and soybean leaf and flower abscission zones but very little in rooting hypocotyls and apical buds. Additionally, abscission cellulase mRNA is found in soybean leaf and flower abscission zones, but not in rooting hypocotyls or apical buds. This shows that abscission-specific cellulase does not contribute significantly to cellulase activity in rooting or apical buds.

TRANSGENIC TOBACCO PLANTS EXPRESSING AN EPITOPE-TAGGED DNA BINDING PROTEIN;

Lauren McHenry and Mark Guiltinan; Department of Horticulture, The Pennsylvania State University, University Park, PA 16802

The phytohormone, abscisic acid (ABA), is involved in seed maturation and in the osmotic stress responses of many plants. A DNA binding protein (EmBP-1) of the basic leucine zipper class has been implicated in the mechanism controlling expression of the ABA inducible Em gene of wheat. EmBP-1 binds to a 6 base pair core sequence in the Em promoter known as the G Box (CACGTG). In order to assess the functional role of EmBP-1 *in vivo*, transgenic tobacco plants expressing the dimerization and DNA binding domain of EmBP-1 have been generated. A 10 amino acid epitope tag was fused to the EmBP-1 sequences, allowing the use of a monoclonal antibody directed against the epitope to monitor expression in as little as 10 mg of leaf tissue. Homozygous offspring of these plants are being crossed to a transgenic line containing the Em promoter region, previously shown to be ABA responsive in tobacco, fused to the β-glucuronidase reporter gene. It is anticipated that the truncated EmBP-1 *trans*-protein will compete with the putative tobacco homologue for binding to the Em promoter, rendering it insensitive to ABA induction. In addition, it is possible that the *trans*-protein may bind to and block expression of endogenous tobacco genes which have promoter binding sites similar to the G box sequence. Observed phenotypes of primary and second generation transformants expressing the truncated EmBP-1 protein include reduced vigor and stature, yellowing and necrotic lesions of leaves, brittle stems and leaves, split stems, and extreme drying of leaves and stems when exposed to low ambient humidity.

TRANSFORMATION OF ORCHID EMBRYOS VIA ELECTROPHORESIS:
R.J. Griesbach and J. Hammond; Florist & Nursery Crops, USDA, ARS, Beltsville, MD 20705-2350

The apical meristems of Calanthe orchid embryos were exposed to 1 mg/ml pBI-121 DNA in an electric field. The pBI-121 plasmid contains the uid A marker gene under the control of the 35 S cauliflower mosaic virus promoter. A pipette containing 0.3% agarose and acetate buffer containing the DNA was placed on the one end of the embryo that contained the apical meristem; while the opposite end was in contact with a pipette containing only buffer and agarose. Uptake of the DNA into the meristem was monitored by 4'6-diamidino-2-phenylindole (DAPI) fluorescence. Optimal uptake occurred after 10 min of electrophoresis at 10 volts and 0.5 milliamps. Under these conditions, 55% of the embryos survived the treatment and 57% of those that survived were transformed as measured by GUS-positive staining. Leaves from 6 month old plants that developed from the transformed embryos expressed specific patterns of GUS staining.

PLANT TRANSFORMATION AND CROP IMPROVEMENT: Maud Hinchee, Fred Perlak, Yvonne Muskopf, Dave Stark, Toni Armstrong, Mike Fromm, Chuck Armstrong, Harry Klee, Jeanne Layton, Steve Padgette, Renee Rozman, Xavier Delannay, Rob Horsch and Rob Fraley. Monsanto Co., 700 Chesterfield Parkway North, St. Louis, MO. 63198

Commercialization of transgenic crop plants will become a reality in this decade. Plant biotechnology has stepped from the basic research lab onto the complex path towards the launch of a product. In order to do so, the transformation technology for a crop needs to be highly efficient. The production of transgenic plants should not be a bottleneck in evaluating the efficacy and performance of new genes. Transformation of cotton, canola, potato and tomato has been optimized such that large numbers of transgenic plants can be produced without significant increases in labor. This in turn has allowed for more efficient evaluation of a new trait's performance in the field. Significant progress has been recently made in the development of insect tolerant cotton, potatoes and corn, Roundup® tolerant soybean and canola, improved shelf-life tomatoes, and higher starch potatoes. These technical successes are now taking the necessary steps to become commercial products.

Poster #1

Recombinant Fab Antibody Fragments Which Bind Bean Yellow Mosaic Potyvirus Coat Protein. Leslie M. Palmer and Ramon L. Jordan. USDA, Agricultural Research Service, Plant Sciences Institute, Florist and Nursery Crops Laboratory, Beltsville, MD.

To genetically engineer potyvirus resistance into plants, recombinant DNA clones were developed from a potyvirus coat protein-reactive monoclonal antibody. Potyviruses are the largest group of viruses responsible for diseases in crops. These viruses are divided into groups based on immunological cross-reactivity of coat protein epitopes. cDNAs of a murine monoclonal antibody, which recognizes a coat protein epitope present on bean yellow mosaic potyviruses, were amplified by polymerase chain reaction using primers specific for the Fab region of the IgG1 heavy chain and κ light chain. The resulting 700-bp products were cloned into bacteriophage lambda expression vectors that contain a *lacZ* promoter, ribosomal binding site, and bacterial leader sequence and expressed in *Escherichia coli*. Production of heavy and light chain domains was determined by immunoscreening of plaque lifts.

