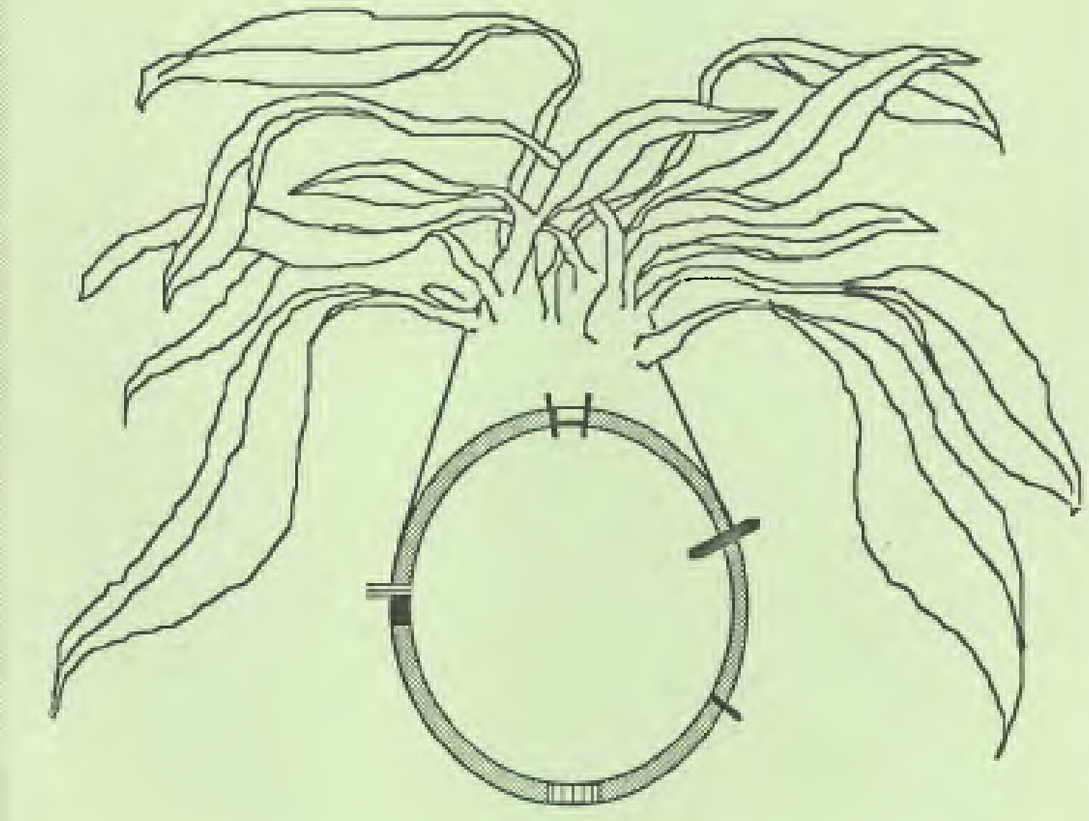
Sue Mischke

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

FOURTH ANNUAL MEETING



AUGUST 20 & 21, 1987 UMBC

FOURTH ANNUAL MEETING

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

AUGUST 20 and 21, 1987

University of Maryland Baltimore County

Catonsville, MD

CONTENTS

INTRODUCTION

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

Since its inception three years ago by Ben Matthews at the USDA, MAPMBS has grown steadily and now has more than 300 members. Attendance and participation at our annual meetings has also increased each year, as has the geographic area served by the meeting; this year we have many participants from New York, New Jersey, North Carolina, Delaware, Pennsylvania, and Virginia. It is now truly a Mid-Atlantic Society! We are all indebted to Ben,and the many people who have worked on the previous three meetings, for taking the time and substantial effort needed to make a good idea become a great reality.

We are proud to host this year's meeting here at UMBC and we look forward to visiting other campuses in the Mid-Atlantic region for future meetings. As always, there have been many people whose efforts were essential to this conference. We especially thank Ben Matthews, Bob Owens, John Watson, Lowell Owens (Program); Joel Chandlee and Sue Mischke (Registration); Janet Slovin (Funding and Workshops); Bob Owens (Abstracts); Rosemarie Hammond (Publicity); Denise O'Donnell (Housing); and Joanna Truitt (University Center Planning). Their efforts and those of all the others involved are greatly appreciated.

J. Lynn Zimmerman and Madeline Wu

Organizers

GENERAL INFORMATION

UMBC: The campus is located just 10 minutes south of Baltimore and approximately 45 minutes north of Washington, D.C. If you have free time after the meeting or would enjoy some night life Thursday evening, you should visit Baltimore's Inner Harbor which contains many excellent restaurants and shops. Other points of interest in the Inner Harbor are the National Aquarium, the Science Center and the Power Plant. The Inner Harbor is easily reached by going north on I-95 and following the signs.

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

There will be a reception immediately following Dr. Goldberg's lecture on Thursday and all are invited to attend; it will be held in the back of the Ballroom.

MEALS: If you pre-registered, your lunches have been prepaid and will be provided in the Faculty/Staff Dining Room (about a 5 min. walk from the Ballroom). You will need to present your meal pass on both days. If you did not pre-register, you will not prepay you lunches (and we were not able to include you in our count to the Food Services people) and are, therefore, on your own for lunch. There is a cafeteria on the first floor of the University Center or you may choose to veture off campus.

If you pre-registered for the Barbeque, you will have received a special ticket for that event. The Barbeque will be held on Thursday at approximately 6:00 pm on the first floor of the University Center (outside,weather permitting).

WORKSHOPS: The two special interest workshops will be held concurrently on Friday from 11:00 - 12:00. The sites of the workshops will be determined by the number of interested participants and will be announced Friday am.

Plant Cell
ed. Bob Goldberg
1st issue - Next July
(ASPP)
$45/yr to members
$60/yr - 2 journals

EXHIBITORS AND SPONSORS

We wish to thank the 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 with your research.

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Special thanks tothe Rohm and Haas Company and E.I. duPont de Nemours Company for their sponsorship of this year's meeting. The gift from Rohm and Haas allowed us to lower student registration fees and that of duPont helped sponsor our keynote address by Dr. Goldberg.

- Eric Heydemann (Zeiss?)

Mid-Atlantic Plant Molecular Biology Society
Fourth Annual Meeting

PROGRAM SCHEDULE

Thursday, August 20, 1987

8:00-9:00 Registration

9:00-11:30 Session I--Gene Regulation I pp.1-6

Convenors--J. Lynn Zimmerman and Lowell Owens

9:00-9:30 Joseph Ecker--"Molecular Genetics of Plant Stress Responses: Ethylene and Wound-Regulated Genes"

9:30-10:00 Gloria Coruzzi--"Molecular Analysis of the Genes Encoding Chloroplastic and Cytosolic Glutamine Synthetase"

10:00-10:15 BREAK

10:15-10:30 Fred Abeles--"Senescence of Cucumber Leaves and Cotyledons: Induction of a 33-kDa Peroxidase"

10:30-10:50 Jennifer Sorrentino--"Molecular Characteristics of the RDT Maize Controlling Element"

10:50-11:10 Frank Turano--"Isolation of cDNA Clones Encoding Alpha-Galactosidase and the 24 kD Oil Body Membrane Protein of Soybean"

11:10-11:30 Jennifer Schnall--"Temperature-Sensitive Variants in Carrot Somatic Embryogenesis"

11:30-1:00 LUNCH

1:00-3:50 Session II -- Plant/Microbe Interactions pp.7-12

Convenor--R.A. Owens

1:00-1:30 Olen Yoder--"Virulence Genes from Plant Pathogenic Fungi"

1:30-2:00 Carole L. Cramer--"The Phenylalanine Ammonia-Lyase Small Multigene Family: "Gene Structure and Differential Induction by Stress"

2:00-2:15 BREAK

2:15-2:35 Rosemarie Hammond--"Mutational Analysis of Potato Spindle Tuber Viroid"

2:35-2:55 Candace Collmer--"Structure-Function Analyses Using Infectious Transcripts from Cloned cDNAs of Cucumber Mosaic Viral Satellites"

2:55-3:15 Merelee Atkinson--"Evidence for Ion Channel Involvement in Hypersensitivity of Tobacco to Pathogenic Pseudomonads"

3:15-3:30 H-C. Huang--"Molecular Cloning and Characterization of a Pseudomonas syringae pv. syringae Gene Cluster That Enables Saprophytic Bacteria to Elicit the Hypersensitive Response in Tobacco"

3:30-3:50 Business Meeting

4:00-5:00 KEYNOTE ADDRESS: Dr. Robert B. Goldberg, UCLA

"Gene Regulation in Higher Plants"

5:00-6:00 Reception for Dr. Goldberg

6:00 BARBEQUE AND SOCIAL

Friday, August 21, 1987

9:00-10:40 Session III-Molecular Biology of Organelles pp.13-16

Convenor--Benjamen Matthews

9:00-9:30 Marvin Edelman--"Chloroplast Ribosomal Protein Genes: Some Unusual Features"

9:30-10:00 Neil Hoffman--"Translocation of Proteins into the Chloroplast"

10:00-10:20 Pablo Scolnik--"Chloroplast Differentiation and the Control of Nuclear Gene Expression"

10:20-10:40 Greg Silk--"Rapid Light Regulation of Chloroplast Transcript Levels in Chlamydomonas reinhartii"

