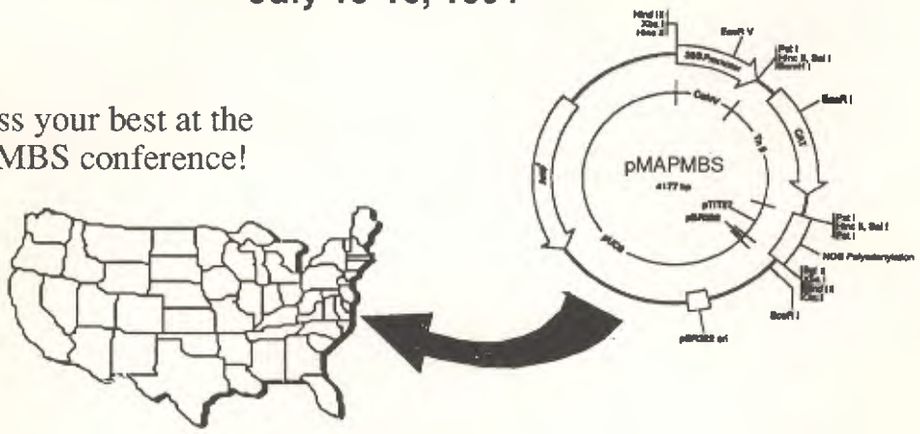
Tucker

Eighth Annual Meeting

Mid-Atlantic Plant Molecular Biology Society

Loyola College, Baltimore, Maryland
July 15-16, 1991

Express your best at the MAPMBS conference!



Keynote Address:

Tuan-hua David Ho

Dept. of Biology, Washington University, St. Louis, MO

"Hormonal Regulation of Gene Expression in the Aleurone Layers of Cereal Grains"

Monday, July 15, 1991, 4:30-5:30 p.m.

Meeting Sessions and Invited Speakers

Plant/Microbe Interactions:

Mike Wilson (Rutgers University), "Implications of Plant Virus Nucleocapsid Structure for Infection"
Richard Broglie (DuPont), "Transgenic Plants with Enhanced Resistance to *Rhizoctonia solani*"

Regulation:

Peter Goldsbrough (Purdue University), "Regulation of Gene Expression by Ethylene During Carnation Petal Senescence"
Gloria Coruzzi (Rockefeller University), "Molecular Mechanisms Controlling Amino Acid Biosynthesis in Plants"
Tony Gatenby (DuPont), "Molecular Chaperones and Rubisco Assembly"

Cloning Strategies:

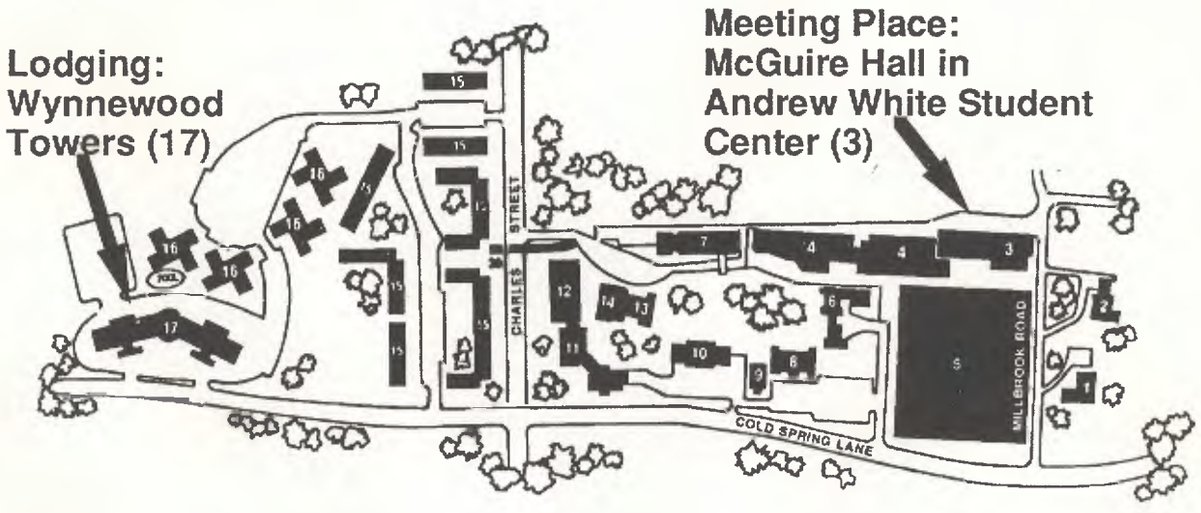
Ken Feldman (University of Arizona), "Dwarfs and Other T-DNA Insertion Mutants in *Arabidopsis*"
Patrick Masson (University of Wisconsin), "Transpositional Behavior of the *Spm* Corn Transposable Element in Heterologous Plants"

Organelles:

Maureen Hanson (Cornell University), "Plant Mitochondrial Gene Expression"
Karen Kindle (Cornell University), "Chloroplast Transformation of *Chlamydomonas reinhardtii*"

CONTENTS

Campus Map



INTRODUCTION

Welcome to the eighth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to bring together area scientists for the exchange of ideas and information in plant molecular biology. These meetings are designed to be accessible and affordable so that the greatest number of students, post-docs and other scientists may attend to participate in presentations and discussions.

We are pleased to host this year's meeting at Loyola College. Many people have been involved in organizing this meeting and we wish to express our thanks to them for the time and effort invested in this meeting. We also wish to thank the sponsors listed below, whose generous contributions allow us to provide a high quality and affordable meeting.

Dave Straney
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GENERAL INFORMATION

LOYOLA COLLEGE

Loyola College is a private, 4-year liberal arts college established in 1852 by the Jesuit fathers. It is the ninth oldest among the 28 Jesuit colleges and universities in the United States. The undergraduate full and part-time enrollment is approximately 3,000 students. If you have free time, there is many activities both on and off the campus. On campus, there is: an outdoor swimming pool behind Winwood towers open 11 am -7 pm, tennis courts outside the meeting rooms, athletic facilities with operating hours posted in the lobby of Winwood Towers. Baltimore has many attractions to offer including inner harbor, museums and a zoo. A listing of Baltimore sites is copied in the back of this book.

Parking is available on any Loyola lot. Overnight parking is only allowed for those staying in Loyola housing.

SESSIONS

All plenary sessions, poster sessions, and coffee breaks will be held in McGuire Hall of the Andrew White Student Center. Lunches and the banquet will be held in the upper level of the Grand Market Place which adjoins the meeting room. The reception preceding Dr. Ho's talk will be held in McGuire Hall.

MEALS

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. Breakfast is not included with the meeting, however, anyone wishing breakfast can buy tickets at registration for the lower level of the Grand Market Place where a full breakfast is served for $4.00.

VENDORS

The following companies will be exhibiting their products during the meetings in the rear of McGuire Hall. We are grateful for their interest in our meeting and hope that they continue to meet with us to show their latest products.

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MAPMBS MEETING SCHEDULE

Monday, July 15, 1991

8:00 AM Registration

9:00 AM Opening Remarks - Dave Straney

PLANT/MICROBE INTERACTION
(Moderator: John Hammond)

9:15 AM Mike Wilson, Jane Osbourn, Roger Beachy, John Shaw, Xiaojie Wu, Lynne Watkins, and Karl Mundry (Rutgers University), "Implications of Plant Virus Nucleocapsid Structure for Infection"

9:45 AM John Hammond and Kathy Kamo (USDA/ARS/FNCL), "Virus Resistance Through Expression of Coat Protein and Antisense RNA of Bean Yellow Mosaic Virus in Transgenic *Nicotiana benthamiana*"

10:05 AM Richard Broglie, Karen Broglie, Ilan Chet, and Nicole Benhamou (DuPont), "Transgenic Plants with Enhanced Resistance to *Rhizoctonia solani*"

10:35-11:00 AM **COFFEE BREAK & POSTER SETUP**

REGULATION
(Moderator: John Watson)

11:00 AM Peter Goldsbrough, Amir Drory, Ky Young Park, Kaschandra Raghothama, Hong Wand, and William Woodson (Purdue University), "Regulation of Gene Expression by Ethylene During Carnation Petal Senescence"

11:30 AM Mark Tucker, Gail Matters, Elizabeth Kemmerer, Susan Baird, and Roy Sexton (USDA/ARS/PMBL), "Hormonal and Tissue-Specific Regulation of Cellulase Gene Expression"

11:50-1:00 PM **LUNCH**

REGULATION
(Moderator: Janet Slovin)

1:00 PM Gloria Coruzzi, Tim Brears, Fong-Ying Tsai, and Gabrielle Tjaden (Rockefeller University), "Molecular Mechanisms Controlling Amino Acid Biosynthesis in Plants"

1:30 PM Xia Lin, Xin-Hua Feng, and John Watson (Univ. of Maryland, College Park), "Differential Accumulation of Transcripts Encoding Protein Kinase Homologs in Greening Pea Seedlings"

1:50 PM Jerry Cohen, Allen Wright, Michael Sampson, Gerald Neuffer, Lech Michalczuk, and Janet Slovin (USDA/ARS/PHL), "*De novo* Indole-3-Acetic Acid Biosynthesis in the Maize Mutant Orange Pericarp, a Tryptophan Auxotroph"

2:10 PM Tony Gatenby, Gail Donaldson, Paul Vitanen, George Lorimer, and Saskia van der Vies (DuPont), "Molecular Chaperones and Rubisco Assembly"

2:40 PM Nestor Apuya and Lynn Zimmerman (Univ. of Maryland, Baltimore), "Differential Gene Expression in Carrot Somatic Embryos is Accomplished Through Translational Control"

3:00 PM Lauren McHenry, H Tai, JA Couch, H Dodo, DB Furtek, and PJ Fritz (Penn. State Univ.) Genome Characterization and Nucleotide Sequence of cDNA and Genomic Clones Encoding Major Seed Proteins of Cocoa"

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

KEYNOTE ADDRESS
(Introduction: Susan Koehler)

4:30 PM Tuan-hua David Ho (Washington University), "Hormonal Regulation of Gene Expression in the Aleurone Layers of Cereal Grains"

5:30-6:30 PM **SOCIAL HOUR**

6:30 PM **DINNER**

Tuesday, July 16, 1991

CLONING STRATEGIES
(Moderator: Rose Hammond)

9:00 AM Ken Feldman (University of Arizona), "Dwarfs and Other T-DNA Insertion Mutants in *Arabidopsis*"

9:30 AM Ben Matthews, Greg Wadsworth, Jane Weisemann, Barbara Wilson, Cleo Hughes, and Gregg Silk (USDA/ARS/PMBL), "Cloning of Soybean cDNAs Encoding Genes Involved in Amino Acid Biosynthesis and Nitrogen Distribution"

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

11:00 AM Patrick Masson (University of Wisconsin), "Transpositional Behavior of the *Spm* Corn Transposable Element in Heterologous Plants"

11:30 AM Sandra Russell, Joyce Hoopes, and Joan Odell (DuPont), "Manipulation of Transgenes: *LoxP-Cre* Mediated Marker Excision"

11:50 AM Business meeting

12:00-1:00 PM **LUNCH**

ORGANELLES
(Moderator: Betsy Kemmerer)

1:00 PM Maureen Hanson (Cornell University), "Plant Mitochondrial Gene Expression"

1:30 PM Eliot Herman (USDA/ARS/PMBL), "Multiple Origins of Intravacuolar Protein Aggregates of Plant Cells"

1:50 PM Karen Kindle and David Stern (Cornell University), "Chloroplast Transformation of *Chlamydomonas reinhardtii*"

2:20 PM Tim Fawcett, Roshni Mehta, Dan Porath, and Autar Mattoo (USDA/ARS/PMBL), "Membrane Translocation and Degradation of Ribulose-1,5-Bisphosphate Carboxylase Occurs Independent of Cross-Linking at Cysteine-247"

2:40 PM Closing Remarks - Mark Tucker

POSTER SESSIONS

(Monday 3:20-4:30 PM & Tuesday 8:00-10:00 AM)

Poster (page)

1 (30) "Preliminary Pathogenesis-Protein Characterization of *Theobroma cacao*". T. Synder and P. Fritz (Penn State)

2 (31) "Replication of Cucumber Mosaic Virus Satellite RNA *in vitro* by an RNA-Dependent RNA Polymerase from Virus Infected Tobacco". Gusui Wu, J.M. Kaper, and E.M. Jaspers (USDA/U. Maryland/Leiden U.)

