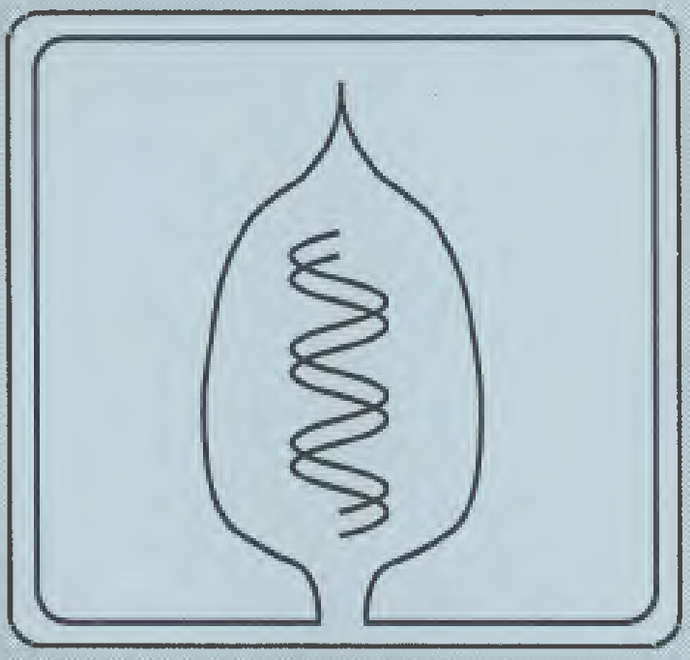
MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

FIFTH ANNUAL MEETING



AUGUST 4 & 5, 1988

UNIVERSITY OF MARYLAND

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INTRODUCTION

Welcome to the fifth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The Society was formed to provide a forum for the free exchange of ideas and information concerning plant molecular biology for researchers at universities, research institutes, and industrial laboratories. The Society's goal is to ensure scientists in the Mid-Atlantic region of a high-quality, accessible, and affordable plant molecular biology meeting each year. The society particularly wishes to encourge graduate student and postdoctoral participation in presenting oral reports, posters and contributing to the discussions.

Since the inception of the Society by Ben Matthews at USDA Beltsville and its first meeting in 1984, we have seen a steady increase in attendance and participation at the annual meeting. Each year, MAPMBS grows geographically as well. This year, we have participants coming from as far away as Massachusetts, Connecticut, Ohio, and Kentucky. The original idea that led to the formation of MAPMBS is clearly an excellent one, and the Society is maturing very well. All of us who participate in the Society are indebted to Ben and his colleagues at USDA for laying the groundwork for MAPMBS.

I am pleased to host this year's meeting at the University of Maryland College Park. No scientific conference organizes itself, and I want to thank those people listed on next page and Clay Detlefsen (Campus Guest Services) for their efforts. In particular, I express my sincere gratitude to Ben Matthews, Ellen Reardon, and Janet Slovin for their diligence and enthusiam for the task.

John C. Watson
Organizer

MAPMBS COMMITTEES FOR THE 1988 MEETING

Organizing Committee

Todd Cooke
Beth Gantt
John Hammond
Rosemarie Hammond
Steve Hutcheson
Shain-dow Kung
Ellen Liberman
Ben Matthews
Autar Mattoo
Sue Mischke
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Registration

Ben Matthews
Sue Mischke

Funding

Ellen Reardon

Abstract Booklet

Steve Hutcheson
John Watson

GENERAL INFORMATION

UMCP: UMCP is the largest campus in the University of Maryland system, with approximately 38,000 students and a vast array of academic departments. The campus is located just 4 miles from the Washington D.C. border and 8 miles from the White House, and about 45 minutes south of Baltimore and west of Annapolis. If you have post-meeting free time or desire some non-college town night life on Thursday evening, Washington, Baltimore, and Annapolis offer many delightful experiences.

SESSIONS: All plenary sessions will be held in the Grand Ball Room of the Adele H. Stamp Union. All poster sessions and breaks will be in the Grand Ball Room Lounge and the Prince Georges Room (which adjoin the Grand Ball Room). The exhibitors will have their displays in these two rooms as well. Please take time to visit the exhibitors. The social hour following Dr. Larkins' address will be in the Grand Ball Room Lounge.

MEALS: If you pre-registered, your lunches were prepaid and will be provided in the back portion of the Grand Ball Room. You will need to present your lunch tickets (found in the back of your name badge) on both days.

If you did not pre-register, you did not prepay for lunches and could not be included in our count for the campus catering sevice. Alas, you are on your own for lunch. But do not dispair, since the Union contains a variety of eating establishments on the floor below the Grand Ball room. There are also many nearby places to eat off-campus.

If you pre-registered for the dinner, you will find a dinner ticket with your lunch tickets in your name tag. The dinner will be Thursday at 6:00 p.m. in the Grand Ball Room.

SPECIAL DEMONSTRATIONS: Several of the vendors will present special demonstrations to highlight the unique features of their products. These will occur during Session IV on Friday morning. The time each vendor will have their Special Presentation is in the Program Schedule.

SPONSORS

We are particularly grateful to the following organizations for their generous financial support. Without their support, this meeting possible would not have been possible. The International Society for Plant Molecular Biology is sponsoring the Keynote Address by Dr. Brian Larkins. The other sponsors have contributed to our general operating fund, thus allowing us to reduce the registration fees for participants.

International Society for Plant Molecular Biology

Center for Agricultural Biotechnology of the Maryland Biotechnology Institute

Rohm and Haas Company

E.I. duPont de Nemours and Company

EniChem Americas

Department of Botany, University of Maryland

BioRad Chemical Division

EXHIBITORS

We wish to express our thanks to the following exhibitors for their displays and financial support, which helped make this meeting possible. Please visit with the technical representatives to find out how they can help you in your research activities.

Baltimore Instrument Co.

Beckman Instruments

Fisher Scientific

Hoefer Scientific

Hydro Service & Supplies

Image Systems

International Biotechnologies Inc.

Protein Databases Inc.

PROGRAM SCHEDULE

Thursday, August 4, 1988

8:00 - 9:00 REGISTRATION

9:00 SESSION I - GENE REGULATION I pp. 11 - 16
Convenor - John Watson

9:00 - 9:30 **J. Lynn Zimmerman,** "The Influence of Heat Shock and Other Stresses on Somatic Embryo Development in Carrot."

9:30 - 10:00 **Glenn Galau,** "Ovule Abscission and the Embryo Predesiccation Program."

10:15 - 10:20 **Break**

10:20 - 10:40 **Peter Morgens,** "Comparison of High and Low Quality Peach Fruit Cultivars by Differential Hybridization of a cDNA Library."

10:40 - 11:00 **Fred B. Abeles,** "Ethylene Induced Peroxidase from Cucumber Cotyledons."

11:00 - 11:20 **Robert C. Elliot,** "Molecular Analysis of *Fed* I Gene Expression."

11:20 - 11:40 **Michael S. Dobres,** "A Developmentally Regulated Bud Specific Transcript in Pea has Sequence Similarity to Seed Lectins."

12:00 - 1:00 LUNCH

1:00 SESSION II - ORGANELLES pp. 17 - 19
Convenor - Ellen Reardon

1:00 - 1:30 **Carl Price,** "Chromoplasts are Not Gerontoplasts."

1:30 - 2:00 **Bruce Kohorn,** "Transport of Proteins to the Chloroplast: Genetic Approaches."

2:00 - 2:20 **Nancy Speer Kirkpatrick,** "Alteration of the Plastid Genome in a Naturally Occurring Chimeric Mutant *Hosta* Leaf."