10:40-11:00 BREAK

11:00-12:00 WORKSHOPS

12:00-1:00 LUNCH

1:00-2:00 POSTERS AND EXHIBITS

2:00-4:45 SESSION IV--Gene Regulation II

Convenor--John Watson

2:00-2:30 Robert Ferl--"Characterization of Nuclear Factors that Regulate the Expression of Alcohol Dehydrogenase Genes"

2:30-3:00 Lon Kaufman--"Phytochrome and Cryptochrome Regulation of Nuclear Genes in Peas"

3:00-3:15 BREAK

3:15-3:45 John Watson--"Light Regulation of Nuclear Ribosomal RNA Expression in Peas"

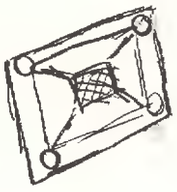
3:45-4:00 Charles Moehs--"Histones of Arabidopsis thaliana"

4:00-4:15 Mahmoud Rifaat--"Genetic Basis of Maize B-Glucosidase Multiplicity"

4:15-4:30 Kamaruzaman Mohammed--"Degradation of Zeins in Germinating Corn Kernels"

4:30-4:45 Gail Dinter-Gottlieb--"Viroids and Introns: Probing the Relationships"

POSTER ABSTRACTS



MOLECULAR GENETICS OF PLANT STRESS-RESPONSES: ETHYLENE- AND WOUND-REGULATED GENES, Joseph R. Ecker, Plant Sciences Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Understanding an organism's response to stress is a fundamental problem in biology. The unifying theme in the stress response is that perturbation of the environment leads to rapid and specific activation of gene expression. In the course of evolution, plants have developed unique and effective mechanisms for resistance to a variety of stresses such as infection and wounding; the "immune response" of plants. A major goal of our laboratory is to understand the molecular and cellular basis of a plants response to these stresses.

One of the earliest detectable events in the plant-stress response is a rapid increase in ethylene biosynthesis. This gaseous plant "stress" hormone may be a signal for plants to erect defense mechanisms against potential pathogens. We have initiated a molecular genetic approach to dissect the mechanisms controlling the induction and expression of ethylene- and wound-regulated genes in carrot (1,2) and Arabidopsis. The effect of ethylene of four plant genes (phenylalanine ammonia-lyase, PAL; 4-coumerate CoA: ligase, 4-CL; chalcone synthase, CHS and hydroxyproline-rich glycoproteins, HRGP) involved in three defense response pathways was examined. Blot hybridization analysis of mRNA from ethylene-treated carrot roots revealed a dramatic increase in the levels of PAL, 4-CL, CHS and certain HRGP mRNAs. The effect of ethylene on HRGP accumulation was different from that of wounding. Ethylene induces two HRGP mRNAs; where as, wounding leads to accumulation of an additional HRGP mRNA. These results indicate that at least two distinct plant stress signals, ethylene and a wound signal, can affect the expression of HRGP genes.

As a first step toward extending these studies to Arabidopsis, we have isolated HRGP-homologous sequences from Arabidopsis cDNA and genomic libraries. Preliminary results indicate the presence of 6-7 genes homologous to a carrot HRGP gene. Studies of the expression of HRGP genes in response to stress, as well as, several strategies for the identification of Arabidopsis wound and ethylene-response mutants will be discussed.

(1) Ecker, J.R. & Davis, R.W. (1987) In: Molecular Biology of Plant Growth Control, UCLA symposia on Molecular and Cellular Biology, New Series (Fox & Jacobs, eds.) pp. 133-143.
(2) Ecker, J.R. & Davis, R.W. (1987) Proc. Natl. Acad. Sci. USA, 84, in press.

MOLECULAR ANALYSIS OF THE GENES ENCODING CHLOROPLASTIC AND CYTOSOLIC GLUTAMINE SYNTHETASE: Gloria M. Coruzzi, Scott V. Tingey*, Elsbeth L. Walker, Janice W. Edwards, and Fong-Ying Tsai. The Rockefeller University, New York, NY 10021-6399. *present address: E.I DuPont de Nemours Co., Wilmington, DE 19898.

Glutamine synthetase (GS) is the major enzyme responsible for ammonia assimilation in higher plants. Distinct isoforms of GS are located in the chloroplastic and cytosolic compartments of a cell. We have characterized the GS polypeptides, primary translation products, and mRNAs which are present in Pisum sativum. Three GS subunit polypeptides occur in peas, which are distinct in size 44, 38, and 37 kd. The 44 kd GS polypeptide is localized to the chloroplast stroma. Levels of the chloroplastic GS_2 polypeptide increase upon greening of etiolated pea leaves. There are two types of cytosolic GS polypeptides (GS_1 and GS_n) which are 38 and 37 kd in size, respectively. The role of plant GS in assimilation of ammonia produced in nitrogen-fixing root nodules is evidenced by the induced synthesis of three charge variants of the 37 kd GS_n polypeptides which are expressed most specifically, but not exclusively in nodules. There are at least four different GS mRNAs as evidenced by a characterization of GS cDNA clones. Hybrid-select translations reveal that there are three distinct GS primary translation products (49, 38, and 37 kd). Three full length GS cDNA clones have been shown to encode these GS gene products. cDNA clone pGS185 encodes a 49 kd GS polypeptide which has been shown by in vitro chloroplast uptake to be a precursor to the 44 kd chloroplast stromal GS_2 polypeptide. Northern blot analysis has shown that GS_2 mRNA is expressed specifically in leaves and that light affects the steady state level of this GS mRNA. Two full length cDNA clones pGS299 and pGS341 encode cytosolic forms of GS which correspond to the 38 and 37 kd GS primary translation products, respectively. The levels of pGS341 mRNA is induced 10-fold in nodules compared to leaves or uninoculated roots. Southern blot analysis of nuclear DNA, and analysis of GS genomic clones, has shown that the distinct GS mRNAs for chloroplastic and cytosolic GS are encoded by a family of homologous but distinct nuclear genes. Future studies are directed at identifying the cis-acting DNA elements of the GS gene promoters which are involved in the differential expression of the distinct members of this gene family.

SENESCENCE OF CUCUMBER LEAVES AND COTYLEDONS: INDUCTION OF A 33-kDa PEROXIDASE. F. B. Abeles and L. J. Dunn. USDA-ARS, Appalachian Fruit Research Station, PO Box 45, Kearneysville WV 25430; R. E. Dinterman and J. Schmidt USAMRIID, Fort Detrick, Frederick MD 21701.

During natural senescence of cucumber leaves and ethylene induced senescence of cotyledons, a number of new protein bands appear in SDS-PAGE gels. Two of these proteins were purified on the basis of their association with a red chromophore. The absorption maximum of the chromophore was 398 nm with smaller peaks at 498 and 649 nm. The molecular weights of these proteins, 33-kDa and 60-kDa, were determined by means of chromatography on Sephacryl S-200 and mobility in SDS-PAGE. The 33-kDa and 60-kDa red proteins appeared to be different polypeptides because they had different amino acid compositions. The observation that ^{35}S-Na_2SO_4 was incorporated into these proteins during ethylene enhanced senescence was consistent with the idea that they represent newly synthesized proteins. Using a radial immuno assay for the 33-kDa red protein, the protein was evident two days after the treatment of cucumber cotyledons with 20 mM ethephon. CNBr was used to cut the 33-kDa protein into two polypeptides. One fragment was analyzed with a gas phase protein sequencer and indicated that the fragment contained the following sequence of amino acids; DLVSLSGAHTFGRSRNRFFSH. A protein homology search indicated a 63.2% homology to horseradish peroxidase, and 73.3% homology to turnip peroxidase. Using guaiacol as a substrate the 33-kDa protein exhibited peroxidase activity. Using an in-vitro translation system and total RNA from cucumber cotyledons, the formation of a number of translation products, some in the 33-kDa range was observed. These data indicate that 33-kDa peroxidase was induced during normal and ethylene induced senescence, and may serve as a model for ethylene regulated protein synthesis.

MOLECULAR CHARACTERISTICS OF THE RDT MAIZE CONTROLLING ELEMENT. J. J. Sorrentino[1], C. O'Reilly[2], Zs. Schwarz-Sommer[3], H. Saedler[3], and N. S. Shepherd[4], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, [2]Sunderland Polytechnic, Sunderland, UK, [3]Max-Planck-Institut, Cologne FRG, [4]CR&D DuPont Experimental Station, Wilmington, DE.

Dotted, Dt, was the first of the maize controlling elements to be genetically defined. Dt is a transposable element which can regulate, in trans, the expression of several alleles at a second locus al (anthocyaninless) on chromosome three. It has been hypothesized that a responding element, rDt, controlled by the Dt element, is present at the al locus in these alleles.

To identify rDt, we cloned two alleles, al and am-1: Cache, which are anthocyaninless (stable nulls) in the absence of Dt, but in response to Dt express anthocyanin pigment in somatic sectors. These were compared to the wildtype A1 structure. A 0.7 Kb DNA insertion which has molecular characteristics common to eukaryotic transposons was identified in each allele, within the coding region of A1. The insertion is absent in a revertant A1 allele derived from al, and is thought to be the rDt element. A short inverted-repeat (IR) at the ends of rDt shares sequence homology with certain other eukaryotic transposon IR's, but not with IR's of the Ac/Ds or Spm controlling elements. The flanking sequence at the insertion sites suggests that rDt generates a target site duplication on integration, but that this integration is by an "imprecise" molecular mechanism.