3 (32) "Structure Domain of the 5' Half of Cucumber Mosaic Virus Satellite RNA Codetermines the Induction of Lethal Tomato Necrosis". Gusui Wu, M.E. Tousignant, and J.M. Kaper (USDA/U. Maryland)

4 (33) "A Cocoa Seed Protein with Trypsin Inhibiting Activity". H.W. Dodo, H. Tai, P.J. Frits, and D.B. Furtek (Penn State)

5 (34) "Characterization of the Soybean Aspartate Aminotransferase Isozyme-Gene System". Gregory J. Wadsworth and Benjamin F. Matthews (USDA, Beltsville)

6 (35) "Soybean Genes Encoding Aspartokinase and Homoserine Dehydrogenase". Jane M. Weisemann, Barbara J. Wilson, Thomas E. Devine, and Benjamin F. Matthews (USDA, Beltsville)

7 (36) "Analysis of Heat Shock Gene Expression in Transgenic Carrot Cells Containing an Antisense Gene". Cheol Ho Hwang and J. Lynn Zimmerman (U. Maryland)

8 (37) "Sensitivity of Petunia Seeds to Adenine Analogs". Sunita K. Agarwal and Dennis A. Schaff (U. Delaware)

9 (38) "Hormonal Regulation of Cellulase During Adventitious Rooting in Soybean". Elizabeth C. Kemmerer and Mark L. Tucker (USDA, Beltsville)

10 (39) "Molecular and Structural Studies of Soybean Oilbody Ontogeny". Deborah S. Loer, Andrzej Kalinski, Jane M. Weisemann, Benjamin F. Matthews, and Eliot M. Herman (USDA, Beltsville)

11 (40) "Putative Senescence Related Proteins in *Phaseolus vulgaris* as Detected by *in vivo* and *in vitro* Translation" Henry C. Butcher, Michael A. Wolfe, Nipa R. Doshi, Robert J. Mitkus, and Thomas A. Hensing (Loyola)

12 (41) "Does PCH313 Encode the Ethylene Forming Enzyme?". Ann Callahan, Ken Nichols, Reuben Cohen, Deanna Fishel, Linda Dunn, and Peter Morgens (USDA, Kearneysville)

Poster (page)

13 (42) "Ethylene and Polyamines Regulate Wound-Inducible Tomato Fruit ACC Synthase". N. Li, B.L. Parsons, D. Liu, and A.K. Mattoo (USDA, Beltsville)

14 (43) "Restriction Fragment Length Polymorphism Analysis of *Capsicum*". James P. Prince, Fernando Loaiza-Figueroa, Edmond Pochard, and Steven Tanksley (Cornell/INRA)

15 (44) "Effect of Nucleases Released From Germinating Pollen on Foreign DNA During Pollen Electrotransformation". Sally Van Wert and James A. Saunders (USDA, Beltsville)

IMPLICATIONS OF PLANT VIRUS NUCLEOCAPSID STRUCTURE FOR INFECTION

T. Michael A. Wilson[1], Jane K. Osbourn[1,2], Roger N. Beachy[3], John G. Shaw[4], Xiaojie Wu[4], Lynne H. Watkins[1], and Karl W. Mundry[5]

[1]Center for Agricultural Molecular Biology, Cook College, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903 USA, [2]Department of Virus Research, John Innes Institute, AFRC Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, United Kingdom, [3]Scripps Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037 USA, [4]University of Kentucky, Lexington, KY 40546 USA, [5]Biologisches Institut, Universitat Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80, Federal Republic of Germany

Destabilizing events required for and leading to the initiation of co-translational disassembly of rod-shaped plant RNA viruses in vitro have revealed a micro-instability at the 5' end of tobacco mosaic virus particles. Brief (≤15 secs) treatment of U-^{32}P-labelled TMV (vulgare or U2 strain) with 1% sodium dodecyl sulphate (SDS) exposed 2.5% (160 nts) of the RNA in susceptible virions. Uncoated RNA included distinctive, RNase T1-resistant oligonucleotides spanning the untranslated leader and first AUG codon on either viral RNA. The amount of ^{32}P-5'-oligonucleotide marker recovered remained constant over 15 mins in SDS. Additional RNase T1-sensitive oligonucleotides appeared only after 1-2 mins in SDS. Release of coat protein (CP) subunits from virions "destabilized" by ultracentrifugation at pH 7.2-9.2 was quantified using L-^{35}S-methionine-labelled particles of TMV (U2 strain). ^{35}S-CP recovery and virus particle translation results were consistent with increasing numbers of virions uncoating for approx. 200 nts. In the presence of sparsomycin (SPN), the TMV (*vulgare*) leader and first AUG (Ω; 70 nts) bind two 80S ribosomes. Electron microscopy of pH 7.5-'washed' TMV particles incubated in SPN-treated wheat germ extract or rabbit reticulocyte lysate, showed ≈10% with two bound ribosomes, confirming that Ω (at least) must have been fully uncoated. Nucleocapsids in these complexes were shortened by 9-10nm (i.e. 192-217 nucleotides uncoated). The template activities of virions pre-treated at pH 7.2-9.2 were destroyed by RNaseH when short (10-19 nt) cDNAs were hybridized to sequences at, or immediately 3' to, the first AUG codon. Computerized structure predictions of native TMV RNA suggest that Ω has an unfolded configuration. Several "random" leader sequences also lacking G-residues base-paired extensively with internal portions of TMV RNA. In contrast, a C-to-G transversion of Ω remained unpaired. We propose that the 5'-leader of TMV RNA interacts weakly with CP subunits, probably due to the absence of G-residues, while the precise sequence provides a low secondary structure to assist in recruitment and "scanning" of 40S ribosomal subunits.

To address the role of RNA base sequence in triggering release of the 5' leader sequence (Ω) for efficient recruitment of plant ribosomes, chimaeric mRNA molecules encoding E. coli ß-glucuronidase with a variety of different leader sequences have been constructed. These T7 transcripts can be encapsidated in TMV coat protein (CP) in vitro and PEG-inoculated or electroporated into protoplasts to assess the extent of nucleocapsid disassembly in response to varying 5' leader sequences.

Work with protoplasts from CP-transgenic tobacco lines has already revealed that over 90% of Ω-GUS pseudovirus particles fail to uncoat and release their messenger RNA in CP-expressing cells in vivo. Using double-labelled TMV particles, we have also demonstrated that the formation of "striposome" complexes in vivo is severely inhibited in CP-transgenic tobacco protoplasts. Further experiments have shown that inhibition of uncoating is insufficient alone to account for the protected phenotype.

Extensive analyses of a wide variety of 5' leader sequences have also revealed details of the mechanism of translational enhancement by the native TMV leader (Ω). The utility of native plant RNA viral leader sequence, or derivatives thereof, has been widely adopted in several biotechnological applications. Studies reveal that a subsequence within the TMV leader is responsible for its enhancing effect in 70S (prokaryotic) ribosome systems.

VIRUS RESISTANCE THROUGH EXPRESSION OF COAT PROTEIN AND ANTISENSE RNA OF BEAN YELLOW MOSAIC VIRUS IN TRANSGENIC NICOTIANA BENTHAMIANA: J. Hammond and K.K. Kamo; USDA-ARS, FNCL, Beltsville, MD 20705-2350.

Agrobacterium-mediated transformation was used to introduce the coat protein (CP) gene and a 3' antisense construct of bean yellow mosaic virus (BYMV) into Nicotiana benthamiana. Regenerated plants were analyzed for the presence of BYMV sequences by PCR, and seedling populations further examined. R1 plants with either of two CP constructs expressed up to 20% of the level of CP in an active BYMV infection, as estimated by indirect ELISA. Protection against BYMV is being determined in homozygous R2 populations; one antisense transformant showed a high degree of protection against virus inoculation (only one of eight plants becoming infected). Both CP constructions conferred some protection, with variation between transformants and between populations. In most cases symptom severity was reduced, and some plants showed apparent recovery from infection in upper leaves. Several populations of BYMV-infected antisense- and CP-transgenic plants developed a distinct pattern of interveinal chlorosis that was not observed in non-transgenic control plants; this pattern was typically seen in those plants that apparently recovered from symptom expression in subsequent growth, but was not limited to such plants.

TRANSGENIC PLANTS WITH ENHANCED RESISTANCE TO *RHIZOCTONIA SOLANI*. Richard Broglie, Karen Broglie ,Ilan Chet[1], and Nicole Benhamou* E.I. Du Pont de Nemours & Co., Agricultural Products Department, Wilmington, DE. 19880. * Universite Laval, Departement de Phytologie, St Foy, Quebec, Canada.

Chitin is an important component of the cell wall of many pathogenic fungi. One method by which plants protect themselves against potentially pathogenic organisms is by the production of lytic enzymes, such as chitinase, which enable them to partially hydrolyze the cell walls of invading pathogens. In healthy, uninfected plants, chitinase levels are low or undetectable. However, treatment with ethylene, oligosaccharide elicitors, or infection results in an increase in chitinase mRNA levels and an increase in enzyme activity. Transgenic tobacco plants containing a chimeric gene composed of a bean chitinase promoter fused to the coding region of the reporter gene β-glucuronidase (GUS) have been used to study chitinase gene expression in response to fungal infection. Our results indicate that gene activation is greatest in tissues immediately surrounding the site of fungal infection and coincides with the induction of endogenous defense genes. We have also produced transgenic tobacco and canola plants which express a bean chitinase gene modified so that the native inducible promoter is replaced by a high level, constitutive promoter. These transgenic plants exhibit increased chitinase enzyme activity and increased resistance to the pathogenic fungus *Rhizoctonia solani*. The resistant phenotype is characterized by a delay in the development of disease symptoms and an increased ability to survive in infested soil. Ultrastructural analysis of infected transgenic plants show that invading hyphae of *R. solani* suffer from poorly developed cell walls leading to cell disruption and protoplasm leakage.
([1]present address: Faculty of Agriculture, The Hebrew University, Rehovot, Israel).

callose plugs prevent further spread of pathogen.
- region 2 - conserved seq two chitin
-200
delete -453 lower expression but still reg. - 240 loose ethyl regulation

REGULATION OF GENE EXPRESSION BY ETHYLENE DURING CARNATION PETAL SENESCENCE. Peter B. Goldsbrough, Amir Drory, Ky Young Park, Kaschandra G. Raghothama, Hong Wang and William R. Woodson: Department of Horticulture, Purdue University, West Lafayette, IN 47907