When the heavy chain and light chain constructs were combined in a single vector and expressed in *E. coli*, a protein of the expected size for an assembled Fab molecule (52-55 kDa) was obtained by non-denaturing Western blot analysis. These constructs were shown to bind coat protein subunit of bean yellow mosaic virus in an indirect antigen-coated plate enzyme-linked immunosorbent assay.

Development of transgenic plants expressing these Fab fragments capable of binding potyvirus coat protein will provide new information useful to understanding viral pathogenesis and developing new virus control strategies.

Poster #2

THE NUCLEOTIDE SEQUENCE OF A CUCUMBER MOSAIC VIRUS STRAIN DEFICIENT IN SATELLITE RNA REPLICATION.

P.B. McGarvey*, F. Cellini#, M. Tousignant*, and J.M. Kaper*.
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Metopontum Agrobios, Metoponto, Italy.

Satellite RNAs are small RNA molecules found in association with many plant viruses. Satellite RNAs are dependent on the viral genome for their replication and spread. The plant virus in turn does not require the satellite RNA for any known function. The presence of satellite RNA can modulate the disease symptoms produced by its "helper" virus in a specific host plant. Usually this results in a reduction in virus titer and disease symptoms. However, certain satellite variants can cause more severe diseases in certain host plants.

The cucumber mosaic virus strain Ixora (CMV-Ix) is unable to support the replication of most but not all CMV satellite RNAs (Kaper et al. 1990, Res. Virol. 141, 487-503). We have cloned and sequenced full length cDNA copies of RNAs 1-3 from CMV-Ix. A comparison of the nucleotide sequences of this strain with other CMV strains that replicate all the CMV satellites tested will be presented along with biological data that indicate what sequence variations in CMV-Ix may be responsible for the observed changes in its ability to support the replication of CMV satellite RNAs.

Poster #3

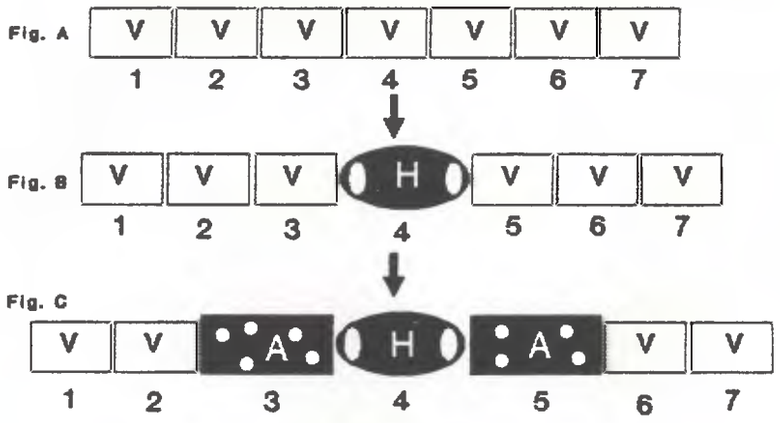
AGROBACTERIUM-MEDIATED COTRANSFORMATION AND INHERITANCE OF MULTIPLE GENETIC MARKERS: Hema Bandaranayake and Paul J. Bottino; Department of Botany and Maryland Agricultural Experiment Station, University of Maryland College Park, MD 20742.

Agrobacterium-mediated transformation was carried out using the binary vector pEND4K::ALS. This vector carries the NPTII gene for kanamycin resistance, and the mutant tobacco ALS gene encoding a protein resistant to the herbicide chlorsulfuron. Leaf disks of *Nicotiana tabacum* var Maryland 201, 609 were precultured for two days, cocultivated with bacteria for two days, and placed on selection medium containing 50 mg/ml kanamycin. Shoots which formed were excised and rooted on medium containing kanamycin. When the shoots were rooted, they were transferred to soil in the greenhouse. A recallusing assay on the primary transformants was carried out in the presence of either kanamycin or chlorsulfuron. Self and cross pollinated progeny obtained from the plants were screened for resistance to kanamycin or chlorsulfuron. We found that results from the recallusing assay agreed completely with the inheritance data. That is, kanamycin resistant plants produced kanamycin resistant progeny as a dominant trait, and chlorsulfuron resistant plants gave chlorosulfuron resistant progeny as a dominant trait. PCR analysis of some of the transgenic plants indicated stable integration of the NPTII gene in the plants. Self pollinated plants gave progeny with a 3:1 ratio of resistant to sensitive for both markers. Cross pollinated plants gave 1:1 ratios of resistant to sensitive plants for both markers. Segregation for kanamycin resistance was 3:1 in over 80% of the plants indicating that a single gene insertion had taken place. Of the originally selected kanamycin resistant plants, 73% were also chlorsulfuron resistant indicating a high cotransformation frequency. Seventy five percent of these plants segregated 3:1 for both genes. Additional experiments with chlorsulfuron selection are under way.

Poster #4

AKINETE DIFFERENTIATION AND GERMINATION: Shane Hardin and Bob Fisher; Department of Biology, Virginia Commonwealth University, Richmond, Virginia 23284-2012.

