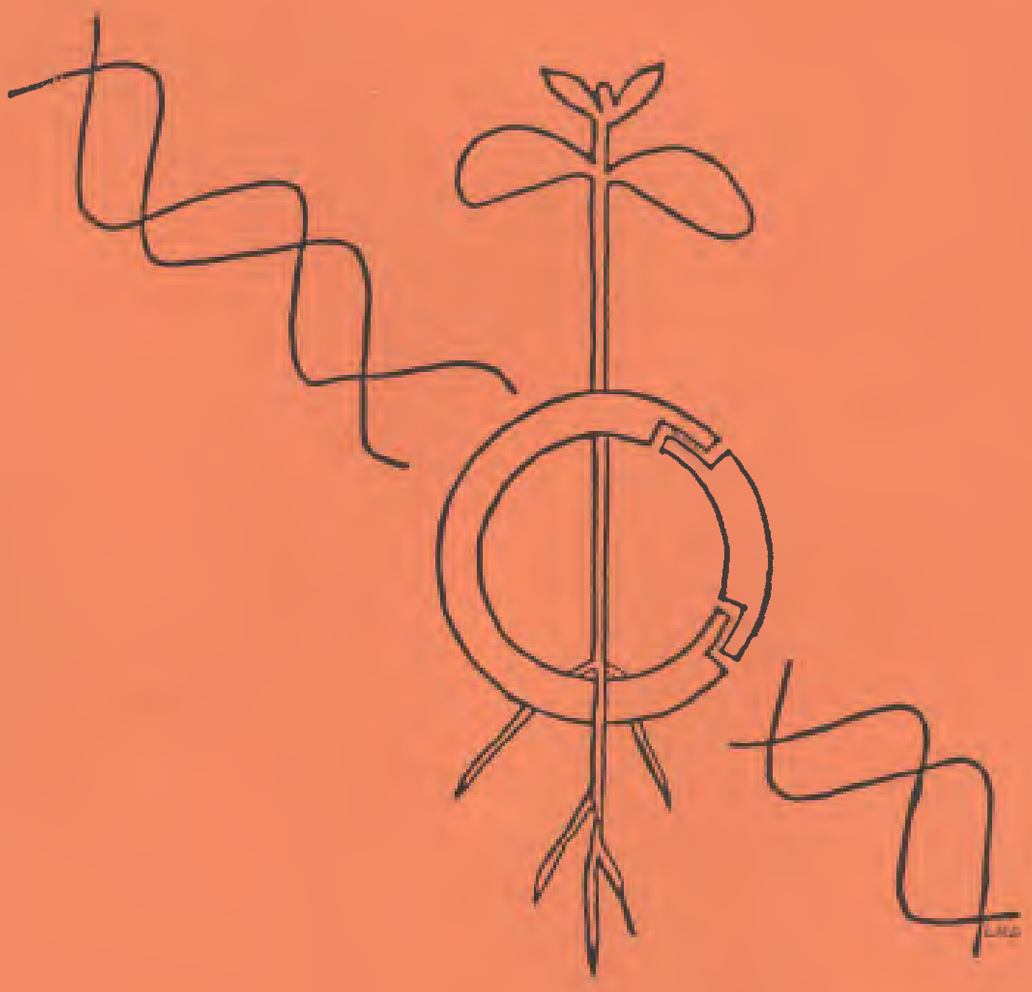
MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY MEETING



AUGUST 22 & 23, 1985

SECOND ANNUAL MEETING

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

AUGUST 22 AND 23, 1985

UNITED STATES DEPARTMENT OF AGRICULTURE

BELTSVILLE, MARYLAND

CONTENTS

INTRODUCTION

Welcome to the second annual meeting of the Mid-Atlantic Plant Molecular Biology Society. One of the goals of the society is to promote communication among the scientists in this region. The people involved in the initial organization of the society hope that this will help scientists thinking about using, or just beginning to use, molecular biological techniques, as well as facilitate transfer of information (and perhaps clones) among long time workers in the field.

The general topic of plant molecular biology covers an enormous number of fields. This becomes readily apparent by quick perusal of the titles of the talks we will hear today and tomorrow. The common thread is the use of certain techniques to answer questions about biological problems. Not everyone uses these techniques in the same way, and new applications and technologies pop up all the time. We hope you come away from these two days knowing not only new molecular biology but new and different biological systems being researched, and new people you can call with questions.

The society is extremely flexible; we have no real ground rules. At a brief business meeting on Friday we will discuss what the orgainizing committee has done re our tax situation and what our plans are or should be for next year's meeting. Everyone is invited to help out and provide advice and personal views.

The society owes a special thanks to the speakers, the exhibitors, and those who put a tremendous amount of effort into getting this meeting together, especially:

Lynn Zimmerman and Autar Mattoo, Organizing Vice-Chairpeople

Lorin DeBonté, Posters and Abstracts Committee (and all the little details)

Patsy Rhodes, Registration Commmittee

Candace Collmer, Sally McCammon, Ken Leto, Ben Matthews, Program Committee

Lynn Zimmerman, Exhibitors Committee

Jerry Cohen and Lowell Owens, Mailings and Signs

and many others who helped out in lots of ways with all the unexpected details.

Janet P. Slovin
Chairperson
Organizing Committee

GENERAL INFORMATION

All sessions will be held in building 003 of the USDA in Beltsville. The oral presentations will take place in the auditorium on Thursday and Friday. The poster sessions will be held in conjunction with lunch on Thursday and Friday in the upper and lower lobby of the auditorium and extending down the hallways on either side. Refreshments will be available during coffee breaks, either in the lobby or outside, weather permitting.

Information will be available at the registration desk for suggestions for eating in the Beltsville and downtown Washington D.C. area. Maps will also be available to help you get around. We originally planned to do an informal buffet lunch outside on the Beltsville USDA grounds but there were too few responses to our enquiry on the registration form so that it became impractical for us to provide lunch services. Our alternative suggestion is that you pick up something at one of the local fast food places and bring it back so we can eat on the lawn together as a group. Jerry's Sub-Shop is adjacent to the USDA and has a wide assortment of food. Menu's will be available at the registration desk so you can have your order in mind before going there (if you choose to). Hopefully this will speed things up a little. We are sorry for any inconvenience this causes.

EXHIBITORS

We would like to acknowlege the exhibitors at this meeting whose displays enhance the value of the meeting and whose financial assistance made this meeting possible. We encourage you to visit with the technical representives during the sessions to find out how they can further help you with your research.

The exhibitors are (in alphabetical order):

BIORAD

BOEHRINGER MANNHEIM BIOCHEMICALS

HYDRO SERVICE AND SUPPLIES INC.

NIKON INC.

REGIONAL SCIENTIFIC ASSOCIATE, INC.

SCHLEICHER AND SCHUELL

CARL ZEISS, INC.

