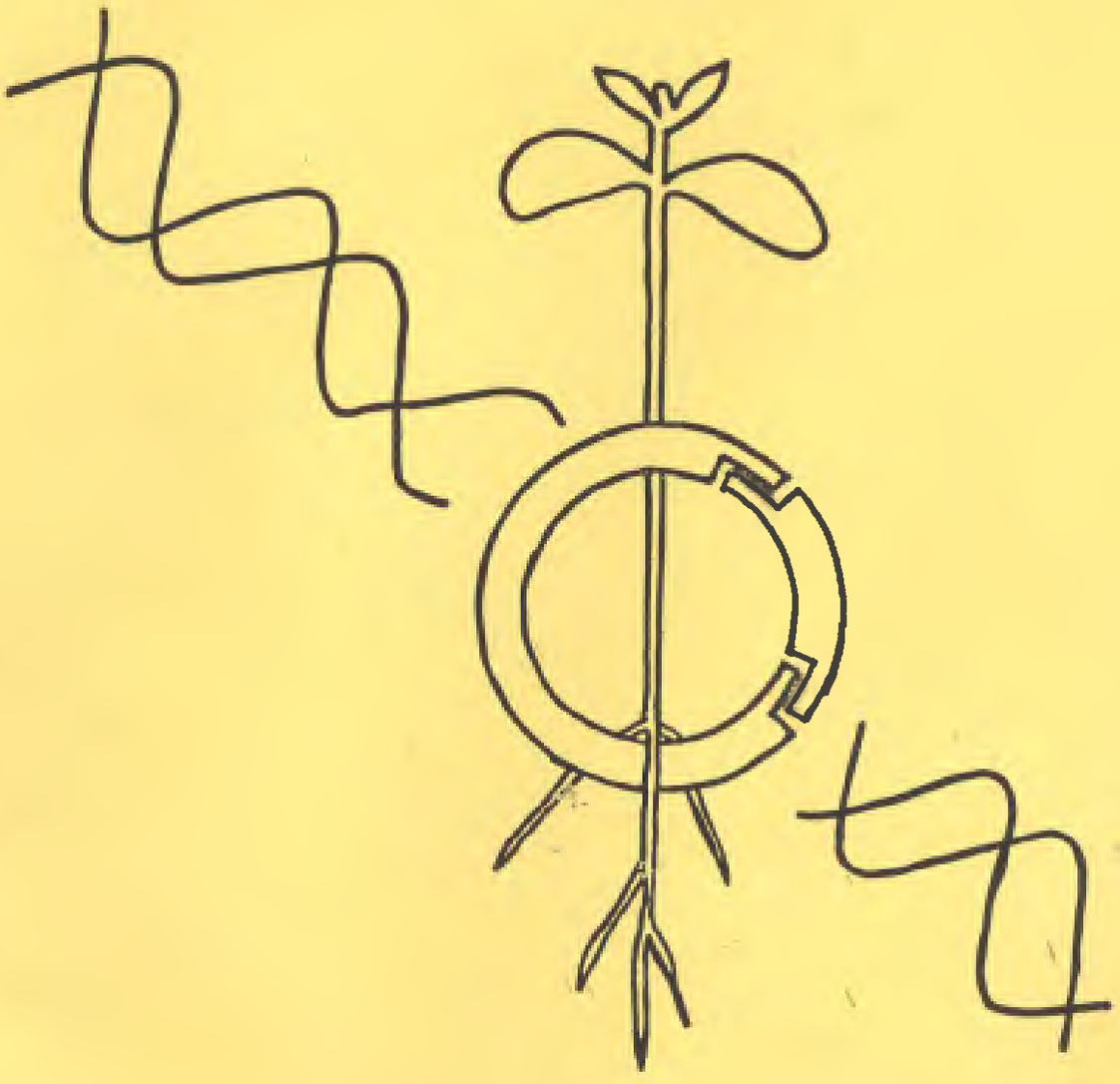
MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY MEETING



October 18 & 19, 1984

FIRST ANNUAL MEETING

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

October 18 & 19, 1984

THE NATIONAL ARBORETUM

WASHINGTON, D.C.

CONTENTS

INTRODUCTION

Welcome to the first 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. Hopefully, the society will ensure scientists 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 discussions. This will be promoted at our next meeting by offering monetary prizes for best oral report and best poster presented by graduate students.

The first year has been dedicated to setting up the society and its first meeting. If the society is to continue, participation by members in running the society and meeting is needed. This society is extremely flexible; we have no ground rules. Therefore, everyone is encouraged to help out and provide advice and personal views. On Friday at 12:00 noon there will be a business meeting, which the entire membership is encouraged to attend. Let's discuss freely the Mid-Atlantic Plant Molecular Biology Society and its future. The society owes a special thanks to the speakers, exhibitors, and those deeply involved in organizing this meeting, especially Sue Mischke (Registration Committee and mailings); Lorin DeBonte (Posters and Abstracts Committee, all the little details); Janet Slovin, Autar Mattoo, Jerry Cohen, Rob Griesbach (Program Committee); Freddi Hammerschlag, Murray Spruill (Finance Committee); Jim Saunders (Publicity Committee; breaks, and lunch), and others too numerous to mention.

Benjamin F. Matthews
Chairman
Organizing Committee

GENERAL INFORMATION

All sessions will be held in the Administration Building of the National Arboretum, Washington, D.C. The oral presentations will take place in the auditorium on Thursday and Friday. The poster session will be held on Thursday, in the lower level conference room from 12:30 pm to 2:00 pm. Refreshments provided by Ace Scientific Supply Co., Inc., will be available in this lower level room during the morning breaks.

At the registration desk information will be made available pertaining to dining out in the Washington area, and some local information. If you are interested in the catered lunch, please pay while in the lunch line.

EXHIBITORS

We would like to acknowledge the exhibitors of 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 representatives during the sessions to find out how they can further help you in your research.

The exhibitors are located in the lower level conference room.