2:20 - 2:40 **Break**

2:40 - 3:15 POSTERS, EXHIBITS

3:15 - 3:45 BUSINESS MEETING

4:00 - 5:00 KEYNOTE ADDRESS: *BRIAN LARKINS*

"Structure, Synthesis, and Engineering of Maize Storage Proteins."

SPONSORED BY THE
INTERNATIONAL SOCIETY FOR PLANT MOLECULAR BIOLOGY

5:00 - 6:00 SOCIAL HOUR

6:00 DINNER

Friday, August 5, 1988

8:30 SESSION III - GENE REGULATION II pp. 20 - 23
Convenor - Lowell Owens

8:30 - 9:00 **Ann Smigocki**, "Cytokinin Gene Fused with a Strong Promoter Enhances Shoot Organogenesis and Zeatin Levels in Transformed Plant Cells."

9:00 - 9:30 **Richard Broglie**, "Analysis of an Ethylene Responsive Promoter in Transgenic Tobacco Plants and by Transient Expression in Bean Protoplasts."

9:30 - 9:50 **Break**

9:50 - 10:10 **Xin-Hua Feng**, "Introduction and Expression of T-DNA Phytohormone-Biosynthesis Genes in *Nicotiana* Genetic Tumors."

10:10 - 10:30 **David F. Kendra**, "Effect of Thidiazuron in Cytokinin Bioassay Systems."

10:30 - 12:00 SESSION IV - POSTERS AND EXHIBITS
SPECIAL DEMONSTRATIONS BY EXHIBITORS

POSTERS pp. 29 - 41

A. Abdul-Baki et al., "Uptake of DNA by Electroporation of Pollen."

G.R. Bauchan et al., "Utilization of DNA Probes for Identifying Alfalfa Chromosomes."

B.M. Chereskin et al., "Maize Glutamine Synthetase Gene Structure."

R. Cohen et al., "Protein and RNA Populations of Ripening Peach Fruit from Melting Flesh and Non-Melting Flesh Cultivars."

R.J. Daines et al., "Progess in the Genetic Manipulation of Maize."

J. DeRobertis et al., "Use of Electroporation for Transient Assays and Stable Transformation of Carrot."

B.F. Matthews et al., "Purification and Interconversion of Forms of Homoserine Dehydrogenase Isoenzymic Forms from Cell Suspension Cultures of *Daucus carota*."

W.C. Mitchell and J.J. Gaynor, "Spatial and Temporal Expression of Endochitinase in Maize."

S. Mischke, "Plasmids in the Filamentous Fungus *Gliocladium virens*."

T.J. Pedersen et al., "Molecular Analysis of the Expression of the Gene Encoding Ferredoxin I (*Fed* I) - Pea Nuclei Contain Factors that Bind Specifically to the *Fed* I Promoter."

L. Solberg and I.M. Sussex, "Expression of Endopeptidase Involved in Bean Seed Storage Protein Mobilization."

J.P. Slovin et al., "*Lemna gibba*: A Tool for Studying Developmental Biochemistry and Molecular Biology."

F.J. Turano et al., "Verification of a Homoserine Dehydrogenase Specific Monoclonal Antibody."

SPECIAL DEMONSTRATIONS BY EXHIBITORS:

10:30 - 10:45 **International Biotechnologies Inc., Richard Vento.**
"Automated Reading of Sequencing Gels and Mobility Sizing by Digitization."

10:45 - 11:00 **Fisher Scientific, Paula Blanchard.**
"An Isothermically-Controlled Electrophoresis System."

11:00 - 11:15 **Image Systems, Dale Gibson.**
"Introduction to the Nikon FXA Computer-Operated Microscope."

11:15 - 11:30 **Hydro Service and Supplies, Natalie Mason.**
"Is 18 megaohm good enough?"

11:30 - 11:45 **Baltimore Instrument Co., Jill Curtis.**
"A New Microscope System from Carl Zeiss."

11:45 - 12:00 **Protein Databases Inc., Mary Pingitore**
"2-D Electrophoresis and Quantification Analysis of Proteins Using Protein Databases' Discovery Analysis Systems."

OTHER PARTICIPATING EXHIBITORS:

Beckman Instruments, Karen de Pianelli

Hoeffer Scientific, Christopher Conway

12:00 - 1:00 LUNCH

1:00 SESSION V - PLANT / MICROBE INTERACTIONS pp. 24 - 28
Convenor - John Hammond

1:00 - 1:30 **Robert Shepherd**, "Pathogenic and Host Range Responses Associated with Gene VI of Caulimoviruses."

1:30 - 2:00 **Marc Orbach**, "Genetics of Host Cultivar Specificity in the Rice Blast Fungus."

2:00 - 2:20 **Break**

2:20 - 2:50 **Steven Hutcheson**, "Elicitation of Plant Resistance Reactions by *Pseudomonas syringae*."

2:50 - 3:10 **Anne E. Simon**, "Turnip Crinkle Virus Satellite Domains Involved in Virulence and Processing."

3:10 - 3:30 **John Hammond**, "Comparison of the Nucleotide and Amino Acid Sequences of the Coat Protein Genes of Bean Yellow Mosaic Virus and Other Potyviruses."

THE INFLUENCE OF HEAT SHOCK AND OTHER STRESSES ON SOMATIC EMBRYO DEVELOPMENT IN CARROT. J. Lynn Zimmerman, Kamel El-Darwish, Cheol Ho Hwang and Cynthia O'Carroll, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228

We have characterized the heat shock response of carrot cells in culture, both during undifferentiated growth and during the developmental program of somatic embryogenesis. In general, the response of carrot cells to heat shock closely resembles that of other plants (most notably soybean) with respect to the range of new proteins produced and the timing and temperature of induction. However, we have determined that developing somatic embryos, particularly those at the globular stage of embryogenesis, respond to heat shock differently. We have investigated the physiological effect of heat shock on the progression of somatic embryogenesis, and have determined that there is a narrow window of development, during the globular stage, that is extremely sensitive to heat shock; exposure of embryos at this stage to heat shock leads to the rapid and irreversible degeneration of the embryos. No other stage of somatic embryo development is similarly affected. We have further determined that only a very specific portion of the globular stage is sensitive; specifically the window of sensitivity is only about 2-4 hours in duration. Finally, we have established that the normally sensitive globular embryos can be protected from the effects of heat shock by "preshocking" the embryos for two hours either 24 or 48 hours prior to the sensitive period. These observations together indicate that this sensitive period may represent a critical juncture in the development of somatic embryos.

OVULE ABSCISSION AND THE EMBRYO PREDESICCATION PROGRAM: D. Wayne Hughes and Glenn A. Galau; Department of Botany, University of Georgia, Athens, GA 30602.

Recent studies in our laboratory with cloned mRNA markers have identified a new stage in cotton embryogenesis that initiates with ovule abscission and terminates just prior to embryo desiccation. Although we conservatively define this stage as the postabscission stage, it is our belief that a substantial part of it is devoted to preparing the embryo for subsequent desiccation and hence it may be considered a predesiccation program. These results grow out of attempts to understand the regulation and function of 18 cloned Lea (Late embryogenesis-abundant) mRNAs that are induced when storage protein mRNAs disappear at the time of ovule abscission, 8 days before desiccation. Several lines of evidence suggest that LEA proteins are desiccation protectants or repair proteins.