PROGRAM SCHEDULE

THURSDAY, AUGUST 22

8:15	Assemble posters		
SESSION I - CONVENOR - Lynn Zimmerman			PAGE
8:30-8:45	Introduction		
8:45-9:40	Barry Marrs	A bacterial reaction center assembly mutant.	1
9:40-10:00	Gordan Inamine	Light regulated synthesis of the large subunit of ribulose 1,5-bis-phosphate carboxylase in pea: evidence for control at the level of translation.	2
10:00-10:20	S.-D. Kung	The study of chloroplast terminators by gene fusion techniques.	3
10:20-10:40	COFFEE*		
10:40-11:00	Eleanore Wurtzel	DNase I hypersensitive sites at the Shrunken locus in maize.	4
11:00-11:20	Andrzej Kalinski	Barley α-amylase gene family. De novo synthesis of nucleases in aleurone layers.	5
11:20-12:15	Robert Fluhr	Tissue-specific and light regulated expression of monocot and dicot photo-synthetic genes in transformed plants.	6
12:15-1:15	LUNCH		
1:15-1:40	POSTERS		
SESSION II - CONVENOR - Lila Vodkin			
1:40:-2:35	Judy Strommer	Effects of a transposable element on gene expression.	7
2:35-2:55	Patsy Rhodes	The soybean transposable element Tgm1 and related sequences.	8
2:55-3:15	COFFEE*		
3:15-3:35	Nancy Shepherd	Maize transposable elements.	9
3:35-4:50	Steve Dellaporta	Molecular cloning of the R locus of maize using transposon tagging.	10

FRIDAY, AUGUST 23

*Refreshments during breaks provided by:
Botany Dept., U. of M., College Park, Graduate Student Organization
Fruit Laboratory, USDA

A BACTERIAL REACTION CENTER ASSEMBLY MUTANT: W. J. Jackson, R. C. Prince, and B. L. Marrs, Exxon Research and Engineering Company, Annandale, New Jersey 08801.

The photosynthetic bacterium Rhodopseudomomas capsulata is an excellent system for the study of photosynthesis at the molecular level. The membrane-bound protein complexes that catalyze the cyclic electron flow characteristic of bacterial photosynthesis are well described for this organism, and a variety of useful genetic tools have been developed. All of the genes for chlorophyll-binding proteins have been cloned and sequenced, and techniques for site-directed mutagenesis and host-of-origin gene expression are operational. An operon encoding the genes for two antenna and two reaction center proteins has been described.

We have recently characterized a mutant that appears to synthesize normal reaction centers but cannot grow photosynthetically because they are not inserted in the membranes in a functional orientation. Membranes from the mutant show normal flash-induced chlorophyll oxidation and quinone reduction, but the normal interactions with the cytochromes are absent, as is the light-induced carotenoid band shift. Membrane integrity and carotenoid competency are demonstrable by salt-induced band shifts. The reaction centers in the mutant membranes are unusually accessible to exogenous cytochrome c, and they are unusually sensitive to proteolytic degradation. The mutation that causes this phenotype maps in or near the antenna (LH1) genes, and the mutant does not accumulate LH1 polypeptides or 880nm-absorbing species. The LH1 apoproteins appear to be made and degraded. We interpret these observations to suggest that reaction centers may be assembled in an unusual environment, but that they require interactions with the closely-associated LH1 antenna complex for correct orientation in the membrane.

LIGHT REGULATED SYNTHESIS OF THE LARGE SUBUNIT OF RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE IN PEA: EVIDENCE FOR CONTROL AT THE LEVEL OF TRANSLATION. G. Inamine, B. Nash, H. Weissbach and N. Brot. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The regulation of ribulose 1,5-bisphosphate carboxylase (RuBPCase) expression by light has been studied. The specific activity of this enzyme in whole leaf tissue is increased 30-50-fold when dark grown plants are exposed to light for 24-48 hr. The gene for the large subunit (LS) of this enzyme is encoded in the chloroplast (cp) genome and we have examined how the level of LS mRNA correlates with the increase of RuBPCase activity. Although there is a 30-50-fold increase in carboxylase activity after dark grown plants are exposed to light, the level of LS mRNA, measured either by hybridization to an LS DNA probe or *in vitro* translation, increases at most 3-5 fold in the light. The results of Sasaki *et al.*, *FEBS Lett.* **173**, 31-35 (1984) showing that the cp genome copy number also increases ~3 fold in the light have been corroborated. This could account for the observed increase in LS mRNA. There is also no evidence of enzymatically inactive LS protein in dark grown plants. The above results suggest that the light induced increases in LS synthesis in peas is due primarily to regulation at the level of translation.

THE STUDY OF CHLOROPLAST TERMINATORS BY GENE FUSION TECHNIQUES: Kung, S.D., S. Acada, S. Mongkolsuk, and P.S. Lovett, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228

Chloroplast genomes are organized into many transcriptional units. Each unit contains single or multiple genes and requires defined signals to initiate transcription at one end and to terminate it at the other. Such signals are known as promoters and terminators. The promoters and terminators are regions of DNA containing recognition signals for initiation and termination of transcription by RNA polymerases. Currently, our knowledge on chloroplast promoters is rapidly advancing. In contrast, there is virtually no information available on the chloroplast terminators. This study was designed to analyze the structure and function of some Nicotiana chloroplast terminators by using gene fusion techniques.

Several terminator-probe plasmids were employed in this experiment. When the chloroplast fragments exhibiting terminator function were sequenced, they invariably contain the dyad symmetry capable of forming stem-loop structures. On the other hand, if the DNA sequences which have the potential of forming stem-loop structures are analyzed, they invariably function as terminators. Thus, a structure-function relationship of chloroplast terminator as tested in the procaryotic system is established.

The chloroplast terminators studied are Rho independent. The estimated efficiency in terminating the expression of cat gene is about 90% as analyzed by cat gene activity. It is interesting to note that there is an orientational difference in function of the rbcL terminator when tested in the procaryotic system.

DNASE I HYPERSENSITIVE SITES AT THE SHRUNKEN LOCUS IN MAIZE

Eleanore Wurtzel, Frances Burr, and Benjamin Burr. Biology Dept., Brookhaven National Laboratory, Upton, NY, 11973.

The relationship between local chromatin structure and gene expression in higher plants, is poorly understood. Therefore, we developed a method for analyzing local chromatin structure of the maize Shrunken (Sh) gene, which is developmentally regulated and expressed in a tissue-specific fashion. Sh is a unique gene mapping to chromosome 9. Sh encodes the enzyme, sucrose synthetase, an important enzyme involved in starch synthesis, catalyzing the cleavage of sucrose to UDP-glucose and fructose. Sh is highly expressed in endosperm tissue, where starch accumulates during the course of endosperm development and seed maturation. Local chromatin structure was probed by analyzing DNase I hypersensitivity. We are able to demonstrate that the active Sh gene in the endosperm, is DNase I hypersensitive. In addition, we have mapped DNase I hypersensitive sites for the first time in a higher plant gene. These sites map at the 5' end of the Sh gene, and span a region of 2.6 kb. Both point mutations and insertional mutations occur at Sh which affect gene expression. Their effect on the DNase I hypersensitive sites are being examined.

BARLEY α- AMYLASE GENE FAMILY. DE NOVO SYNTHESIS OF NUCLEASES IN ALEURONE LAYERS.