Petal senescence in carnation is dependent on the ability to perceive and respond to ethylene. Senescence is accompanied by changes in petal mRNA populations, and these changes can be prevented by treatments that inhibit either ethylene biosynthesis or perception; in contrast exposure of mature flowers to ethylene induces both changes in gene expression and petal senescence. We have cloned a number of genes that are expressed during petal senescence. Two of these genes encode enzymes involved in ethylene biosynthesis, ACC synthase and ethylene forming enzyme (EFE). The ACC synthase cDNA represents a gene that is specifically expressed in floral tissue during the ethylene climacteric and not in response to auxin or wounding. Ethylene stimulates the accumulation of ACC synthase mRNA in petals as expected based on the autocatalytic nature of ethylene production in senescing flowers. EFE mRNA follows a similar pattern of expression in petals. A number of other senescence-related cDNAs have been isolated. These can be divided into two classes, one containing genes that are strictly regulated by ethylene and a second that is responsive to ethylene but is also controlled by temporal factors. Transcriptional precesses play a critical role in regulating the expression of these genes. Genomic sequences encoding a number of senescence related genes have been isolated and are being used in a variety of experiments to determine the elements that regulate tissue specificity and ethylene responsiveness.

pSR120 = pTOM13 (EFE)
similar expression to other
ethylene induced genes, ACC tox.
- SAM synthase not ethylene
regulated
- other ACC synthase gene in carnation

HORMONAL AND TISSUE-SPECIFIC REGULATION OF CELLULASE GENE EXPRESSION. Mark Tucker, Gail Matters, Elizabeth Kemmerer, Susan Baird, Roy Sexton, USDA, ARS, PSI, Plant Molecular Biology Lab, Beltsville, MD

We have identified and sequenced a cDNA clone of a bean abscission cellulase mRNA. The tissue- and cell-specific accumulation of cellulase mRNA was examined using RNA gel blots, tissue prints, and *in situ* hybridization. *In situ* hybridization indicates that all cells in the abscission fracture plane, regardless of cell type, accumulate cellulase mRNA. Experiments with 2,5-norbornadiene, a competitive inhibitor of ethylene action, show that ethylene is required not only to initiate cellulase gene expression in abscission but also to maintain its expression. Auxin applied prior to an ethylene treatment blocks abscission and cellulase mRNA accumulation.

An abscission-specific cellulase gene has been selected from a bean genomic DNA library. Two, overlapping genomic clones were identified, which encompass the entire cellulase coding region and 12 kbp of 5' upstream DNA. The bean abscission cellulase upstream sequence was compared to upstream gene sequences for avocado fruit cellulase, bean chitinase, and the tomato fruit expressed E8 gene. Sequence similarities with the bean cellulase gene were found for each of these ethylene induced genes. Exonuclease III was used to make deletions through the 5' upstream region of the bean cellulase gene, and these deletions were fused to a β-glucuronidase reporter gene. These promoter constructs are being analyzed in bean using a particle bombardment transient assay and in transgenic tomato plants.

A study of cellulase gene expression in soybean has been initiated. The emphasis in soybean is on adventitious root initiation (see Elizabeth Kemmerer's poster). Of particular interest in this project is that the increase in cellulase enzyme activity associated with root initiation is stimulated by auxin. Auxin has the opposite effect on cellulase accumulation during leaf abscission. Experiments are in progress to determine if the same cellulase gene or different genes are expressed in soybean root initiation and leaf abscission.

MOLECULAR MECHANISMS CONTROLLING AMINO ACID BIOSYNTHESIS IN PLANTS. Gloria Coruzzi, Tim Brears, Fong-Ying Tsai, Gabrielle Tjaden; The Rockefeller University, 1230 York Ave., New York, N.Y. 10021-6399

We have been investigating the molecular mechanisms which regulate amino acid biosynthesis in plants. In particular, we are studying the regulation of genes encoding enzymes involved in the biosynthesis of glutamine and asparagine, the principle amino acids utilized to assimilate and transport nitrogen in most higher plants. Our studies on the glutamine synthetase (GS) gene family in *Pisum sativum* have uncovered four nuclear genes for GS: one gene encoding the chloroplast isoenzyme (GS2) and three genes encoding cytosolic GS isoenzymes (GS1, GS3A, GS3B) (1,2). Expression studies have shown that the light-induced expression of the nuclear gene for chloroplast GS2 is mediated in part via phytochrome and in part via changes in chloroplast metabolism (e.g. photorespiration) (3). The expression of the genes for cytosolic GS3A and GS3B are both induced in two organs where large amounts of ammonia are assimilated into glutamine for intercellular transport (e.g. nitrogen-fixing root nodules and cotyledons) (4). Analysis of transgenic plants containing GS promoter-beta glucuronidase (GUS) fusions revealed that the GS2 promoter functions predominantly in photosynthetic cell types which correlates with its role in the reassimilation of photorespiratory ammonia. By contrast, the GS3A promoter functions exclusively in the phloem of transgenic tobacco, underscoring the function of this isoform in the generation of glutamine for intercellular transport. The non-overlapping patterns of expression of chloroplast GS2 and cytosolic GS3A suggest that these isoenzymes serve distinct roles in plant nitrogen metabolism (5). Our studies on the genes encoding the enzyme asparagine synthetase (AS1 and AS2) have revealed that AS gene expression is negatively regulated by light via phytochrome (6). This finding correlates with the fact that asparagine is utilized to transport nitrogen in situations where carbon skeletons are limiting (e.g. in the dark). AS gene expression is also induced coordinately with GS gene expression in nodules and cotyledons of germinating seeds (6). We have begun to define the cis-elements and trans-acting factors involved in the regulation of GS and AS genes. In particular, we are interested in determining how light via phytochrome activates the expression of GS2 while concomitantly repressing the expression of AS1. In addition, we are investigating the molecular basis for the induction of GS and AS gene expression in nodules and cotyledons. Our continuing studies on the GS and AS gene families should uncover the molecular basis for the coordinate regulation of genes encoding enzymes in a common metabolic pathway in plants.

1. Tingey, S.V., Walker, E.L., and Coruzzi, G.M. (1987) *EMBO J.* **6**, 1-9
2. Tingey, S.V., Tsai, F.-Y.,Edwards, J.W., Walker, E.L., and Coruzzi, G.M. (1988) *J. Biol. Chem.* **263**, 9651-9657.
3. Edwards, J.W., and Coruzzi, G.M. (1989) *Plant Cell* **1**, 241-248.
4. Walker, E.L., and Coruzzi, G.M. (1989) *Plant Physiol.* **91**, 702-708.
5. Edwards, J.W., Walker, E.L., and Coruzzi, G.M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3459-3463.
6. Tsai, F.-Y., and Coruzzi, G.M. (1991) *EMBO J.* **9**, 323-332.

DIFFERENTIAL ACCUMULATION OF TRANSCRIPTS ENCODING PROTEIN KINASE HOMOLOGS IN GREENING PEA SEEDLINGS:

Xia Lin, Xin-Hua Feng, and John C. Watson. University of Maryland, Department of Botany and Center for Agricultural Biotechnology, College Park, MD 20742-5815.

As a first step toward understanding the role protein kinases may play in photoregulated leaf development, we have examined transcripts encoding putative protein kinases in the garden pea. Degenerate oligonucleotides, corresponding to conserved regions within the catalytic domain of known protein-serine/threonine kinases, were used as primers for the polymerase chain reaction to amplify cDNA synthesized from poly(A)$^+$ RNA purified from the apical buds of 7 d-old pea seedlings. Five partial cDNAs were obtained and designated PsPK1 through PsPK5 in order of decreasing length. The deduced amino acid sequences show that each member of the PsPK series is different in length, and, although their sequences are quite similar overall, each has a unique sequence. Moreover, each member of the PsPK series has structural features typical of members of the protein-serine/threonine kinase family. All are equally similar to cyclic nucleotide-dependent protein kinase and protein kinase C. The similarity to second messenger-dependent protein kinases suggests to us that the pea homologs may be involved in signal transduction. DNA gel blots show that each PsPK cDNA is likely to be encoded by a single gene within the pea genome. RNA blot analyses show that the PsPK transcripts accumulate differentially during greening of etiolated seedlings. PsPK3 and PsPK5 transcripts show a large and rapid decline during de-etiolation. In contrast, the level of PsPK4 RNA increases steadily during de-etiolation whereas PsPK1 and PsPK2 transcripts show little change during the greening period. The differential alterations in transcript abundance we observe may reflect changes in the protein kinase forms present during photoregulated leaf development in the garden pea.

De Novo INDOLE-3-ACETIC ACID BIOSYNTHESIS IN THE MAIZE MUTANT *ORANGE PERICARP*, A TRYPTOPHAN AUXOTROPH.

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The maize (*Zea mays* L.) mutant *orange pericarp* is a seedling lethal which lacks tryptophan synthase B subunit activity. This phenotype is caused by the mutation of two unlinked loci, orp1 and orp2. The mutant accumulates indole and can be rescued by treatment with tryptophan. The orange kernel color is due to indolic products which accumulate in the pericarp. We have used this mutant to test the hypothesis that tryptophan is the precursor to the plant hormone indole-3-acetic acid (IAA). IAA levels in aseptically grown mutant seedlings (10 d) were determined by quantitative mass spectrometry to be approximately 50 times that of the normal seedlings. Mutant and normal embryos were grown on media containing stable isotope labeled precursors, or 30% D_2O. All embryos grown with 30% D_2O contained aromatic ring-deuterated IAA, indicating *de novo* synthesis from early precursors prior to entry into the shikimic acid pathway. The mutant seedlings showed preferential incorporation of deuterium into IAA versus tryptophan, whereas the reverse was true in the normal seedlings. The mutant and normal both incorporated ^{15}N from anthranilate into IAA, but only the normal incorporated anthranilate into tryptophan. These results support our findings in *Lemna gibba*, that there is very low incorporation of label from L-tryptophan into IAA (and no incorporation of D-tryptophan) and these results now establish that indole-3-acetic acid can be produced *de novo* in higher plants without tryptophan as an intermediate.

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MOLECULAR CHAPERONES AND RUBISCO ASSEMBLY: Anthony A. Gatenby, Gail K. Donaldson, Paul V. Viitanen, George H. Lorimer and Saskia van der Vies: Central Research and Development, E.I. DuPont de Nemours & Co., Wilmington, DE 19880-0402.

Chaperonins are a conserved class of proteins found in bacteria, chloroplasts and mitochondria. In Escherichia coli, the heat-shock regulated cpn60 (GroEL) and cpn10 (GroES) chaperonins together facilitate the assembly of various forms of ribulose bisphosphate carboxylase/oxygenase (Rubisco). The assembly of dimeric, octameric and hexadecameric forms of Rubisco therefore require the chaperonins in vivo (1). In subsequent experiments, it has been demonstrated that the in vitro reconstitution of active dimeric Rubisco from chemically denatured polypeptides depends on the presence of purified chaperonins (2). Using this in vitro system it has been possible to characterize in detail the interactions of unfolded Rubisco with chaperonins (3)

Chaperonins isolated from bacteria or eukaryotic organelles can support in vitro Rubisco reconstitution, and this has formed the basis of an assay to identify the cpn10 homologue in organelles (4). Thus, both chloroplasts and mitochondria possess the same two proteins (cpn60 and cpn10) that are used in bacteria for the folding and assembly of bacteriophages, Rubisco and other proteins. In addition to Rubisco assembly, chaperonins may have a general role in chloroplasts in the folding of numerous proteins. Using isolated chloroplasts, it has been observed that many proteins that are imported into the plastid will form a stable complex with the endogenous chaperonin cpn60 (5). These binary complexes may be formed between chaperonins and incompletely folded imported proteins, and may represent an important intermediate step in the folding pathway of plastid proteins.