The behavior of 47 cloned mRNAs defines 5 gene expression programs associated with early ABA levels and cotyledon, maturation, postabscission, and germination stages. Ovule abscission normally terminates the maturation program and induces the postabscission program, of which Lea mRNAs are the major component. Excised maturation stage embryos also terminate the maturation program and express the postabscission program during culture. However, the postabscission program appears to be turned off in planta about 2 days prior to desiccation, and embryos at that time cannot maintain them in culture. Remarkably, slow desiccation specifically induces the Lea mRNAs in maturation stage embryos but then these are degraded upon rehydration. Thus, late postabscission, mature, and in vitro-desiccated maturation stage embryos are equivalent in that they all have high amounts of postabscission mRNAs but cannot maintain them upon subsequent culture. It is unclear what terminates the postabscission program. Some state of desiccation is suggested, but autoregulation of the program is also possible and is not yet excluded.

Besides being competent to express the postabscission program upon excision, postcotyledon-stage embryos are also always competent to express the germination program in culture; they appear to require only excision and high embryo water potentials. Similar regulation of maturation, postabscission, and germination programs appears to occur in other species as well.

Our evidence suggests that the predesiccation program consists of termination of maturation, rapid induction of postabscission-active genes, and continued repression of germination-active genes. According to our model, ovule abscission signals the embryo to prepare for desiccation by expressing Lea genes. We hypothesize that loss of a maternally supplied repressor is the essence of the postabscission signal.

COMPARISON OF HIGH AND LOW QUALITY PEACH FRUIT CULTIVARS BY DIFFERENTIAL HYBRIDIZATION OF A cDNA LIBRARY

Peter Morgens, Ann Callahan, Ralph Scorza, Seth Mante, John Cordts, and Reuben Cohen. USDA-ARS, Appalachian Fruit Research Station, Kearneysville, West Virginia 25430.

We are studying gene expression during peach fruit development. Peach genes whose levels of expression differ between the high fruit quality cultivar 'Suncrest' and the low fruit quality cultivar 'Bailey' have been isolated. RNA populations from the two cultivars during fruit development were compared by in vitro translation. The most differences in RNA populations between the two cultivars occur 30 days after bloom. A collection of cDNA clones was constructed in a lambdoid vector from 'Suncrest' fruit RNA collected thirty days after bloom. This cDNA library has been screened in duplicate with single stranded radioactive cDNAs derived from 'Suncrest' and 'Bailey' fruit tissue. Five clones that preferentially hybridized to the RNAs from 'Suncrest' were further purified and isolated. Dot blot analyses indicate that the expression of the most highly expressed cloned sequence is up to five times greater in 'Suncrest' than in 'Bailey'. Levels of expression of the cloned sequences were compared in five cultivars by dot blot analysis Some of the genes appear to be developmentally regulated.

ABSTRACT

ETHYLENE INDUCED PEROXIDASE FROM CUCUMBER COTYLEDONS

Morgens, P., Callahan, A., Dunn, L. J., and Abeles, F. B. USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 24530

Ethylene induced senescence of cucumber cotyledons was accompanied by a fifty-fold increase in a cationic, 33-kDa, pI 8.9 peroxidase (33-CPO) over a 2 day period. Other plant hormones such as IAA, abscisic acid, gibberellic acid, and cytokinin and senescence inducing factors such as methyl jasmonate did not induce 33-CPO. A CNBr fragment of 33-CPO was sequenced with a protein sequencer and found to be homologous with amino acid sequences in horseradish, turnip, and tobacco peroxidase. Using a 20 base polynucleotide probe based on this sequence, cDNA clones thought to represent peroxidase from mRNA isolated from ethylene treated cucumber cotyledons were isolated. mRNA homologous to these clones was induced by ethylene by 9 hours. The function of 33-CPO during senescence is not known. The observation that methyl jasmonate induced senescence without inducing peroxidase is evidence that peroxidase is not involved in chlorophyll catabolism.

MOLECULAR ANALYSIS OF *FED I* GENE EXPRESSION.

Robert C. Elliott, Lynn F. Dickey, Brian Fristensky, and William F. Thompson, Departments of Botany and Genetics, North Carolina State University, Raleigh, NC 27695-7612.

Ferredoxin I is a soluble electron transport protein involved in the photosynthetic reduction of NADPH. In pea plants , it is encoded by a single copy gene, *Fed I.* Expression of this gene is under the control of phytochrome, and steady state levels of the *Fed I* mRNA increase substantially afterplants have been exposed to light. We have isolated, mapped, and sequenced a *Fed I* genomic clone and have found several sequences upstream of the coding region that are similar to sequences that confer light-regulation on RbcS genes. There are also similarities to the consensus upstream sequences of Cab genes. Expression of an intact pea *Fed I* gene with normal flanking sequences is regulated by light in the leaves of transgenic tobacco plants. A construct containing a GUS reporter gene under the control of a 500 bp promoter fragment from the pea *Fed I* gene is expressed in transgenic tobacco plants, but is not light regulated under the light regime we used Additional constructs designed to determine the role of mRNA and 3' flanking sequences in *Fed I* light responses have been made and transferred to tobacco. Preliminary data from these plants should be available shortly.

A developmentally regulated bud specific transcript in pea has sequence similarity to seed lectins. Michael S. Dobres* and William F. Thompson. Department of Botany, North Carolina State University, Raleigh, NC 27695-7612.

We report a striking example of organ and stage specific gene expression in pea (*Pisum sativum L.*). A transcript corresponding to a previously isolated cDNA clone, pEA207 (Thompson *et al.*, Planta, 158, 487-500, 1984) accumulates in the actively growing bud of the pea plant but is either absent or present at very low levels in the expanded leaves below the bud. The deduced amino acid sequence of pEA207 shows 49% similarity to the phytohemagglutinin lectin sequence of kidney bean (*Phaseolus vulgaris*) and 37% similarity to that of the major pea seed lectin sequence. It is also similar to seed lectins from five other legumes. All of the residues directly involved in metal binding by lectins are present in this sequence. Metal binding has been shown to be required for the sugar binding activity of lectins.

* As of September 1st 1988: Department of Biotechnology and Bioscience, Drexel University, Philadelphia, PA 19104.

Chromoplasts Are Not Gerontoplasts

C. A. Price

Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759

Chromoplasts are one of a half-a-dozen or so differentiation states of plastids and are common in flowers and fruits of higher plants. Chromoplasts are typically formed from chloroplasts and are characterized by an accumulation of carotenoids and an absence of the photoynthetic apparatus. Conventional wisdom has held that chromoplasts are a product of irreversible differentiation, a kind of molecular end game. This was also our view when we began to inquire if the plastid genome plays an active role in the formation of chromoplasts.

We chose as our test system fruits of the sweet pepper, *Capsicum annuum* L. The advantages are that the genetics of fruit color in pepper is well characterized; numerous cultivars are available; and the chromoplasts can be isolated intact. All genes known to affect fruit color in *Capsicum* spp. are Mendelian. We first constructed a gene map of plastid DNA in *C. annuum* and determined that the restriction patterns of chromoplast and chloroplast DNA are indistinguishable (Gounaris et al., Curr. Genet. 11:7-16). We then looked for possible chromoplast-specific transcripts by probing Southern blots of plastid DNA with cDNA from chromoplasts. We found none, but were surprised by the abundance of plastid transcripts in chromoplasts: hardly a region of the plastid genome was not represented (Gounaris & Price, Curr. Genet. 12:219-224).

We wondered if the transcripts in the chromoplast could be active as mRNAs or were merely relics of pre-existing chloroplasts. We tested this idea by supplying isolated chromoplasts with [^{35}S]-Met and analyzing the products. To our considerable surprise, chromoplasts incorporated [^{35}S]-Met into protein at substantial rates and into a wide variety of polypeptides (Hadjeb & Price, Plant Physiol., *in press*). Incorporation by chloroplasts from green fruits, unfortunately, was too feeble to provide comparisons of the translation products.