Our laboratory has been concentrating on cell differentiation and pattern formation as manifested in the filamentous, heterocystous, nitrogen-fixing cyanobacteria; particularly those in the genus Anabaena. We have studied the structural, biochemical, and are starting to study the molecular genetic changes that take place when cyanobacterial vegetative cells differentiate into heterocysts or akinetes. We can regulate cyanobacterial cell differentiation by controlling culture growth conditions. When grown on ammonium (Fig. A) only vegetative cells (V) develop. When ammonium is removed and nitrogen is available only as N_2 (Fig. B) some vegetative cells differentiate into heterocysts (H) and form a spacing pattern along the filament. When phosphate is removed from the growth medium (Fig. C) a third cell type differentiates; the akinete (A).



The akinete cell has a limited stress resistance function allowing for survival under adverse conditions and is also capable of germinating. The major focus has been on discerning gene regulatory changes which occur during akinete differentiation and germination. The levels of chlorophyll a, total protein, phycobiliproteins and nitrogenase activity have been investigated. Analysis of water soluble protein fractions by SDS-PAGE and a two dimensional procedure have been utilized at various stages to characterize polypeptide gene products specific to either process. It is hoped that these analyses will eventually identify the gene expression patterns responsible for the phenotypic cell variations. We have just entered into the molecular genetic studies and look forward to sharing ideas with our conference colleagues.

Poster #5

MICROSURGICAL AND HORMONAL ANALYSES OF AN ARABIDOPSIS MUTANT ALTERED IN ORGAN ELONGATION

Roxanne H. Fisher[1], Kathy Barton[2], Jerry D. Cohen[3], R. Scott Poethig[2], Todd J. Cooke[1] [1]Dept. of Botany, Univ. Maryland, College Park, MD 20742;[2] Dept. of Biology, Univ. Pennsylvania, Philadelphia, PA 19104;[3] Horticultural Crops Quality Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705

We have isolated gordo, an EMS-generated nuclear recessive mutant of Arabidopsis thaliana. In gordo all multicellular organs and the plant body itself exhibit radial expansion instead of uniaxial elongation. If grown in culture, gordo develops a stunted inflorescence but fails to set seed. Wild-type and gordo embryos were dissected out of developing seeds in order to perform hormonal and microsurgical studies. Wild-type heart embryos treated with NAA mimic the gordo phenotype, but other auxins and auxin antagonists have no significant effect on the length to width ratios of wild-type and gordo embryos. The roots of gordo torpedo embryos elongate much more following surgical separation from the apex than those roots which remain attched to the apex. We are currently investigating the metabolisms and concentrations of ethylene and auxin using mutant and wt seedlings. This work was supported by USDA-CRGO-89-37261-4791

Poster #6

EFFECTS OF GENOMIC METHYLATION ON RAPD PATTERNS IN BRASSICA OLERACEA: Steven J. Gagliardi[1], C. Lan[2], and Fang-Sheng Wu[1]; [1]Department of Biology, Virginia Commonwealth University, Richmond Virginia, USA; [2]Department of Biology, Fu Jen Catholic University, Taiwan, R.O.C.

Two day old Brassica oleracea seedlings were given a single 24 hour exposure to 5-Azacytidine (5-Ac), a synthetic analog to the nucleotide cytidine. The genomic DNA was isolated from 5 day old seedlings and stored at -20°C. This DNA was then subjected to Random Amplified Polymorphic DNA (RAPD) analysis using the Coy model 50/60 Tempcycler. The PCR protocol included three programs of: 1) 1 cycle of 94°C (3 min.), 30°C (4.5 min.), and 72°C (2 min.); 2) 35 cycles of 94°C (1.15 min.), 30°C (4.5 min.), and 72°C (2.15 min.); and 3) 1 cycle of 95°C (2 min.), 30°C (5 min.), and 72°C (10 min.). The samples were then stored at +4°C until analyzed via agarose gel electrophoresis.

A novel pattern was observed in the RAPD fingerprint when comparing the control (untreated) to the 5-Ac treated DNA. Generally, the fragments observed ranged from greater than 1 Kb to as small as 75 bp with alternate banding patterns appearing. One primer, OPL-16 (Operon Technologies Kit L), yielded a single intense band at about 500 bp while the control yielded no bands. Another primer, OPL-11 yielded two bands present at 700 and 750 bp which again were absent in the control DNA. Primer OPL-2 plus the control DNA contained a light band at 300 bp which was absent from the treated DNA. Some, but not all of the primers used yielded similar results. From these data it appears probable that the methylation status and possibly the secondary structure of the DNA play a role in the RAPD analysis of genomic DNA.