The exhibitors are:	Representatives:
ACE SCIENTIFIC SUPPLY CO., INC.	Laura Dresdner
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CARL ZEISS, INC.	Jim Schindele

PROGRAM SCHEDULE

THURSDAY, OCTOBER 18

8:30 Assemble posters
9:00 Introduction

SESSION I - CONVENOR - Benjamin Matthews			PAGE
9:05	Andrew Binns	Are host hormone autonomy systems induced by the T-DNA?	1
10:00	Lowell Owens	Genotypic variability of soybean response to Agrobacterium strains harboring the Ti or Ri plasmids.	2
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10:40	BREAK*		
11:00	Stephanie Curtis	Structure and expression of cyanobacterial photosynthesis genes regulated during nitrogen fixation.	4
12:00	LUNCH		
12:30	POSTER SESSION		

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2:00	Charles Arntzen	The molecular characterization of an herbicide receptor protein in photosynthetic membranes.	5
3:00	Kenneth Leto	Nuclear mutation leads to an accelerated turnover of chloroplast encoded 48,000 dalton and 34,500 dalton polypeptides in thylakoids lacking photosystem II.	7
3:20	Benjamin Matthews	Variation of organelle DNAs in carrot and their wild relatives.	8
3:40	Madeline Wu	Characterization of one Chlamydomonas chloroplast DNA replication origin.	9

FRIDAY, OCTOBER 19

*Refreshments during breaks provided by:
Ace Scientific Supply Co., Inc.

ARE HOST HORMONE AUTONOMY SYSTEMS INDUCED BY THE T-DNA?

Andrew N. Binns
Department of Biology
University of Pennsylvania
Philadelphia, PA 19104

The molecular basis of the crown gall disease has, to a large extent, been elucidated. Virulent strains of Agrobacterium tumefaciens transfers a portion (at least) of the Ti plasmid into competent host cells. This T-DNA is subsequently inserted into the nuclear DNA and is actively transcribed. Extensive molecular, genetic and biochemical analysis has shown that a 9kb section of the T-DNA from various Ti plasmids is quite conserved in terms of sequence and function. Of the six genes identified, three have been assigned functions: genes 1 and 2 (tms 1+2) are believed to encode enzymes that convert trypotphan to indoleacetamide and subsequently to indoleacetic acid, while gene 4 (tmr) codes for an enzyme (isopentenyl transferase) that synthesizes isopentenyl adenosine, a cytokinin. Together these three activities can account for the uncontrolled growth observed in crown gall tumors.

In our laboratory we are examining possible host involvement in the tumorous state. Specifically, some plants (e.g. N. glauca, N. glutinosa) can respond in a virulent fashion to Agrobacaterium strains mutated in one or both of the genes specifying IAA biosynthesis. We have shown that the resultant tumors can grow continuously in culture on hormone free medium, and contain IAA in amounts equivalent to wild type tumors. We are conducting experiments to determine whether other T-DNA genes are involved in auxin biosynthesis or, rather, induce host auxin autonomy systems. In addition, results will be presented which indicate physiological differences between the hypothesized host auxin autonomy and the gene 1 and 2 directed auxin autonomy.

GENOTYPIC VARIABILITY OF SOYBEAN RESPONSE TO AGROBACTERIUM STRAINS HARBORING THE Ti or Ri PLASMIDS.

Lowell Owens and Dean Cress, Tissue Culture and Molecular Genetics Lab, USDA, ARS, Beltsville, MD 20705.

Twenty four diverse cultivars of soybean (Glycine max (L.) Merrill) and three lines of its annual wild progenitor G. soja Sieb. and Zucc. were tested for their response to Agrobacterium strains harboring either the Ti plasmid from A. tumefaciens or the Ri (root-inducing) plasmid from A. rhizogenes following uniform wounding and inoculation. Based upon gall weight at eight weeks post-infection, three G. max cultivars (Biloxi, Jupiter and Peking) and one G. soja line (Plant Introduction (PI) 398.693B) were judged highly susceptible to the Ti-plasmid strain A348, ten genotypes moderately susceptible, 11 weakly susceptible and two nonsusceptible. Of 26 genotypes inoculated with the Ri-plasmid strain R1000, only seven responded in a clearly susceptible fashion by forming small, fleshy roots at internodal infection sites. Cotyledons excised from one- or three-day old seedlings of Peking and Biloxi cultivars also formed galls when infected in vitro with agrobacteria harboring either the Ti or Ri plasmid. Tumor lines established from cotyledon and stem galls induced by the Ti-plasmid strain A348 exhibited the T-DNA borne traits of phytohormone-independent growth and octopine synthesis. Additionally, DNA isolated from cultured tumors hybridized with labelled T-DNA probe. Tumors incited by A. tumefaciens tmr 338 were rhizogenic when cultured in vitro and remained so through many subcultures of their unorganized tissue.

The Electro-fusion and culture of tobacco protoplasts.

James A. Saunders, Lee Ann Roskos, and Kin Lin Chao, Tobacco Laboratory, Plant Genetics and Germplasm Institute, Beltsville Agriculture Research Center, USDA, Beltsville, MD 20705.

Protoplasts isolated from Nicotiana rustica and Nicotiana tabacum leaf tissue can be fused to themselves and to each other by electrofusion procedures. The procedure involves the alignment of the isolated protoplasts in a non-ionic medium with low voltage sine wave current. Subsequent fusion of adjacent calls is accomplished by a rapid non-repetitive D.C. pulse which momentarily disrupts the plasma membrane at the cell-to-cell interfaces. We have successfully cultured the protoplasts in low density cell suspension cultures through several cell divisions using Kao-8-P media. The electrofusion apparatus can be easily manufactured using commercial function genetators with little or no modification. The specific voltage frequency and duration of the electric currents must be optimized for each cell type to obtain viable fusion products. This procedure has the potential to be an important tool in the transfer of foreign materials across cell membranes.

STRUCTURE AND EXPRESSION OF CYANOBACTERIAL PHOTOSYNTHESIS GENES REGULATED DURING NITROGEN FIXATION.

Stephanie E. Curtis, Dept. Genetics, Box 7614, North Carolina State University, Raleigh, NC 27650

The research in my laboratory is directed toward understanding the regulation of nitrogen fixation in Anabaena 7120, a filamentous cyanobacterium (blue-green alga). We are especially interested in the differentiation of heterocysts, specialized cells which develop in response to nitrogen starvation, and in which nitrogen fixation exclusively occurs. Among the biochemical and morphological changes accompanying heterocyst differentiation are rearrangements in the photosynthetic apparatus, including loss of Photosystem II and Calvin cycle activities. We have focused on the control of photosynthesis in heterocysts by looking at the regulation of genes encoding photosynthetic polypeptides. These genes have been isolated with the use of heterologous photosynthetic gene probes from plant chloroplast DNAs. The structure, organization and expression of genes encoding certain Calvin cycle, Photosystem II and ATPase complex polypeptides will be discussed.

THE MOLECULAR CHARACTERIZATION OF AN HERBICIDE RECEPTOR PROTEIN IN PHOTOSYNTHETIC MEMBRANES: C. J. Arntzen*, David Kyle, Carl Gilbert, Kathy Williams, and John Williams. MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan.