ISOLATION OF cDNA CLONES ENCODING ALPHA-GALACTOSIDASE AND THE 24 kD OIL BODY MEMBRANE PROTEIN OF SOYBEAN. Frank J. Turano, Charles Cohen, Benjamin F. Matthews and Eliot Herman. USDA-ARS Plant Molecular Genetics Laboratory, Beltsville, MD 20705

Alpha-galactosidase (AG) degrades raffinose-type oligosaccharides during germination and early seedling growth in legume cotyledons. AG has been localized on the Golgi apparatus and protein bodies of developing soybean cotyledons. The 24 kD oil body membrane protein (OBMP) is one of four abundant proteins that is contained in the oil body membrane of developing cotyledons.

Recombinant clones containing immunologically related proteins to AG and the 24 kD OBMP were identified from a lambda gt11 cDNA library. The library was constructed from poly (A)+ RNA which was isolated from midmaturation soybean cotyledons (150 mg). The library was separately screened with AG and 24 kD OBMP antisera. The AG and 24 kD OBMP cDNA's comprised 0.01% and 0.1% of the library, respectively. These percentages correlate to their total protein composition in developing seeds.

AG from mature soybean seeds is a tetramer of 160 kD and is composed of subunits of 38 and 40 kD. The AG clone contains a DNA insert of 1,000 bp which could code of a protein of 37 kD. The OBMP clones contain inserts of approximately 600 bp which could code for a protein of 22 kD.

Phylogenetic Studies and Ethylene Inducibility of Endochitinase in Higher and Lower Plants. *William C. Mitchell and John J. Gaynor, Department of Biological Sciences, Rutgers University, Newark, NJ 07102.*

Chitinase is a putative defense protein that is capable of hydrolyzing both chitin and peptidoglycan. This enzyme is ethylene inducible, is present in most organs and tissues, and is thought to function as a defense against fungal and bacterial pathogens. Immunological screening of higher and lower plant groups has revealed that this enzyme is widely distributed and, in most cases, induced by stress ethylene. Chitinase was detected in all higher plants screened and had a molecular weight between 26 and 37 kilodaltons. This enzyme is also present in gymnosperms but occurs as a larger variant (41 to 68 kd). Chitinase is absent, however, from lower vascular plants (*i.e.*, bryophytes, psilophytes, lycopods). Ferns and dicotyledonous plants regulate chitinase with ethylene whereas that of gymnosperms and most monocotyledonous plants appears to be constitutive. Detailed studies of primary bean leaves has demonstrated that chitinase is steadily accumulated during normal development, even in the absence of stress or exogenously applied ethylene. In addition, aged primary bean leaves (*ca.* 40 days old) retain their ethylene inducibility. The fact that chitinase appears to be ubiquitous in higher plants, and that it is immunologically conserved, lends support to the view that chitinase functions as a defense protein in higher plants.

Did Immuno blot.

TEMPERATURE-SENSITIVE VARIANTS IN CARROT SOMATIC EMBRYOGENESIS

Jennifer A. Schnall[1], Todd J.Cooke[1], and Dean E. Cress[2], Department of Botany, University of Maryland, College Park 20742 and Agricultural Biotechnology, Rohm and Haas Company, Spring House, PA 19477.

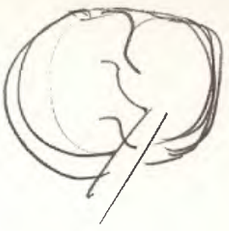
Conditional mutants are powerful tools which can be used to gain insight into the genetic regulation of complex phenomena such as plant development. Using a filtration procedure, we have isolated 21 temperature-sensitive variants of somatic embryogenesis in carrot cell culture. These variants produce normal plantlets at the permissive temperature (24°C) and the altered phenotype at the restrictive temperature (33°C). The variant phenotypes fall into six basic classes: no growth (ng-1 to ng-4), callus formation (cf-1), globular stage block (gb-1 to gb-9), oblong stage block (ob-1 to ob-3), lateral growth (lg-1 to lg-3), and root formation (rf-1). The no growth class fails to grow in embryo inductive medium (MS medium lacking 2,4-D) at 33°C, while it proliferates as callus in callus inductive medium (MS medium containing 2,4-D) at 33°C. The callus formation variant forms callus in both media at 33°C. Some of the globular stage block variants display greatly enlarged globular embryos at 33°C, while others produce average-sized globular embryos which give rise to secondary globular embryos in temperature-shift studies. The oblong stage block class forms embryos possessing axial elongation and no cotyledons. The lateral growth variants appear to represent heart and torpedo embryos from which several lateral projections arise. Finally, in the root formation variant, embryos of any stage grown at 24°C produce numerous roots when they are shifted to 33°C. The temperature-sensitive periods revealed from temperature-shift experiments performed on the variants allow us to begin to assess the timing of gene action during plant embryogensis. We hope to analyze several of these variants on the molecular level.

VIRULENCE GENES FROM PLANT PATHOGENIC FUNGI: O. C. Yoder, Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853.

The ability of some fungi to attack plants, penetrate their surfaces, and colonize their tissues is under rigorous genetic control. In an attempt to understand the biochemical nature of the plant/fungus interaction genes that are involved in fungal virulence are being defined and cloned so that their structures, mechanisms of regulation, and the metabolic functions of their products can be determined. Only a few such genes have yet been cloned. These include two genes encoding cutinase, which may be necessary for fungal penetration of the plant cuticle, a gene controlling production of pisatin demethylase (PDA), an enzyme required by one fungus to be virulent toward its host, and six genes whose products are unknown but which are expressed only during the formation of the infection structures needed for penetration of the plant epidermis. The PDA gene and the penetration genes will be discussed. PDA was isolated from the genome of the pea pathogen Nectria haematococca by transforming protoplasts of Aspergillus nidulans, which does not have the gene, with a cosmid library of DNA fragments from N. haematococca. The gene was recovered from an A. nidulans transformant that expressed it and subcloned. It can be expressed not only in A. nidulans, a nonpathogen, but in Cochliobolus heterostrophus, a pathogen of corn but not pea. The penetration genes were recovered from a lambda library of genomic DNA from the bean pathogen Uromyces appendiculatus. In this fungus, infection structures are specifically induced by physical contact with the host stomates. Labeled cDNA probes were used to identify genes in the lambda library that are expressed during differentiation of infection structures but not in undifferentiated cells. Analysis of transcript production by each of these genes indicate that some are entirely specific for the differentiated state, some are upshifted during differentiation, and at least one is expressed intermittently during differentiation.

THE PHENYLALANINE AMMONIA-LYASE SMALL MULTIGENE FAMILY: GENE STRUCTURE AND DIFFERENTIAL INDUCTION BY STRESS, Carole L. Cramer[1], Michel Dron[2], Xiaowu Liang[2], and Chris J. Lamb[2]; 1. Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; 2. Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Phenylalanine ammonia-lyase (PAL) is the first enzyme and a key regulatory step in the phenylpropanoid pathway which produces isoflavoid phytoalexins (antibiotics), anthocyanin pigments, lignins, and cinnamic acid esters (wound protectants) in higher plants. In bean (Phaseolus vulgaris), PAL is induced as part of the plant defense response during fungal infection, treatment with fungal elicitor, or wounding. In addition, PAL is developmentally regulated in response to light and hormones. We have previously described the isolation of a PAL cDNA clone using RNA from elicitor-induced bean cell cultures and have shown that PAL induction involves the rapid activation of PAL gene transcription. Genomic Southern hybridization using the PAL cDNA clone suggests that the bean genome contains four PAL genes. We are interested in a) determining the role of differential gene expression in regulating PAL induction by such a diverse array of chemical and environmental signals, and b) identifying regulatory sequences important in plant stress and disease resistance. Several PAL genes have been isolated from a bean genomic library and analyzed. One gene (PAL3) has been sequenced in its entirety, contains a single intron (~450bp), and encodes a protein of 77.5kd. Sequence comparisons with partial sequences of PAL1 and PAL2 suggest a relatively divergent family with about 55% nucleic acid homology in exon 1, 75-85% homology in exon 2, and complete divergence of sequences (and intron size) in untranslated regions. Expression of PAL isozymes and isogenes has been studied using chromatofocussing, in vitro translation and gel electrophoresis, S1-nuclease protection experiments and Northern hybridizations with gene-specific oligonucleotide probes. PAL3 is induced by wounding and infection but not by light and thus appears to be a "stress-specific" gene. In addition, a short region in the 5'-regulatory region of PAL3 shows substantial homology to an analogous sequences of a bean chalcone synthase gene coordinately expressed during the defense response.



Mutational Analysis of Potato Spindle Tuber Viroid

Rosemarie W.Hammond, Robert A. Owens and T.O.Diener

Microbiology and Plant Pathology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD. 20705

Their small size (246-375 nucleotides) and ability to replicate autonomously make viroids uniquely suitable for studies of the relationship between RNA structure and biological function. The inactivation of infectious potato spindle tuber viroid (PSTV) cDNAs by nucleotide substitutions in the terminal loops, pathogenicity domain, or central conserved region dramatically demonstrates the effect of small structural changes on viroid infectivity and emphasizes the importance of their highly base-paired, rod-like native structure for viroid function. In one case, restoration of base-pairing in the native structure by introduction of a second, compensating mutation also restored cDNA infectivity. In addition, successive mutations have been introduced into a region associated with pathogenicity to examine their effect on viroid virulence. We hope that mutational analysis of PSTV will also allow assessmant of the role of RNA recombination in viroid replication and evolution.

Central conserved region — pair to make chains of viroids — palindromic

Circular

indep repl.