Poster #7

USE OF POLYMERASE CHAIN REACTION AND SEROLOGY FOR DETECTING HEAT SHOCK PROTEINS IN HEAT-SENSITIVE AND TOLERANT TOMATO GENOTYPES: Magdy S. Montasser, Aref A. Abdul-Baki, and Benjamin F. Matthews, Plant Sciences Institute, USDA, ARS, Beltsville, MD 20705-2350

Five-week-old intact tomato (*Lycoperison esculentum* Mill) plants of two heat-tolerant genotypes 'CLN 475" and 'CL 1131' and two heat-sensitive genotypes 'Duke' and 'Long Keeper' were subjected to a heat shock treatment for 4 hr at 39C. Total nucleic acid (TNA) was extracted from leaves of heat treated and untreated plants. The TNA extracts were reverse-transcribed before using in a polymerase chain reaction (PCR) amplification technique which utilizes specific primers to amplify the heat shock protein (hsp) gene sequence in the extracts. The PCR product of hsp70 was observed only in TNA extracted from leaves of the two heat-tolerant genotypes and was not detected in leaf extracts of the two heat-sensitive genotypes. These results were further confirmed by Southern and Northern blot hybridizations. The expressed protein of hsp70 from leaves and roots of the same tested plants was serologically detected by enzyme linked immunosorbent assay (ELISA) using specific monoclonal antibodies. Heat shock protein 70 was detected in the leaves and roots of all genotypes tested before the heat treatment irrespective of genotype tolerance to heat. Levels of hsp70 in leaves of heat-tolerant genotypes before heat treatment were lower than those of the heat sensitive genotypes. Heat treatment increased the levels of hsp70 in leaves of the two heat-tolerant genotypes by 347 to 686% respectively, whereas, the increase in the leaves of the two heat sensitive genotypes was about 38 and 65% respectively. In contrast, hsp70 levels in roots of heat-tolerant and heat-sensitive genotypes were about the same before the heat treatment. Heat treatment increased the levels of hsp70 in the roots of the two heat-tolerant genotypes by only 12 to 92%, respectively, whereas, the increase in the roots of the two heat-sensitive genotypes was 115 and 194%, respectively. These results suggest the possibility of a gene regulating the hsp70 gene family.

Poster #8

Application of the Diffusible Factor Hypothesis in the Induction of α-amylase During Seed Germination in Two Cultivars of Rice.

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The induction of amylase activity was investigated in two different cultivars of rice to evaluate the application of the diffusible factor hypothesis to this cereal. This hypothesis suggests that gibberellin (GA) is transported from the embryo, during germination, to the endosperm portion of the seed, where it induces the secretion and synthesis of α-amylase for the degradation of endosperm starch. We tested this hypothesis because in rice, in contrast to barley, previous studies emphasized the scutellum as the initial important site for amylase induction. Application of the classical diffusible factor hypothesis, which was derived from studies of barley, and which stresses the requirement for the embryo for enzyme induction in the endosperm, has not been tested in rice. It has been assumed that because the embryo is the site of gibberellin synthesis, that the induction in endosperm amylase activity depends on an embryonic source for the hormone. We used dissection studies to investigate the requirement for the embryo and GA in the induction of amylase activity in the endosperm tissues of two rice cultivars, Nortai and Lemont. We measured α-amylase activities in various tissues using spectrophotometric assays, and monitored amylase isoenzymes through isoelectric focusing techniques. As reported by others, we observed that the initial first site of amylase activity in rice is the embryo. However, Nortai and Lemont showed differences in the dependence of the endosperm on the embryo for the induction of α-amylase activity. The cultivars differed in isozyme profiles and in their sensitivity to GA in amylase induction. It appears that the diffusible factor hypothesis applies to rice, but the induction of some α-amylase activity in the endosperm depends on an inducible factor in that tissue.

Poster #9

PHOTOSYSTEM I PROTEIN PsaL cDNA CLONE FROM MAIZE.

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A maize cDNA clone encoding the Photosystem I protein PSI-L has been isolated. The insert of the cDNA clone is 776 bp in length and contains an open reading frame of 633 bp. The deduced sequence of the precursor polypeptide is 211 amino acids in length with a molecular mass of 22,300. The polypeptide contains a typical transit peptide of about 42 amino acids, yielding a mature polypeptide of about 169 amino acids (molecular mass = 17,864). Several hydrophobic domains which could represent potential membrane spanning regions are found in the deduced amino acid sequence of the putative mature polypeptide. The maize PSI-L polypeptide is about 86% identical with the barley precursor protein. The mature polypeptide is highly similar (151 out of 169 identical amino acids) while the transit peptide is less conserved. Northern blot analysis shows that the maize PsaL mRNA is about 960 bases in length. The level of PsaL mRNA is regulated by both white and red light.

Poster #10

DELETION ANALYSIS OF A POLYUBIQUITIN GENE (UBI-1) PROMOTER FROM MAIZE: Donna M. Fox and Alan H. Christensen; Department of Biology, George Mason University, 4400 University Drive, Fairfax, VA 22030.

A cassette containing 0.9 kb of the maize polyubiquitin gene (Ubi-1) promoter and the entire 5′ untranslated region directs a high level of reporter gene expression when this chimeric construct is electroporated into maize protoplasts. A 5′ deletion analysis of this promoter fragment was undertaken to identify regulatory regions which contribute to this efficient expression. Eighteen promoter mutants, fused to the reporter gene chloramphenicol acetyl transferase (CAT), were electroporated into maize BMS protoplasts and the levels of CAT expression were analyzed. The data indicate that the deletion of sequences to -460 results in a level of activity similar to that of the "full-length" (899 bp) promoter. Further deletion to -380 results in a 40% loss of activity suggesting the loss of a positive regulatory element. Deletion of bases to -273 restores activity to approximately 90% of that obtained with the "full-length" promoter construct suggesting the elimination of a negative regulatory region. Retention of a 161 bp region of the promoter is sufficient to confer high levels of promoter activity. Thirty percent of promoter function can be attributed to the presence of the TATA box (-42) and partial deletion of this essential regulatory region (-24) virtually eliminates reporter gene expression.