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DIFFERENTIAL GENE EXPRESSION IN CARROT SOMATIC EMBRYOS IS ACCOMPLISHED THROUGH TRANSLATIONAL CONTROL

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We have isolated two genes whose expression is enhanced in the globular stage of somatic embryogenesis of carrot. These two genes encode a translation Elongation Factor (EF-1α) and the β-subunit of the mitochondrial ATP synthase (ATP-2) in carrot, and were identified by screening a cDNA library constructed from polysomal mRNA from globular embryos. A comparison of polysomal mRNA from callus cells, globular embryos and plantlet stage embryos reveals that the abundance of the EF-1α mRNA in globular embryo polysomes is 2X greater than in polysomes of callus cells and 20X greater than in polysomes of plantlet stage embryos. A parallel analysis of the ATP-2 gene reveals that this mRNA is 3X greater in polysomes of globular embryos than in callus polysomes and 30X greater than in plantlet polysomes. At the same time, however, the abundance of either mRNA is equivalent in the total RNA from callus cells, globular and plantlet stage embryos. These results reveal that the developing globular embryo is capable of selectively translating specific mRNAs presumably required for or important in early embryogenesis, and that these mRNAs would never be identified by differential screening using total RNA. Moreover, they highlight the potential significance of translational level control in directing the important developmental program of plant embryogenesis.

GENOME CHARACTERIZATION AND NUCLEOTIDE SEQUENCE OF CDNA AND GENOMIC CLONES ENCODING MAJOR SEED PROTEINS OF COCOA: L McHenry, H Tai, JA Couch, H Dodo, DB Furtek, and PJ Fritz, American Cocoa Research Institute Cocoa Molecular Biology Laboratory, Intercollege Plant Physiology Program, Department of Food Science, Intercollege Genetics Program, and the Biotechnology Institute, The Pennsylvania State University, University Park, PA 16802

Theobroma cacao is a tropical tree which is the unique source of cocoa butter and chocolate flavor used in the manufacture of a variety of cosmetic and food products. Characterization of the cocoa genome and several key seed genes is being undertaken in order to provide a molecular basis for potentially altering characteristics of economic importance. The major storage product of the seed is highly saturated lipid, comprising approximately 50% of the dry weight. Proteins (15-20%) provide additional reserves for the developing seed as well as the precursors of chocolate flavor components. Preliminary estimates from reassociation kinetic data indicate that the cocoa genome is small and contains less repetitive DNA than the majority of angiosperms characterized to date. G+C content is estimated at 40% (T_m=85°C). cDNA and genomic clones encoding the most abundant storage protein of cocoa seeds have been sequenced, as well as a cDNA encoding the second most abundant protein, a protease inhibitor with high homology to the soybean trypsin inhibitor (Kunitz) class. The latter cDNA encodes a 221 amino acid protein of 23kDa, including a 26 residue signal polypeptide. The storage protein appears to be glutamine-rich and its gene has a potential open reading frame which could encode a protein of 61kDa. Four introns of between 90 and 113 base pairs have been identified. The majority of seed mRNA during mid-seed development encodes the storage protein and Southern analysis indicates that only one or two copies of the gene are present per haploid genome. A third seed cDNA encoding an enzyme involved in fatty acid desaturation, stearoyl-ACP desaturase, has been cloned using a heterologous probe from castor bean. Partial sequence data indicates high homology (79% identity, 30% of coding region presently sequenced) to the castor bean cDNA.

The phytohormone abscisic acid (ABA) regulates seed maturation and desiccation tolerance in a variety of temperate angiosperms. While cocoa seed development does not culminate in dormancy, nor does the seed exhibit extensive desiccation, the levels of ABA in the seed mimic that of temperate species and exogenously applied ABA inhibits premature germination of cultured immature seeds. The effect of ABA on mRNA accumulation of cocoa seed genes is currently under investigation.

HORMONAL REGULATION OF GENE EXPRESSION IN THE ALEURONE LAYERS OF CEREAL GRAINS

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The aleurone layers of germinating cereal grains respond to gibberellic acid (GA_3) by synthesizing and secreting a group of hydrolytic enzymes, including α-amylases, proteases, and nucleases. All these enzymes have been purified, characterized and their physiological roles are well understood. There are two groups of α-amylase isozymes with markedly different specific activities toward their substrates. The major proteases capable of degrading the storage proteins are two classes of cysteine-endoproteases. These cysteine endoproteases are synthesized as large precursors which have to be post-translationally processed both intra- and extra-cellularly to form the final products. The nuclease, which possesses DNase, RNase and 3'-nucleotidase activities, is first targeted for the vacuoles and eventually released upon cell death. Gibberellic acid transcriptionally activates α-amylase genes in this tissue while abscisic acid (ABA) suppresses this expression. The nuclear extracts from aleurone cells have been assayed for proteins which bind to sequences in α-amylase promoters. Two sequences, TAACAGA (element I) and TATCCATGCAGTG (element II) have been shown to interact with proteins in the nuclear extracts. A GT box-like element (AGCGGTATT) as well as other DNA elements are also able to interact with nuclear proteins. To test the significance of these interactions, various derivatives of α-amylase promoters, including a set of linker-scan deletions which eliminate or disrupt sites of protein/DNA interaction, have been generated and fused to the reporter gene β-glucuronidase. Similarly, oligonucleotides that have been shown to interact with nuclear proteins have been fused to a minimal promoter/β-glucuronidase construct. These constructs were expressed transiently in aleurone cells following particle-bombardment mediated delivery into aleurone cells. GUS expression from constructs containing as little as 350bp of promoter sequence were appropriately regulated by GA and ABA. A 30bp of promoter sequence encompassing element I and II abolished GA_3-dependent expression. A linker-scan mutation in element II, TCCATCTAGA, retained GA_3-responsive expression, but at a reduced level (20%), indicating that this element could serve as a general enhancer for gene expression. Potential applications of the cereal aleurone system in biotechnology will also be discussed.

DWARFS AND OTHER T-DNA INSERTION MUTANTS IN **ARABIDOPSIS**:
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We have now generated more than 12,000 transformants of *Arabidopsis thaliana* using the seed infection/transformation protocol (MGG 208:1, 1987). Segregating seed families from 8,000 of these have been screened under several environmental regimes for visible alterations in phenotype. More than 1,000 putative mutants were noted. The majority of these mutant lines are segregating in a manner that indicates that they are due to a T-DNA insert (K. Feldmann, Plant Journal, in press, 1991). The visible mutants fall into seven classes including seedling-lethal, reduced fertility, size variant, pigment, embryo-defective, physiological, and dramatic or morphological. The physiological mutants include the late flowering, deviated wax layer, ethylene-affected, and all of the mutants that will be isolated using various selective agents. The dramatic mutants are those that have a lesion in a developmental pathway. Included in this class are the flower, root, trichome, root hair, leaf shape, meristem, and dwarf mutants. Dwarf plants are those that have a reduced stature (5-8 cm), short dark green leaves, and a robust stem. All, except one, have reduced fertility due to a failure of the stamens to elongate. Allelism tests are in progress. Two of the dwarfs have a twisted phenotype and the roots do not respond in a wild-type manner to gravity. Three of the dwarfs are smaller in stature than the rest. One dwarf accumulates large amounts of anthocyanins such that the rosette is purplish green. We have not been able to get this mutant to flower. Under etiolated conditions this mutant has a phenotype similar to *det2* (Chory et al., Plant Cell 3:445 1991). Three other dwarfs are similar in phenotype to a previously characterized dwarf (Feldmann et al., Science 243:1351). Tests for cosegregation of the insertion marker (kanamycin resistance) with the mutation has been completed on nine of the mutants (Yewen Wu, unpublished data). The results for six of the mutants, that had a normal Mendelian ratio for the Kan^R marker and the mutation, indicate that the mutation is due to a T-DNA insert. A line that was segregating 40:1 (wt:mutant) was shown not to be due to a Kan^R marker. Results from two other lines that were segregating 1:4:1 and 1:2:2 (Kan^R, mutant: Kan^R, wt:Kan^S, wt) also show that these lines are not due to a functional insert. A tenth line has yet to be tested. In this talk I will review the transformation system, show examples of mutants, and update our progress on the dwarf mutants.

CLONING OF SOYBEAN cDNAs ENCODING GENES INVOLVED IN AMINO ACID BIOSYNTHESIS AND NITROGEN DISTRIBUTION

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In soybean aspartate is an amino acid which is central to carbon and nitrogen distribution and hydrogen shuttles. Also, aspartate is the precursor of asparagine and the essential amino acids lysine, threonine, methionine and isoleucine. Aspartate can be synthesized from oxaloacetic acid and glutamate by **aspartate aminotransferase (AAT)**. In soybean this enzyme exists as multiple isoenzymes, which are differentially expressed spatially and temporally. Aspartate can be converted into asparagine by **asparagine synthetase (AS)** for use in protein synthesis or nitrogen transport. Aspartate also can be metabolized into lysine, threonine and methionine by a complex, branched pathway. The first enzyme of this pathway is **aspartokinase (AK)**, which is feedback regulated by lysine and threonine. Threonine and methionine are synthesized from aspartate through the pathway branch-point enzyme **homoserine dehydrogenase (HSDH)**. HSDH is feedback inhibited by threonine in soybean. In carrot and soybean AK and HSDH activities can exist on the same protein molecule forming a bifunctional enzyme. cDNA clones encoding AAT and the bifunctional AK-HSDH were identified by screening soybean cDNA libraries using heterologous carrot cDNA probes produced by this laboratory. cDNA clones encoding three different soybean AATs and one bifunctional AK-HSDH have been identified and partially or completely sequenced. Hybridization patterns of AK-HSDH to Southern blots of digested soybean genomic DNA suggest that AK-HSDH is comprised of a small family of genes. Attempts to identify cDNAs encoding AS and **dihydrodipicolinic acid synthase (DS)**, the branch point enzyme leading to lysine synthesis, are underway using PCR reactions with primers designed from portions of published pea AS and wheat DS sequences.

TRANSPOSITIONAL BEHAVIOR OF THE *Spm* CORN TRANSPOSABLE ELEMENT IN HETEROLOGOUS PLANTS: Patrick H. MASSON, Laboratory of Genetics, University of Wisconsin-Madison, 445 Henry Mall, Madison, WI 53706

The *Spm* family of corn transposable elements is composed of both autonomous and defective elements. The autonomous elements are capable of promoting their own transposition as well as the transposition of other elements located elsewhere in the genome. The defective (*dSpm*) elements are not capable of promoting their own transposition. However, they can respond to the transposase function encoded by an autonomous element. The autonomous element is 8.3 kb-long and contains two long open-reading-frames (ORF1 and ORF2). It codes for at least four alternatively spliced transcripts named *tnpA*, *tnpB*, *tnpC* and *tnpD*. *tnpA* is about 100 times more abundant than the three other *Spm*-encoded transcripts in maize plants containing a fully active autonomous element. While *tnpA* and *tnpB* are monocistronic, *tnpC* and *tnpD* are dicistronic. Their first ORF is encoded by ORF1 and ORF2, while their second ORF is identical to ORFA.