The triazine herbicides inhibit the growth of weeds by blocking photosynthetic electron transport. Research in our laboratory, as well as at a number of other locations throughout the world, has been focused in recent years on the identity of the triazine receptor protein, the molecular determinants of herbicide binding, and mechanisms of target-site mediated herbicide resistance which are determined by this protein.

The triazine receptor protein is a polypeptide of approximately 32 kilodaltons which serves a functional role in the photosystem II complex of chloroplast membranes. Triazine-binding results in displacement of a bound plastoquinone (designated as Q_B) which acts as a secondary electron acceptor for the photosystem II complex. The herbicide receptor protein is, therefore, now designated as the Q_B protein. This polypeptide is encoded on chloroplast DNA and is synthesized as a larger size-class precursor. The protein undergoes rapid turnover in chloroplasts, especially at high light intensities due to a damage process termed "photoinhibition".

In purple photosynthetic bacteria, a 31.5 kilodalton polypeptide of the reaction center complex has been identified as a triazine herbicide receptor. In functional comparisons, the mode of action of triazine herbicides in these bacteria is identical to that in the photosystem II complex of higher plant chloroplasts in that herbicide binding displaces a bound quinone (also designated Q_B) of the L subunit of the reaction center.

The analysis of herbicide binding and herbicide receptor proteins in photosynthetic membranes has been aided by characterization of herbicide-resistant mutants. Naturally occurring weed biotypes which are resistant to the triazine herbicides have been found to have a subtle change in the primary sequence of the Q_B protein. A single base change in the chloroplast-localized gene coding this protein results in a serine to glycine conversion in the two resistant weed species thus far characterized. In a triazine-resistant photosynthetic bacterium, the amino acid sequence of the L protein (derived from the DNA sequence of the cloned gene encoding the L subunit) also showed the change of a single amino acid in comparison to the susceptible wild type. A gene transfer vector has been

*Current Address: Du Pont Experimental Station, Building 402, Room 3149, Wilmington, Delaware 19898

successfully utilized to transform herbicide-susceptible bacteria to resistance using a segment of the L subunit gene. These latter data conclusively demonstrate that the L subunit of the reaction center is the herbicide receptor protein. The availability of a transformation system which confers herbicide resistance allows new efforts at site-directed modification of the polypeptide which should give a thorough characterization of the molecular determinants for both the quinone and herbicide binding sites in photosynthetic membrane polypeptides.

NUCLEAR MUTATION LEADS TO AN ACCELERATED TURNOVER OF CHLOROPLAST-ENCODED 48kDa AND 34.5kDa POLYPEPTIDES IN THYLAKOIDS LACKING PHOTOSYSTEM II.

Kenneth J. Leto(1), Erin Bell(2), and Lee McIntosh(2)
(1) DuPont Co., Central Research and Development, Wilmington, De. 19898
(2) Michigan State University, E. Lansing, Mi. 48824

The green nuclear maize mutant hcf*-3 lacks organized photosystem II reaction centers. Thylakoids from this mutant show reduced accumulation of the 34.5kDa atrazine binding protein and lack a 48kDa photosystem II chla-reaction center protein. Both of these polypeptides are encoded on chloroplast DNA. We find that the loss of these polypeptides is due to their increased turnover in hcf*-3 membranes as indicated by the following: (A) Wild type and hcf*-3 plastids contain equal message levels for the 34.5kDa polypeptide as determined by dot blot analysis of total chloroplast RNA probed with pZmc 427. Thus, loss of this polypeptide is post transcriptional. (B) Both the 48kDa and the 34.5kDa polypeptides are initially synthesized and inserted into hcf*-3 stroma membranes in normal amounts, but; (C) following insertion, both the 34.5kDa and the 48kDa polypeptides turn over prematurely in mutant thylakoids. This accelerated turnover occurs before or during the process which normally leads to the enrichment of these polypeptides in the grana. The accelerated turnover of the 34.5kDa polypeptide in hcf*-3 plastids is not due to photoinhibition, since accelerated turnover is also seen in the dark. Rapid turnover of polypeptides which fail to assemble into complexes may be a general feature of thylakoid biogenesis.

VARIATION OF ORGANELLE DNAs IN CARROTS AND THEIR WILD RELATIVES.

B. F. Matthews[1], P. J. Larivee[1,2], L. R. DeBonte[1], R. W. Fisher[2].[1] USDA Tissue Culture and Molecular Genetics Laboratory, Beltsville, MD 20705, and[2] Dept. of Biology, Virginia Commonwealth University, Richmond, VA 23298.

A system is being developed to study somatic cell hybridization and organelle transfer using Daucus carota subsp. sativus var. Danvers (DAN), D. capillifolius (CAP), D. carota subsp. gummifer (GUM) and D. pusillus (PUS). These species are morphologically different and were examined for heterogeneity in organelle DNAs. The chloroplast (ct) DNAs of DAN and CAP were very similar while GUM and PUS were increasingly different. Maps of the 160kb ctDNAs from DAN and PUS were produced by single and double restriction digestion using the endonuclease restriction enzymes SmaI, XhoI and SalI. Furthermore, probes of known genes were used to localize several genes by hybridization techniques. The genes encoding the large subunit of ribulose-bisphosphate carboxylase (rbcL) and the 32KD photosystem II protein (psbA) were localized in the large, 88-91 kb single-copy region, while the genes encoding the 16S and 23S rRNAs were located within an inverted repeat unit of approximately 16kb. The 4.5S + 5S rRNAs extend to the 38-41 kb small single-copy region. Although restriction site variation between the ctDNAs of DAN and PUS exists the positional arrangement of the genes is preserved.

The restriction patterns of the mtDNAs from these Daucus species are very different. There was a closer relationship of DAN to CAP mtDNA than to that of GUM or PUS. This confirms the relationship found using the ctDNAs. The sizes of the mtDNAs range from 386 to 468 kb. No variation in ctDNA or mtDNA restriction fragment patterns was found when organelle DNAs from cells cultured under various cell culture conditions or lengths of time were examined.