Poster #11

GENE STRUCTURE OF APRT IN *Arabidopsis thaliana*

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The APRT gene codes for the purine salvage enzyme adenine phosphoribosyltransferase (APRTase, EC 2.4.2.7) that catalyzes the conversion of adenine and PRPP to AMP and PPi. APRTase activity has been reported for a variety of organisms from E. coli to plants to man. APRT genes and/or cDNAs have been isolated in mouse, human, hamster, *Drosophila melanogaster* , *Escherichia coli* , *Arabidopsis thaliana* and *Brassica juncea.* We report the genomic sequence of the APRT gene of *Arabidopsis thaliana* . The sequence of the transcribed region is 1.6 kb in length. Comparision with the cDNA sequence of *Arabidopsis* reveals 6 exons and 5 introns. The exons code for 183 amino acids. The mouse, human and hamster genomic sequences each consist of 5 exons and 4 introns with the exons coding for 180 amino acids. Also, in mouse, human and hamster the largest intron is intron 2 while in Arabidopsis intron 3 is the largest intron.

Arabidopsis thaliana plants deficient for the enzyme APRTase were isolated by Moffatt and Somerville (1988). We are characterizing the cDNA and the genomic DNA corresponding to mutant APRT gene. We designed PCR primers from the cDNA sequence of *Arabidopsis thaliana* (Moffatt et. al. 1992), to amplify the coding region of the cDNA . The same primers are also being used to amplify the transcribed region of APRT from the genomic DNA. A comparision of the nucleotide sequences of the mutant and wild type APRT cDNAs show that the mutant cDNA sequence lacks exon 3. The absence of exon 3 in the mutant may be the explanation for the deficiency of APRTase activity in this mutant. At present we are involved in characterizing the genomic DNA of the APRT deficient mutant. This will allow us to understand the aberrant mRNA in the mutant.

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Poster #12

CLONING OF SOYBEAN ASPARAGINE SYNTHETASE GENE.

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Asparagine synthesis is mediated by the enzyme asparagine synthetase (AS) and involves the ATP-dependent transfer of the amide group of glutamine to aspartate in the presence of Mg^{2+} producing asparagine, glutamate, AMP, and PPi. Asparagine is the major nitrogen transport amino acid found in plants faced with conditions of excess ammonia. Even though asparagine plays an important role in nitrogen transport in plants, AS has not been characterized extensively or purified to homogeneity in plants. Also, extensive studies regarding AS expression and regulation has been limited. Our laboratory is interested in defining the AS system at the DNA and protein level. In efforts to determine how this system relates to nitrogen distribution and utilization throughout the soybean plant. Degenerate oligonucleotide primers were synthesized to highly conserved regions of the AS gene of P. sativum and human. These primers were used in a polymerase chain reaction (PCR) with soybean cDNA to amplify a portion of the AS gene. The amplification generated a product of about 0.83 Kb which corresponded to the predicted fragment size. This product was used to screen a soybean cDNA library synthesized from mRNA extracted from dark-grown cotyledon. Preliminary comparison of the DNA sequences of these cDNAs with that of P. sativum AS indicated that the 5' end of this soybean AS is highly homologous with P. sativum. To obtain the 3' end of this gene, the 3' RACE and Clone AMP systems (Gibco, BRL Life Technologies, Inc., Gaitherburg, MD. USA) were employed. The amplification generated a predominant fragment of 1.8 Kb which hybridized strongly to the 5' end of AS gene. The cloning was performed using the Clone AMP system and we are currently in the process of screening these putative RACE clones.

Poster #13

USE OF PCR IN THE MOLECULAR CLONING OF THE SOYBEAN GENE ENCODING DIHYDRODIPICOLINATE SYNTHASE (DS)

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In plants, the first committed step in the pathway to lysine synthesis is the condensation of aspartate B-semialdehyde and pyruvate, catalyzed by the enzyme dihydrodipicolinate synthase (DS). DS activity undergoes feedback inhibition by lysine and may control lysine synthesis. Cloning of the soybean DS gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the DS gene may be an important way of increasing lysine synthesis in soybeans and other crop plants. The gene which encodes DS has been cloned from monocots, but it has not been previously cloned from dicots. We have cloned the DS gene of soybean (G. max var. Century) using PCR. The sequence of the PCR primers was based on the published sequences of the maize (1) and wheat (2) DS genes. PCR products were amplified from soybean genomic DNA and cDNA. The PCR products were cloned in the plasmids pCR1000 (Invitrogen Corp.) and later Bluescript (Stratagene). A 340 bp cDNA PCR clone and a 420 bp genomic DNA PCR clone have been sequenced. The cDNA PCR product (340 bp) has 90% amino acid and 76% DNA identity with the maize gene. Inverse PCR (3) was used to amplify the uncloned flanking sequences. Genomic DNA was cut with restriction enzymes and ligated to form circular pieces of genomic DNA. These ligated fragments were then amplified using primers that diverged from the region of known sequence. Inverse PCR of Century genomic DNA cut with TaqI yielded a 675 bp PCR product that has been cloned in Bluescript and has been sequenced. A genomic library of Century DNA in lambda GEM11 was constructed using genomic DNA that had been partially digested with Sau3A. 15 kbp fragments were size selected and ligated into lambda GEM11. The genomic PCR clone was used to screen approximately 500,000 plaques and three DS clones were purified. These clones have been characterized by restriction mapping. Several subclones of these phage have been sequenced. Several small clones have also been isolated from cDNA libraries. Southern blots show that the soybean genome contains a small number of DS genes. Although the soybean gene hybridizes to the maize DS gene, the level of homology is not high enough for the soybean probe to detect the maize DS gene in genomic Southern blots. Northern blots indicate that the soybean DS mRNA is present at only very low levels.