STRUCTURE-FUNCTION ANALYSES USING INFECTIOUS TRANSCRIPTS FROM CLONED cDNAS OF CUCUMBER MOSAIC VIRAL SATELLITES. C.W. Collmer and J.M. Kaper. Microbiology and Plant Pathology Laboratory, Agricultural Research Service, U.S. Dept. of Agriculture, Beltsville, Maryland, U.S.A.

Complete cDNA copies of two variants of the cucumber mosaic virus satellite CARNA 5 (=CMV-Associated RNA 5) have been cloned in the transcription vector pPM1. These two naturally-occurring satellites are capable and incapable, respectively, of inducing a lethal necrotic disease of tomato upon coinoculation with the genomic RNAs 1, 2, and 3 of cucumber mosaic virus. Uncapped transcripts synthesized in vitro from the two linearized, recombinant plasmids are infectious and each induces appropriate symptoms upon coinfection with cucumber mosaic viral RNAs on tomato plants. While the infecting transcripts contain 4-5 extra nucleotides at their 3' termini, progeny CARNA 5s isolated from infected plants correspond to their natural CARNA 5 counterparts. Potential protein-coding regions within the two CARNA 5s were disrupted by site-directed mutagenesis to test their possible involvement in some stage of the satellite replication cycle and specifically, for D-CARNA 5, in tomato necrosis induction. A D-CARNA 5 mutant that lacked an initiation codon for its first open reading frame (ORF I) still induced tomato necrosis upon coinfection with CMV RNAs, disproving the involvement of an ORF I-encoded polypeptide in necrosis induction. Attempts to test the biological necessity for an internal ORF of S-CARNA 5, with mutants either lacking the AUG initiation codon or containing a premature translation termination codon, were foiled by instability and/or reversion of the progeny CARNA 5s. Results from these experiments document the great care necessary for the analysis and interpretation of in vivo tests of CARNA 5 mutants.

Linear
Req. helper virus for repl.

EVIDENCE FOR ION CHANNEL INVOLVEMENT IN HYPERSENSITIVITY OF TOBACCO TO PATHOGENIC PSEUDOMONADS.

Merelee M. Atkinson, L.D. Keppler and C.J. Baker, USDA Microbiology and Plant Pathology Lab, Bldg 010, HH 5, Beltsville, Maryland 20705.

Disease resistance in higher plants is closely associated with hypersensitivity of host tissue to the invading pathogen. Hypersensitivity is characterized by rapid host cell death and localization of the infection. Understanding the molecular mechanism for hypersensitivity will thus provide critical information about the molecular basis for disease resistance and plant-microbe recognition.

The hypersensitive response of tobacco to Pseudomonas syringae pv. syringae proceeds through the activation of an electroneutral K^+/H^+ exchange across the host plasma membrane. The exchange leads to a severe loss of K^+, a disruption of pH regulation and the destruction of the plasma membrane H^+ gradient in host cells. These consequences lead or contribute to host cell death.

The rapidity and specificity of K^+/H^+ exchange suggest that it is mediated by an ion channel. Exchange activity has been demonstrated in purified tobacco plasma membrane vesicles. This activity is destroyed by N,N'-dicyclohexylcarbodiimide (DCCD), a covalent protein modifier which blocks most known H^+ channels. These results provide evidence that the exchange response is mediated by an ion channel in the plasma membrane. Current research is focused on the use of ^{14}C-DCCD to identify the putative ion channel.

binds covalently to hydrophobic protein, binds to carboxyl (interferes w/ Antiporter)

Cation/proton Antiport

K^+/H^+ - plants, animal, E.coli

Na^+/H^+ - animal / Ecoli

Elicidor → older transport protein / damage exchange

Nigricin —

Not working w/ pure elicitor. Using whole bact. cell ext. Strain doesn't produce Syringomycin.

MOLECULAR CLONING AND CHARACTERIZATION OF A PSEUDOMONAS SYRINGAE PV. SYRINGAE GENE CLUSTER THAT ENABLES SAPROPHYTIC BACTERIA TO ELICIT THE HYPERSENSITIVE RESPONSE IN TOBACCO. H.-C. Huang, I. Yucel, S. W. Hutcheson, and A. Collmer, Department of Botany, University of Maryland, College Park, MD 20742.

The cosmid pHIR11 contains 31 kb of Pseudomonas syringae pv. syringae 61 DNA and complements Tn5 mutations in this bacterium affecting the elicitation of the hypersensitive response (HR) in tobacco leaves and the exchange response (XR; K^+ efflux/H^+ influx) in suspension-cultured tobacco cells. Transconjugants of the compatible (wild fire disease-causing) strain P. syringae pv. tabaci and the saprophytes P. fluorescens, P. putida, and Escherichia coli containing this clone elicited typical HR symptoms in tobacco leaves. The first two transconjugants also elicited a strong XR in suspension-cultured tobacco cells. These results indicate that pHIR11 contains a functional cluster of plant reaction genes that are expressed in a variety of bacteria. We have mutagenized pHIR11 with TnphoA, a Tn5 derivative that generates gene fusions producing hybrid proteins composed of alkaline phosphatase (which is only enzymatically active if exported out of the bacterial cytoplasm) fused to the amino-terminal sequence of a target protein. Analysis of the HR, XR, and alkaline phosphatase phenotypes of these cosmid mutants in P. fluorescens and P. syringae pv. tabaci should provide insights into the functional loci within this gene cluster and the possible role of exported proteins in elicitation of plant responses.

Some homology w/ P. tabaci
P. syringae
not P. fluorescens

pHIR11 - 31 kb of P.s.syringae 61 chromosome

Genes 12 for H.R. response

(but just a little & 1g piece of DNA)

CHLOROPLAST RIBOSOMAL PROTEIN GENES: SOME UNUSUAL FEATURES.

Marvin Edelman
Department of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel.

Anatomy of the rps12 gene (Hillel Fromm, Barbara Koller, Esra Galun). The rps12 gene codes for chloroplast ribosomal protein S12. In the tobacco chloroplast genome, exons II and III of this gene are separated by an intron of 536 bases and are present in two copies in the inverted repeat region, while exon I is located in the large single copy region at a distance of 90kb and 126kb from the two copies of exons II and III. Hybrids between cloned rps12 DNA fragments and chloroplast RNA were analyzed by electron microscopy. The exon I region and the exons II,III region of the gene are separately transcribed as parts of two different polycistronic pre-mRNAs. However, in the most abundant transcripts exon I is covalently linked to exon II. These data indicate that maturation of rps12 pre-mRNAs in chloroplasts of tobacco involves trans splicing.

Frame keeping during translation (Pierre Goloubinoff, Edward Trifonov). Along with the basic information for amino acid coding, the great majority of genes, including chloroplast ones, carry an additional 3-base periodic pattern: viz., a high ratio of guanine residues in the first, as opposed to the second, position of their triplet codons. A complementary, 3-base periodicity of cytosine has been found in universally conserved regions of 16-18S rRNAs. These regions are in contact with mRNA during translation and are thought to participate in frame keeping operations during protein synthesis. Analysis of the Nicotiana chloroplast genome revealed that the ribosomal protein genes, as a class, are uniquely guanine-aperiodic. Thus, this class of chloroplast genes indicates the existence of an additional frame keeping mechanism for peptide elongation.

=GI/GII RATIO

Trifonov JMB 194: 643 1987

GI/GII = 1.6 – 2.6 for various organisms

non-random, universal

TRANSLOCATION OF PROTEINS INTO THE CHLOROPLAST

Neil E. Hoffman, Mark J. Hand, and Anthony R. Cashmore, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Most chloroplast proteins are synthesized in the cytoplasm as larger molecular weight precursor molecules containing an N terminal extension called the transit peptide which is cleaved off during the translocation process. Import can be studied *in vitro* using isolated chloroplasts and radiolabelled precursor polypeptides synthesized by *in vitro* transcription and translation of individual nuclear genes or chimaeric genes constructed using recombinant DNA technology. Using this approach it has been demonstrated that the transit peptide is necessary and sufficient to direct a polypeptide from the cytoplasm to the chloroplast. We are interested in defining polypeptide domains which specify intrachloroplast sorting. To address this question we have isolated nuclear genes encoding chloroplast proteins compartmentalized within the stroma (small subunit of RUBISCO), granal lamellae (Photosystem (PS) II chlorophyll a/b binding protein (CAB), and stromal lamellae (PS I CAB, PS I core complex II protein). Our approach is to switch domains of the various proteins and to determine where the chimaeric precursor protein assembles, i.e. in the stroma or in PS I or PS II within the thylakoid membrane. The PS I and PS II CAB polypeptides are attractive for this study because they contain two large stretches of amino acid homology at the N terminal and C terminal ends in addition to divergent sequences in the transit peptide and center of the protein. A structural analysis of PS I and PS II CAB polypeptides emphasizing sequences possibly involved in sorting will be presented as well as preliminary results on defining sequences which mediate intrachloroplast sorting.