Spm is functional in transgenic tobacco where it catalyses its own transposition as well as the transposition of other defective elements located elsewhere in the genome. A visual assay for *Spm* transposition in transgenic tobacco was developed, based on the restoration of GUS gene expression upon *dSpm* transposition. Using this assay, we showed that *tnpA* and *tnpD* are necessary and sufficient to promote *dSpm* transposition in tobacco plants. The molecular characterization of several empty donor fragments left over after *tnpA*/*tnpD*-promoted *dSpm* transposition in tobacco and of the sequences flanking several transposed elements in corn and transgenic tobacco allowed the development of a model for *Spm* transposition.

MANIPULATION OF TRANSGENES: *LOXP*-CRE MEDIATED MARKER EXCISION: Sandra H. Russell, Joyce L. Hoopes, and Joan T. Odell. Du Pont, Central Research and Development and Agricultural Products, Wilmington, DE 19880-0402

Stably introduced *loxP* and *cre* elements derived from bacteriophage P1 were previously shown to be active in the plant genome resulting in site-directed recombination (Odell et al. MGG (1990) 223:369). *LoxP*, a 34 bp DNA sequence, is a substrate for the Cre recombinase protein. Cre was used to activate an inactive gene, that was integrated into the tobacco genome, by excising an intervening sequence bounded by *loxP* sites. We now demonstrate that two linked traits can be introduced into the plant genome, and then loss of one of the traits in re-transformants or in F1 and successive generations can be controlled by the introduction of *cre*. A sulfonylurea resistant acetolactate synthase (ALS) gene bounded by *loxP* sites was used as the selectable marker to produce transgenic tobacco and Arabidopsis plants. A β-glucuronidase (GUS) gene was linked to the ALS gene. Deliberate loss of the ALS selection marker through Cre-mediated excision was demonstrated by loss of resistance to sulfonylurea herbicides and molecular analysis, while the GUS gene remained intact. Factors affecting the efficiency and heritability of the controlled trait loss were analyzed. These and the previous results demonstrate that the *loxP*-Cre system allows manipulation of a transgene to either an active or inactive state; a transgene can exist in one expression state for any number of generations until *cre* is introduced, which then causes an alteration in the expression of the transgene.

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recombination repeats

PLANT MITOCHONDRIAL GENE EXPRESSION. Maureen R. Hanson; Genetics and Development, Biotech. Bldg., Cornell University, Ithaca, NY 14850

Gene expression in plant mitochondria involves several types of RNA processing (1). Optional group II introns are present in plant mitochondrial cytochrome oxidase subunit II (*coxII*) genes (1,2). Group II introns also appear to function in the trans-splicing of NADH dehydrogenase subunit I (*nad1*) genes (1). In *Petunia* mitochondria, one cis-splicing and three trans-splicing events are needed to produce a mature *nad1* transcript (3). The five exons of *nad1* are scattered around the 443 kb genome of fertile *Petunia* line 3704 (3,4), some over 100 kb and in the opposite orientation with respect to others.

Foremost among the unusual aspects of RNA processing in plant mitochondria is the phenomenon of RNA editing (1). At multiple locations within cDNA clones, Ts are found where C would be predicted from the genomic sequence. Transcripts of Petunia ATP synthase subunit 9 (*atp9*) are among the most highly edited which have been detected: 10 editing events occur within 75 codons, and the stop codon is created by editing (5). By analyzing precursor transcripts of *coxII* and *nad1*, we have determined that editing can occur before cis- and trans-splicing (6). Editing intermediates are found, indicating that editing is not strictly coincident with transcription. No obvious polarity of the editing process is observed. As sequences including Cs that are edited have no obvious similarity that distinguish them from those that are not edited, how Cs are selected for editing remains an important question.

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MULTIPLE ORIGINS OF INTRAVACUOLAR PROTEIN AGGREGATES OF PLANT CELLS

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The central vacuole of plant cells occupies a majority of the cell's total volume. The vacuole consists of a single unit membrane that encloses the vacuolar sap, a solution of diverse small molecules and acid hydrolases. Ultrastructural analysis of plant cells frequently show aggregates of protein within the vacuole. There are a variety of functional roles of the intravacuolar protein aggregates which have been elucidated by the combined approaches of immunocytochemistry and ultrastructural analysis. Vacuoles are used by many different cells and organs for sequestering aggregates of newly synthesized proteins as transient nitrogen/carbon reserves. Developmental programming or environmental cues are important in the pattern of deposition and subsequent mobilization of storage proteins. These proteins which enter the vacuole after transport through the endomembrane system must possess vacuolar targeting sequence(s). Proteins are also sequestered within the vacuole as the consequence of autophagy and endocytosis and may be subsequently degraded. Proteins which enter the vacuole through autophagy or endocytosis do not have to possess vacuolar targeting sequences. As a consequence, intravacuolar protein aggregates may contain the constituents derived from multiple cellular compartments.

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CHLOROPLAST TRANSFORMATION OF *Chlamydomonas reinhardtii.*
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Chloroplast transformation in *Chlamydomonas* usually results in homologous recombination between the transforming DNA and sequences in the chloroplast genome. Taking advantage of this tendency, we have developed a cotransformation approach that can be used to identify transformants with alterations that cannot be selected directly because they do not change the phenotype of the cell. We have used this cotransformation approach to generate specific mutations in the chloroplast genome.

Mutations that remove the 3' untranslated region of the *Chlamydomonas* chloroplast *atp*B gene, including a region that has the potential of forming a stable stem-loop structure, prevent the accumulation of a discrete size class of *atp*B mRNA. This truncated gene functions well enough to permit phototrophic growth, but *Chlamydomonas* chloroplast transformants carrying such mutations do not grow well phototrophically, accumulating only about 30% of wild-type levels of *atp*B mRNA. The *atp*B mRNA is heterogeneous in size, and accumulation of the β subunit of chloroplast ATPase is only half of wild-type levels (Stern, Radwanski, and Kindle, *Plant Cell* **3**: 285-297). Some transformants that had been bombarded with the deleted stem-loop *atp*B gene grew better than the majority class under selective conditions, and analysis of these strains indicates that they transcribe the gene at a high rate, ranging from 2-5 fold higher than wild-type. Although they still do not accumulate a discrete size mRNA, the accumulation of RNA and protein are at or above normal levels. The increased transcription appears to be due to the high copy number of the transforming plasmid in the chloroplast compartment. Quantitative DNA blot analysis indicates that in some variants there are >500 copies of the plasmid per chloroplast. I will describe the characterization of these transformants, including the form of the plasmid DNA copies and additional recombination events in the chloroplast genome.

MEMBRANE TRANSLOCATION AND DEGRADATION OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE OCCURS INDEPENDENT OF CROSS-LINKING AT CYSTEINE-247: Timothy W. Fawcett, Roshni A. Mehta, Dan Porath and Autar K. Mattoo; Plant Molecular Biology Laboratory, Beltsville Agricultural Research Center, USDA/ARS, Beltsville, MD 20705.

The degradation of ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) was examined under conditions of oxidative stress in *Spirodela*, wheat and intact wheat chloroplasts. In all of them rubisco underwent a rapid translocation to the chloroplast membrane prior to its degradation; however, the kinetics of the translocation and degradation were different. In addition, dimerization between large subunits of rubisco (LS) most probably via cysteine-247 also occurred during the oxidative stress imposed, indicating hypersensitivity of the domain encompassing that amino acid residue to oxidative damage. To determine if LS dimerization via disulfide linkage in the rubisco holoenzyme is required for membrane translocation and/or degradation, two strains of *Chlamydomonas* were utilized. *C. reinhardtii*, like most higher plants, contains a cysteine residue at position 247 while *C. moewusii* contains a serine at that position. Thus, *C. moewusii* is a natural point mutant which we used to address this question. Although no dimerization of LS was observed in *C. moewusii*, translocation and degradation of rubisco did occur under oxidative conditions indicating that rubisco dimerization and degradation are independent processes.

Poster #1

PRELIMINARY PATHOGENESIS-PROTEIN CHARACTERIZATION OF THEOBROMA CACAO: T. Snyder and P. Fritz, American Cocoa Research Institute Cocoa Molecular Biology Laboratory, Intercollege Plant Physiology Program, Department of Food Science, The Pennsylvania State University, University Park, PA 16802

Theobroma cacao is a tropical tree crop grown within 20° of the equator. This tree is the only source of cocoa and cocoa butter, making it an important cash crop for many developing countries. *T. cacao* is susceptible to some viral and many fungal pathogens. Fungal diseases of cacao can cause losses of 80% or more annually worldwide. During the past several decades tropical research stations have been screening cocoa for disease resistant phenotypes. Although some resistant clones are recognized, little information about the molecular basis of disease resistance is available.

During the past decade, several pathogenesis-related proteins associated with disease resistance have been identified as beta-1,3-glucanases and chitinases. Concentrations of beta-1,3-glucanases and chitinases have been demonstrated in a number of plant species to increase dramatically in response to fungal infection and wounding. These enzymes have fungitoxic, hydrolytic activity that directly degrades fungal cell walls and releases oligosaccharides that are elicitors of defense responses in plants. Resistant plants often accumulate these hydrolytic enzymes, and other defense compounds such as phytoalexins, at a much faster rate than the susceptible plants.

This research addresses some of the initial reponses of *T. cacao* to pathogen attack. Proteins were extracted from healthy, infected or wounded tissue and analyzed by SDS-PAGE and Western blotting. Dr. Richard Broglie of E.I. Du Pont de NeMours Co. generously provided anti chitinase sera used in the Westerns.

Fully expanded leaves on four week old seedlings were used for wounding and *Phytophthora palmivora* inoculation (causal organism of cocoa black pod disease). Inoculated plants were grown in a high humidity tent for 7-10 days and leaves were harvested when symptoms began to develop. Pod material was taken from the greenhouse or from the Hershey Foods Corporation plantation in Belize. Protein extracts from bean leaves were used as a positive control in Western blots.

Results from Western blotting demonstrate the crossreactivity of a 26 Kd constitutive protein from *T. cacao* pod tissue with the antisera from bean chitinase. No cross reaction is found when the Westerns are incubated with non-immune rabbit serum. These results indicate a protein of 26 Kd in *T. cacao* pods is immunologically reactive with the bean chitinase. Protein analysis, PCR and cDNA screening are being used to isolate the first defense related gene(s) from *T. cacao*.

Poster #2

REPLICATION OF CUCUMBER MOSAIC VIRUS SATELLITE RNA IN VITRO BY AN RNA-DEPENDENT RNA POLYMERASE FROM VIRUS INFECTED TOBACCO; Gusui Wu[1,2], J.M. Kaper[1] and E.M.J. Jaspars[3], [1]Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705; [2]Dept. of Botany, University of Maryland, College Park, MD 20742; [3]Dept. of Biochemistry, Leiden University, The Netherlands.