The accumulated wisom of plant genetics provided a crucial test of the "relic" hypothesis: Several recessive alleles in *C annuum* control the pigmentation of immature fruits independent of the pigmentation of mature fruits. Through Robert A. Morrison of DNA Plant Technology, we were able to obtain cultivars in which the immature fruits are "sulfur white" and the mature fruits are red. In other words chromoplasts can be formed from leucoplasts as well as chloroplasts.

When chromoplasts of var. VS-12, a variety in which fruits change from white to red, were incubated with [^{35}S]-Met, label was incorporated into protein more rapidly than by the precursor leucoplasts and at rates similar to that of normal proplastids. Both kinds of plastids synthesized a wide range of polypeptides. A few were specific to leucoplasts and chromoplasts.

We conclude that the informational apparatus of plastids remains active during chromoplast development: transcripts continue to accumulate; the transcripts include functional mRNAs; and the chromoplasts remain *capable* of protein synthesis. Whether any plastid gene is in fact expressed in the mature chromoplast is almost certainly determined by controls operating after the level of transcription.

Transport of Proteins To The Chloroplast: Genetic Approaches.

Bruce D. Kohorn, David Murray, Andrea Auchinchloss, Elaine Walker. Dept. Botany, Duke University, Durham, NC.

We are developing a genetic selection to isolate nuclear mutations in Arabidopsis that disrupt the transport of cytoplasmically synthesized proteins to the chloroplast. Recovered mutations should affect molecules that interact with LHCP during its transport. We have used genetic transformation and the targeting signals found within LHCP to route an enzyme marker to the chloroplast. As this enzyme marker must be present in the cytoplasm for the plant to survive under selective conditions, only those plants defective in protein sorting can survive.

We are also investigating a number of existing mutants of Arabidopsis that disrupt chloroplast function. One lesion, ch-1, affects the assembly of LHCP into a chlorophyll-protein complex but not LHCP itself, and we are "walking" to this locus.

Another genetic approach provides a method for the isolation of DNA clones that encode proteases. The LHCP transit peptide has been fused to an internal region of a functional yeast transcription factor, gal4. This chimeric protein is fully active in yeast, and the absence or presence of this activity is stringently selectable. We have transformed into this yeast strain a plant cDNA library that should express a protease that cuts the transit peptide. The protease would therefore also cleave the chimeric transcription factor and destroy its function.

Distinct groups of amino acids within LHCP are also required for its transport to the chloroplast and its integration into the photosynthetic membrane. This assembly process can be fully reconstituted in vitro using isolated chloroplasts and LHCP synthesized from a cloned gene. We have changed or deleted specific regions of the protein by DNA mutagenesis and asked how the import and assembly process is affected. These studies are used to define portions of the protein that are important for either import, integration into the membrane, or stability in a chlorophyll complex.

ALTERATION OF THE PLASTID GENOME IN A NATURALLY OCCURRING CHIMERIC MUTANT HOSTA LEAF. by Nancy Speer Kirkpatrick, William A. Tozier, and Kenneth G. Wilson, Department of Botany, Miami University, Oxford, Ohio.

We are analyzing the green and white leaf tissue of a naturally occurring chimeric mutant of Hosta undulata var. albomarginata. Previous genetic and ultrastructural studies have indicated that variegation in the genus Hosta is caused by mutations in the plastid genome. In order to verify this we have isolated total DNA from green and white leaf tissue of the same leaf and cut it with nine different restriction enzymes, BamHI, BglI, ClaI, EcoRI, HindIII, PstI, PvuII, SalI, and XhoI. We then hybridized these to BamHI and PstI clones from wheat chloroplast DNA (cpDNA) kindly provided by Tristan A. Dyer (C.M. Bowman & T.A. Dyer, 1986, Cur. Gen.10:931-941). We have detected a difference in the restriction patterns of BglI and PvuII digested DNA from white leaf tissue hybridized to the BamHI wheat cpDNA clone B(10-18). This clone is very close to the border of one of the inverted repeats (IR) in the large single copy region and contains the photosystem II genes psbC and psbD. In BglI cut DNA from green leaf tissue, clone B(10-18) hybridized to two bands 8.5kb and 7.5 kb in size. The same clone hybridized only to an 8.5 kb fragment in BglI digested DNA from white leaf tissue. Clone B(10-18) hybridized to three bands, 8.1 kb, 7.1 kb, and 4.5 kb, in PvuII digested green leaf tissue. The same clone hybridized to two bands, 0.8 kb and 0.6 kb in white leaf tissue digested with PvuII. We suggest that a significant deletion has occurred in this area of the chloroplast genome in white leaf tissue of Hosta undulata var. albomarginata. We are now in the process of cloning the cpDNA in this region from both the white and the green leaf tissue. We will also complete the restriction maps of the chloroplast genomes of the green and white leaf tissue of this Hosta species.

CYTOKININ GENE FUSED WITH A STRONG PROMOTER ENHANCES SHOOT ORGANOGENESIS AND ZEATIN LEVELS IN TRANSFORMED PLANT CELLS. A. C. SMIGOCKI AND L. D. OWENS, TISSUE CULTURE & MOLECULAR BIOLOGY LABORATORY, AGRICULTURAL RESEARCH SERVICE, U.S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND 20705, U.S.A.

The isopentenyl transferase gene (ipt) associated with cytokinin biosynthesis in plants was cloned from a tumor-inducing (Ti) plasmid carried by Agrobacterium tumefaciens and placed under the control of promoters of differing activities, the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) promoter. These promoter-gene constructs were introduced into wounded Nicotiana stems, leaf pieces, and cucumber seedlings by A. tumefaciens infection. Shoots were observed in the infection site on all responding genotypes of Nicotiana plants infected with the 35S promoter construct (35S-ipt), whereas only 41% responded similarly to infection with the unmodified gene. Furthermore, shoots were observed 19 days after infection with the 35S-ipt gene, but not until 28 to 45 days with the unaltered ipt gene. Shoots were more numerous (>40) on galls incited by 35S-ipt and were up to six times taller than shoots induced by the native gene. On cucumber, shoots were observed only on galls incited by the 35S-ipt construct. These galls were on the average 7.5 times larger than those incited by the NOS promoter construct (NOS-ipt) or the unmodified ipt gene. Zeatin and zeatinriboside concentrations averaged 23 times greater in the 35S-ipt transformed shoots than in ones transformed with the native ipt gene. These results suggest that a more active promoter on the ipt gene can enhance or change the morphogenic potential of transformed plant cells by increasing their endogenous cytokinin levels.

ANALYSIS OF AN ETHYLENE-RESPONSIVE PROMOTER IN TRANSGENIC TOBACCO PLANTS AND BY TRANSIENT EXPRESSION IN BEAN PROTOPLASTS

Richard Broglie[1], Karen Broglie[1], Dominique Roby[2], and John J. Gaynor[2]. E. I. du Pont de Nemours & Co., Agricultural Products Department, Wilmington, DE 19898, USA[1], and Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA[2].

Production of chitinase, a lytic enzyme found in most higher plants, is a well characterized host response to infection by pathogens. This enzyme is also induced by elicitors and the plant stress hormone, ethylene. In bean, chitinase is encoded by a small gene family, and is regulated at the transcriptional level. In order to investigate the nature of this control, the expression of a chimeric gene comprising the 5' flanking sequences of a bean endochitinase gene, fused to the reporter gene GUS, was studied in bean protoplasts. 1-Aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ethylene, and a fungal elicitor isolated from *Colletotrichum lagenarium*, caused a high and transient expression of the reporter gene. A two-fold induction of GUS activity was observed within 6 hours of incubation with ACC, and a 20- to 50-fold induction was reached after 48 hours. Additionally, an analysis of deletion mutants for this putative ethylene promoter in transgenic tobacco plants is currently underway. These mutants should permit us to further define and map the *cis*-acting elements required for ethylene-induced transcription.