Characterization of one Chlamydomonas chloroplast DNA replication origin. M. Wu, X. M. Wang and C.H. Chang. Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Maryland 21228, USA

Chloroplast (Ct)DNA, isolated from a synchronous culture of Chlamydomonas reinhardii, was digested with restriction endonucleases and examined in the electron microscope (EM). Restriction fragments containing displacement loops (D-loops) were photographed and measured to determine the position of replicated sequences in relation to the restriction enzyme sites. D-loops (the putative replication origins) were located at two positions on the physical map of chloroplast DNA. One replication origin was mapped at about 10 kb upstream of the 5' end of a 16s rRNA gene. The second origin was spaced 6.5 kb apart from the first origin and was about 16.5 kb upstream of the same 16s rRNA. Replication is initiate with the formation of a D-loop resulting from the synthesis of one daughter strand. After a short initial lag phase, corresponding to the synthesis of 350 $\pm$ 130 bp of one daughter strand, DNA synthesis then proceeds in both directions. CtDNAs from two species of Chlamydomonas have been purified. Clone libraries containing the CtDNA EcoRl restriction fragments have been constructed in E. coli plasmid pBR 325. The EcoRl restriction fragments containing D-loops which marked the replication origin of CtDNA were identified in both species of Chlamydomonas. The cloned fragments were compared by restriction endonuclease analyses and by heteroduplex analyses in the EM to detect the homologous regions. The relative position of the D-loop region and the homologous regions between the two fragments was determined. The D-loops were located within one short homologous region of 0.42 kb in length between the two cloned EcoRl restriction fragments. The homologous regions was subcloned in pBR 322. The coding capacity of this homologous region and its adjacent regions were examined by Northern blot analyses, our result suggests that the 0.42 kb homologous region cross hybridize with a small stable transcript. A portion of the DNA sequences in the homologous region was determined, we detected extensive AT rich regions, direct repeats, inverted repeats and secondary structures.

PLANT GENE STRUCTURE. Joachim Messing, University of Minnesota, St. Paul, MN 55108.

DNA sequencing with restriction fragments has been a powerful method of obtaining information about gene structure. When the purification method of these fragments has been changed from a physical separation technique to a cloning step, the sequencing of particular large genomes like Cauliflower mosaic virus or lambda DNA has become more economical. It has been particularly useful to combine the cloning into vectors derived from bacteriophage M13 and the chain termination technique of DNA sequencing. An important feature of this combination is the application of a universal primer which is used to target the DNA sequencing reaction to the cloned DNA.

We have used this approach to determine the nucleotide sequence of a number of cDNA and genomic clones representing genes of the zein multigene family of maize. The results have been used to compare them to other plant gene sequences and to ask the question what potential signal sequences for regulatory events can be observed. The strategy for this type of comparison is based on what has been learned from functional tests in other eukaryotic systems.

Specific Molecular Changes During Tomato Fruit Ripening and in Response to Wounding Stress

AUTAR K. MATTOO[1,2], HIRA L. NAKHASI[3] AND JAMES D. ANDERSON[2]

[1]Department of Botany, University of Maryland, College Park, Md. 20742

[2]Plant Hormone Laboratory, USDA/ARS, BARC(W), Beltsville, Md. 20705

[3]Division of Biochemistry and Biophysics, Office of Biologics, FDA, Bethesda, Md.

A marked rise in the biosynthesis of ethylene characterizes early events during fruit ripening and several stresses, such as wounding. Our aim is to unravel the common processes that are associated with ethylene production during fruit ripening and upon wounding stress. During tomato fruit ripening specific gene transcripts appear or disappear as revealed by *in vitro* translation of poly A^+ RNA from different developmental stages. Similarly, differences in mRNA populations were found between unwounded and wounded ripe fruit. These data suggest ripening- and stress-specific regulation of gene expression. In order to understand molecular changes during these processes, a cDNA library of total poly A^+ RNA from wounded fruit was constructed. Using differential hybridization strategy, ripening- and stress-specific cDNA clones were isolated. One of these clones, called pTC'2, having a 580 bp cDNA insert was selected as a probe to follow changes in its mRNA during ripening and wounding using northern blot and dot blot techniques. Two RNAs (5.1 and 2.2 kb) hybridized to the pTC'2 cDNA. The steady state level of the smaller, 2.2 kb RNA decreased as ripening progressed but increased slightly upon wounding; the larger RNA was produced constitutively. In addition, fruits carrying a ripening inhibitor gene, *rin*, do not ripen and also were unable to decrease the level of the developmentally regulated, smaller RNA. Studies are in progress to characterize the protein products that are coded for by the two differentially regulated mRNAs and their possible relation to ethylene biosynthesis.

ISOLATION AND CHARACTERIZATION OF A GENE ENCODING DIAMINOPIMELATE DEHYDROGENASE FROM GLYCINE MAX. L. K. Wenko*, R. W. Treick*, and K. G. Wilson+. *Department of Microbiology, and+Department of Botany, Miami University, Oxford, Ohio 45056.

A detailed characterization of the lysine biosynthetic pathway in plants is yet to be completed. It is, however, assumed that the diaminopimelic acid pathway exists in the plant kingdom as commonly described for Escherichia coli.

Misono and Soda have isolated and characterized the enzyme diaminopimelate dehydrogenase from Bacillus sphericus and bacterial species other than E. coli. This enzyme catalyzes the reversible oxidative transamination of tetrahydrodipicolinate directly to meso-diaminopimelate. This alternative shortens the classic nine step diaminopimelic acid pathway by three enzymatic steps. Modification and refinement of lytic complementation, a technique previously utilized in bacterial systems, facilitated the isolation of a functional gene from a Glycine max nuclear gene library. The isolated gene codes for the enzyme meso-diaminopimelate dehydrogenase. The coding capacity for the enzyme was originally contained on a 6.6kb fragment in a Charon 4-A soybean gene bank. Subcloning of the 6.6kb fragment resulted in the recombinant plasmid pMW75. Subsequent investigation revealed the enzyme was coded for on a 4.05kb Eco-Sal restriction fragment of the original 6.6kb fragment. This 4.05kb fragment is contained in the recombinant plasmid pLW14. One region of homology was observed upon hybridization of pMW75 to EcoR1 digested soybean DNA. Homologous sequences were also observed in triticum DNA. Maximum enzymatic activity of the clonal enzyme was observed at a pH of 8.0. The cloned soybean enzyme has an apparent molecular weight of 67,000.

Mezo-diaminopimelate dehydrogenase activity has been demonstrated in developing soybean embryos, but not in leaf or tissue culture preparations. These data suggest that the gene is expressed when lysine is needed for storage protein synthesis.