The satellite RNA of cucumber mosaic virus (CMV) with the designation CARNA 5 (for CMV-Associated RNA 5) comprises a group of sequence related variants. Although CARNA 5 is essentially sequence unrelated to the CMV tripartite RNA genome, it depends on the latter's presence for replication. An RNA-dependent RNA polymerase (RdRp) was purified from tobacco tissue infected with a satellite-free preparation of CMV strain S which yielded a sufficiently pure RdRp so as to be completely dependent on an exogenous viral RNA template [Quadt and Jaspars (1991) FEBS Letters 279:273-276]. Synthetic activity of this CMV RdRp was determined using the genomic RNAs of CMV, tomato aspermy virus (TAV), and peanut stunt virus (PSV) (all tricornaviruses and members of the cucumovirus group), as well as two CARNA 5 variants significantly different in length and the satellite PARNA 5 (for PSV-Associated RNA 5) as templates. The purified CMV RdRp recognized both CARNA 5 variants and completed a full replication cycle by synthesizing both minus and plus strands when only the plus strand was offered as template. The enzyme failed to replicate PARNA 5. This finding is consistent with previous *in vivo* studies that showed no replication support of PARNA 5 by CMV. The CMV RdRp also recognized and utilized both TAV-RNA and PSV-RNA as templates, but with TAV-RNA ^{32}P label was incorporated equally strongly as with the homologous CMV-RNA, while PSV-RNA was synthesized with lesser efficiency. This is the first unequivocal evidence that the RdRp encoded by a plus stranded RNA virus, with a high degree of specificity for catalyzing the replication of its homologous RNA, can also replicate its encapsidated, sequence-unrelated satellite RNA. The specificity with which CMV RdRp replicated different sequence-unrelated RNA templates suggests that the site of their recognition requires secondary or higher level structural organization.

Poster #3

STRUCTURE DOMAIN OF THE 5' HALF OF CUCUMBER MOSAIC VIRUS SATELLITE RNA CODETERMINES THE INDUCTION OF LETHAL TOMATO NECROSIS; Gusui Wu[1,2], M.E. Tousignant[1] and J.M. Kaper[1], [1]Microbiology and Plant Pathology Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705; [2]Dept. of Botany, University of Maryland, College Park, MD 20742.

Different variants of the satellite RNA of cucumber mosaic virus (CMV), CARNA 5 (for CMV-Associated RNA 5) modify viral disease symptoms in tomato in varying ways, from inducing plant death at one extreme to complete amelioration of symptoms at the other. All CARNA 5 variants that cause lethal necrosis have a conserved sequence domain within their 3' half, with the exception of Y-CARNA 5 which contains this 3' conserved sequence domain but does not induce lethal tomato necrosis. Y-CARNA 5 is also unusual in that its 5' half harbors a region where 100 nucleotides have substituted a stretch of 70 others, present in most variants, with the two nucleotide blocks essentially unrelated in sequence. To determine whether these biological and structural anomalies were related, we have constructed chimeric CARNA 5 cDNAs in which the 5' half of Y- CARNA 5 was exchanged with those of a prototype necrogenic D-CARNA 5 or non-necrogenic S-CARNA 5 lacking the 70 for 100 nt replacement. Control chimera retaining the 5' half of Y-CARNA 5 but possessing the 3' halves of D-or S-CARNA 5 were also constructed. Chimeric CARNA 5 transcripts from the clones were assayed on tomato by co-inoculation with a helper CMV-1, their replication analyzed by gel electrophoresis and Northern blot hybridization, and the sequences of their PCR generated cDNAs confirmed. All control chimera containing the 5' half of Y-CARNA 5 did not induce tomato necrosis, even if their 3' half possessed the conserved sequence domain of necrogenic D-CARNA 5. Chimera where the 5' half of Y-CARNA 5 was replaced by those of either D- or S-CARNA 5 caused lethal necrosis in tomato. This result indicates that the 100 for 70 nucleotide exchange in the 5' half of Y-CARNA 5 is responsible for its lack of necrogenicity and that structure domains in both halves of CARNA 5 codetermine its ability to induce tomato necrosis with a given helper CMV.

Poster #4

A COCOA SEED PROTEIN WITH TRYPSIN INHIBITING ACTIVITY:
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The cocoa tree, *Theobroma cacao*, is a very important crop to the chocolate and pharmaceutical industries because its seeds are the sole source of cocoa powder and cocoa butter. The tree is also important to many developing countries whose economic welfare depends heavily on the export of cocoa seeds and cocoa butter.

To study and characterize cocoa seed proteins and their functions, water-soluble cocoa seed proteins were extracted from mature cocoa beans and purified. Polyacrylamide gel electrophoresis and immunochemical analysis indicates the size of one of the major seed proteins to be about 21 kD.

The sequence of the first twenty NH_2-terminal amino-acids of the protein were determined. A synthetic oligonucleotide probe was designed, and used to screen a cocoa seed cDNA library constructed from poly (A)+ mRNA. A 983 bp clone was isolated and sequenced. The deduced amino acid sequence of the clone shows 38% homology with a barley alpha-amylase subtilisin inhibitor, and with protease inhibitors of the Kunitz familly.

These inhibitor activities have never before been demonstrated in *Theobroma cacao*; therefore the 21 kD seed protein was assayed for serine proteinase and alpha-amylase inhibiting activities. The protein is a potent inhibitor of bovine trypsin, with an inhibiting capacity twice as high as Kunitz soybean trypsin inhibitor. However, it did not exhibit inhibiting activity toward bovine pancreas alpha-chymotrypsin, subtilisin Carlsberg, and barley malt alpha-amylase.

Poster #5

CHARACTERIZATION OF THE SOYBEAN ASPARTATE AMINOTRANSFERASE ISOZYME-GENE SYSTEM. Gregory J. Wadsworth and Benjamin F. Matthews, USDA/ARS, Plant Molecular Biology Laboratory, 10300 Baltimore Ave, Beltsville, MD. 20705

Aspartate aminotransferase (AAT) plays multiple metabolic roles in plants including nitrogen assimilation, hydrogen shuttles, and amino acid biosynthesis. Plants characteristically have multiple isozymes of aspartate aminotransferase encoded by multiple genes. In soybean we can distinguish five different isoforms electrophoretically, named according to their mobility from slowest to fastest (1-5). Two of the isozymes are spatially and temporally limited in their expression. AAT-1 is detected only in the postgermination cotyledon and AAT-3 is found only in green tissues such as green leaves. Organelle fractionation on sucrose gradients indicates the AAT isozymes are localized in different subcellular compartments. AAT-1 is localized in the glyoxysome, AAT-4 is localized in the mitochondria, and AAT-5 is localized in the plastids. AAT-2 and AAT-3 do not sediment with membrane bound organelles in the sucrose gradients and may be cytosolic isozymes. We have isolated three soybean cDNA clones which hybridize to a carrot AAT cDNA. Preliminary restriction and sequence analyses indicate that these three clones represent three different soybean AAT genes. These cDNA clones hybridize to soybean genomic blots in simple but unique patterns, indicating there is one or very few copies of each of these AAT genes. One of these clones, pAAT-17 has been completely sequenced. It contains an open reading frame which encodes a 55.5 kilodalton protein which shows homology to the mitochondrial AAT isozyme of mouse and other vertebrates. At the N terminal of the predicted protein there is a region which resembles the transit peptide of nuclear encoded mitochondrial and chloroplast proteins. We have cloned and expressed in E. coli the region of the pAAT-17 open reading frame with homology to the mature mitochondrial AAT of vertebrates. The expressed polypeptide has AAT activity and comigrates with the chloroplast form of soybean AAT during electrophesis.

Poster #6

SOYBEAN GENES ENCODING ASPARTOKINASE AND HOMOSERINE DEHYDROGENASE. Jane M.Weisemann, Barbara J. Wilson, Thomas E. Devine and Benjamin F. Matthews.
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Aspartokinase (AK) is the first enzyme in the pathway for synthesis of the amino acids lysine, threonine, and methionine from aspartate. Homoserine dehydrogenase (HSDH) catalyzes the third step in the branch of the same pathway leading to formation of threonine and methionine. In soybean (Glycine max) two forms of AK and four forms of HSDH have been identified. We have isolated several cDNA clones. The DNA sequence of one clone has been partially determined. The clone appears to encode a bifunctional AK-HSDH protein with homology to carrot AK-HSDH and E. coli AK-HSDH. A fairly complex hybridization pattern is observed when this clone is used to probe soybean genomic DNA on Southern blots. The pattern suggests that soybean AK-HSDH may be part of a small family of related genes. One AK-HSDH gene has been mapped to a soybean linkage group containing genes for seed coat color, resistance to soybean cyst nematode race 3, and the 34 kDa seed thiol protease.

Poster #7

Analysis of heat shock genes expression in transgenic carrot cells containing an antisense gene

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We introduced an antisense gene targeting the low molecular weight (lmw) hs genes into carrot cells by *Agrobacterium* mediated hypocotyl transformation to study the role of lmw heat shock proteins in plant cells and embryos. The antisense gene corresponding to hsp17 was stably integrated into the genome and was shown to be transcribed at a similar rate in both callus cells and globular embryos of transgenic lines. However antisense transcripts are only detected, by northern analysis, in globular embryos which were known to produce a very low level of lmw hs transcripts in normal carrot cell lines. In addition, transgenic globular embryos accumulate unique extra bands of larger sizes for hsp17 transcripts that may result from inhibition of correct processing of the hsp17 messages by the antisense transcripts. Coincidently, globular embryos of the transformed line appear to produce a reduced level of lmw hs proteins compared to nontransformed lines.

We are currently investigating the possibility of the inhibition of message processing by antisense transcripts and are evaluating the thermotolerance of the antisense containing cells and embryos.

Poster #8

SENSITIVITY OF PETUNIA SEEDS TO ADENINE ANALOGUES:
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Adenine phosphoribosyltransferase (APRT) is one of the enzymes associated with the purine salvage pathway that catalyzes the conversion of adenine to its nucleotide form AMP. Purine nucleotides are essential precursors for nucleic acid synthesis as well as important compounds for energy metabolism. The APRT enzyme, in general, does not show a high degree of specificity for the exact structure of adenine and can also act on adenine derivatives including the cytokinins. The uptake of both purine and cytokinin bases is accompanied by their rapid conversion into nucleotides catalyzed by APRT. In the present investigation, we have used a seed germination system of Petunia to study the uptake of the adenine analogues 2,6-diaminopurine, 2-fluoroadenine, and 6-methylpurine. Petunia seeds were imbibed in solutions containing the adenine analogues and allowed to germinate. The APRT enzyme utilizes the adenine analogues as substrates and converts them into their corresponding nucleotide forms. The nucleotide forms of these adenine analogues are toxic and therefore inhibit seed germination. This system can be used to detect the presence or absence of functional APRT enzyme in plants since APRT is the only enzyme known to catalyze the conversion of adenine and its analogues into their nucleotide forms. Plants that lack APRT activity cannot convert adenine to AMP and must depend on the *de novo* pathway for the production of AMP which is not as efficient as the purine salvage pathway. Since they lack APRT activity, they cannot convert the base analogues into their toxic nucleotide forms and, therefore, survive in their presence. The data obtained in this investigation indicate that the order of seed sensitivity is 6-methylpurine > 2-fluoroadenine > 2,6-diaminopurine.

Poster #9

HORMONAL REGULATION OF CELLULASE DURING ADVENTITIOUS ROOTING IN SOYBEAN. Elizabeth C. Kemmerer and Mark L. Tucker. USDA/ARS, Plant Mol. Biol. Lab., BARC-West, Beltsville, MD 20705.