A. Kalinski and G. R. Chandra, Seed Research Laboratory, USDA-ARS, Belsville, MD 20705

Barley aleurone cells have been used for many years as a system for studying hormonal regulation of gene expression. In the absence of gibberellic acid these cells synthesize essentially no α- amylase. After addition of gibberellic acid total protein synthesis increases, and in vivo, 60% of the protein synthesized is α - amylase.

Our long term goals are to characterize the organization of aleurone layers chromatin in relation to the expression of α- amylase genes.

We have found that during the procedure of aleurone nuclei isolation from the imbibed tissue, DNA in chromatin is degraded by endogenous nuclease(s) to oligo- and mononucleosomes. The DNA repeat size equals about 220 bp. Degradation of aleurone chromatin to nucleosomes is generated by endogenous nuclease synthesized de novo in the tissue during the second day of imbibition of deembryonated seeds.

No endogenous nuclease action is detected during nuclei preparation from imbibed embryos. The size of the monomer in embryo chromatin obtained after micrococcal digestion of nuclei is the same as in aleurone chromatin and equals 220 bp.

Imbibition of deembryonated seeds in 1μM GA_3 stimulates the activity of aleurone nucleases - much more specific degradation of nuclear DNA was found on agarose gel.

We compared Southern blots of embryo and aleurone DNA's using nick translated α - amylase clone 103 DNA. At least five embryo DNA bands in a Hind III digest hybridized to the radioactive DNA probe. In the aleurone layer autoradiograms particular DNA bands are less visible, especially after treatment of the tissue with GA_3. The presence of smearing on these autoradiograms might be a result of the action of endogenous nucleases causing single-strand nicks in the region of α- amylase genes.

TISSUE-SPECIFIC AND LIGHT REGULATED EXPRESSION OF MONOCOT AND DICOT PHOTOSYNTHETIC GENES IN TRANSFORMED PLANTS.

R. Fluhr, F. Nagy, G. Morelli, G. Lamppa, P. Moses, and N.-H. Chua
Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA

Our laboratory is interested in regulatory sequences that are involved in transcriptional control of major photosynthetic genes. We have chosen as examples nuclear genes encoding the small sub unit (rbcS) of ribulose-1,5-bisphosphate carboxylase and the chlorophyll a/b-binding protein (Cab). Our immediate objective is to define DNA sequence elements that confer their tissue-specific and light-regulated expression. Both the rbcS and Cab polypeptides are encoded by a small multigene family (Coruzzi et al., 1983; Broglie et al., 1983). We have isolated from peas, a set of four genomic clones encoding five members of the rbcS gene family. The complete nucleotide sequence of three gene members (rbcS-E9, rbcS-3A and rbcS-3C) and their expression patterns in peas have been investigated (Coruzzi et al., 1984; R. Fluhr et al., unpublished results). All three rbcS genes are light-regulated and differentially expressed among different plant organs.

To define DNA sequences required for their regulated expression, we first inserted the rbcS-E9 gene into the Ti-plasmid of Agrobacterium tumefaciens and transferred the gene into petunia cells by in vitro transformation. To define 5' DNA sequence elements needed for light-regulation we constructed a series of 5' deletion mutants of rbcS-E9 by Bal31 digestion. With these deletions we could show that a 33 bp cis-regulatory element is sufficient to confer lightactivated transcription (Morelli et al., 1985). The rbcS-3A and rbcS-3C genes have been transferred to petunia plants. Their expression is tissue-specific and light-regulated. We have shown that under appropriate conditions petunia transgenic plants exhibit phytochrome control of the pea rbcS genes, suggesting that the cis-regulatory elements necessary for their control are present (R. Fluhr, submitted).

Many of our most important crop plants are monocotyledons, including wheat, corn, rice and barley. Although transformation systems have been reported, regeneration of monocotyledons is usually blocked at the callus stage. As a result, it has not been possible to identify the regulatory domains of monocotyledonous genes in a homologous nuclear background. To circumvent these difficulties we asked if monocotyledonous genes can be expressed and correctly regulated in dicotyledons. We have introduced a wheat gene (whAB1.6) encoding the major chlorophyll a/b binding protein (Cab) of the light-harvesting complex into the genomes of tobacco. We obtained light-regulated and organ-specific expression of a monocotyledonous gene in transgenic dicotyledonous plants (Lamppa et al., 1985).

Coruzzi, G., Broglie, R., Cashmore, A.R., and Chua, N.-H. 1983. J. Biol. Chem. 258, 1399-1402.

Broglie, R., Coruzzi, G., Lamppa, G., Keith, G., and Chua, N.-H. 1983. Biotechnology 1, 55-61.

Coruzzi, G., Broglie, R., Edwards, C., and Chua, N.-H. 1984. EMBO J. 3, 1671-1679.

Morelli, G., Nagy, F., Fraley, R.T., Rogers, S.G., and Chua, N.-H. 1985. Nature 315, 200-204.

Lamppa, G., Nagy, F., and Chua, N.-H. 1985. (submitted).

EFFECTS OF A TRANSPOSABLE ELEMENT ON GENE EXPRESSION

Judy Strommer, The University of Georgia, Department of Genetics, Biological Sciences Building, Athens, Georgia, USA, 30602.

We are interested in the evolution of mobile genetic elements in plants. Clues to evolutionary history are provided by characteristics such as host range, structural heterogeneity and transpositional behavior. Alternatively, an element newly introduced into a population allows us to observe the short term evolutionary consequences directly, at several levels. Mu is a rare and nearly homogeneous mobile element of unknown origins. We are analyzing its success as a colonizer and its effect on genes, chromosomes, individual plants and populations of maize. Most of what we have learned applies to the first two parameters: its transpositional behavior as it relates to successful dispersal of the element and its effects on gene expression, especially at the Adh1 locus.

THE SOYBEAN TRANSPOSABLE ELEMENT Tgm1 AND RELATED SEQUENCES -
Patsy Rhodes and Lila Vodkin; Plant Molecular Genetics Lab;
Agricultural Research Service; Beltsville MD, USA 20705

The soybean insertion element Tgm1 (transposon Glycine max) typifies a distinct class of plant transposable elements which includes representatives known to be mobile in snapdragon (Tam1; Tam2) and maize (Spm/En, Suppressor-mutator/Enhancer). The complete sequence of Tgm1 contains 3550 base pairs (bp) and has an extensive border which consists of various forms of a prototype 54 bp repeat unit. The repeating unit is composed of a stem-loop structure and interhairpin sequence. Seven nucleotides (ACATCGG and its complement) maintained within each stem also appear as a subset of inverted repeats found at nearly equal distances from the Tgm1 target site in the flanking lectin gene. The dyad symmetries which contain this sequence may be involved in target gene selection. Furthermore, a striking resemblance between the interhairpin portion of the repeating unit and the last few nucleotides at the 5' and 3' ends of Tgm1 where a transposase would be expected to act, suggests some relationship between the repeat unit format and transposition.

Since Tgm1 is part of a family of moderately repetitive DNAs in the soybean genome, it should be possible to compare the Tgm1 repeat unit and flanking target region with analogous areas in related soybean elements. For this purpose, isolates from a soybean library have been obtained and are being examined through targeted sequencing at the termini.