CHARACTERIZATION OF LECTIN GENES AND A TRANSPOSABLE ELEMENT IN SOYBEAN: Vodkin, L. O., Rhodes, P. R., and Goeken, R. M., Bldg. 006, Plant Genetics and Germplasm Institute, Agricultural Research Service, Beltsville, MD. 20705

Lectin, a carbohydrate-binding tetrameric protein, normally constitutes up to 5% of the total protein in protein bodies of cotyledon cells in soybean (Glycine max). The DNA sequence for the single gene, Le1, coding for lectin has been determined. It has a 32 amino acid signal sequence, encodes a 1000 bp message, and does not contain any introns. A naturally occurring mutation in some soybean cultivars results in the absence of lectin protein. Data showing that the basis of this mutation is a large insertion of DNA into the coding region of the lectin gene will be summarized. The 3.6 kb insertion, designated Tgm1, has the structural features of a transposable element including inverted repeat termini and duplication of a small region of the target gene. In contrast to the simple structure of the target gene, Tgm1 is complex and highly structured. No large open reading frames are present; but an extensive border of repeating dyad symmetries each having a 7bp sequence similarity to the lectin gene is a characteristic feature of the element. The termini and repeating unit format of Tgm1 denote a distinct class of eukaryotic elements which include Tam1 in snapdragon and suppressor-mutator (Spm) in corn. Glycine soja, a non-domesticated relative of soybean, also contains an element resembling Tgm1 which interrupts the lectin gene in this species.

THE CURRENT STATUS OF VIROID RESEARCH: T. O. Diener and R. A. Owens, Plant Virology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland 20705

Since the formulation of the viroid concept 13 years ago, knowledge of these subviral pathogens has accumulated at an exponential rate. Modern RNA sequencing methods have allowed the rapid elucidation of their unique structural properties. Three viroid groups can be distinguished on the basis of nucleotide homologies (1) the potato spindle tuber viroid (PSTV) group, to which also belong the tomato planta macho, tomato apical stunt, citrus exocortis, chrysanthemum stunt, and (probably) the Columnea viroids; (2) coconut cadang-cadang viroid; and (3) avocado sunblotch viroid. The molecular basis of viroid function is still largely unknown but viroids have structural similarities to transposons and introns. A (ds) cDNA representing the complete sequence of PSTV has been constructed, cloned, and its infectivity in tomato plants verified. The construction of deletion and substitution mutants of PSTV is now possible. These may permit identification of specific viroid regions involved in host specificity, replication, or disease induction. Consensus now exists that viroids are (1) not translated, (2) replicated by host enzymes from RNA templates, (3) replicated by a rolling circle-type mechanism, resulting in the synthesis of oligomeric strands of PSTV and cPSTV. The molecular mechanism of viroid pathogenesis may involve interference with m-RNA synthesis and/or processing. The metabolic aberrations responsible for symptom expression are unknown, but a disease-associated M_r 70,000 protein has been identified and purified. This protein occurs in infected cells as a dimer of M_r 140,000; serological evidence indicates that it is a host-specific and not a viroid-coded protein: it occurs in small amounts in healthy plants, it accumulates in tomato plants infected with any one of four viroids, but not in other host species infected with some of the same viroids. The protein is associated with pathogenicity and not with viroid replication per se: it is not detectable in symptom- less, viroid-infected tomato plants and its subcellular distribution does not correlate with that of the viroid.

SEQUENCE HOMOLOGIES BETWEEN THE SATELLITE OF PEANUT STUNT VIRUS AND VIROIDS, INTRONS, AND RNASE P: Candace Whitmer Collmer, A. Hadidi, and J. M. Kaper, Plant Virology Laboratory, Plant Protection Institute, U.S.D.A., Beltsville, MD 20705.

The complete nucleotide sequence of the satellite of peanut stunt virus (PSV-Associated RNA 5 or PARNA 5) has been determined. PARNA 5 is a linear RNA containing a 5' cap, a 3' hydroxyl group, and two open reading frames which extend most of the length of its 393 nucleotides. Although the 5' and 3' ends are homologous with those of CARNA 5, the satellite of the related cucumber mosaic virus, little other homology exists between these linear RNAs. In contrast, PARNA 5 has several regions of 90% sequence homology with various plant viroids, including sequences of the conserved central region of most viroids. Such homologies suggest a common origin with viroids coupled with specific adaptation as a linear RNA.

A possible origin for both PARNA 5 and viroids from introns of eukaryotic RNAs is suggested by the presence in each of six regions highly conserved across both nuclear and mitochondrial introns. The established involvement of the conserved intron sequences in forming a secondary structure essential to proper splicing suggests a similar role for the PARNA 5 sequences, perhaps in the processing of PARNA 5 multimers to monomers at some stage of its replication.

Finally, there are striking homologies between PARNA 5 and the RNA of RNase P, a tRNA processing enzyme. Each has a very similar and unusual nucleotide composition, containing over 33% G and less than 20% U, with long runs of G and several purine-rich regions. Such features may be involved in the recognition between PARNA 5 and its helper virus, suspected to have a tRNA-like 3' terminus.

IN VITRO TRANSLATION OF SEVERAL SATELLITE RNAS OF CUCUMBER MOSAIC VIRUS: Maria Avila-Rincon, C. W. Collmer, and J. M. Kaper, Plant Virology Laboratory, Plant Protection Institute, U.S.D.A., Beltsville, MD 20705.

CARNA 5 is a small, 334-339 nucleotide, linear, satellite RNA associated with cucumber mosaic virus (CMV) upon which it depends for replication. CARNA 5 occurs as a population of variants with different nucleotide sequences that contain open reading frames capable of coding for different polypeptides. The presence of CARNA 5 generally ameliorates CMV symptom expression; however, some CARNA 5s elicit a lethal tomato necrosis disease. Whether these effects are mediated at the nucleic acid level or through polypeptide translational products remains to be determined.

Three different CMV satellite RNAs, (S_1)CARNA 5 from CMV strain S, (1)CARNA 5 from CMV strain 1 and (n)CARNA 5 from CMV strain D or WT, have been purified by propagation in particular host plants and elution from polyacrylamide gels capable of resolving CARNA 5 sequence variants. Each was translated in a wheat germ protein synthesis system. Translation of only (S_1)CARNA 5, a non-necrotic satellite RNA, produces the two small polypeptides previously described by Owens and Kaper (Virology 80, 196-203, 1977) for CARNA 5 isolated from CMV-S. Similar results are obtained with (S_1)CARNA 5 isolated from virions or from its double stranded form. This CARNA 5 has been sequenced and contains one open reading frame that could code for a 5.4K polypeptide, the estimated size of its larger translational product. Tryptic peptide maps of (S_1)CARNA 5 *in vitro* translation products show that these polypeptides are not related to CMV-S coat protein and that they are closely related to each other.