Exogenously applied auxin induces adventitious rooting in seedling cuttings. One of the enzymes involved in this process is cellulase, which breaks down cell wall components in the stem, allowing the new roots to emerge. We are studying the hormonal regulation of cellulase enzyme(s) and gene(s) active during adventitious rooting in soybean. Cellulase enzyme activity increases 10-fold in hypocotyls from the day the soybean seedlings are cut and treated with auxin to the day of maximum hypocotyl swelling and splitting, after which the cellulase enzyme activity decreases as the emerging roots elongate. Typically, 40 to 60 adventitious roots emerge after auxin treatment. Epicotyl and leaf tissues, which do not swell or form roots, show no distinct pattern of cellulase activity. Explants treated with water, reacting only to endogenous auxin, show a similar pattern of cellulase enzyme activity accumulation but at half the level of activity. Hypocotyls of water treated explants do not swell or split, and form only a few roots at the lower cut end. Explants treated with auxin and AVG (an ethylene synthesis inhibitor) show no swelling, splitting or root emergence and no change in cellulase activity in the hypocotyls, indicating that auxin-induced ethylene production plays an important role in adventitious rooting. The addition of ethylene to the auxin and AVG treated explants correlates with a return of hypocotyl swelling and splitting, some rooting and cellulase enzyme activity. Preliminary RNA gel blot analyses indicate that cellulase mRNA levels also correlate with hypocotyl swelling, splitting and root emergence. Studies at the enzyme activity and transcript levels are being performed with anti-auxin agents. We have isolated and sequenced 688 bp of a soybean cellulase gene encompassing 241 bp of coding sequence and 447 bp of 3' flanking sequence. Compared with other cellulases, the partial coding sequence of the soybean cellulase clone displays 55.6% and 57.8% nucleotide sequence identity with avocado fruit and bean abscission cellulases, respectively. At the deduced amino acid level, the partial soybean clone shares 55.0% and 48.1% identity with avocado fruit and bean abscission cellulases, respectively.

Poster #10

MOLECULAR AND STRUCTURAL STUDIES OF SOYBEAN OIL BODY ONTOGENY. Deborah S. Loer, Andrzej Kalinski, Jane M.Weisemann, Benjamin F. Matthews and Eliot M. Herman. USDA/ARS, Plant Mol. Biol. Lab., Beltsville, MD 20705-2350

Soybean seed oil accumulates in the cytoplasm as droplets of triglycerides bounded by a monolayer of phospholipids in which a few distinct membrane proteins (oleosins) are embedded. We are interested in structure-function aspects of the oleosins in relation to the developmentally regulated mechanisms of oil body assembly and mobilization. Also, the expression of oil body membrane protein genes is directly correlated with the accumulation of oil bodies during seed maturation. Therefore, oleosins are useful *in situ* probes for studies on the regulation of reserve triglyceride synthesis and accumulation. We have isolated the major oil body membrane protein of soybean, the 24 kDa oleosin. The isolated protein was used to elicit polyclonal antibodies that were then used to conduct biochemical and structural studies on the assembly and mobilization of oil bodies. To begin a molecular approach, we isolated and characterized two cDNA clones for 24 kDa soy oleosin. Nucleic acid sequence analysis shows a full-length open reading frame encoding a polypeptide of 23,366 Da. The two cDNA clones differ in both their nucleic acid sequence and predicted amino acid sequence, indicating that soybean oleosin can be expressed as at least two isoforms. The deduced protein structure of soybean oleosin indicates that it has a relatively amphipathic aminoterminal region, a hydrophilic carboxyterminal region, and a hydrophobic central region that has extensive homology with the hydrophobic regions of oleosins characterized from other plants. However, there is little homology between 24 kDa soy oleosin and maize or carrot oleosin in the hydrophilic regions of the molecule.

Poster #11

PUTATIVE SENESCENCE RELATED PROTEINS IN PHASEOLUS VULGARIS AS DETECTED BY IN VIVO AND IN VITRO TRANSLATION: Henry C. Butcher, Michael A. Wolfe, Nipa R. Doshi, Robert J. Mitkus, and Thomas A. Hensing; Biology Department, Loyola College, Baltimore, MD 21210.

We are continuing our studies on the molecular biology of senescence in bean. Intact potted plants were placed in darkness for varying periods of time (0-8 days) to allow senescence processes to occur. In vivo translation was effected by applying ^{35}S-methionine to the abraded upper surface of primary leaves. After several hours, the treated leaves were frozen in liquid nitrogen, the proteins extracted in buffer and the extract electrophoresed. In vitro translation used a commercial wheat germ translation kit and mRNA purified from primary leaves of experimental plants. We will exhibit autoradiograms from both procedures showing at least two proteins of interest which appear in dark-treated leaves and which electrophorese at approximately 26 and 66 kDa.

Poster #12

DOES PCH313 ENCODE THE ETHYLENE FORMING ENZYME?: Ann Callahan, Ken Nichols, Reuben Cohen, Deanna Fishel, Linda Dunn, and Peter Morgens; USDA-ARS-Appalachian Fruit Research Station, 45 Wiltshire Rd., Kearneysville, WV 25430.

The control of ethylene evolution in fruit could be a means to regulate the ripening process since ethylene is involved in the regulation of several ripening genes. The pTom13 gene may encode the ethylene forming enzyme (EFE) in tomato. If it encodes EFE then there should be a strong correlation between the rates of ethylene evolution and the amount of RNA accumulated. Two experiments were set up to examine the correlation of RNA accumulation with ethylene evolution using pch313 (a pTom13 heterologous gene in peach). The first involved measuring ethylene evolution and the amount of pch313 RNA on individual peach fruit during ethylene evolution and softening. The rate of ethylene evolution was higher in one cultivar of fruit but the amount of pch313 RNA was less. The second experiment utilized fruit from climacteric species and non-climacteric species which do not have an increase in ethylene during ripening. When pch313 RNA was measured during ripening it was found that pch313-related RNA was present in all species tested but the increase in pch313 was not necessarily correlated with ethylene evolution. This suggests that there may be other forms of regulation acting or pch313 does not encode the EFE.

Poster #13

ETHYLENE AND POLYAMINES REGULATE WOUND-INDUCIBLE TOMATO FRUIT ACC SYNTHASE. N. Li, B. L. Parsons, D. Liu and A. K. Mattoo. Plant Mol. Biol. Lab, ARS/BARC, Beltsville, MD 20705.

We have studied regulation of the wound-inducible 1-aminocyclopropane-1-carboxylic acid (ACC) synthase transcript in tomato fruit using a oligonucleotide probe homologous to published cDNA sequences. The probe hybridized to a 1.8 kb transcript that increased upon wounding in early-red and red-ripe fruit. Ethylene was found to positively regulate the level of this transcript using several approaches. First, the transcript level was found to increase as tomato fruit ripened, which paralleled the rise in ethylene production. Second, an inhibitor of ethylene perception, 2,5-norbornadiene, inhibited the ethylene-induced accumulation of the transcript. Autocatalytic ethylene production may thus result from induction of ACC synthase gene. We also found that polyamines, putrescine, spermidine and spermine, that have anti-senescence properties and also inhibit the development of ACC synthase activity, inhibit the wound induction of the ACC synthase transcript. The inhibition by spermine was greater than that caused by putrescine or spermidine. These data indicate that polyamine-mediated inhibition of ethylene biosynthesis involves suppression of ACC synthase transcript level. Further, we obtained a 1.6 kb tomato DNA fragment when oligonucleotide sequences from 3' and 5' ends of published tomato ACC synthase cDNA sequence were used as primers to amplify homologous sequences from total tomato (cv. Pik-Red) RNA using polymerase chain reaction. The 1.6 kb fragment was cloned into a pCR 1000 vector and expressed in INVαF' *Escherichia coli*. The soluble extracts of transformed cells contained considerable ACC synthase activity (46-64 units per h per mg protein). The ACC synthase expression was further substantiated by the detection of 73-79 nmol of ACC per ml of the extracellular medium.

Poster #14

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF *CAPSICUM*: James P. Prince[1], Fernando Loaiza-Figueroa[2], Edmond Pochard[3], and Steven D. Tanksley[1]

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A molecular map of pepper (*Capsicum sp.* L) has been constructed with 192 markers in an interspecific F2 cross (*C. annuum* X *C. chinense*) by using restriction fragment length polymorphisms and isozymes. Eleven large linkage groups representing the twelve haploid chromosomes of pepper, two linked by pseudolinkage, were formed. Nine additional small linkage groups were formed. Twenty-six markers showed no linkage at all. Twenty-five markers showed significant deviation from expected Mendelian ratios and did not cluster in the genome. Restriction enzymes that cut DNA into larger fragments detected more polymorphism than those cutting DNA into smaller fragments, and clones hybridizing to larger fragments of DNA or to more individual fragments showed more polymorphism than the other clones. Pepper shows a scrambling of the order of clones compared to tomato and potato and has a higher degree of methylation.

DNA samples of twenty-five accessions of pepper (*Capsicum sp.*) from various regions of Mexico and the two parents currently in use for restriction fragment length polymorphism (RFLP) mapping were cut with *Eco*RI and probed with 43 tomato genomic clones and 3 pepper genomic clones in order to assess the amount of genetic variation present. The banding patterns detected were used to estimate genetic distances among the accessions. A dendrogram from cluster analysis and a principal component analysis diagram of the genetic distance matrix are presented. The cluster analysis is almost identical to a previously published cluster analysis based on isozyme data and the correlation between genetic distance measured by RFLPs versus genetic distance measured by isozymes is good (R^2= 0.438, p=0.0001). Information on interfertility along with the genetic distance data is used to select seven sets of parents for future RFLP mapping in pepper.

Poster #15

EFFECT OF NUCLEASES RELEASED FROM GERMINATING POLLEN ON FOREIGN DNA DURING POLLEN ELECTROTRANSFORMATION.

Sally L. Van Wert and James A. Saunders, USDA, Plant Sciences Institute, Bldg 9 , Rm 5, Beltsville MD 20705.

Pollen electrotransformation is a technique being developed in our lab to genetically modify plants by the electroporation of foreign DNA into germinating pollen. The reported release of DNA nucleases from germinating pollen grains implied that foreign DNA added to germinating pollen would be degraded within a few minutes. We added plasmid pBI221 DNA (20μ g) to germinating tobacco (*Nicotiana gossei*), corn (*Zea mays*) and alfalfa (*Medicago sativa* L.) pollen (4 mg) and observed the effect of released nucleases on the DNA by agarose gel electrophoresis. Isolated DNA from the pollen/pBI221 suspensions showed increased degradation of pBI221 as incubation time with the pollen increased. Electroporation immediately after the addition of pBI221 had little ellect on the amount of degradation. The addition of increasing amounts of EDTA or $MgSO_4$ to the pollen suspension after germination but before the addition of pBI221, followed by electroporation, resulted in decreasing DNase activity. Electroporated tobacco pollen/pBI221 suspensions were placed on the stigmas of emasculated flowers and viable, transformed seed was produced. It was concluded that nucleases released from germinating tobacco pollen would not degrade all the foreign DNA in the time period necessary for the uptake of DNA during pollen electroporation. The potential for pollen electrotransformation in corn and alfalfa is currently under investigation.