INTRODUCTION AND EXPRESSION OF T-DNA PHYTOHORMONE-BIOSYNTHESIS GENES IN *NICOTIANA* GENETIC TUMORS

Xin-Hua Feng, Paul J. Bottino, and Shain-dow Kung
Center for Agricultural Biotechnology and Department of Botany
University of Maryland
College Park, MD20742

To characterize the molecular and physiological aspects of genetic tumors in *Nicotiana,* T-DNA phytohormone biosynthesis genes were introduced into tumorous wild-type and a nontumorous mutant of amphidiploid *N. glauca* x *N. langsdorffii*. It was confirmed that the hormone genes were integrated into the plant genomes. Variations in morphology was observed in different transformed tissues. The cytokinin-biosynthesis gene can restore the shoot-forming capacity in the mutant, indicating that there might be a mutational defect in cytokinin biosynthesis in the mutant. The genetic tumor tissues produced variations in morphology even when transformed by the same gene, particularly the genes *iaaM* and *iaaH*. Some of the variations no longer showed a shooty teratoma unless cytokinin was added to medium. These variations might be attributed by the interruptions of genes for genetic tumorigenesis and/or position effects due to the introduction of T-DNA genes, which is currently under investigations.

EFFECT OF THIDIAZURON IN CYTOKININ BIOSASSAY SYSTEMS

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Our laboratory is interested in characterizing the mechanisms involved in the control of plant cell division. In many cases auxin and cytokinin are required for continuous cell proliferation *in vitro*. The cellular and biochemical processes that they control have not been precisely defined. Although cytokinins are promoters of cell division in plant tissue cultures, they also affect a wide range of other physiological properties. These include: retardation of senescence, promotion of seed germination, promotion of leaf and cotyledon expansion, control of apical dominance, initiation of shoot meristems in cultured tissues, and induction of pigment biosynthesis. Cytokinin-active compounds can be grouped into two structurally distinct classes, the N^6-substituted adenine derivatives and the diphenylureas. Virtually all of the naturally occurring cytokinins are N^6-substituted derivatives of adenine. Modification of the N^6-side chain or the purine ring significantly alter the biological activity of the compounds. A structurally unrelated urea compound, N,N'- diphenylurea (DPU) displays cytokinin-like activity in several cytokinin bioassays. Although DPU is a weakly active cytokinin, other phenylurea derivatives, including N-phenyl-N'- 1,2,3-thiadiazol-5-ylurea , Thidiazuron, possess high activity.

We are investigating the physiological and biochemical properties of thidiazuron in several cytokinin-dependent bioassay systems. i.) Thidiazuron stimulated shoot formation in 1^{o} explants from pith and leaf disks of *Nicotiana tabacum* var. H425 and cloned cytokinin- dependent H425 leaf callus cultures at concentrations between 0.1 and 10.0 µM. ii.) Betacyanin production in *Amaranthus cruentus* L. seedlings is a cytokinin-dependent process. The dose response curve for thidiazuron-induced betacyanin formation was shifted to lower concentrations than for the natural cytokinin, *trans*-zeatin riboside. Maximum activity was observed at 0.1 - 1.0 µM and 10.0 - 100.0 µM for thidiazuron and t-ZR, respectively. iii.) The cytokinin-antagonist, PMPP, [3-methyl-7-(pentylamino)-pyrazolo-(4,3-d) pyrimidine] can inhibit growth and cell division of both cytokinin-autonomous and cytokinin-dependent cultures. Addition of exogenous cytokinin can overcome the inhibitory effect suggesting that the antagonist and the cytokinin compete for the same cellular receptor. Incorporation of 1.0 µM PMPP in the growth medium markedly inhibits growth of H425 cytokinin-dependent leaf callus. Simultaneous addition of thidiazuron to the medium with PMPP counteracts this inhibition.

These results show that thidiazuron exhibits cytokinin activity in all the bioassays so far examined. Such observations are a paradox since the structure of thidiazuron would suggest it should not bind to any theoretical cytokinin receptor. We are testing the hypothesis that thidiazuron functions by causing an accumulation of naturally occurring adenylate cytokinins. Enzyme immunoassays using monoclonal antibodies for t-ZR will be described.

PATHOGENIC AND HOST RANGE RESPONSES ASSOCIATED WITH GENE VI OF CAULIMOVIRUSES

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ABSTRACT

A single gene of the caulimoviruses may be largely responsible for the pathogenic effects of these viruses. This gene (gene VI) is the only one of six major genes that is expressed as a separate RNA transcript. It specifies a protein (P62) that accumulates in infected plants and probably makes up a major component of virus inclusion bodies. Information from mutagenesis of gene VI and pathogenic analysis of hybrid virus strains of cauliflower mosaic virus (CaMV) indicate that gene VI is mainly responsible for disease induction. In addition, gene VI transformations of plants (using a Ti plasmid-Agrobacterium system) have shown that gene VI expression is accompanied by chlorosis and mottling similar to virus infections. These experiments suggest that P62 perturbs some process in plants to cause disease.

In addition, it has also been found that gene VI determines the host response to infection (hypersensitive versus compatible reactions) and virus host range. Moreover, this gene appears to be the major factor in host adaption by these viruses as shown by recent experiments with figwort mosaic virus (FMV). When this virus was adapted to a new host it was found that a strain with a greatly modified form of gene VI predominated in the new host.

GENETICS OF HOST CULTIVAR SPECIFICITY IN THE RICE BLAST FUNGUS: Marc J. Orbach, Barbara Valent, John E. Hamer, and Forrest Chumley. Central Research and Development Department, The Dupont Company, Wilmington, DE 19898.

The Ascomycete fungus *Magnaporthe grisea* (anamorph *Pyricularia oryzae* Cav.) causes the disease known as rice blast, and is also a pathogen of other grasses. Hundreds of races of the fungus have been described based on their unique pattern of infection of different rice cultivars. Our goal is to understand the molecular basis of cultivar specificity in the *M. grisea* - rice interaction. In addition to the multitude of races, several features of *M. grisea* make it a good system to study host cultivar specificity. It can be grown and crossed on defined medium. Mutant strains can be isolated by standard techniques and the segregation of the mutations can be assessed by tetrad analysis. A transformation system has been developed allowing for the cloning of genes by complementation.

To understand host cultivar specificity in *M. grisea*, crosses between different strains of the fungus have been performed. A backcrossing scheme has been used to discern the differences between a field isolate, O-135, that infects both rice and weeping lovegrass, and a fertile laboratory strain, 4091-5-8, that infects weeping lovegrass. Progeny from this cross all infect weeping lovegrass, but only a few produce lesions on any of three rice cultivars tested. Progeny strains that produced no visible symptoms of infection on rice were backcrossed to the rice pathogen O-135 through five generations. We were able to discern two types of genes segregating among the progeny of this backcrossing regime. Variations in the size of lesions produced by virulent progeny strains demonstrate that quantitative pathogenicity factors are segregating. In addition, four unlinked single genes ("avirulence genes") that determine virulence in an all or nothing manner towards one of the three rice cultivars, CO39, M201 or Yashiro-mochi were identified. Using the *M. grisea* transformation system we will attempt to clone these avirulence genes by complementation in *Magnaporthe*.