ABSTRACT

Chloroplast differentiation and the control of nuclear gene expression

Pablo A. Scolnik, Giovanni Giuliano* and Glenn Bartley

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About 70% of the plastid proteins are coded for by nuclear genes and a different set of polypeptides are synthesized according to whether the cells contain chloroplasts, chromoplasts, amyloplasts or etioplasts. Thus, a mechanism must exist that coordinates plastid development with the expression of nuclear genes that code for plastid proteins. Carotenoids are chloroplast pigments that protect photosynthetic membranes against photooxidation. In the absence of these pigments, chloroplast development is arrested at an early stage. Therefore, plant carotenoid mutants can be used to study the effects of chloroplast differentiation on nuclear gene expression. Using the tomato *ghost* mutant, which is blocked in carotenoid biosynthesis, we have drawn the following conclusions: 1) the *ghost* mutation blocks carotenoid biosynthesis at the level of dehydrogenation of phytoene; 2) the mutation is somatically unstable, resulting in the formation of both white and green leaves; 3) chloroplast differentiation is blocked in white leaves but proceeds normally in green leaves, 4) tissue culture can be used to propagate plants that develop to maturity supported by the photosynthetic green sectors; 5) steady state mRNA levels for *SSU* and *LHCP*, two families of nuclear genes that code for chloroplast proteins, are sharply decreased in white leaves but reach wild-type levels in green leaves; 6) *SSU* and *LHCP* mRNA levels in white leaves, although very low, are still regulated by light, implying that light and chloroplast differentiation control nuclear gene expression through different pathways; 7) experiments with isolated nuclei indicate that the regulatory effects observed take place at the transcriptional level. These results will be discussed in the context of our current knowledge of the regulatory interactions between chloroplasts and nuclei in plants.

Ultimately, an understanding of the influence of carotenoids on chloroplast differentiation and nuclear gene expression will require the cloning of genes for the enzymes of the biosynthetic pathway. This task is considerably complicated by the fact that enzymes for carotenoid biosynthesis are membrane-bound and become rapidly inactivated when extracted. Thus, we decided to use photosynthetic bacteria in a "reverse genetics" approach to obtain basic biochemical information about these enzymes. The organism of choice is the photosynthetic bacterium *Rhodobacter capsulatus*. We have now mapped all the genes for carotenoid biosynthesis in this organism and characterized the enzyme phytoene dehydrogenase.

1987 Mid-Atlantic Plant Molecular Biology Meeting

RAPID LIGHT REGULATION OF CHLOROPLAST TRANSCRIPT LEVELS IN CHLAMYDOMONAS REINHARTII.

G. W. Silk and Madeline C. Wu, Department of Biological Sciences, University of Maryland, Baltimore County, Catonsville, Maryland.

We have determined that the steady state levels of a chloroplast transcript in the single-celled alga C. reinhartii cc-125+ is regulated by light. An EcoRI fragment, designated RO3 in the nomenclature of Rochaix (1978), contains the 3' end of this light-regulated transcript as well as the 5' end of a smaller transcript. The large transcript accumulates when the algae are kept in the dark for several hours before harvest, while the concentration of the smaller transcript is unchanged. The concentration of the larger transcript is increased at least three-fold by placing the algae in darkness for several hours before harvest. A light-induced decrease in the concentration of the larger transcript is observed in as little as three hours. These effects are observed in phototrophic synchronous cultures at different points in the life cycle. The dark-specific accumulation of the larger transcript was also observed in log-phase auxotrophic cells. These findings indicate that the life cycle does not regulate the accumulation of the larger transcript.

CHARACTERIZATION OF NUCLEAR FACTORS THAT REGULATE THE EXPRESSION OF Alcohol dehydrogenase GENES: Robert J. Ferl, Anna-Lisa Paul and Beth Laughner. Department of Botany, University of Florida, Gainesville FL 32611.

The regulated association of the transcription complex with the promoter DNA is the fundamental basis for gene expression. Transcription initiation requires the binding of RNA polymerase, and that binding is facillitated by the prior or concommitant binding of other transcription factors. In several gene systems, the proteins associated with the regulatory process are being characterized. One of the prominent examples is the Drosophila heat shock system, where the binding behavior and binding sites of the regulatory proteins have been elucidated.

Our laboratory has recently focussed on identifying regions of the 5' flanking DNA of the maize Adh1 gene that interact with such regulatory components. The anaerobically inducible DNase I hypersensitive site from position -40 to -100 and the constitutively exposed region with several DNase I hypersensitive sites from position -150 to -400 drew attention to the region just 5' to the gene.

This region of DNA was investigated in vivo by using genomic sequencing coupled with dimethyl sulfate footprinting. DMS serves as a chemical probe for detecting the binding sites of regulatory proteins, since the interaction of proteins with specific sequences can alter the reactivity of a given guanine residue, either by enhancing or suppressing the DMS modification reaction. Points of close in vivo contact between the DNA and regulatory proteins appear as weak (protected) bands or strong (enhanced) sequence bands as compared to the corresponding bands in naked DNA control lanes.

In aerobic cells, factors were detected bound to sequence elements labelled Region B (located from position -117 to -145). This conclusion is further supported by the relative resistance to DNase I digestion in nuclei demonstrated by this region. The footprints in the Region B are clearly separated into distinct subregions B1 and B2, suggesting that B1 and B2 are the binding sites for two separate proteins. Upon anaerobic induction, the footprint of the factor at B1 persists. However, the footprint at B2 specifically changes at the G residue on the bottom strand at position -141. In vivo this change could be the result of an alteration in protein conformation.

The transcriptional activation of the the Adh1 gene is further characterized by the recruitment of distinct factors which bind to regions A (from position -100 to -108) and C (from position -186 to -190).

The actual data on the binding sites of regulatory proteins to the Adh1 promoter provide the basis for a model for the regulation of the Adh gene by the action of transacting factors. The model will be presented and compared to similar models that we have derived for the maize Adh2 and Arabidopsis Adh genes.

PHYTOCHROME AND CRYPTOCHROME REGULATION OF NUCLEAR GENES IN PEAS: Lon S. Kaufman, Kathy A. Marrs, John C. Watson and William F. Thompson. Dept. Biolical Sciences, Univ. of Illinois at Chicago, Dept. of Botany, University of Maryland, Dept. of Botany, North Carolina State University.

The process of leaf development is light regulated in many plants including peas. Phytochrome, a red and blue light receptor and cryptochrome, a blue light receptor are responsible for the major portion of the light induced events in leaf development. Many of the events occurring during light induced leaf development require the activation of nuclear genes. In order to better understand the role of light, photoreceptors and gene expression during leaf development we have been studying the photoregulation of twelve nuclear encoded transcripts.

All of theses transcripts show altered steady state levels as a result of light induced leaf development; and therefore either increase or decrease during the developmental process. These transcripts include those coding for the LHCP, the small subunit of RUBISCO, plastocyanin and several unidentified polypeptides. The parameters describing the phytochrome regulation these transcripts have been described previously.

We are currently examining the role of cryptochrome in the regulation of these same transcripts. To test for cryptochrome regulation plants are grown for seven days in continuous red light. Growth in red light serves to fully saturate any phytochrome response. This is necessary as phytochrome is capable of responding to blue light. The blue light treatment consists of a single pulse of light given six days after planting. Under these growth conditions only five of the transcript show a definitive response to cryptochrome.

Transcripts showing a blue light response include those coding for the LHCP and plastocyanin. The former and one unidentified transcript (pEA 215) increase as a result of the blue light treatment, while plastocyanin and one unidentified transcript (pEA 25) decrease as a result of the blue light pulse. In all four cases the final level, that is continuous red plus the blue light pulse is approximately that achieved in continuous white light.

The final transcript (pEA 207) is a dark abundant transcript. It shows no response to the continuous red light. The level of pEA 207 in continuous white light is approximately 5-fold less than the dark level. This same decrease in a bundance is achieved in response to the blue light treatment.

Currently we are attempting to determine if these changes in steady state levels are refelctive of changes in the rate of transcription as measured in isolated nuclei.

LIGHT REGULATION OF NUCLEAR RIBOSOMAL RNA GENE EXPRESSION IN PEAS: John C. Watson*, Lon S. Kaufman†, and William F. Thompson‡. *Dept. of Botany, Univ. of Maryland, College Park, MD 20742; †Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, IL 60680; ‡Depts. of Botany and Genetics, North Carolina State Univ., Raleigh, NC 27695.

Prominent features of the cytosine methylation and DNase I hypersensitivity patterns of the *Pisum sativum* cv. Alaska nuclear rRNA genes have been determined. The extent to which particular features of these patterns change during light-regulated seedling development have also been determined. Cytosine methylation within the CCGG sequence was studied using the restriction enzymes *Hpa*II and *Msp*I and gel blot hybridizations of the restriction digests with DNA probes derived from different portions of a cloned rDNA repeat. With an indirect end-labelling technique, a map of 23 cleavable *Hpa*II and/or *Msp*I sites in genomic rDNA was constructed that covers ~90% of the rDNA repeat including the entire non- transcribed spacer (NTS) region and most of the rRNA coding sequences. One notable feature of the map is that the most prominent *Hpa*II site, located about 800 base pairs upstream from the 5'-end of the mature 18S rRNA, is cleaved only in one of the two most abundant rDNA length variants (the short variant). With a gel blot assay specific for cleavage at this site, we estimated the *Hpa*II sensitivity of DNA preparations from several stages of pea seedling development. While methylation is generally low in young seedlings, DNA obtained from the apical buds of seedlings is highly methylated. Further, the methylation level of rDNA within the pea bud decreases as the buds are allowed to develop under continuous white light.

To examine the rDNA chromatin of pea plants grown with or without exposure to light for the presence of DNase I hypersensitive sites and possible developmental changes in their distribution, isolated nuclei from pea seedlings were incubated with various concentrations of DNase I. To visualize the hypersensitive sites, DNA purified from these nuclei was restricted and analyzed by gel blot hybridization. We find that several sites exist in both the coding and noncoding regions of rDNA repeating units. Several of the sites in the NTS region are present in the light but are absent in the dark. Conversely, the hypersensitive sites within the mature rRNA coding regions are present in the dark but absent in the light. Of the two major rDNA length variants, the sites in the NTS region that appear during the light treatment occur only in the short variant.