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AREA ATTRACTIONS

Baltimore offers its visitors everything from major league baseball to theatre and music. Among its attractions are:

*the **Inner Harbor** - this rejuvenated center of the city, located on the waterfront, includes:

-**National Aquarium in Baltimore** - nationally acclaimed for its presentation of sharks, tropical fish and large game fish, reptiles, birds, etc. 576-3800
-**Maryland Science Center/Davis Planetarium/IMAX** - visitors are encouraged to see, do and touch to better understand the wonders of science. 685-2370
-**World Trade Center/Top of the World** - the tallest pentagonal building in the world provides a panoramic view of the city and mini-museum. 837-4515
-**U.S. Frigate Constellation** - the first commissioned ship of the U.S. Navy and oldest American warship continuously afloat. 539-1797
-**Harborplace** - a shopping bonanza of over 140 shops, restaurants and eateries.
-**The Gallery** - Baltimore's newest retail complex with four floors of upscale shops and eateries
-**Federal Hill** - public park and lookout point over Baltimore
-**The Baltimore Maritime Museum** - located in the submarine that sank the last ship of WWII. 396-3854

***Fort McHenry National Monument and Historic Shrine** - at this star-shaped Fort, during a battle with the British in 1812, Francis Scott Key wrote our national anthem. 962-4290

***B&O Railroad Museum** - houses the most extensive collection of railroad memorabilia in the United States. 237-2387

***The Johns Hopkins Hospital and Medical Institutions**

***Babe Ruth House** - a museum of Babe's mementos and memorabilia, including a life-size wax figure and slide show. 727-1539

***Lexington Market** - established in 1782, this building houses aisles of stalls offering the freshest and finest fruits, vegetables and meats. 685-6169

***Edgar Allen Poe House and Grave** - This tiny house includes the garret chamber where Edgar Allen Poe lived and wrote from 1832-1835. The Westminster Churchyard contains graves of many famous Marylanders, including Poe's. 396-7932

***Walters Art Gallery** - this world-renowned museum houses Renaissance and Baroque paintings and sculpture, Egyptian artifacts, medieval armor and Faberge jeweled eggs. 547-9000

***Peabody Conservatory of Music** - established in 1857, this famed school of music has impressive interiors and a celebrated 5-tier library of ornate iron balconies overlooking a central courtyard. 659-8100.

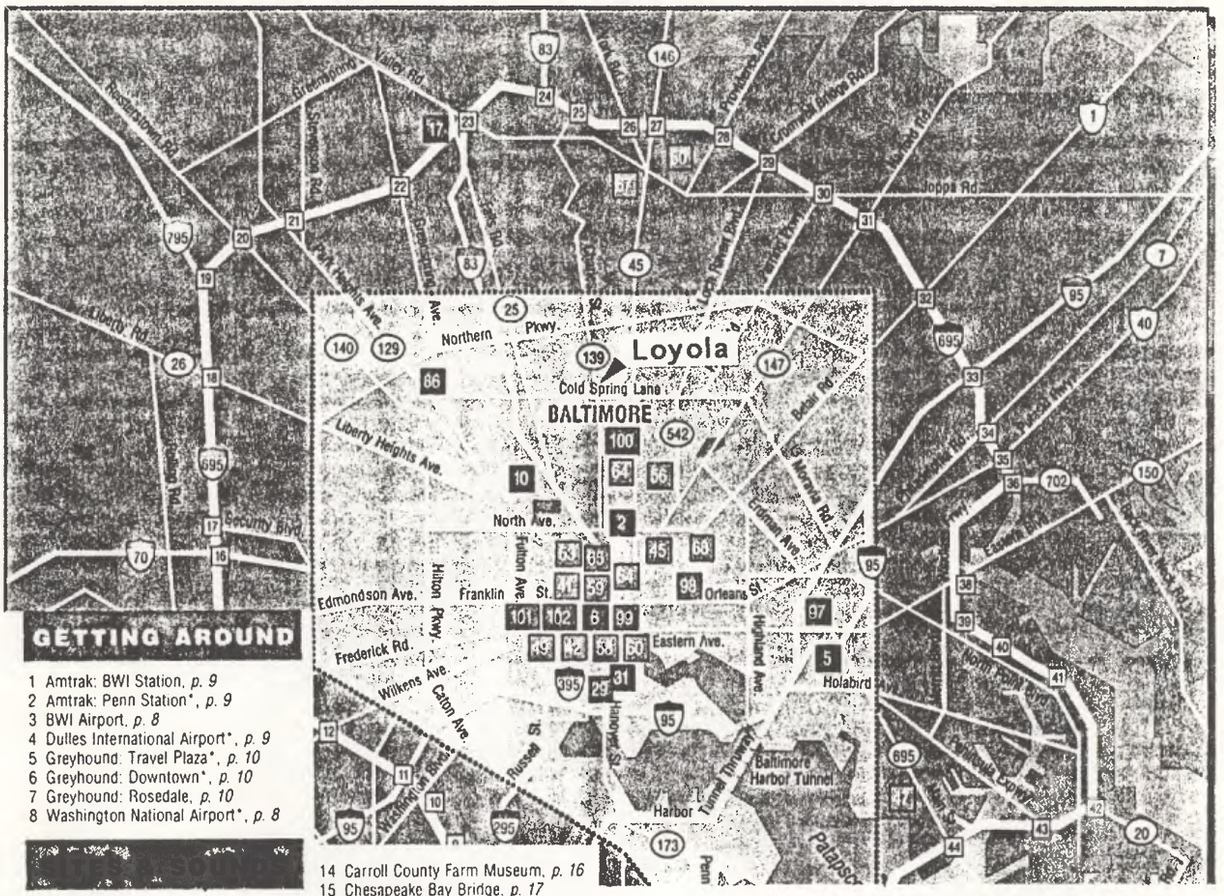
***Shot Tower** - built in 1828, this 234 foot-high tower produced lead shot by pouring molten lead through a sieve at the top and allowing droplets to harden as they fell into vats of cold water at the bottom. 539-8942

***Little Italy** - this ethnic neighborhood filled with restaurants is a must for lovers of Italian food.

***Fells Point** - Baltimore's original shipbuilding and maritime center, dating back to 1730. The area now includes pubs, restaurants, galleries and antique shops.

***Baltimore Zoo and Children's Zoo** - home to over 1200 animals. 396-7102

.....and much, much more. Most of the above information was taken from a brochure published by the Baltimore Convention Bureau. For your own brochures about Baltimore's attractions, call the Baltimore Office of Promotion & Tourism at 301-752-8632.



GETTING AROUND

1 Amtrak: BWI Station, *p. 9*
2 Amtrak: Penn Station*, *p. 9*
3 BWI Airport, *p. 8*
4 Dulles International Airport*, *p. 9*
5 Greyhound: Travel Plaza*, *p. 10*
6 Greyhound: Downtown*, *p. 10*
7 Greyhound: Rosedale, *p. 10*
8 Washington National Airport*, *p. 8*

WHAT'S AROUND

9 Ballestone Manor, *p. 12*
10 Baltimore Zoo*, *p. 18*
11 B&O Railroad Station Museum, *p. 13*
12 Bollman Truss Bridge, *p. 17*
13 Boordy Vineyards, *p. 15*
14 Carroll County Farm Museum, *p. 16*
15 Chesapeake Bay Bridge, *p. 17*
16 Cider Mill Farm, *p. 16*
17 Cloisters Children's Museum*, *p. 18*
18 Fire Museum of Maryland, *p. 14*
19 Fort Meade Museum, *p. 14*
20 Hampton Nat'l. Historic Site, *p. 12*
21 Heritage Society of Essex and Middle River, *p. 14*
22 Historic Savage Mill, *p. 16*
23 Howard Co. Courthouse, *p. 13*
24 Howard Co. Historical Society, *p. 13*
25 Howard Co. Library, *p. 13*
26 Jericho Covered Bridge, *p. 17*
27 Kimmey House, *p. 18*
28 Maryland Midland Railway, *p. 13*
29 Maryland Science Center*, *p. 18*
30 Montbray Wine Cellars, *p. 15*
31 National Aquarium*, *p. 18*
32 Oella-Benjamin Banneker Site, *p. 16*
33 Thomas Viaduct, *p. 17*
34 Union Mills Homestead, *p. 16*
35 Waverly Historical Site, *p. 12*
36 W. Maryland Railway Museum, *p. 13*
37 Whitemarsh Cellars, *p. 15*
38 Woodhall Vineyards, *p. 15*

HAPPENINGS

39 Annapolis Summer Garden, *p. 25*
40 Anne Arundel Comm. College, *p. 28*
41 Arena Players*, *p. 25*
42 Baltimore Arena*, *p. 26*
43 Capital Centre*, *p. 27*
44 Catonsville Comm. College, *p. 28*
45 Center Stage*, *p. 25*
46 Colonial Players, *p. 25*
47 Dundalk Comm. College*, *p. 28*
48 Essex Comm. College, *p. 28*
49 Festival Hall/Convention Cntr.*, *p. 23*
50 Goucher College*, *p. 28*
51 Howard Co. Arts Council/Rockland Arts Center, *p. 25*
52 Howard Co. Comm. College, *p. 28*
53 J. Meyerhoff Symphony Hall*, *p. 24*
54 Lyric Opera House*, *p. 23*
55 MD Hall for the Creative Arts, *p. 25*
56 Memorial Stadium*, *p. 26*
57 Merriweather Post Pavilion, *p. 25*
58 Morris A. Mechanic Theatre*, *p. 24*
59 Peabody Conservatory of Music*, *p. 25*
60 Pier Six Concert Pavilion*, *p. 24*
61 RFK Memorial Stadium*, *p. 27*
62 Slayton House, *p. 25*
63 The Little Theater on the Corner, *p. 25*
64 Theatre Hopkins*, *p. 25*
65 Theatre Project*, *p. 25*
66 Timonium State Fairgrounds, *p. 22*
67 Towson State University*, *p. 28*
68 Vagabond Players*, *p. 25*
69 Villa Julie College, *p. 28*

GREAT OUTDOORS

70 Annapolis Farmers' Market, *p. 36*
71 Anne Arundel Co. Farmers' Market, *p. 36*
72 Baltimore Co. Farmers' Market, *p. 36*
73 Carroll Co. Farmers' Market, *p. 36*
74 Cedar Lane Park, *p. 31*
75 Centennial Park, *p. 31*
76 Downs Park, *p. 33*
77 Freestate Raceway, *p. 35*
78 Greenway Gardens/Arboretum, *p. 32*
79 Gunpowder Falls State Park, *p. 30*
80 Hashawha Environmental Cntr., *p. 32*
81 Irvine Natural Science Center, *p. 32*
82 Laurel Race Course, *p. 35*
83 Liberty Reservoir, *p. 34*
84 Loch Raven Reservoir, *p. 34*
85 Patapsco Valley State Park, *p. 30*
86 Pimlico Race Course*, *p. 35*
87 Prettyboy Reservoir, *p. 34*
88 Rockburn Branch Park, *p. 31*
89 Rocky Point Park, *p. 33*
90 Rocky Gorge Reservoir, *p. 34*
91 Sandy Point State Park, *p. 33*
92 Schooley Mill Park, *p. 31*
93 Severn Run Natural Environmental Area, *p. 30*
94 Soldiers Delight Natural Environment Area, *p. 33*
95 Southwest Area Park, *p. 31*
96 Triadelphia Reservoir, *p. 34*

GETTING HELP

97 Francis Scott Key Medical Cntr.*, *p. 38*
98 Johns Hopkins Hospital*, *p. 38, 39*
99 MIEMSS/Shock Trauma Cntr.*, *p. 38*
100 Union Memorial Hospital*, *p. 39*
101 U. of MD Cancer Center*, *p. 38*
102 U. of MD School of Pharmacy*, *p. 39*