We are also analyzing the genome structure of *Magnaporthe* in several ways. Middle repetitive DNA elements in Magnaporthe have been isolated (called MGR for *Magnaporthe grisea* repeat) and shown to segregate in a Mendelian manner in crosses, like genetic markers. Polymorphisms associated with MGR could be useful markers for the construction of a physical map of the *M. grisea* genome. We have begun a direct physical characterization of the genomes of different M. grisea strains using CHEF gel electrophoresis. Variations in the sizes of the chromosome DNAs have been detected. Through Southern hybridization of CHEF gel blots, we are developing an electrophoretic karyotype.

ELICITATION OF PLANT RESISTANCE REACTIONS BY *PSEUDOMONAS SYRINGAE*: Steven W. Hutcheson; Department of Botany, University of Maryland, College Park, MD 20742.

The inducible resistance reactions associated with the necrotic hypersensitive response (HR) are thought to play a key role in preventing the colonization of resistant plant tissue by potentially pathogenic bacteria, such as *Pseudomonas syringae*. The bacterial signals that trigger the HR in resistant plants have not been established. It is thought that multiple, apparently-inducible, bacterial genes function in the formation of a presently undefined signal (elicitor) that then causes a defensive response by plant cells.

The model system we are using to investigate the bacterial induction of resistance reactions is the interaction of *P. syringae* with tobacco, a nonhost plant. We have reported previously the isolation of a cosmid clone from a gene library of *P. syringae* pv. *syringae* that contains an apparently complete set of the bacterial genes necessary to trigger the HR. This cosmid, containing a 31 kb insert, conferred upon saprophytic bacteria, such as *E. coli*, the ability to elicit the HR. Mutational analysis and subcloning has now shown that most of the insert DNA is necessary for the elicitation of the HR. The genes that function in HR elicitation are also necessary for *P. syringae* to parasitize compatible host tissue and appear to be organized in at least two complementation groups. An early idea that this clone contains host range determinants in addition to parasitism factors has not been supported by recent work; over-expression of the cloned genes, possibly due to vector-related artifacts, can account for the effects of this clone on the host range of recipient *P. syringae* pathovars. Studies employing a bioassay for the HR elicitor suggest that the environmental signals controlling the expression of the genes that function in HR elicitation may be the available nitrogen source. We are presently employing *lux* fusions to test whether any of the cloned genes are regulated in a similar manner. The function of the cloned genes remains elusive. Recently, we have observed that *P. syringae* mutants containing insertions in the cloned genes are unable to incorporate exogenous methionine into protein. This suggests that the *P. syringae* genes that function in HR elicitation and host parasitism may encode amino acid transport functions.

TURNIP CRINKLE VIRUS SATELLITE DOMAINS INVOLVED IN VIRULENCE AND PROCESSING. Anne E. Simon and Clifford D. Carpenter. Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

We are developing the turnip crinkle virus (TCV) satellite system as a model to study how small pathogenic RNAs interact with their helper virus and host plants to produce disease symptoms. TCV supports a small family of satellite RNAs, one of which (sat-RNA C) intensifies the symptoms of TCV when co-inoculated on turnip or mustard, while two other satellites (sat-RNAs D and F) are avirulent. Sat-RNA C (355 b) differs from sat-RNA D (194 b) and sat-RNA F (230 b) mainly by the presence of a 3′-domain which is homologous to the 3′-end of the TCV genome (1). To construct a functional map of sat-RNA C, we constructed a chimeric satellite composed of the virulent and avirulent satellites, as well as inserted and deleted sequences at various sites in the virulent sat-RNA C cDNA template. Mutated satellite RNA was synthesized *in vitro*, and assayed for infectivity and symptom production after inoculation with the helper virus on turnip. The chimeric satellite, constructed from the 5′ 155 bases of avirulent sat-RNA F ligated to 200 bases of the 3′-domain of sat-RNA C, produced severe symptoms, which demonstrates the 3′-domain of sat-RNA C determines virulence. Small insertions and deletions (up to 60 bases) in the 5′ two thirds of the molecule generally had no detectable effect on infectivity or symptom production (2). Since insertions or deletions in the 3′ terminal 100 nucleotides of sat-RNA C (the region implicated in symptom production) destroy the infectivity of the satellite, we have introduced single base point mutations into nearly 50% of the 3′ 100 nucleotides of sat-RNA C in order to further define the virulence determining region. We have also identified a region of sat-RNA C involved in satellite processing. Deletion of nucleotides 79-84 resulted in the accumulation of large amounts of the dimeric form of the satellite. Only trace amounts of sat-RNA the size of the inoculated (monomeric) transcripts were found. We have not observed self-processing by *in vitro* synthesized sat-RNA C dimers under a variety of conditions, implying that sat-RNA C is processed by a mechanism which differs from that used by circular satellites.

1. Simon, A.E. and Howell, S. H. (1986) EMBO J. 5, 3423-3428.

2. Simon, A.E. et al., (1988) EMBO J. in press.

COMPARISON OF THE NUCLEOTIDE AND AMINO ACID SEQUENCES OF THE COAT PROTEIN GENES OF BEAN YELLOW MOSAIC VIRUS AND OTHER POTYVIRUSES: John Hammond[1] and Rosemarie W. Hammond[2]; USDA-ARS, [1]Florist and Nursery Crops Laboratory, and [2]Microbiology and Plant Pathology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705.

The sequence of cDNA clones representing the 3'-terminal 1035 nucleotides of the bean yellow mosaic virus (BYMV) RNA has been determined. These clones include the coat protein gene and 3'-non-coding sequences. One clone expresses a fusion protein that was detected with antisera and monoclonal antibodies reactive with the BYMV coat protein. The nucleotide and deduced amino acid sequences were compared to those of several other potyviral coat protein genes. The deduced amino acid sequences were found to be more highly conserved than the nucleotide sequences. The major differences between amino acid sequences were at the amino terminus of the coat protein, which is exposed on the virion surface, and to which most virus-specific antibodies are produced. The conservation of internal amino acid sequences is probably necessary for capsid structure; similarities in structure are also indicated by hydropathy plots of the amino acid sequences. Conservation of internal amino acid sequences also explains why polyclonal antisera used in antigen-coated forms of enzyme-linked immunosorbent assay (ELISA) reveal relationships between viruses that are not obvious in double antibody sandwich (DAS) forms of ELISA using the same sera. Antigen-coating of polystyrene ELISA plates leads to partial disruption of the virion and thus exposes internal sequences, whereas the amino terminus is the primary target of antibodies trapping undissociated virus in DAS-ELISA, and conserved sequences are less exposed.

UPTAKE OF DNA BY ELECTROPORATION OF POLLEN

Aref Abdul-Baki[1], James A. Saunders[2], and Benjamin F. Matthews[1]
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Electroporation of plant protoplasts has become a popular method for the transfer of specifically cloned genes into isolated protoplasts. Plants obtained from the transformed protoplasts involve lengthy sterile culture and regeneration procedures. Many economically important plant species cannot be regenerated consistently from protoplasts. To circumvent this problem we are developing a procedure which does not involve cell culture or plant regeneration. In this procedure, cloned DNA is taken into germinating pollen grains by electroporation. The pollen is then applied to the stigmas of receptive emasculated flowers, and seed resulting from the cross is collected and screened for transformants. Our results show that germinating pollen grains from tobacco are capable of taking up radioactively labelled DNA by electroporation. When the electroporation treatment is applied within one hour of pollen gemination, the pollen can withstand field strengths up to 11.25 Kvolts/cm without showing adverse effects from the electroporation treatment. The viability of the pollen grain does not appear to be adversely affected by the electroporation treatment. In addition, pollen treated in this manner is capable of producing viable seed when applied to the stigmas of compatible flowers. This procedure offers a wide applicability for gene transfer because it is not limited by host range.