(n)CARNA 5 and (1)CARNA 5, although less efficient than (S_1)CARNA 5, each direct the *in vitro* synthesis of products migrating to unique positions in SDS-polyacrylamide gels containing 8M urea. Two dimensional tryptic peptide maps of these products should establish the relationship among the protein products of all three CARNA 5s, whose nucleotide sequences show extensive homology. Experiments using hybrid-arrested translation are in progress to prove definitively that the products obtained *in vitro* are directed by CARNA 5 and not by contaminant genomic RNA fragments.

EPITOPE ANALYSIS OF THREE ILARVIRUSES USING MONOCLONAL ANTIBODIES. Ramon Jordan[1], J. Aebig[2], and H.-T. Hsu[2]. USDA-ARS[1], Beltsville, MD 20705, USA and American Type Culture Collection[2], Rockville, MD 20852, USA

Seven monoclonal antibodies (McAbs) were utilized to evaluate epitope distribution on isolates of apple mosaic virus (ApMV), Prunus necrotic ringspot virus (PNRV) and Danish plum line pattern virus (DPLV). Initial investigations included determining virus serogroup specificity, affinity and binding activity of each of the McAbs in several different ELISA procedures. The McAbs can differentiate at least 5 different serogroups of ApMV and 3 different serogroups of PNRV (including DPLV). The binding activity for most of the McAbs was high when tested against virus adsorbed to polyvinyl chloride microtiter plates. However, two of the ApMV-specific McAbs did not react to virus bound to rabbit anti-virus immunoglobulin-coated plates. The McAbs were purified by salt fractionation, and sephacryl and DEAE Affi-gel blue column chromatography before labelling with biotin for use in an avidin-biotin ELISA-based competitive inhibition assay. The results obtained from analysis of McAb isotype and virus serogroup reactivity coupled with data from the competitive inhibition assays were used to attempt to assign site specificity as defined by each of the McAbs. The analysis of these data suggest that at least 6 different antigenic sites can be delineated by the seven McAbs used in this study, and that one of the McAbs recognizes an epitope common to ApMV, PNRV and DPLV. Results and analysis of the binding characteristics of the McAbs to detergent- or enzyme-treated virions and capsid protein as evaluated in ELISA, dot-blot and western-blot assays will also be presented.

ELECTRIC FIELD-INDUCED EFFECTS ON PROTOPLASTS. Sue Mischke and Lowell Owens, Tissue Culture and Molecular Genetics Lab, PPHI, USDA-ARS, Beltsville, MD 20705.

The realization of potentials offered by genetic engineering rests on the development of fundamental techniques, including that of gene transfer. Current methods are not particularly efficient and a number of investigators are devoting their efforts toward devising practical methods of DNA delivery and cell fusion. Among the most recently developed techniques is methodology based on the observation that one or several electric pulses of microsecond duration and kV/cm strength will induce membrane pores. Possible applications of this technique to plant genetic engineering include transformation by creating pores through which DNA can pass, or "electroporation", entrapment in the cytoplasm of plant protoplasts of bacteria carrying vector-borne genes, and "electrofusion" of protoplasts from different origins to achieve new genomic combination leading to previously unattainable hybrids with new properties. Despite the promise of the technique, little of practical significance has been achieved thus far. The vast majority of experiments have been performed with artificial lipid bilayers or red blood cell ghosts and scant attention has been paid to mesurement of cell viability. From successes that have been claimed it is clear that optimum conditions must be found for each system empirically.

The achievement of electrofusion depends upon two processes, each controlled by an electric signal. A sine wave is used to align the cells, which are suspended in a non-electrolyte solution, in the 100-500 micron space between two electrodes. The formation of "pearl chains" of cells depends upon a number of parameters including signal frequency, voltage and wave shape; solution conductivity, cell concentration and time. Experiments performed exposing $4x10^5$/ml Nicotiana tabacum suspension-cell protoplasts in 0.7M mannitol, to a sine wave of 100 V/cm at variuos frequencies demonstrated superior alignment against the electrodes of frequencies between 10^5 and 10^7 Hz. Protoplasts aligned poorly at lower frequencies.

The second electric signal necessary for fusion is a short electric pulse which induces membrane pores. It is believed that fusion occurs when apposed pores are generated in the flattened contact zones of membranes, establishing channels which lead to cytoplasmic continuity between adjacent cells. The induction of permeability was examined independently of cell fusion in N. tabacum protoplasts. A double staining method distinguishes reversible (nonlethal) electric field-induced poration from mechanical breakdown that leads to cell death. Based on phenosafranin staining alone, permeability appears to be related to field strength for a 10 msec pulse. Fluorescein diacetate staining reveals that viability has a strong inverse dependence on field strength, and when this is taken into account, the percent of viable electroporated cells does not reproducibly increase with increasing field strength. When the osmolarity of the medium is reduced to 0.5M, the increase in permeability predicted by the electro-mechanical compression model of pore formation favored by Zimmerman (1982, BBA 694:227) is not observed. Preliminary data suggests that 20 mM Ca^{++} increases survival by stabilizing the membrane against electroporation.

Board 2

GENETIC DIFFERENCES IN THE THEORETICAL MAXIMUM SPECIFIC ACTIVITY OF RUBP CARBOXYLASE/OXYGENASE

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Large, significant, and reproducible differences have been observed in the theoretical maximum specific activity (SAmax) of the enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) among a number of crop species. These differences indicate a hitherto unexplored potential for increasing the rate of carbon fixation in crop plants through alteration the chloroplast genome. Species examined include spinach (2.8 micromoles CO_2-fixed/mg RuBisCO·min), soybean (1.6), tobacco (1.9), and wheat (3.8). The SAmax measured in crude leaf extracts or purified enzyme is similar. RuBisCO concentration is estimated from the irreversible-binding of the labelled substrate-analog, 2-carboxy-arabinitol-1,5-bisphosphate. Differences in other kinetic properties, Km(CO_2) and Km(RuBP), and the ratio of RuBisCO protein to total leaf protein (ca. 0.35) are much smaller and probably not significant among C_3-crop species. The SAmax appears to be genetically determined because it is similar among related species, but very different among distantly related genera. Preliminary data indicate that the SAmax is determined by the large subunit, which is coded by the chloroplast genome.

Board 3

REGULATORY STUDIES IN PLANT PRIMARY AND SECONDARY METABOLISM: PURIFICATION OF SHIKIMATE DEHYDROGENASE AND CHALCONE SYNTHASE.