MAIZE TRANSPOSABLE ELEMENTS

Dr. Nancy S. Shepherd
E. I. du Pont de Nemours and Company

The phenomenon of DNA transposition was first identified by Dr. Barbara McClintock in Zea mays L. ssp. mays (corn). Although a few of the plant DNA elements have been well characterized genetically, the molecular analysis is still in its infancy. To date, only a few of the DNA elements have been cloned (e.g. Ac-Ds; Spm (En); Mutator). Each of these elements has a different molecular structure and seems to have unique biological characteristics. The cloned maize transposable elements have proven to be particularly useful in the cloning of genes into which they have inserted. This process has been termed "gene tagging". We have recently cloned the A1 gene of maize (a gene involved in anthocyanin biosynthesis) by the use of gene tagging methods. The method of isolation of the A1 gene and its general application will be discussed.

Nevers, P., Shepherd, N. S., and Saedler, H. "Plant Transposable Elements" In: Advances in Botanical Research, 1985. In press.

O'Reilly, C., Shepherd, N. S., Pereira, A., Schwarz-Sommer, Zs., Bertram, I., Robertson, D. S., Peterson, P. A., and Saedler, H. "Molecular Cloning of the a1 locus of Zea mays using the transposable elements En and Mul. EMBO Journal 4, 877-882. 1985.

MOLECULAR CLONING OF THE R LOCUS OF MAIZE USING TRANSPOSON TAGGING.

Steve Dellaporta, Cold Spring Harbor Lab, P. O. Box 100, Cold Spring Harbor, NY, 11724.

BACTERIAL PECTIC ENZYMES AND PLANT DISEASE: MULTIPLE FORMS AND ACTIVITIES.

A. Collmer, M. M. Atkinson*, C. J. Baker*, J. H. Payne, J. L. Ried, D. L. Roeder, C. Schoedel, and R. Schuurink. Department of Botany, University of Maryland, College Park, MD 20742 and *Plant Pathology Laboratory, USDA, Beltsville, MD 20705.

Microbial pectic enzymes, because of their destructive effects on parenchymatous plant tissues, have long been studied as potential determinants in many plant diseases. Recently, pectic enzymes (and their products) have been found to trigger defense reactions in plants. We have been using the soft-rotting enterobacterium Erwinia chrysanthemi, which produces five isozymes of pectate lyase, to explore both of these activities of pectic enzymes. As a step toward understanding the functional basis of pectate lyase isozyme multiplicity in E. chrysanthemi and to aid exploration of the multiple effects of pectate lyase on plant tissues, we have cloned the encoding pel genes into Escherichia coli where they are used as tools in marker exchange mutagenesis and as sources of purified individual isozymes. The marker exchange experiments suggest that some of the E. chrysanthemi pectate lyase isozymes are redundant; mutants unable to produce two of the isozymes are little altered in their ability to utilize pectate or cause soft-rot disease. A homogeneous pectate lyase isozyme has been shown to be able to inhibit the hypersensitive response and the normal disease response of tobacco to various bacterial phytopathogens and to activate a transient plasmalemma K^+/H^+ exchange in suspension-cultured tobacco cells. The significance of these findings will be discussed in the context of general research on plant-bacterium interactions.

MOLECULAR BIOLOGICAL APPROACHES TO SPIROPLASMA GENETICS AND DISEASE DETECTION. S. McCammon[1], R. E. Davis[1], M. Konai[1], I.-M. Lee[2], E. Stanbridge[3], B. Kirkpatrick[4], and R. Jordan[5]. [1]Plant Virology Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705; [2]Department of Botany, University of Maryland, College Park, MD 20742; [3]Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717; [4]University of California, Berkeley, CA 94720; Florist and Nursery Crops Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705.

Plant pathogenic spiroplasmas are motile helical cell-wall-less prokaryotic microorganisms that inhabit only the phloem of diseased plants and induce symptoms including stunting, yellowing, little leaf, abnormal flowers and fruits, wilting, and necrosis. They are related to the mycoplasmas and are transmitted in nature by leafhoppers. Much new knowledge of spiroplasmas and of the pathogenic mycoplasma-like organisms (MLO's) in plants could be gained by improved detection, identification, and cultural methods, and by investigations of the molecular genetics of these pathogens. For example, we have used a 900bp cDNA probe from the 23s ribosomal RNA sequence from Mycoplasma hyorhinus to identify cloned DNA from Spiroplasma citri and the peach yellow leaf roll strain of Western X-infected leafhoppers (Collandus montanus). Identification of spiroplasmas routinely uses serology. The specificty of serological tests has been facilitated by the development in our laboratories of monoclonal antibodies (McAbs) to group 1 spiroplasmas. We have characterized McAbs which react specifically with strains of S. citri alone and others which react only with strains of corn stunt spiroplasma. Using the latter McAbs, we have detected corn stunt spiroplasma in diseased plants by ELISA.

In preliminary studies of the genetics of spiroplasmas, antibiotic resistant spiroplasmas and also strains of corn stunt spiroplasma which are defective in helicity and motility have been isolated in our laboratories. Such variants should prove useful in genetic analysis of spiroplasma. An additional factor potentially of use for genetic analysis is that there are several groups of viruses which infect spiroplasmas. We have developed a transfection system using the single-stranded closed circular DNA from a rod-shaped group 1 virus, C1/TS2, in which a 1×10^5 fold increase in infectivity was obtained using a 50% polyethylene glycol treatment. The double-stranded replicative form of the virus also transfected spiroplasma and has sites which are susceptible to a single cut by each of several restriction enzymes. This system should facilitate the development of a cloning vector and gene transfer system in spiroplasma.

Site-specific mutagenesis of viroid cDNAs: A single nucleotide substitution can abolish infectivity.

R. W. HAMMOND[3], R. A. OWENS[1], R. C. GARDNER[4], M. C. KIEFER[5], S. M. THOMPSON[1], and D. E. CRESS[2]
[1]Plant Virology and [2]Tissue Culture and Molecular Genetics Laboratories, U. S. Department of Agriculture, Beltsville, MD 20705; [3]Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228; [4]Calgene Inc., Davis, CA 95616; and [5]Department of Botany, University of Maryland College Park, College Park, MD 20742.

Viroids, the smallest autonomously replicating pathogenic agents known, are small, unencapsidated, and covalently closed circular single-stranded RNA molecules with extensive secondary structure. As the focus of plant viroid research has begun to shift from *in vitro* structural studies to the relationship between viroids' structure and their various biological functions, genetic approaches to viroid/host interaction have become increasingly important. Viroids appear ideally suited for detailed structure/function analyses because disease induction appears to result from direct interaction between the viroid and its host.

As part of our plan to use site-specific mutagenesis to systematically study structure/function relationships in potato spindle tuber viroid (PSTV), we have examined the effect(s) of alterations in regions previously implicated in viroid replication by comparative sequence analysis. A number of different techniques, including directed mutagenesis with sodium bisulfite and oligonucleotide-directed mutagenesis, were used. Because most of the alterations produce C-->U transitions in PSTV, effects upon its rod-like "native" structure should be minimal.