UTILIZATION OF DNA PROBES FOR IDENTIFYING ALFALFA CHROMOSOMES:
G. R. Bauchan; D. A. Schaff; and B. F. Matthews; USDA/ARS, Germplasm Quality and Enhancement Lab., and the Plant Molecular Biology Lab., Beltsville, MD.

Production of a karyotype requires the identification of homologous pairs of chromosomes. Alfalfa has 16 chromosomes at the diploid level. The chromosomes are small (5-8 microns in length) and 4 of the 8 pairs of chromosomes are metacentric and relatively the same length, thus a standardized karyotype has not been established. Molecular biological techniques have been employed to identify individual chromosomes. Southern blots using alfalfa genomic DNA were run to test for hybridization of selected heterologous probes. Heterologous DNA was labeled with biotin to produce non-radioactive probes. Probes were hybridized *in situ* to diploid CADL alfalfa chromosome squashes. Streptavidin-horseradish peroxidase complex was used to localize these probes on the chromosomes. Specific bands were observed on the Southern blot and on individual alfalfa chromosomes. This demonstrated that these probes identified specific sequences of alfalfa DNA. Alfalfa cDNA and genomic libraries were constructed from cell suspension cultures. These libraries will supply additional probes. Refinement of this technique is underway to identify all 8 pairs of chromosomes for the construction of a standardized alfalfa karyotype.

MAIZE GLUTAMINE SYNTHETASE GENE STRUCTURE: Barbara M. Chereskin, Joachim Messing and *D. Peter Snustad. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ 08855 and *Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108.

A size fractionated cDNA expression library made from maize Black Mexican Sweet tissue culture cells was used to transform a Δgln A *E.coli* strain. Clones expressing glutamine synthetase (GS) were identified by marker rescue. These clones were able to grow on minimal plates without glutamine supplementation. Two of the clones were sequenced and were shown to differ only in the position of the poly A tail. Although homologous to published sequences for GS in other plant and mammalian systems, a unique feature of the maize GS is an upstream minicistron containing 28 codons and separated from the translational start for GS protein by 37 nucleotides. Western blots have demonstrated that expression of the maize GS in the bacterial glutamine auxotroph is higher when this minicistron is not present. This minicistron is also present in a genomic clone isolated from maize inbred line A188. Sequencing of this genomic clone has shown it to be nearly identical to the sequence of the cDNA clones and organized into 13 exons spanning more than 4 kb. Genomic southerns probed with the GS cDNA clone indicate only one gene for GS in a number of inbred lines. However two anti-GS cross reacting proteins are detected in Western blots of total leaf proteins, presumably the chloroplastic and cytosolic forms of the enzyme. The possibility that these two homologous proteins are generated from one gene is currently under investigation.

PROTEIN AND RNA POPULATIONS OF RIPENING PEACH FRUIT FROM MELTING FLESH AND NON-MELTING FLESH CULTIVARS

Reuben Cohen*, Ann Callahan, Linda Dunn, and Peter Morgens. USDA-ARS, Appalachian Fruit Research Station, Kearneysville, West Virginia 25430

Total proteins from ripening series of the melting flesh, freestone cultivar 'Loring' and the non-melting flesh, clingstone cultivar 'Suncling' were compared by SDS-PAGE. A 38 kD ripening-specific protein is present at a higher level in 'Suncling' than in 'Loring'. Translatable RNAs were isolated from the same samples. Analysis of *in vitro* translation products by SDS-PAGE indicated that an RNA encoding a 41 kD polypeptide is present earlier in ripening in 'Suncling' than in 'Loring'. To examine RNA populations more sensitively, a ripe fruit cDNA library has been constructed from a melting flesh cultivar and is being analyzed by differential hybridization with 'Loring' and 'Suncling' cDNA probes. We are comparing gene expression in melting flesh, freestone and non-melting flesh, clingstone peaches to identify genes influencing flesh firmness and texture.

PROGRESS IN THE GENETIC MANIPULATION OF MAIZE

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ABSTRACT

The introduction of exogenous DNA into protoplasts and subsequent regeneration of fertile transformed plants has not been reported in maize. Toward this end, we have utilized expression of EPSP synthase (encoded by the *aroA* gene of *Salmonella typhimurium*) and antibiotic resistance (encoded by the *neo* gene of *Escherichia coli*) to test conditions for the introduction, expression and integration of foreign genes in maize. Type II callus from A188XB73 was used to initiate embryogenic suspension cultures which yielded division competent protoplasts. These protoplasts were used to establish electroporation conditions which resulted in transient expression of *aroA* and in stably transformed callus which survived selection on G418. In separate experiments, with the same suspension cultures, a protocol was developed allowing the regeneration of plants from single protoplasts. Experiments have begun to couple these transformation and regeneration protocols and to extend these efforts to commercially important genotypes.

USE OF ELECTROPORATION FOR TRANSIENT ASSAYS AND STABLE TRANSFORMATION OF CARROT: J. DeRobertis, C. Browning and D. Pierce; Enichem Americas, 2000 PrincetonPark Corporate Center, Monmouth Junction, NJ 08852.

We have used an embryogenic suspension culture of carrot (Daucus carota) to develop a protoplast transient assay model system for gene expression in dicot cells. Electroporation has been used as the method for direct DNA uptake into the protoplasts. Electroporation parameters have been optimized by analysis of expression of gene cassettes carrying the bacterial chloramphenicol acetyltransferase (CAT) gene. The HPLC method for CAT assays gives very quantitative and reproducible results and avoids the use of radioactively labeled substrates. We have also used electroporation of protoplasts to produce stably-transformed cell lines from which regenerated carrot plants were obtained. The gene cassettes for these latter experiments carried the bacterial neomycin phosphotransferase (NPTII) gene. Methods were developed for culturing of protoplasts, selection of transformed cells on kanamycin and regeneration of transgenic carrot plants. Stable transformation of callus and plants was confirmed by NPTII assays for expression of the cassette and by analysis of carrot DNA for the presence of the cassette.

PURIFICATION AND INTERCONVERSION OF FORMS OF HOMOSERINE DEHYDROGENASE ISOENZYMIC FORMS FROM CELL SUSPENSION CULTURES OF DAUCUS CAROTA.

Benjamin F. Matthews and Marne Farrar. USDA-ARS, Plant Molecular Genetics Laboratory, Beltsville, MD 20705

Homoserine dehydrogenase has been purified to apparent homogeneity from Daucus carota cell suspension cultures by selective heat denaturation, ion exchange and gel filtration chromatography, and electrophoretic techniques. The native form of homoserine dehydrogenase is composed of subunits having a molecular weight of 85,000+/-5000. The native enzyme can exist as multiple isoenzymic forms, having apparent molecular weights ranging from 180,000 to approximately 720,000.

Multiple forms of homoserine dehydrogensase (HSDH) have been identified by manipulating in vitro environmental conditions. Two distinct forms of HSDH have been examined. One form (Mr=240,000) is highly sensitive to feedback inhibition by threonine and is stimulated 2.6 fold by K^+. The other form (Mr=190,000) is not inhibited by threonine and is only stimulated 1.5 fold by K^+. These two forms appear to interconvert and can polymerize under certain conditions to form higher molecular weight aggregates ranging in size up to 720,000.

SPATIAL AND TEMPORAL EXPRESSION OF ENDOCHITINASE IN MAIZE. William C. Mitchell and John J. Gaynor, Department of Biological Sciences, Rutgers University, Newark, NJ 07102.