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The reactive response of plants to infection and environmental stress involves the coordinate expression of the phenylpropanoid and flavonoid pathways. Phenylpropanoids are the precursors of lignin, one of the most important structural components in plants. Flavonoids have been shown to participate in the defense mechanism of plants against microbial infections The aromatic amino acid phenylalanine that is formed by the shikimate pathway is a precursor for both classes of compounds. Shikimate dehydrogenase, an enzyme of the aromatic amino acid biosynthetic path, and chalcone synthase, the key enzyme of flavonoid biosynthesis were purified by a rapid method, utilizing HPLC. The isolated proteins are being used to elicit antibody responses in rabbits. The antisera will be used for subcellular localization and genetic investigations.

Board 4

GENE EXPRESSION INVOLVED IN TOMATO FRUIT RIPENING: A. Callahan, F. Abeles, L. Dunn, W. Hershberger, J. Pyle, and P. Morgens. West Virginia University-USDA, Appalachian Fruit Research Station, Rt. 2 Box 45, Kearneysville, WV 25430.

While the literature reports many changes involved in fruit ripening, the regulation of the process has not been elucidated. It is known to be associated with ethylene production. We have recently begun to study the regulation of genes that are either turned on or off during the ripening process in hopes of understanding the coordination involved in the developmental change. We are studying the problem at the levels of enzyme activity, protein synthesis, and RNA synthesis. We have been looking at differences in total proteins, in vivo labelled proteins, and in vitro translated proteins. These techniques all show differences but the differences are not consistent. These preliminary results indicate that the differences are not due simply to changes in levels of transcription.

We are in the process of building cDNA libraries from different stages of ripening fruit and developing antibodies to some of the proteins reported to have changes in activity during the ripening process (i.e. polygalacturonase, β-1,3-glucanase, cellulase, and an RNase). We hope to isolate two classes of genes whose products change during ripening: 1) those known to change in enzyme activity and 2) those that change in their relative levels of RNA. These may or may not be overlapping classes. We hope that the coordinated study of these genes and their respective products will further our knowledge of these developmental events.

Board 5

EXPRESSION OF PROLAMIN BY THE BACTERIUM ESCHERICHIA COLI:
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The prolamin of corn (Zea mays L.), zein, is a group of alcohol soluble proteins which make up 55% of the total endosperm proteins of corn seed. The lack of 2 essential amino acids and the high content of zein make corn a low protein nutritional quality grain for nonruminants. An understanding of the regulation of zein expression would help in the improvement of the nutritional quality of corn. Being interested in the study of zein at molecular level, we constructed an expression library of developing corn kernels using pUC8 plasmid as vector and E. coli strain DH1 as host. The expression library was screened by nonradioactive immunological probes to detect the expression of γ-zein and α-zein. When anti-γ-zein antibody was used as the probe, 23 colonies gave positive signals. In comparing the lengths of cDNA inserts of the 23 colonies, we found them to vary between 250 and 900 base pairs. However, when antibody against α-zein was used, very few colonies gave positive reaction. The library was also screened by colony-hybridization with 32-P-labeled α-zein and γ-zein DNA probes. Based on immunological and hybridization screening of the library and other evidences, we concluded that α-zein was toxic to E. coli cells whereas γ-zein and its fragments were well expressed. To our knowledge this is the first report that a protein encoded by a nuclear genome of a higher plant was expressed in a bacterium.

Board 6

PRODUCTION OF MONOCLONAL ANTIBODY SECRETING HYBRIDOMA CELL LINES TO PRUNE DWARF VIRUS BY IN VIVO AND IN VITRO IMMUNIZATION. Ramon L. Jordan, Nancy Elliott, and H. T. Hsu. USDA, Beltsville, MD 20705 and American Type Culture Collection, Rockville, MD 20852.

Fifty-five hybridoma cell lines secreting monoclonal antibodies (McAbs) to prune dwarf virus (PDV) have been produced and evaluated. Forth-three cell lines were generated by a standard in vivo immunization protocol. Twelve additional hybridomas were developed by an in vitro immunization procedure. Spleen cells from a BALB/c mouse previously injected with 20 ug PDV were cultured (in vitro) in the presence of 100 ug PDV antigen. The spleen cells were sensitized for 4 days in thymocyte conditioned serum-free media prior to fusion with NS1 or P3.658 myeloma cells. Analysis of the immunoreactivity and relative binding characteristics of the McAbs with intact and distrupted immunogen and related and unrelated viruses in double antibody, sandwich and indirect ELISA procedures will be presented.

Board 7

ALKALOID ENHANCEMENT IN CATHARANTHUS ROSEUS IN TISSUE CULTURE: Daniel J. Prochaska,[1] Paul C. Burkhouse,[1] Carolyn D. Mitchell,[1] Kenneth G. Wilson,[1] and Mary B. Fields,[2] [1]Department of Botany, Miami University, Oxford, OH, and [2]Department of Biology, Ursinus College, Collegeville, PA.

Suspension cultures of Catharanthus roseus (L.) G. Don were mutagenized with MNNG (0.03 mM) at LD_{40} for 24 hours. Variants of the mutagenized and non-mutagenized cells resistant to normally toxic levels of 5-methyl tryptophan, tryptophan and tryptamine were isolated. Lines were selected for enhanced fluorescence, indicative of alkaloid production, after growth in white light.

Some suspension cultures were treated with an alkaloid inducer, e.g. methyl viologen (1-1'-dimethyl 1-4-4'-bipyridinium), in concentrations ranging from 1.8-18 ppm in liquid media. The treatment was applied to suspension cultures in media with and without hormones. Both treatments showed significant increases in total specific alkaloid production (OD.mls/mg protein) as measured spectrophotometrically. Fluorescing cells were prevalent following treatment with methyl viologen at low concentrations. Ultrathin layer plating techniques allowed for further selection of the highly fluorescing cell lines which yielded uniformly high fluorescing suspension cultures.

Parallel subcultures of these strains and wild types grown on plates containing either 2,4-D (2,4-dichlorophenoxyacetic acid) or 2,3-D (2,3-dichlorophenoxyacetic acid) were then compared for fluorescence and alkaloid production.

Board 8

CRYOPRESERVATION OF RICE CELL CULTURES: A.T. LEVY, S.C. Jong and E.E. Davis, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, and D.W. Schaeffer, USDA/ARS Cell Culture/Nitrogen Fixation Lab., Rm. 116, Bldg. 011A BARC-W, Beltsville, Maryland 20705, USA

Cryogenic freezing with liquid nitrogen is presently the most feasible method to preserve plant tissues or cells cultured in vitro and maintain genetic characteristics. Because of the large number and range of plant tissue cultures available, the perspective has been to research the most simple methods of cryopreservation first in the hope that only a few may need more complex methods.