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Poster #14

IDENTIFICATION OF TRANSCRIPTS EXPRESSED IN THE CORN ROOT PRIMARY RESPONSE TO ENVIRONMENTAL NITRATE

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When plant roots encounter nitrate in their environment, a series of complex physiological and biochemical events occur, including changes in nitrate transport and assimilation, metabolic activity, and root growth. In order to further characterize this response at the molecular level, we are interested in defining the earliest or primary molecular changes which occur in roots exposed to nitrate. In corn, the rapid, transient, and protein synthesis-independent expression of transcripts for nitrate reductase (NR) and nitrite reductase (NiR) is among these primary events. Corn roots exposed to nitrate in the presence of protein synthesis inhibitors also show increased levels of other transcripts important in nitrate assimilation. Among these transcripts, glutamine synthetase (GS) and glutamate synthase (GOGAT) have also been identified as primary response genes in roots, but not in leaves. cDNA libraries were constructed with RNA isolated from the roots of nitrate and cycloheximide (CHX) treated plants. Primary response to nitrate clones were selected with two "subtractive" cDNA probes: one synthesized using RNA isolated from nitrate and CHX treated roots minus RNA from untreated roots; and a second with RNA isolated from roots treated with nitrate alone minus RNA from untreated roots. Several hundred hybridizing plaques have been identified and isolated with these probes, and clones have been identified whose whose expression is induced by nitrate and/or CHX. Clones for NR, NiR, GS and GOGAT are being identified by hybridization with their respective cDNAs. Other cDNAs are being further characterized by northern, genomic southern and DNA sequence analyses.
Supported by USDA Grant CRCR-9002408.

Poster #15

NUCLEOTIDE SEQUENCE AND ORGANIZATION OF SOYBEAN CHALCONE SYNTHASE MULTI-GENE FAMILY:

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Chalcone synthase is the first committed enzyme in the flavonoid biosynthesis pathway, catalyzing the condensation of a molecule of *p*-coumaroyl-CoA with three molecules of malonyl-CoA to give rise to naringenin chalcone. Various physiological roles for flavonoid compounds have been documented. Anthocyanins are well known as pigments in flowers and fruits. Strong absorption of UV light by flavonoids and their accumulation in the epidermal layer of leaf tissues in many plant species, support the idea that flavonoids function as UV-B protectants for plants. It is also proposed that flavonols such as quercetin function as competitive IAA transport inhibitors. In legumes, additional features of flavonoids have been discovered. Isoflavonoids accumulated after biological stress function as a strong phytoalexin. Isoflavonoids derived from host plants are also involved in the establishment of rhizobium -legume symbiosis.

We have isolated seven members of the chalcone synthase gene family by screening a genomic library of soybean cv. Williams. Nucleotide sequences of the genes show that all have two exons splitted by an intron of 121-445 bp at the same site. Exon I has 178 bp and exon II has 989 bp of protein coding sequence in each gene, constituting an open reading frame of 1167 bp encoding a polypeptide of 388 amino acids. In spite of sequence diversity among those genes in the 5' non-translated region, consensus sequence exists for TATA box at about 100-130 bp upstream of ATG initiation codon for every gene. These common features of the members of gene family argue in favor of an evolutionary pressure to maintain their basic integrity, suggesting that there are no pseudo genes among them and that each of them is quite possibly a functional gene. Among these genes, gene 1 expression was reported to be induced by elicitor and UV light in cotyledons of young seedlings and exon II of gene 7 has a high similarity with the partial cDNA clone expressing in nodules.

Based on sequence similarity of the seven genes, the multi-gene family can be classified into three sub-families; sub-family A is composed of genes 1-5, sub-family B contains gene 6, and sub-family C contains gene 7 and possibly another related gene. In the sub-family A, genes 1, 3 and 4 are clustered in a span of about 10 kb genomic sequence. It is of interesting to note that nucleotide sequences of genes 4 and 5 share the identical 3' non-translated region and intron, and 99% similarity in the translated region, while there is a substantial sequence diversity in the 5' non-translated region. There is possibly another cluster of 1, 3 and 5 in the soybean genome, suggesting the presence of two parental diploids in the present day tetraploid soybean.