Simultaneous C-->U transitions at positions 92 and 103 within the upper portion of the central conserved region appear to be lethal unless the host is given the opportunity to excise the alterations from a longer-than-unit-length RNA transcript. Although we cannot assign precise roles to the individual mutations until the corresponding revertants have been constructed, the change at position 103 would destabilize the stem of secondary hairpin I. Position 92 is located within its single-stranded loop.

A single C-->U transition at position 284, just outside the lower portion of the central conserved region, was also lethal when recombinant pUC9 plasmids were used as inoculum. When inoculation of the altered PSTV cDNA was mediated by the *Agrobacterium tumefaciens* Ti plasmid, PSTV progeny with the unaltered "wild-type" sequence was obtained. This apparent sequence reversion at the RNA level can be explained by the high error frequency characteristic of RNA synthesis and use of a systemic bioassay for PSTV replication.

These and other results to be presented have clarified the requirements for viroid cDNA infectivity and emphasize the importance of an appropriate bioassay system for screening mutant viroid cDNAs.

VIRUS-BASED VECTORS FOR PLANTS: WHETHER AND WHITHER?

Robert M. Goodman, Calgene, Inc., 1920 Fifth Street, Davis, CA., USA, 95616

The single greatest potential contribution that plant viruses could make to the study of gene expression in plants would be the development of a versatile viral-based vector from which cloned genes could be expressed and their expression analyzed. There are numerous possible strategies, several of which have been outlined in the literature previously and many of which are based on the elegant manipulations now possible with animal virus systems. Limited success in expressing a very small coding region in a cleverly made deletion mutant strain of CaMV have been reported. Attempts are underway in various laboratories around the world, with both CaMV and geminiviruses, to construct host/virus systems in which either the virus is integrated into and expressed from the chromosome or a deletion mutant is made dependent for its replication on necessary viral gene functions expressed in *trans* from a gene integrated into the chromosome of the host. The bipartite nature of the geminivirus genome suggests the obvious possibility of constructing a third genome component carrying the sequences necessary for coordinate regulation of genome replication and appropriate expression of a foreign gene. Such a component could then be combined with an appropriately modified viral strain (perhaps attenuated in symptom production) or host plant (perhaps expressing in *trans* from the chromosome the necessary genes for viral replication) to allow easy study of gene expression, promoter function, and perhaps eventually be used to produce useful products in plants. Rational vector development and knowledge about plant gene expression in general using plant viral systems still await the availability of additional basic knowledge about virus replication and expression. In part, progress has been slow due to some constraints imposed by the systems in which one needs to work. In part, it may also be due to the relatively recent discovery of the plant DNA viruses. The DNA genome of caulimoviruses was reported in 1967 and that of geminiviruses in 1977. It is nevertheless also the case that the level of effort, and the level of reseach funding which drives that level of effort, has been much less in the case of basic studies on plant viruses compared to that focused over the past forty years on bacterial and animal viruses.

MASS SPECTROMETRY FOR MOLECULAR BIOLOGISTS.

Robert Cotter, Dept. Pharm.; John's Hopkins School of Medicine,
725 N. Wolfe Street, Baltimore, MD., 21205.

pBR322 - Library

The Mitochondrial Genome of Carrot (Daucus)

Lorin R. DeBonte and Benjamin F. Matthews
U.S. Department of Agriculture, ARS, PPHI
Tissue Culture & Molecular Biology Laboratory
Beltsville, Maryland 20705

The mitochondrial genomes of D. carota subsp. sativus, D. carota subsp. gummifer, D. capillifolius and D. pusillus have been compared using DNA restriction fragment digestion patterns. The overall restriction enzyme patterns are not highly conserved in fragment homology as in the chloroplast genomes of these species. The sizes of the Daucus mitochondrial genomes examined range from 328 to 468 kb. The Daucus mitochondrial genome has been stable in cell suspension cultures maintained more than 18 years and through biochemical selection pressure and in cells from cell suspension cultures regenerated into plants and placed back into culture. In somatic hybrids of D. carota and D. capillifolius (Matthews and Widholm, 1985) mitochondrial DNA rearrangement has been observed. To better understand this rearrangement we are determining the organization of the Daucus mitochondrial genome by chromosome walking using a Charon 35 library containing sized fragments of a BamHI partial mitochondrial DNA digest. The order of the ribosomal RNA genes in the mitochondrial genome is being established by RNA-DNA hybridization using the 26S, 18S and 5S ribomsomal RNAs. The genes encoding subunit II of cytochrome C oxidase (COXII) and subunit 9 of the Fo ATPase (ATPase-9) have been located by DNA-DNA hybridization using known probes from maize and petunia. The ATPase-9 gene is located near the rRNA gene region. Both the ATPase-9 and COXII genes appear as single copies. These studies will be used in the analysis of mitochondrial DNA rearrangements obtained through somatic hybridization of selected Daucus species.

Matthews, B. F. and J. M. Widholm. 1985. Mol Gen Genet 198:371-376.

A POSSIBLE MOLECULAR MECHANISM INVOLVED IN THE GENERATION OF CYTOPLASMIC MALE STERILITY

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Cytoplasmic male sterility (CMS) is a mitochondrially inherited trait resulting in the lack of pollen production. It is used extensively in the manufacture of hybrid seed. In Petunia, a specific mitochondrial genome arrangement has been shown to correlate with CMS. This region has an open reading frame formed by the fusion of three different mitochondrial loci. The 5' flanking region, containing the promoter, and the N-terminal transmembrane segment are from the wild type ATP synthase subunit 9 gene. This is connected, in frame, with an aberrant cytochrome oxidase subunit 2 segment. Due to several small deletions and tandem repeats the copper binding site has been removed but possibly the cytochrome c binding site has been maintained. This is followed by a highly hydrophilic unidentified open reading frame containing 4 copies of a tandem repeat. S1 nuclease protection studies show that this gene is transcribed only in sterile lines but not in fertile lines. The steady state mRNA levels increase in reproductive tissues relative to leaves. Models consistent with this gene fusion being involved in the cause of CMS will be presented.

SELECTION AND CHARACTERIZATION OF HERBICIDE-RESISTANT MUTANTS OF NICOTIANA TABACUM

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Mutants resistant to the herbicides chlorsulfuron and sulfometuron methyl were isolated from haploid cell cultures of Nicotiana tabacum cv. Xanthi. Fertile diploid plants regenerated from ten resistant cell lines were analyzed genetically. In all cases resistance resulted from a single semidominant nuclear mutation. Linkage studies demonstrated that the mutations occurred at either of two unlinked genetic loci, SuRA and SuRB. One mutation, S4, when present in a homozygous state, increases the tolerance of cultured cells and of whole plants for chlorsulfuron at least 100 times. Acetolactate synthase (ALS) activity in extracts of normal cells is inhibited by very low concentrations of chlorsulfuron, whereas both SuRA and SuRB mutants possess a herbicide-insensitive form of ALS activity. The resistance phenotype cosegregates with the herbicide-resistant ALS activity through genetic crosses. These results indicate that ALS is the site of action of these two sulfonylurea herbicides in higher plants and that in N. tabacum resistance is accomplished by production of an altered form of either of two ALS isozymes. An even more highly resistant cell line was derived from cells homozygous for a mutation at the SuRB locus. Biochemical and genetic studies of regenerated plants suggest that this enhanced resistance results from the occurrence of a second mutation genetically linked to the S4 mutation.