Basic endochitinase is found in four divisions of higher plants including ferns, cycads, conifers and angiosperms. Flowering plant leaf endochitinases are small basic proteins, 26 to 37 kd in molecular weight, which are capable of hydrolyzing both chitin and peptidoglycan. These enzymes are ethylene inducible in dicotyledonous plants, are present in most organs and tissues, and are thought to function as defense proteins against fungal and bacterial pathogens. In monocotyledonous plants, regulation of endochitinase by ethylene is species specific and ranges from completely insensitive to low-level inducible.

The endochitinase of young corn (*Zea mays* cv. Golden Bantam) seedlings displays a unique tissue specific and developmental expression. Immunoblotting and radiometric enzymatic assays have demonstrated the presence of endochitinase in roots, mesocotyl, coleoptile, stem and leaves. In contrast to dicotyledonous plants, however, the relative amounts of endochitinase are low and unresponsive to exogenous ethylene. Coleoptile endochitinase is developmentally regulated and increases dramatically between three and nine days post-imbibition, whereas mesocotyl endochitinase levels are constant over the same period. The increase in the coleoptile is insensitive to ethylene as well as mechanical wounding. Immunoblotting has demonstrated that there are two chitinases (34.5 and 36.6 kd) in both coleoptile and mesocotyl. Seeds contain a uniquely smaller endochitinase which is not found in seedlings. The level of primary leaf endochitinase is also developmentally regulated and increases with leaf age. The 34.5 kd endochitinase appears to be constitutive in young leaves and low-level ethylene inducible in older leaves, whereas the 36.6 kd endochitinase remains ethylene insensitive in older primary leaves.

PLASMIDS IN THE FILAMENTOUS FUNGUS *GLIOCLADIUM VIRENS*

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Gliocladium virens is a biocontrol antagonist of soilborne plant pathogens such as *Pythium ultimum* and *Rhizoctonia solani* More than 70% of the twenty *G. virens* strains examined contain one or more plasmids in their mitochondria. It is of interest to determine the nature and function of these plasmids since they may contain genetic material related to biocontrol capabilities. Strain G-1 has a single plasmid present in high copy number which has been shown by nuclease digestions to be double-stranded DNA. This plasmid appears to exist in a circular form with a size of 3.2 + .5 kb. Experiments are in progress to clone and map this plasmid and to determine its relationship to plasmids in other *G. virens* strains.

MOLECULAR ANALYSIS OF THE EXPRESSION OF THE GENE ENCODING FERREDOXIN I (*FED I*) - PEA NUCLEI CONTAIN FACTORS THAT BIND SPECIFICALLY TO THE *FED I* PROMOTER.

Thomas J. Pedersen, Michael J. White, Robert C. Elliott, Lynn F. Dicky and William F. Thompson, Department of Botany, North Carolina State University, Raleigh, NC 27695-7612, USA.

Ferredoxin I is a soluble electron transport protein involved in the photosynthetic reduction of NADPH. In pea plants, it is encoded by the single-copy gene, *Fed I*. Expression of this gene is under phytochrome control and steady-state levels of the *Fed I* mRNA increase substantially after plants are exposed to light. We have isolated, mapped and sequenced a *Fed I* genomic clone and have found several sequences upstream of the coding region that are similar to sequences experimentally shown to confer light-regulation on the genes that code for the small subunit of ribulose bisphosphate carboxylase. Using the gel retardation and DNase I protection assays we have begun a detailed analysis of transcriptional mechanisms that may play a role in the regulation of *Fed 1* expression. When pea nuclear extracts were assayed, a DNA-binding activity specific for this upstream region of the pea *Fed I* gene was observed. Although this activity is resistant to heat inactivation, it is sensitive to digestion by proteinase K, suggesting that it is proteinaceous.

Expression of Endopeptidase involved is bean seed storage protein mobilization: Lorraine Solberg and Ian M. Sussex; Yale University, Department of Biology, Box 6666, New Haven, CT 06511.

The cotyledonary reserve protein and starch of *Phaseolus vulgaris* are mobilized during seedling growth to supply the seedling with amino acids and sugars. During mobilization of phaseolin, the major storage protein of bean, an initial endoproteolytic cleavage occurs, followed by complete digestion of the protein. The endopeptidase which catalyses the initial endoproteolytic cleavage has been purified (Boylan, M. and Sussex, IM, 1987). It is a 30kD cysteine endopeptidase with pH optimum of 5.0 and maximal activity during the sixth day post imbibition. Polyclonal antibody to the endopeptidase has been used to probe western blots of cotyledon protein extracts which show the endopeptidase present from the fifth to the ninth day post-imbibition.

Boylan, M. and Sussex, I.M. (1987). Purification of an endopeptidase involved with storage-protein degradation of *Phaseolus vulgaris* cotyledons. Planta 170, 343-352.

LEMNA GIBBA: A TOOL FOR STUDYING DEVELOPMENTAL BIOCHEMISTRY AND MOLECULAR BIOLOGY. [1,2]J.P. Slovin, [2]B. Baldi, and [2]J.D. Cohen, [1]Dept. Botany, U. MD. College Park, and [2]USDA-ARS Plant Hormone Lab., Beltsville, MD 20705.

We are continuing to develope a new system, using the small aquatic monocot Lemna gibba G-3, to study the metabolism of the plant hormone, indole-3-acetic acid (IAA). Our approach has involved the use of classical techniques such as rapid kinetic labeling (under sterile conditions to avoid fungal or bacterial metabolites) and to isolate and characterize mutants in the biochemical pathway.

We have obtained many different kinds of mutants of Lemna by various techniques, including tissue culture, and whole plant mutagenesis. By chemical mutagenesis of only 700 intact plants (in one petri dish) we obtained 36 morphological or pigmentation variants. One such variant has low or no chlorophyll b and very low or no steady state levels of LHCP II.

We have regenerated Lemna from tissue culture and obtained several variant lines. One line, jsR-1, is approximately 1.5 times the size of the Parental LIne (PL), and has approximately the same amount of DNA per nucleus as PL. R-1 is especially useful to us because it contains up to 100 times the amount of free IAA as PL, and unlike PL, it contains no detectable IAA-conjugates. Using stable isotope labeled compounds, high resolution mass spectrometry, and jsR-1, we are developing methods to measure the kinetics of labeling in indolic compounds related to the biosynthesis of IAA. We are also using R-1 to study the processes by which plants regulate the formation and hydrolysis of IAA-conjugates. [NSF DMB-86-17171].

VERIFICATION OF A HOMOSERINE DEHYDROGENASE SPECFIC MONOCLONAL ANTIBODY

Frank J. Turano, Ramon L. Jordan and Benjamin F. Matthews

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Most of our understanding of the structure and regulation of enzymes involved in the biosynthesis of the essential amino acids methionine, threonine, lysine and isoleucine is from research conducted in bacterial systems. In order to gain a better understanding of key enzymes involved in the biosynthesis of the aspartate family amino acids in plants, we have prepared antibodies specific to carrot homoserine dehydrogenase (HSDH). The antibodies will be used to characterize and purify large quantities of the enzyme and identify recombinant cDNA clones.

Mouse polyclonal antibody was prepared from the 240-kD form of carrot HSDH. Western blot analysis performed in parallel with enzyme activity stains was used to verify the specificity of the mouse anti-HSDH antibody to carrot HSDH. Western blot analysis of various in vitro forms of carrot HSDH showed that the antibody reacted with the various polymerized forms of HSDH. The antibody also cross-reacted with soybean HSDH.

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