Poster #16

ORGANIZATION AND EXPRESSION OF PHENYLALANINE AMMONIA-LYASE GENES IN TOBACCO (*NICOTIANA TABACUM)*

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Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5.) catalyzes the first reaction in the general phenylpropanoid pathway leading to the production of flavonoids, coumarins, lignins and other phenolic compounds which are widely distributed among higher plants. The significance of these compounds in plants, as flower pigments, UV-B protectants, IAA-transport inhibitors, cell wall components, and phytoalexins is well documented. To investigate the regulation of PAL biosynthesis, the gene was isolated from a tobacco genomic library and the complete nucleotide sequence was determined (tpal1). The tpal1 consists of two exons separated by an intron. This 1933 bp intron was found to be located in the codon for amino acid 133 by determining the sequence of a corresponding cDNA. Exon I, 398 bp, and exon II, 1750 bp, together encode a polypeptide of 715 amino acids. There is a TATA box 145 bp upstream of the initiation codon and a polyadenylation signal 201 bp downstream from the stop codon.

Southern blot analysis of genomic DNA fragments, using various parts of tpal1 as probes, indicated the presence of a small gene family of at least four genes in tobacco genome. One of them corresponds to the gene sequenced (tpal1). Another gene (tpal2) is highly homologous to tpal1. The other two genes (tpal3 and4) are less homologous to tpal1. *N. tomentosiformis* and *N. sylvesteris,* which are believed to be parental species of tobacco, appeared to contain tpal1 and 3, and tpal2 and 4, respectively. These results suggest the presence of two distinct types of PAL genes in each of these *Nicotiana* species.

The expression of the gene was examined using RNA gel blots. In mature tobacco plants, PAL transcript levels were significantly higher in flowers and roots than in leaves and stems. Our evidence also shows that PAL expression is developmentally regulated in flowers and leaves.

Poster #17

PRIMARY STRUCTURE AND EXPRESSION OF S-RNASE HOMOLOGS IN *ARABIDOPSIS THALIANA*. Pauline A. Bariola, Crispin B. Taylor, Michael T. Verburg and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.

Changes in ribonuclease activities in plants are associated with numerous developmental and environmental phenomena. The RNases best characterized molecularly are the S-RNases, which are involved in self-incompatibility in some plants. We have previously reported the identification of S-RNase homologs in a self-compatible plant, *Arabidopsis thaliana* [Taylor and Green, Plant Physiol. **96**:980]. Nucleotide sequences of the cDNAs for the homologs, designated RNS1, RNS2, and RNS3, have been obtained, and the amino acid sequences of the corresponding proteins have been deduced. An alignment of these protein sequences with those of S-RNases and other similar RNases from self-compatible plants ("S-like RNases") revealed strong similarities, but several significant differences were also apparent. A computer-generated genealogy of these sequences shows that the S-RNases and S-like RNases form two divergent groups. RNS1 and RNS3 are closely related to RNase LE, which is secreted from tomato cells upon starvation for phosphate [Jost et al., Eur. J. Biochem. **198**:1]. The expression characteristics of the RNS genes under senescence- and stress-related conditions will be discussed.

Poster #18

CLONING AND CHARACTERIZATION OF GENES EXPRESSED DURING CARROT SOMATIC EMBRYOGENESIS

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The early events of plant embryogenesis are quite important for pattern formation, but so far little is known about the process at the molecular level. Although carrot somatic embryogenesis has been used as a model system in attempt to isolate embryo specific genes, the success has been very limited. The lack of success may be due to the cloning strategies which relied mostly on a comparison of total transcripts present in callus cells and somatic embryos. We have taken a new approach to this problem. A cDNA library was constructed from **polysomal mRNA** of globular embryos of carrot. Two kinds of screening methods were used to isolate clones preferentially expressed during embryogenesis. In a differential screen, the library was screened with cDNA probes from globular polysomal mRNA and from seedling polysomal mRNA. In a subtractive screen, a cDNA probe made from globular polysomal mRNA was first hybridized with an excess amount of seedling polysomal mRNA to remove common sequences, and then was used to screen the library. By using polysomal mRNA, clones whose expression is regulated at the translational as well as transcriptional level can be isolated. Moreover, by comparing genes expressed in embryos with seedlings instead of callus, we can bypass the problem that the difference in gene expression between callus and embryos does not appear to be extensive. Using this approach we have isolated 50 clones. Slot blot and Northern blot analysis showed almost all the clones are enhanced in globular embryos compared to seedlings, and fall to different categories of relative abundance. Most of the clones show very similar levels of transcripts in globular embryos and callus; this validates our early suspicions that the globular/callus comparison is inappropriate for identifying globular enhanced clones. DNA sequence analysis on all the clones has revealed that some of the clones are carrot embryo genes previously isolated by other groups, some are homologous clones of known genes from other species, and still some are completely novel clones. These clones are being characterized in greater detail, including developmental RNA blot analysis and *in situ* hybridization.

Poster #19

PHOTOREGULATED EXPRESSION AND SEQUENCE ANALYSIS OF PsPK5, A PROTEIN KINASE HOMOLOG FROM *PISUM SATIVUM* L.