MOLECULAR GENETICS OF SULFONYLUREA HERBICIDE ACTION:

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Sulfometuron methyl (SM), a new and potent broad spectrum sulfonylurea herbicide, inhibits the amino acid biosynthetic enzyme acetolactate synthase (ALS) from diverse sources including bacteria, yeast, and higher plants. An extensive genetic analysis of yeast mutants resistant to SM has led to the identification of three genes which can mutate to yield resistance. Most mutations are in the ILV2 gene which encodes ALS and result in the production of herbicide-resistant forms of the enzyme. Resistance can also be achieved in yeast by amplification of the ILV2 gene by molecular cloning onto a high copy plasmid. The DNA sequence of the wild-type ILV2 gene and of a mutant allele which encodes an SM-resistant ALS has been determined. The mutant allele contains a single nucleotide change resulting in a pro to ser substitution in the protein. The deduced amino acid sequence of yeast ALS shows considerable homology with that of three ALS isozymes of E. coli. This homology persists over a wide evolutionary span. As a result, the yeast ILV2 gene has been successfully used as a heterologous hybridization probe to isolate genes which encode ALS from the cyanobacteria, Anabaena, the green algae Chlamydomonas and the higher plants, Arabidopsis thaliana and Nicotiana tabacum. The tobacco ALS gene was isolated from a herbicide-resistant strain of tobacco. Thus it will be useful as a dominant selectable genetic marker for plant transformation as well as for introduction of herbicide resistance into crop plants.

Board 1

COMPUTERIZED ANALYSIS OF 2-D GEL AUTORADIOGRAPHS OF [^{35}S]-METHIONINE-LABELED TOMATO FRUIT PROTEINS

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Tomato fruit pericarp tissue at the immature green, mature green, breaker, and ripe stages of development were labeled with ^{35}S-methionine applied to the cut surface and vacuum infiltrated. The tissues were frozen in liquid N_2, powdered, and extracted in lysis buffer. The extracts were subjected to 2-D SDS-PAGE, the gels were silver-stained, and autoradiographs of the gels were developed. The autoradiographs were digitized using a television camera, and data were stored on hard disk. Comparison of two autoradiographs was according to the method of Hruschka (Clin. Chem. 30:2037-2039, 1984). Comparisons of autoradiographs of immature-green and mature-green gels showed numerous changes in the labeling of proteins. Likewise, many changes in labeling patterns were evident in comparisons of mature-green vs. breaker and breaker vs. ripe gels. At each transition, labeling of some proteins was constant, labeling of others was either higher or lower, and some new peptides appeared. The results suggest that ripening involves many changes in gene expression.

Board 2

COMPUTERIZED COMPARISON OF TWO AUTORADIOGRAPHS

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We are developing image processing techniques for agricultural applications. Here we trace the digitization, noise and background removal, coarse rubber sheeting, creation of protein lists, fine rubber sheeting, and quantitative representation of the differences between two autoradiographs. The gels were prepared from soybean samples and are typical 2-D PAGE gels.

A natural extension of this process, once the protein lists are available, would be to display changes such as decay or growth rate using several lists instead of just a pair. The procedures used here are in some ways simpler versions of those used in laboratories with more extensive personnel and equipment. Part of our research is to see how far these versions can go with our particular applications. The FORTRAN program runs on an HP-1000F minicomputer and the color monitor is driven by a Grinnell 275 image processor.

Board 3

ATRAZINE BINDING ALTERS DEGRADATION OF THE 32kDa THYLAKOID RECEPTOR IN TRIAZINE SENSITIVE BUT NOT RESISTANT BIOTYPES

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The kinetics of degradation of the rapidly-metabolized 32kDa chloroplast membrane protein in triazine sensitive and resistant biotypes of Solanum nigrum were determined. Light-induced degradation of the 32kDa herbicide target protein was inhibited by diuron but not atrazine in leaves of a resistant biotype, while both herbicides were effective in inhibiting its degradation in the sensitive biotype. Experiments using radiolabeled atrazine and diuron showed a marked reduction in the ability of the resistant biotype to bind atrazine with little effect on its ability to bind diuron. These findings support a model (Mattoo et al, 1984, Proc. Natl. Acad. Sci., USA 81, 1380-1384) linking electron transport to degradation of the 32kDa protein and suggest that both processes are inhibited by binding of herbicide ligand to the 32kDa thylakoid receptor.

Board 4

GENE EXPRESSION DURING RIPENING (TOMATO)

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We are trying to isolate two groups of cDNA's from ripening fruit. The first represents RNAs whose levels change greatly during ripening. The second group encodes proteins whose activities change in ripening. One of the most prominent changes in enzyme activity is that of polygalacturonase. This change is not reflected in our in vivo labelling studies or in an increase in mRNA levels. Our preliminary studies suggest that there may be a sequestering of the protein in the insides of the mature green tomato fruit and then a transfer into the pericarp during ripening. If this is found to be the case, the appearance of ethylene associated with ripening may not be the inducer of polygalacturonase synthesis as is strongly suggested in the literature. We hope to understand more how ethylene affects and/or coordinates this program of ripening by using the two classes of cDNAs to look at the timing of synthesis of individual RNAs. These will be compared with levels of protein synthesis and protein activity.

Board 5

TRANSLATIONAL REGULATION IN PROPLASTIDS OF EUGLENA GRACILIS

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Evidence with a number of systems indicates that translational or post-translational events may be as important as transcription to the regulation of plastid gene expression. Light-induced development of plastids in Euglene gracilis is one such system (cf. J. Biol. Chem. 258.14478-84). We have recently obtained additional evidence for translational regulation in proplastids of Euglene.

Intact proplastids isolated from E. gracilis and supplemented with equimolar ATP and Mg++ exhibit high rates of protein synthesis, allowing the first clear characterization of the products of translation of proplastids. With the exception of polypeptides tentatively identified as EF-Tu and the alpha and beta subunits of CF1, none of the major translation of rbci in proplastids occur at levels similar to those of chloroplasts, sediment with polysomes, and can be translated in heterologous systems, but the intact proplastids appear not to translate the rbci message.

We conclude that proplastids in Euglene contain a fully competent translational apparatus, but exhibit strong selectivity in the massage translated.

Supported in part by grants from the USDA and the Charles and Johanne Busch Memorial Fund.