A close spatial correspondence exists between the DNase I hypersensitive sites and HpaII/MspI sites we have mapped within the NTS region. Moreover, the light-regulated changes in methylation status and DNase I hypersensivity within the NTS occur primarily in the short length variant. These data lead us to believe that the short and long rDNA length variants are differentially regulated and that the sites detected within the NTS represent regions of functional importance for the regulated expression of pea nuclear rRNA genes.

HISTONES OF ARABIDOPSIS THALIANA: Charles P. Moehs, Steven L. Spiker. Department of Genetics, 3513 Gardner Hall, North Carolina State University, Raleigh, North Carolina 27695.

" MAX MAYS "

Higher plants whose histones have been well characterized have all been found to contain several molecular weight variants of H2A and H2B. Because these plants all have large genomes, I decided to ask the question whether a plant with an extremly small genome would also contain such variants. To answer this question, I isolated the histones of Arabidopsis thaliana, a small crucifer one of whose advantages for molecular studies is its small genome. I have characterized the histones of Arabidopsis on a number of different gel systems and have found that it contains several molecular weight variants of both H2A and H2B.

GENETIC BASIS OF MAIZE B-GLUCOSIDASE MULTIPLICITY : M. M. Rifaat and A. Esen. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

B-glucosidase, B-D-glucoside glucohydrolase (EC 3.2.1.21), is an enzyme implicated in the hydrolysis of cellobiose as well as aryl and alkyl B-D-glucosides. In maize, the enzyme is a dimer (pI 5) and encoded by Glu1 locus (Chromosome 10). Another molecular form of the enzyme (pI 8) has been identified in plants homozygous for Glu1 null or active alleles and appears to be encoded by a different maize B-glucosidase locus (Glu3). Three intergenic enzyme molecular forms (isozymes), resulting from the association of Glu1 and Glu3 encoded polypeptides (hybrid enzymes), have been identified in a Glu1 homozygote, suggesting that the Glu3 encoded enzyme is functionally a tetramer. Glu1 heterozygotes exhibit reduced levels of Glu3 expression.

Degradation of Zeins in Germinating Corn Kernels: K.B. Mohammad and A. Esen, Dept. of Biology, Virginia Tech, Blacksburg, Va 24061.

The sequence and mode of zein degradation was investigated in corn kernels during germination. The sequence of degradation of various zeins were as follows based on immunological and electrophoretic data. Gamma-zein (27 kD) was the first zein to be degraded and its degradation was complete by the second day of germination. Beta-zeins (17 and 18 kD) began to be degraded on the second day after germination (DAG), degradation being complete by the seventh day in the case of the 17 kD polypeptide and the fourth day in the case of the 18 kD polypeptide. The 10 kD zein began to be degraded on the fourth DAG and degradation was complete by the eighth DAG. The predominant zein fraction alpha-zein (22 and 24 kD) was degraded gradually beginning on the second DAG and continuing until after the tenth DAG. Products of zein degradation exhibit solubility properties different from that of the intact protein in that they are soluble in aqueous solvents. Preliminary studies performed on de-embryonated kernels (endosperms) indicated that zein proteolysis occurred in such kernels and was enhanced when gibberellic acid was added to the medium. These results suggested that zein degradation was closely linked to starch hydrolysis during germination. The question of whether proteolytic enzymes are present as zymogens in protein bodies or synthesized de novo during seed germination is under investigation.

Viroids and Introns: Probing the Relationship

Gail Dinter-Gottlieb, Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104

While the viroids remain the smallest pathogens known, their relationships to other small RNAs has recently become apparent. The potato spindle tuber viroid (PSTV) shows a 20% sequence homology with the self-splicing Tetrahymena intron (T-IVS), while it is only 11% homologous with coconut cadang-cadang viroid (CCCV). These homologies with the T-IVS extend over the conserved central core of the PSTV group of viroids and the pathogenicity-modulating region of PSTV, and involve the hallmark Group I intron consensus, as well as the conserved "box" regions essential for the self-splicing reactions (1).

PSTV is incapable of self-splicing in vitro, which should not be unexpected, since its rod shape in the absence of proteins has been well-established. On the other hand, deletions of the T-IVS which eliminate the region analogous to the viroid central core and one PM regions do not interfere with self-splicing, presumably because these regions have lost their functions in the intron.

Recently, the delta hepatitis agent was sequenced, and found to contain regions of homology with viroids (2). Further analysis has revealed homologies with the Group I introns as well.

Since RNA molecules are capable of enzyme activities, acting as RNA polymerases, restriction endonucleases and phosphatases (3), and so may have pre-existed DNA molecules. The viroids and the delta agent may be remnants of these early RNA molecules, while introns represent forms which have integrated into cellular genomes.

IDENTIFICATION AND CHARACTERIZATION OF ACTIVE TRANSPOSABLE ELEMENT SYSTEMS IN SOYBEAN- Joel M. Chandlee and Lila O. Vodkin, USDA-PGGI -PMGL, Beltsville, MD 20705 and University of Illinois, Department of Agronomy, Urbana, IL 61801.

This laboratory has been interested in characterizing active transposable element systems of soybean since the identification of an insertion element (Tgm1) in the lectin gene (Le1) of lectin-negative soybean cultivars. Tgm1 exhibits the structural features of known transposable elements and appears to be related to the En/Spm elements of corn and the Tam1 and Tam3 elements of snapdragon. Our goals are to: 1) identify and characterize unstable alleles producing variegated or mottled phenotypes which suggest the involvement of transposable elements; and 2) try and associate the instability with genomic rearrangements of Tgm1-related sequences.

We have accumulated genetic data which implicates transposable element activity in some unstable phenotypes:

1) a mutable allele (r^m) has been identified at the R locus of soybean (responsible for conditioning seed coat color). The allele exhibits both somatic and germinal instability in its expression such that black seeded or brown seeded lines can be derived from our original stock of mottled seeds. This occurs at a relatively high frequency and these lines generally breed true for their respective seed coat color.
2) a spontaneous mutation recovered from a Tgm1 containing line produced a branch having leaf color variegation of green and yellow sectors. Seed derived from this branch segregated 3:1 for green:yellow plants indicating that the germ line was affected by the mutation. No additional somatic sectoring has been observed. This mutation is currently being analysed with regard to transposition of Tgm1 away from the lectin gene.
3) a mutation has been induced by exposure of some seed to a gamma radiation source. This mutant exhibits green and yellow leaf sectors. The somatic variegation is heritable, however no stable yellow derivatives have yet been recovered.
4) F_2, F_3 and F_4 populations from a variety of crosses involving "mutable" lines have been produced and screened for obvious variegated phenotypes. Several plants have been recovered which exhibit newly arisen variegation of flower color, seed coat color and leaf color.

Southern blot analysis of genomic DNAs using a Tgm1-specific probe is being conducted on lines harboring the various "mutable" alleles in order to determine whether there is an association between the instability in the expression of the alleles and genomic rearrangements of Tgm1-related sequences. Results indicate that for several of these alleles, this indeed appears to be the case.

TRANSCRIPTION OF ACETOLACTATE SYNTHASE GENES IN HIGHER PLANTS

Sharon J. Martin & Julie K. Smith, E. I. du Pont de Nemours & Co., Experimental Station, Wilmington, Delaware 19898.

GENE EXPRESSION/
STRUCTURE
ENZYME REGULATION

Acetolactate synthase (ALS) is the first common enzyme in the biosynthetic pathway leading to the essential amino acids leucine, isoleucine, and valine. It is the target enzyme for two classes of structurally unrelated herbicides, the sulfonlyureas and the imidazolinones. Genomic clones encoding ALS have been isolated from the small crucifer Arabidopsis thaliana and from Nicotiana tabacum (tobacco). In the diploid Arabidopsis there is a single ALS gene, while Nicotiana tabacum, an allotetraploid, expresses two unlinked ALS genes, one from each ancestral parent. We have studied the transcriptional regulation of ALS in each of these two model systems. We describe the identification and characterization of ALS transcripts, and report on the tissue specificity and temporal regulation of these genes.

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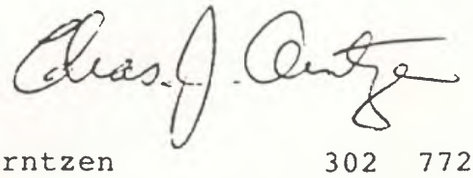

Charles J. Arntzen 302 772-1103

Sharon J. Martin

CONSTRUCTION AND USE OF LAMBDA gt10 LIBRARIES TO ISOLATE GENES EXPRESSED IN CALLI AND SOMATIC EMBRYOS OF CARROT. Martin Stranathan and J.Lynn Zimmerman. University of Maryland Baltimore County Catonsville Maryland.

The development of higher organisms is dictated , at least in part, by the temporal and spatial expression of genes. In an attempt to study the differential gene expression necessary for early embryogenesis of higher plants, we have undertaken an analysis of genes expressed during the in vitro development of undifferentiated callus cells to highly organized somatic embryos. The morphological changes occuring during carrot somatic embryogenesis mimic those observed during in vivo, zygotic embryogenesis. All stages of embryogenesis (globular, heart, torpedo and plantlet) are found in induced tissue cultures. As a first step towards isolating differentially expressed genes (particularly those induced early in embryo development) a cDNA library was constructed in lambda gt10 using polyA mRNA isolated from preglobular stage embryos. This library has been differentially screened using 0 hour and 96 hour cDNA probes. Preliminary results reveal transcripts that both disappear and appear within the first 96 hours of embryogenesis. Further analysis is in progress. A second cDNA library has been constucted from callus suspension cells (non-embryogenic conditions) and screened using pSAc3, a soybean actin gene. The putative actin clones are being further characterized and their expression during development analyzed.