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We previously used the polymerase chain reaction to obtain a suite of partial cDNA clones that encode distinct forms of protein kinase homologs from the garden pea [Lin *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88: 6951-6955]. These partial cDNAs, called PsPK1 through PsPK5, correspond to mRNAs that are differentially expressed during de-etiolation. During greening, PsPK3 and PsPK5 transcripts show a large and rapid decline, PsPK4 RNA increases steadily, and PsPK1 and PsPK2 transcripts show little change. Preliminary evidence indicates that PsPK5 mRNA levels decline more rapidly than PsPK3 mRNA during the initial stages of greening in continuous white light. For further sequence analysis of PsPK5, a series of overlapping cDNA clones were isolated by screening a cDNA library using the partial PsPK5 cDNA as a hybridization probe. The full-length cDNA sequence predicts a polypeptide of 428 amino acids of M*r* 48.6 kDa. The deduced amino acid sequence of the PsPK5 polypeptide possesses all of the invariant amino acids found within the catalytic domain of protein serine/threonine kinases. The putative catalytic domain of the PsPK5 polypeptide is extremely similar to the catalytic domains of both cyclic AMP-dependent protein kinase and protein kinase C. This similarity to second messenger-dependent protein kinases suggests to us that PsPK5 may be involved in signal transduction.

Poster #20

CHANGES IN GENE EXPRESSION ASSOCIATED WITH CHILLING-UNIT ACCUMULATION IN DIFFERENT BLUEBERRY VARIETIES

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There is evidence from several plant species that low, nonfreezing temperatures induce the accumulation of specific proteins in leaves and stems. Only recently, however, have attempts been made to identify changes in gene expression in dormant buds of woody perennials in response to chilling-unit accumulation. For our investigations, we have used dormant blueberry plants representing several varieties with different chilling requirements, placed them in a room maintained at about 4°C, and collected floral buds after every 200-500 hours until the chilling requirements of each variety were met. We also collected floral buds from field plants of the same varieties every 200-300 chilling units. Proteins were extracted from bud samples and analyzed by SDS-PAGE.

Results indicated that certain varieties responded differently to chilling-unit accumulation. Similar changes were noted in the Vaccinium corymbosum cultivars 'Bluecrop' (high-chilling) and 'Gulfcoast' (low-chilling). The concentration of about 3 polypeptides with molecular weights ranging from 60-80 kD increased throughout chilling. The concentration of a 17.5 kD polypeptide increased after about 500-1000 hours and the concentration of a 115 kD polypeptide decreased throughout the treatment. Another change, a decrease in concentration of a 69 kD polypeptide, was noted in the Vaccinium ashei cultivar 'Tifblue' (low-chilling). This change was not observed in the other cultivars examined.

Poster #21

INDUCTION OF 1-AMINOCYCLOPROPANE-1- CARBOXYLATE SYNTHASE IN TOBACCO TREATED WITH AN ETHYLENE BIOSYNTHESIS-INDUCING XYLANASE.

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Abstract

An ethylene biosynthesis-inducing xylanase (EIX) from the fungus *Trichoderma viride* elicits enhanced ethylene biosynthesis in *Nicotiana tabacum* cv *Xanthi* leaf tissues. The increase in ethylene biosynthesis is accompanied by the accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) and an increase in extractable ACC synthase activity. We have isolated a full length clone of a tobacco ACC synthase from a *N. tabacum* cv *Xanthi* cDNA library. The clone shares 88.6 % identity at the amino acid level with published ACC synthase sequence data from tomato and is constitutively expressed at low levels in *E. coli*. This clone was used to probe northern blots of poly $(A)^+$ RNA extracted from EIX-induced *Xanthi* tobacco leaves. Increased levels of a 1.9 kb transcript were visible within 2 h of EIX treatment. Pretreatment of leaves with ethylene (120 nL/L, 14 h) alone had no measurable effect on the responses studied, but enhanced responses induced by EIX (ethylene biosynthesis, ACC content, ACC synthase activity and ACC synthase transcript level).

Poster #22

A WOUND-REPRESSED TOMATO cDNA CLONE ENCODES A GLYCINE-RICH PROTEIN TRANSCRIPT THAT IS EXPRESSED IN VASCULAR TISSUE

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A wound-regulated tomato cDNA was isolated and its expression was characterized. The cDNA hybridizes to a 0.8 kb transcript that is wound-repressible, ethylene-inducible and developmentally-regulated. This cDNA hybridizes to a number of tomato genomic DNA restriction fragments, suggesting that it is one of a small multi-gene family in the tomato genome. In order to identify the gene product and eventually understand the function of this gene, the partial cDNA was sequenced. The cDNA sequence contains an open reading frame that is rich in glycine (61%) and shows significant similarity to a number of plant glycine-rich proteins (GRPs). The deduced protein sequence contains repeats of the amino acid sequence GGGGGYGGGGGYGGGRRQ near the carboxy terminus. Tissue printing was used to determine the location of the tomato GRP transcript. The transcript for this GRP was localized in the vascular bundles of tomato fruit as well as the vascular tissue of tomato stem. Hybridization of stem tissue prints with a rRNA control also resulted in a vascular-specific pattern of hybridization. This indicates preferential loading of RNA from vascular tissue of stem. The vascular localization of the tomato GRP in stem tissue, however, was confirmed by direct isolation of RNA from stem sections either containing or not containing vascular tissue. The homology to another cell wall GRP and the localization of tomato GRP to vascular tissue suggests that this wound-repressed, ethylene-induced cDNA may encode a cell wall protein.

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