Board 6

COMPARISON OF CHLOROPLAST AND CHROMOPLAST DNAs OF ZINNIA ELEGANS BY RESTRICTION ENDONUCLEASE ANALYSIS: David M. Gorzo, Department of Horticulture, University of Maryland, College Park, MD 20742

A protocol was established for the preparation of circular DNA from chloroplasts and chromoplasts of Zinnia elegans. The yellow-flowered variety 'Canary' and the green-flowered mutant 'Envy' were the cultivars used. Plastid isolation included freezing leaf or petal tissue in liquid nitrogen, followed by homogenization in a mannitol buffer, differential sedimentation centrifugation, and density gradient centrifugation on Percoll gradients. The plastids were lysed and the released DNA was purified by phenol extraction, ethanol precipitation, and isopycnic centrifugation on CsCl-ethidium bromide gradients. The DNAs were digested with several different restriction endonucleases and the digests were electrophoresed on agarose gels. Analyses indicate differences in restriction fragment patterns of chloroplast and chromoplast DNAs, suggesting that differentiation of chromoplasts from chloroplasts in Zinnia may involve rearrangements in the structure of the plastome. Similar comparisons of chloroplast DNAs from 'Canary' and 'Envy' leaves and 'Envy' petals indicate no apparent differences in fragment patterns. Size estimation of the Zinnia chloroplast DNA molecule by restriction analysis indicates a size of approximately 130 kbp.

This research was carried out at and partially supported by the USDA's Florist & Nursery Crops Laboratory at Beltsville, MD.

Board 7

ALTERATION IN THE RESTRICTION PATTERN AND CYTOCHROME OXIDASE SUBUNIT II GENE OF THE MITOCHONDRIAL GENOME OF A BIOCHEMICALLY SELECTED RICE LINE

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A comparison of rice mitochondrial DNA restriction patterns was made between an anther culture derived line of the cultivar Assam-5 (B1-2) and an S-aminoethyl-L-cysteine (S-AEC) resistant line (A-23) selected from it. Both lines were maintained as cell suspension cultures from which the mitochondrial DNA were isolated. Alterations in restriction patterns were observed with XhoI, BamHI, SalI and PstI restriction endonucleases. Based on the number of fragments, the percent variation between the B1-2 and its S-AEC resistant line A-23 is approximately 10%. The size of the mitochondrial genome in Assam 5 calculated by fragment addition is approximately 400 kb, without considering band intensities.

Hybridization of the maize cytochrome oxidase subunit II (CoxII) gene to B1-2 and A-23 cell lines demonstrated restriction site variation between the CoxII genes of these lines. Hybridization of the Eco-Bam 5' end of the maize gene to the rice genes showed no variation between the cell lines when hybridized to rice SalI digests. When the Bam-Eco 3' end of the gene was hybridized variation was found between the two lines. In the B1-2 line two fragments of 2.93 and 2.10 kb hybridized while in the A-23 line only the 2.10 kb fragment hybridized. The alteration to the 3' end is under investigation.

Board 8

ELECTROFUSION OF CARROT AND TOBACCO PROTOPLASTS

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Leaf mesophyll protoplasts from several species of Nicotiana and suspension cells from several species of Daucus have been subjected to electrofusion treatments. The procedure involves the initial attraction of the cells to each other by an alternating sine wave current. The subsequent fusion of the protoplasts is achieved by a 30-50 usecond D.C. pulse. Tobacco protoplasts subjected to the electrofusion treatment have been regenerated back into plantlets in tissue culture; however, positive hybrid formation has not been clearly established. Biochemically selectable carrot cell lines have also been subjected to the electrofusion treatments and are currently being used to determine if the regenerated tissue is of hybrid origin.

Board 9

RAPID IDENTIFICATION OF ENVIRONMENTAL ISOLATES USING AGAR DOTS. P. A. W. Martin*, R. S. Travers and C. F. Reichelderfer. USDA, Insect Pathology Lab., Beltsville, MD and Dept. of Entomology, Univ. Maryland, College Park, MD.

It has been recently demonstrated that numerous B. thuringiensis strains, displaying a wide variety of toxicities and biochemical characteristics, could be isolated from most soil samples. B. thuringiensis strains have historically been identified immunologically using flagellar antigens, but this method was too expensive and cumbersome for rapid identification of large numbers of unknown strains. The isolation of over 3000 B. thuringiensis strains from soils of 16 states necessitated the development of a rapid, inexpensive way to identify these isolates. The method we developed used droplets of dots made of agar containing the various media for the different biochemical tests. Thirty-two dots were arranged asymmetrically on a petri dish. An inoculator with the same pattern allowed for simultaneous inoculation of 32 strains. Eight biochemical tests were selected, providing 256 subdivisions within the species designation. Thus, several thousand biochemical tests could be run in a day. Using this method, we discovered 60 new varieties of B. thuringiensis which had some striking differences from known varieties. This method allows for rapid identification and characterization of microbes isolated from a variety of environments.

Board 10

CHROMOSOME-MEDIATED TRANSFORMATION IN PETUNIA HYBRIDA: R.J. Griesbach, Florist & Nursery Crops Lab, USDA, ARS, BARC-w, Beltsville, MD 20705

The vacuoles from suspension-cultured cells of Petunia hybrida were removed by centrifugation. The resulting evacuolated protoplasts were then injected with suspension of chromosomes isolated from Petunia alpicola. P. alpicola is both drought tolerant and resistant to white flies. The goal is to transfer these characteristics into the cultivated petunia.

78% of the injected protoplasts received at least a single chromosome. 57% of these protoplasts contained visible chromosome abberations at mitosis (eg bridges) which suggests that the foreign chromosome might have translocated to a host chromosome. About 5% of the injected protoplasts expressed different protein profiles on polyacrylamide gels.

Experiments are now in progress to determine the extent of genetic transformation by screening for drought tolerance of the regenerated plants and by comparing the favinoid markers between control and transformed cell lines.

Board 11

A NOVEL ALLELE OF THE OPAQUE-2 LOCUS IN MAIZE

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In maize, the zein family of seed storage proteins consists of about 15 to 22 polypeptides belonging primarily to two size classes with molecular weights of 22,000 and 19,000, but exhibiting heterogeneity in charge. Depending on the genetic background, plants homozygous for the standard recessive mutation (o2) at the Opaque-2 (O2) locus show a partial to nearly complete suppression of the 22 kd class of zeins, while leaving the 19 kd class largely unaffected. From the selfed progeny of ethyl methane sulfonate-treated seeds we have obtained a new allele (o676) of the O2 locus that, unlike the standard o2 mutant allele, supresses expression of the 19 kd class of zeins to a greater extent than the 22 kd class. This same phenotype persisted after five generations of backcrossing into the inbred lines R802 and Oh43. Although the heterozygotes of the standard o2 allele and the o676 allele produce opaque seed having reduced zein content, the heterozygous state appears to restore expression of nearly all the individual zein polypeptides to levels which, relative to one another, are similar to that found in wild type kernels. Exceptions to this are one 22 kd zein and one 19 kd zein which, as judged by coomassie blue staining, are produced in heterozygotes at levels exceeding at least twice their relative abundance in wild type kernels. These data suggest that O2 may be a complex locus, since perturbations of different regions of the locus appear to affect individual zeins differently.

(Supported by the U.S. Dept. of Energy and a National Science Foundation Postdoctoral Fellowship to RJS)

POSSIBLE RELATIONSHIP BETWEEN AN INSERTION ELEMENT (TGM1) AND SEVERAL MUTABLE ALLELES OF SOYBEAN - Joel M. Chandlee and Lila O. Vodkin, United States Department of Agriculture, Plant Genetics and Germplasm Institute, Plant Molecular Genetics Laboratory, Beltsville, MD 20705.