Use of Biotin Labelled Proteins to Assess Zein Protein Interactions. David L. Johnson and Karl Pedersen. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061.

Zeins, the heterogenous alcohol soluble storage proteins of maize, are deposited into the endoplasmic reticulum (ER) of developing seeds and are packaged into membrane surrounded vesicles termed protein bodies. A structural model has been proposed for two major size classes having apparent molecular weights of 22,000 and 19,000. Little is known, however, about how these and other minor zein size classes are packaged into protein bodies, that is, whether the proteins are arranged randomly or in a specific dictated by weak interactions among these predominantly hydrophobic proteins.

We are currently investigating the use of biotin labelling as a means of assessing protein - protein interactions. Zeins can be biotin labelled directly presumably through their amino termini, or by using zeins whose tyrosines have been first nitrated with the reagent tetranitromethane followed by reduction of the nitro groups to amino groups. These proteins can be used as probes much as antibodies are used in Western blots. Single species or small sets of zeins bound to nitrocellulose have been used to select out total biotin labelled zeins based upon specific protein - protein interactions. We are presently attempting to determine whether these interactions are random or patterned.

THE ANALYSIS OF THE HEAT SHOCK RESPONSE IN CULTURED CARROT CELLS AND SOMATIC EMBRYOS Cheol Ho Hwang and J. Lynn Zimmerman, Dept. of Biol. Sci., UMBC, Catonsville, Md. 21228

All organisms studied thus far exhibit a characteristic molecular response to a variety of environmental stresses including heat exposure. This "heat shock response" is characterized by the production of a new set of mRNAs and proteins, and is believed to be essential for survival of the stress. In addition, there is good evidence that in several systems, subsets of the heat shock genes are expressed during normal development without heat shock. We have begun to characterize the heat shock response in cultured carrot cells both as a function of heat treatment and as a function of the in vitro development of carrot callus suspension cells into somatic embryos.

SDS-PAGE analysis of heat shock protein accumulation as a function of time and temperature of heat shock shows maximal accumulation of these proteins at 38°C and by 1.5 hr after heat shock. Two dimensional protein gel analysis of heat shock proteins induced at 3 different developmental stages (undifferentiated callus, globular embryo, and heart/torpedo embryo mixture) show some significant changes during somatic embryogenesis. In addition, different protein patterns have been observed when comparing two different cell lines. The significant changes occur primarily in low molecular weight heat shock proteins.

CARROT HEAT-SHOCK GENE EXPRESSION DURING SOMATIC EMBRYOGENESIS
Kamel El-Darwish, Denise C. O'Donnell and J. Lynn Zimmerman, Department of Biological Sciences, University of Maryland, Baltimore County, Catonsville, Md., 21228

We have isolated two carrot genomic clones (in Charon 35) containing sequences hybridizing to transcripts present at high levels in heat-shocked (39°C) carrot callus suspension cells, but undetectable in cultures incubated at control (25°C) temperature. These clones were isolated from a genomic library of total carrot DNA by virtue of homology with a soybean HS-cDNA (provided by F. Schoffl) which hybrid-selects mRNA encoding small (17-18 kDal.) heat-shock proteins. The two cloned carrot DNA regions have different restriction endocnuclease maps, but exhibit significant cross-hybridization under high stringency conditions. Hybridization of the cloned carrot DNAs to total carrot DNA (Southern blot) indicates the presence of a family of related sequences in the carrot genome. Preliminary RNA (Northern) analysis indicates transcripts are about 900 nucleotides long, and heatinducible accumulation of transcripts is significantly (approx. 4-fold) reduced at globular and mixed embryo (heart and torpedo) stages of somatic embryogenesis. These cloned DNAs will be used to generate probe(s) for in situ hybridization to sections of somatic embryos to gain insight to the physiological basis for the apparent reduction in heat-inducible level of transcript(s).

Promoter-Cloning Plasmid Shuttle Vectors for the Cyanobacterium, Synechococcus PCC 7942

Sheldon E. Broedel, Jr.[1&2] and Richard E. Wolf, Jr.[2] Bioscience Department, Martin Marietta Laboratories, 1450 S. Rolling Rd., Baltimore, MD 21227[1] and Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228

Synechococcus PCC 7942 (Anacystis nidulans R2) is an obligate photoautotrophic unicellular cyanobacterium and is a model system for the study of gene expression in cyanobacteria. We have constructed two promoter-cloning plasmid vectors for Synechococcus PCC 7942 that will aid in the identification of transcriptional regulatory sequences. The plasmids replicate in Synechococcus PCC 7942 and E. coli, contain an ampicillin-resistant determinant, and a promoterless reporter gene. One vector, pAN74, is derived from pBR322 and the Synechococcus PCC 7942 plasmid, pUH24. It contains the promoterless nptII gene of the E. coli transposon, Tn5, and transformants with promoters cloned into pAN74 may be selected as kanamycin-resistant (Km^r). The second vector, pAN85, is derived from pUC19 and pUH24. It contains the promoterless luxA and luxB genes of Vibrio fischerii, and transformants with promoters cloned into pAN85 may be screened by bioluminescence. The promoterless reporter genes in both plasmids were arranged such that DNA restriction fragments can be ligated 5' to the translation start site of the reporter gene. Translation terminator codons were situated between the cloned fragment and the translation start site of the reporter genes to prevent translational read-through originating form the cloned DNA. Promoters from E. coli (Ptac17) and Synechococcus PCC 7942 (unidentified, random restriction fragments) were cloned into pAN74 by selection for Km^r Synechococcus PCC 7942 transformants. The promoter-containing restriction fragments were then recloned into the lux vector and Synechococcus PCC 7942 transformants were screened for the production of bioluminescence. These promoter-cloning vectors may be powerful tools for identifying and analyzing transcriptional regulatory signals in Synechococcus PCC 7942.

CIS-ACTING ELEMENTS REGULATING ROOT-SPECIFIC GENE EXPRESSEION IN TOBACCO: Yuri T. Yamamoto and Mark A. Conkling, Department of Genetics, North Carolina State University, Raleigh, North Carolina.

We are interested in the mechanisms regulating organ-specific gene expression in plants. Four tobacco root-specific cDNA clones were cloned by differential hybridization of a root cDNA library with root and leaf cDNA probes. The corresponding genomic clones were isolated. With these clones we are characterizing cis-acting elements which may regulate root-specific gene expression.

We have restriction mapped these genomic clones, determined restriction fragments on which the root-specific cDNAs hybridize and subcloned them into a plasmid vector. One of cDNA clones, B7, has three different corresponding genomic clones. Subclones containing these three sequences were further restriction mapped. DNA-DNA heteroduplexes of two of these genomic clones were also examined under transmission electron microscope. Data from these experiments show that two of these genomic clones are overlapping.

The cDNA B7 is a partial clone. Hybridization to flanking restriction fragments of the corresponding genomic sequence allowed the inference of the orientation and putatively identified the restriction fragment containing the site of transcription initiation. We are now confirming and extending this by S1 nuclease mapping and primer extension experiments. The site of initiation and flanking region will be sequenced. DNA-RNA heteroduplexes will be examined to determine the structure of the gene.

A series of deletions of the 5' flanking region of the gene will be constructed. Each mutant will be fused to a structural portion of a reporter gene, chloramphenicol acetyl transferase (CAT), such that its expression will be regulated by any cis-acting elements. The translational fusion, binary Agrobacterium vector system of G. An will be used, thereby allowing these constructions to be directly transformed into tobacco. The organ-specific expression of the CAT gene will be determined in the regenerated plants. Those constructions exhibiting root-specific expression of the CAT gene must contain the cis-acting elements regulating that expression and will be further analysed by site-specific mutagenesis.

EFFECT OF SPECTRAL LIGHT QUALITY ON TURNOVER OF THE 32 kDa PSII REACTION CENTER PROTEIN

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Turnover of the *Spirodela oligorrhiza* 32 kDa protein was measured in 350, 447, 560, 660, 716, 723, 740nm light and in darkness. The protein was synthesized and degraded under all conditions except 740nm light and darkness. DCMU inhibited degradation at all wavelengths where turnover was seen. The relative rates of degradation of the 32 kDa protein (at equal light quanta) are 350>447=660>560>710>723.

Intermittent light-grown *Spirodela* plants (2min light, 2h darkness) contain less than 10% of the chlorophyll found in continuous light-grown plants. When the rate of 32 kDa protein degradation in these plants was measured in white light, it was comparable to that of continuous light-grown plants. In 350nm light the rate of degradation of the 32 kDa protein appears faster in the intermittent light-grown plants than it is in the continuous light-grown plants.