It has been shown previously that the seed lectin null phenotype (Le-) of soybean (Glycine max) arises from the presence of an insertion element within the coding sequence of the Le1 gene. Both Le1 and the insert (Tgm1) have been sequenced. The insert represents a family of related sequences in the soybean genome. Tgm1 exhibits the structural features of a transposable element; the termini have imperfect inverted repeats of 30 bp and there is a target site duplication of 3 bp within Le1. Also, the termini are similar to known transposable elements from maize (spm-I8) and snapdragon (Tam1 and Tam2). At present, however, there is no evidence for reversion of the Le- to Le+ phenotype or for transposition of Tgm1 to other sites in the genome. Several mutable alleles of soybean are currently being analysed to determine if the unstable nature of the phenotypes they condition correlate with transposition of Tgm1 or a related sequence. Present studies include an analysis of the yellow mutable allele ($Y18^m$) at the Y18 locus which produces yellow patches of leaf tissue among normal green tissue, the ringed mutable allele (r^m) at the R locus which produces a series of concentric black rings upon a brown seed coat, and the allelic series (I, i^k, i^i, i) at the I locus which controls the distribution of pigmentation over the seed coat. Three approaches are being used to detect transposition of Tgm1 or a related sequence: (1) Genetic approach: F_1 and F_2 hybrid seed resulting from crosses between plants bearing the mutable alleles and Le+ and Le- lines are being analysed to determine if any interactions occur to induce transpositional events; (2) Mutagenic approach: Radiation can be used as a form of "genomic stress" to induce mobility of cryptic transposable elements. Le- lines, as well as lines harboring the $Y18^m$ and r^m alleles have been irradiated with gamma radiation (^{60}Co). The M_1 seed are currently being analysed in the field. Additional studies include the propagation through callus culture of those lines containing the mutable alleles as an alternative form of "genomic stress" to induce transposition; and (3) Molecular approach: To detect transpositional events, total genomic DNA is isolated from leaf tissue of plants harboring the mutable alleles, digested with various restriction enzymes and analysed by southern blotting using a probe containing a Tgm1 specific sequence. In the case of the yellow mutable allele, digests of DNA isolated from yellow and green leaf tissue from the same plant are compared. In the other cases, digests of DNA isolated from various isolines containing the alternative alleles (in which the total genomes are >98% isogenic) are compared. The preliminary results from these studies will be presented.

PROTEINS ASSOCIATED WITH THE RHIZOMORPH DEVELOPMENT PROCESS OF A FUNGUS, Armillaria mellea. S.D.Cohen and J.J.Motta, Department of Botany, University of Maryland, College Park, MD. 20740.

Armillaria root disease is caused by a fungal pathogen called Armillaria mellea. This disease affects over 650 different plant hosts. The pathogen produces mycelial strands called rhizomorphs which are the infection structures responsible for the spread of the disease. Rhizomorph development of Armillaria mellea occurs when undifferentiated mycelia grown in a defined media is exposed to a minute amount of ethanol. Synchrony of rhizomorph development has been achieved by utilizing hyphal fragments to generate new cultures in a defined media containing 5 grams/l of glucose. The rhizomorph development process may be divided into four morphological stages, undifferentiated mycelia, aggregation of mycelia, initiation of rhizomorph primordia and rhizomorph elongation. Several inhibitors were dissolved in ethanol and added to undifferentiated mycelia in a defined media to observe the effects on the rhizomorph development process. Cycloheximide concentrations of 5 and 50 ug/ml completely blocked rhizomorph development. Chloramphenicol and puromycin did not have any effect on the development process. Actinomycin D at 20 ug/ml prevented rhizomorph initiation and elongation stages. Proteins were extracted from non-induced and induced freeze-dried mycelia at the rhizomorph elongation stage after ethanol addition. Soluble and insoluble proteins were run on 1-dimensional SDS-polyacrylamide electrophoresis. Significant qualitative and quantitative differences in peptides could be seen on this analysis. We plan to examine the regulation of these peptides during rhizomorph development.

Board 14

THE OCCURRENCE OF DIAMINOPIMELATE DEHYDROGENASE IN ORYZA SATIVA.
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Earlier work demonstrated the presence of diaminopimelate dehydrogenase in Glycine max embryos. Subsequent experimentation resulted in the cloning and expression of a 4.05 kb DNA fragment containing the gene coding for diaminopimelate dehydrogenase from Glycine max. Hybridization experiments demonstrated homology between the 4.05 kb Glycine max fragment and Triticum genomic digests. Diaminopimelate dehydrogenase activity has also been demonstrated in maize endosperm tissue. From these data we postulated the existence of an alternative biosynthetic pathway for the production of lysine in higher plants.

In the present study we will demonstrate diaminopimelate dehydrogenase activity in Oryza sativa extracts. Comparative analysis of the rice dehydrogenase to species occurring in Glycine max, and the bacterium Bacillus sphaericus will be presented.

Board 15

MUTANT T-DNA (ROOTY) ALTERS MORPHOGENETIC POTENTIAL OF CULTURED SOYBEAN TUMOR
Lowell Owens, Tissue Culture and Molecular Biology Lab, USDA, ARS, Beltsville, MD., USA, 20705

Soybean (cv Peking) plants and excised cotyledons formed crown galls with roots in response to infection with Agrobacterium tumefaciens bearing a rooty mutant tumor-inducing plasmid (strain tmr-338::Tn5). When unorganized portions of these galls were cultured in vitro they again regenerated roots. Four of five such tumor lines have continued to exhibit this trait for up to a year. Roots excised from these tumor lines proliferated on agar medium lacking phytohormones and produced octopine. Expression of the T-DNA (transferred-DNA) borne trait of octopine synthesis established that these roots are at least partially comprised of transformed cells. Callus cultures of several tmr tumor lines required cytokinin as the sole phytohormone necessary for growth. This phenotype is consistent with the genotype of the Ti plasmid mutant used to incite these tumors, i.e., its T-DNA region harbors active genes for auxin biosynthesis but a defective gene for cytokinin biosynthesis. Nontransformed callus of Peking soybean failed to regenerated roots when supplied with high concentrations of synthetic auxin (NAA), cytokinin (kinetin) or 2,4-D in the medium. Apparently auxin synthesized endogenously by T-DNA borne genes has an effect on cell morphogenesis that cannot be duplicated by exogenous auxin.

Board 16

WOUND-SPECIFIC GENE EXPRESSION IN TOMATO FRUIT

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Increases in the activities of ACC synthase, chitinase and ribonuclease are some of the early events that characterize wounding stress in tomato fruit. Two-dimensional SDS-PAGE analysis of the in vivo ^{35}S-labeled proteins and the translational products obtained upon priming heterologous cell free systems with poly A^+ RNA revealed the appearance and disappearance of specific gene transcripts and proteins. These data suggest stress-specific regulation of gene expression. A cDNA library of total poly A^+ RNA from the wounded fruit was constructed and using differential hybridization strategy, stress-specific clones were isolated. Some of these cDNA clones have been characterized for insert sizes, identification of the specific mRNA, and protein products translated in vitro by positively selected mRNA. Studies are in progress to identify the clones and mRNA's specific for ACC synthase, chitinase and ribonuclease.

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