Degradation of the 32 kDa protein is postulated to be mediated by a component(s) of the PSII reaction center which can be activated at all the wavelengths involved. Two major candidates present themselves: chlorophyll and plastoquinone. The experiments with intermittent light-grown plants suggest that the size of the chlorophyll antennae is not important for turnover. However, they do not exclude activation of the 'special pair' chlorophyll molecule bound to the 32 kDa and D_2 proteins in the reaction center. On the other hand, the apparent increased rate of 32 kDa protein degradation observed at 350 nm in the intermittent vs. normally grown plants could indicate an unmasking of the plastosemiquinone anion (which has an absorbance maximum at 300-350nm) due to the lower chlorophyll content. Since the plastosemiquinone anion is normally formed during PSII photoreactions, and is apparently also formed during cyclic PSI electron flow (Gaba et al., [1987] Plant Physiol., in press), this molecule may meet the criteria necessary to mediate 32 kDa protein degradation. Discussion will center around these possibilities.

PROGRESS TOWARD cDNA CLONING OF THE 72 AND 58 KD SUBUNITS OF THE TOMATO TONOPLAST H^+ ATPase.

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Antibodies raised against red beet tonoplast H^+-ATPase subunits were shown to cross-react with the appropriate tonoplast H^+-ATPase subunit from tomato. A red tomato fruit cDNA library constructed in the expression vector Charon 16 was screened using these antibodies. Two presumptive cDNA clones corresponding to the 72 kd subunit and three clones for the 58 kd subunit were initially isolated. All putative clones within each subunit set were found to cross-hybridize by Southern analysis. Presumptive clones were used to plaque-purify antibody capable of recognizing the appropriate H^+-ATPase subunit on western blots of 1 and 2 D gels of tonoplast membrane. Isolation of full length cDNA clones for each subunit is in progress.

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VARIATION IN TRICHODERMA MITOCHONDRIAL DNAS

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Trichoderma species have been used for the biological control of various soilborne pathogens. Development of improved strains is hampered by the difficulty in identifying the species of this genus. Identification is complicated by the fact that each species of Trichoderma is actually an aggregate of species. For example, seven different species of Hypocrea (the sexual state of Trichoderma) have been reported to produce an asexual state of the Trichoderma viride form. This implies that T. viride may actually be at least seven different species. No morphological characters have been identified that can separate a Trichoderma species aggregate into groups that correspond to the Hypocrea species. To solve these problems, I am using DNA restriction fragment length polymorphisms (RFLPs) in an attempt to resolve the natural groups within the species aggregates. Initial studies have focused on T. viride and variation in its mitochondrial DNA (mt DNA). Four mt DNA groups within T. viride have been identified to date, which indicates that mt DNA RFLPs should be useful in subdividing the present Trichoderma species. The mt DNAs from the four groups range from 28 to 40 kb in size. Plasmid-like DNAs are present in each of the mt DNA preparations. In some strains the plasmid-like DNAs produce a ladder-like pattern when unrestricted mt DNA preparations are run on an agarose gel. The molecular weights of the bands excludes the possibility that the ladder represents a series of concatamers.

PvuII { Tr-2, Tr-6, Tr-8 unique pattern
HindIII { Tr-3, Tr-7, Tr-13 same pattern

EcoRI { Gli-1, Tr-2, Tr-6, Tr-8 unique
{ Tr-3, -7, -13 same

Tr: all have plasmid, but not Gl 21
2-4 kb (2, 6, 8 Unique)
(3, 7-13 Same)

8, 3, 7, 13 - have ladder

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IN VITRO IMPORT OF A CHLOROPHYLL a/b (CAB) POLYPEPTIDE INTO CHLOROPLASTS OF PISUM SATIVUM

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A nuclear gene (Cashmore, AR PNAS 81:2960:1984) encoding a chlorophyll a/b (CAB) polypeptide from Photosystem II was subcloned into the in vitrc transcription plasmid, SP6. In order to analyze the chloroplast import pathway of this precursor polypeptide, a series of transit peptide/mature coding sequence hybrid constructs with the small subunit of RUBISCO were constructed and analyzed for the ability to import into pea chloroplasts in vitro. These results will be discussed as they relate to a proposed import pathway for this CAB precursor polypeptide.

Theobroma cacao - Phytophthora palmivora: Host-Parasite Interaction

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Theobroma cacao is an important cash crop of the tropics and the only source of cocoa and cocoa butter. The geographical range of this plant is limited to the area within 20° of the equator. Of the various pathogens which infect cacao, Phytophthora palmivora, P. megakarya, P. capsici and P. citrophthora, the causal organisms of black pod, are the most widespread and destructive.

Our research addresses the response of different T. cacao trees when infected by P. palmivora. A selection of 27 five-year-old greenhouse grown trees of variable genetic background from Ecuador and Costa Rica were screened for the presence of constitutive or inducible fungitoxic metabolites.

Leaf material was the major source of host tissue but pods were also tested when available. The host tissue was infected under sterile conditions and incubated in a humid chamber to observe symptom development. Tissue extracts were then tested for biological activity against fungi using Cladosporium, a test organism.

Data collected for each tree were: size of necrotic area and metabolites detected in the bioassay. Significant differences were noted in rates of symptom development. Constituent fungitoxic compounds were detected in controls and in some cases were found in lower amounts after infection, which may indicate that the fungus was breaking down the substance.

In similar tests on T. grandiflora, Ibarra (1984) detected inducible metabolites fungitoxic to Cladosporium. Debost (1987) detected both constituent and inducible fungitoxic phenolic compounds in T. cacao infected with P. megakarya. Since most T. cacao is of variable genetic background, one would expect a variety of responses ranging from increased production to very low production of fungitoxic compounds.

In conclusion, we have found differences in constituent fungitoxic metabolites in a sampling of T. cacao greenhouse trees. Inducible fungitoxic compounds have been detected but the response has been inconsistent. Future work will include identification of the constituent and inducible metabolites, testing their effects on the pathogen, and examination of the question: is production of these metabolites related to increased resistance?

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Ibarra, J., G. Blaha & C. Herail. 1984. Proc. 9th International Cocoa Research Conference.

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R-PRIME PLASMIDS CARRYING TN5-TAGGED GENOMIC DNA OF SOYBEAN RHIZOBIA: *BRADYRHIZOBIUM JAPONICUM* AND *RHIZOBIUM FREDII*

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A kanamycin sensitive derivative of pR68.45, pJB3JI was utilized for R-prime formation by selecting for mobilization of random transposon Tn5 insertions. *In vivo* constructed gene library on R-prime plasmids was used for genetic complementation of several symbiotically defective auxotrophic mutants of *Rhizobium fredii* strain HH303. These mutants which were obtained by Tn5 induced mutagenesis, had auxotrophic requirements uracil (RfK1101), adenosine (RfK1105 and RfK1107), nicotinic acid (RfK1108) and aspartate (RfK1114). The apparent complementation frequencies of these mutations were between 10^{-5} and 10^{-7}. Symbiotic nitrogen fixation was restored in the complemented mutants: RfK1101, RfK1105 and RfK1107. The R-prime plasmids from the bacteroids of each of the complemented mutants were transferred into *Escherichia coli* strain HB101. Restriction enzyme analysis and Southern hybridization studies of these plasmids demonstrated the formation of R-primes carrying Tn5 tagged genomic DNA from *R. fredii* HH303. The mobilization of Tn5 insertions along with genomic DNA from the slow-growing *Bradyrhizobium japonicum* strain I-110 into *E. coli* HB101 was also shown.

MITOCHONDRIAL DNA OF TALAROMYCES FLAVUS, A BIOCONTROL FUNGUS: Sue Mischke and G. C. Papavizas, Soilborne Diseases Laboratory, Plant Protection Institute, Agricultural Research Service, USDA, Beltsville, MD 20705

Talaromyces flavus is the teleomorph, or sexually reproducing form of Penicillium dangeardii Pitt (usually reported as P. vermiculatum Dangeard). This fungus effectively antagonizes Verticillium dahliae, the soilborne pathogen responsible for Verticillium wilt of eggplant, potato and other plants. DNA was isolated from T. flavus and fractions containing mitochondrial and chromosomal DNAs were identified. Mitochondrial DNA appeared to be AT-rich. Restriction endonuclease analysis suggested that the size of the mitochondrial DNA is 30 - 36 kilobases (approximately 20 megadaltons). Preliminary Southern hybridization experiments using a heterologous biotinylated DNA probe located the cytochrome oxidase subunit III gene on a 9.4-9.9 kb Eco RI fragment. Probe detection was by streptavidin-alkaline phosphatase conjugation followed by dyes for colorimetric visualization. Mitochondrial DNA analysis of biocontrol fungi has not been previously analyzed. Restriction fragment length polymorphisms which occur between T. flavus strains may be useful for strain identification in field experiments and for analysis of hybrids in protoplast fusions.

QUANTIFYING HOST RESPONSE TO TML, A CROWN GALL TUMOR GENE

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The T-DNA of Agrobacterium tumefaciens Ti plasmids contains several genes responsible for tumor growth and phenotype. One of these genes, tml (tumor morphology large), affects tumor phenotype in a host-dependent fashion. On "responsive" host plant stems, tml mutant strains of A. tumefaciens induce larger tumors than wild-type strains. In contrast, on "indifferent" host plants, tml- and wild-type strains induce tumors of similar size. Until now, these observations have been qualitative in nature, and have not taken into account tumor age. We describe here a novel, nondestructive, and simple method to quantitatively monitor the size of individual tumors as they grow on a stem. We show that: 1) Nicotiana tabacum is a "responsive" host species, since tml- tumors grow larger than wild-type tumors; 2) tumor sizes are similar for the first 20 to 25 days of growth, then differ significantly through day 60; and 3) N. glutinosa is an "indifferent" host species, since tml- and wild-type tumors are similar in size throughout the growth period.

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