Six established cell cultures (ATCC 54011 to ATCC 54016) of Oryza sativa currently included in the Plant Tissue Culture Bank at the American Type Culture Collection (ATCC) have been successfully frozen for a prolonged period of time with the routine procedure used for preservation of mammalian cells and microbial cultures such as bacteria, fungi and yeasts at the ATCC. Rice cells grown in a modified Murashige and Skoog medium were suspended in 5% dimethyl sulfoxide (DMSO) at room temperature and immediately equlibrated for 30 minutes at 5 C in a Cryo-Med programmable freezer. They were then cooled to -40 C at a rate of 1 C per minute. After holding at -40 C for 30 minutes, the vials were plunged in liquid nitrogen and stored in the vapor phase refrigerator. Thawing was done rapidly in a 37 C water bath. Thawed material was inoculated directly to liquid or semisolid medium. Positive fluorescein diacetate staining was seen in 2 weeks and visible regeneration of callus and suspension cultures was seen in 4 weeks. No pretreatment with the cryoprotectant before freezing and post-treatment with washing or dilution of the cryoprotectant after thawing were required.

Board 9

CRYOPRESERVATION OF SUSPENSION CELLS, CALLUS TISSUES AND PLANTLETS OF CARROT: S.C. Jong, A.T. Levy and E.E. Davis, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, and B.F. Matthews, USDA Cell Culture Lab., Bldg. 011A, Rm. 116, Beltsville, Maryland 20705, USA

Although cryopreservation makes possible the long term storage of plant cells, tissues, organs and embryos, with subsequent regeneration into whole plants, the state of art in this field is still at an experimental and empirical stage. There is no single protocol suitable for all of the cultures already studied. Two carrot cultures, Daucus capillifolius (ATCC 54008) and Daucus carota subsp. sativus)ATCC 54010), were studied to determine the most advantageous method of preserving suspension cells, callus tissues and plantlets. The cultures have been successfully frozen and stored in liquid nitrogen since June of 1983.

Cultures were grown as callus or in cell suspension in a modified Murashige and Skoog medium. Suspension cells, callus tissues and plantlets obtained were frozen in liquid nitrogen by a two-step procedure of slow cooling to -40 C at a rate of 1 C per minute followed by storage in a vapor phase liquid nitrogen refrigerator at -150 C. Both 5% dimethyl sulfoxide (DMSO) and Murashige and Skoog broth with 3,4,-dichlorophenoxyacetic acid were proven to be effective cryoprotective agents. The samples in 2 ml polyethylene vials were frozen within 30 minutes after the addition of the cryoprotectant at room temperature. There were no pretreatments such as pregrowth in medium supplemented with cryoprotectant or gradual addition of the cryoprotectant at ice temperature near 0 C. Thawing was performed in a 37 C water bath with rapid swirling of the vials for about 1 minute just to the point of ice disappearance and the thawed samples were inoculated directly to semisolid or liquid growth medium without washing or diluting of the cryoprotectant. Fluorescein diacetate strains were positive within two weeks. Regeneration of callus tissues from frozen suspension cells and callus was obvious within four weeks. Callus was generated from plantlets in less than eight weeks.

Board #10

ESTABLISHMENT OF A GERMPLASM BANK OF PLANT TISSUE CULTURES: S.C. Jong, A.T. Levy and E.E. Davis, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA

Rapid advances made in recent years in plant tissue and cell culture techniques offer many unique opportunities to study the biochemical, physiological and genetic nature of plants. Plant tissues and cells are inherently totipotent, capable of regenerating into entire plants under defined conditions, and have been proven to be excellent materials for germplasm preservation of both vegetatively and seed-propagated crop species. Plant cells also are excellent sources of chemicals, giving forth an array of valuable pharmaceuticals, dyes and flavors.

Provision has been made for the establishment of a plant tissue culture bank in the American Type Culture Collection. The feasibility of developing such a bank at ATCC is based on cryopreservation studies which indicate that tissue cultures of many plants can be stored under liquid nitrogen without loss of viability and on the availability of excellent facilities for liquid nitrogen storage at ATCC.

The bank will accept well-documented plant tissue cultures with properties useful in research, teaching or industry. Contributors may submit prepared cultures in test tubes or plates which should be sealed with tape or parafilm, and packed in waterproof containers as in shipping microbial cultures. Along with the culture, a completed copy of the submission sheet (blanks can be obtained from ATCC) and other supplemental information should be sent.

Only cultures that can be successfully frozen in liquid nitrogen at ATCC will be accepted. The cultures will be made available to the scientific community for a fee to cover expenses. There are no restrictions on availability and no provisions for informing contributors of requests for their cultures. Contributors may receive subcultures of the cell lines they deposited without charge. ATCC accepts cultures for patent application purposes. In this case certain restrictions on availability can be arranged.

The bank now maintains 48 cultures representing alfalfa, birdsfoot trefoil, blueberries, carrot, celery, corn, lily, pine, rice, soybean, sugarcane, sunflower, tobacco, tomato, walnut, and wheat. Of these, seven are patent application strains.

Board 11

ORGANELLE GENOMES IN SOMATIC HYBRIDS OF LYCOPERSICON: Mary A. O'Connell and Maureen R. Hanson, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22901

Somatic hybrids were formed by polyethylene glycol mediated fusion of protoplasts from mesophyll cells of Lycopersicon esculentum, cultivars UC82B or Petoseed #46, and suspension cells of Lycopersicon pennellii. The somatic hybrid calli were identified on the basis of isozyme differences in the marker enzyme phosphogluco isomerase. Total DNA was prepared from frozen lyophilized samples of somatic hybrid calli. Chloroplast DNA present in the somatic hybrid calli was identified by probing restricted digests of the total DNA with nick translated L. esculentum chloroplast DNA. Three types of plastid genome compositions were detected. Either only L. pennellii, only L. esculentum, or a mixture of the two parental plastids was observed. Cloned fragments of L. pennellii mitochondrial DNA were used to identify the mitochondrial genomes present in the somatic hybrid calli. In contrast to the chloroplast DNA analyses, all but one of the somatic hybrids examined exhibited only the mitochondrial DNA signal of the L. pennellii parent. One somatic hybrid also had a faint signal specific for the L. esculentum mitochondrial genome. Whether or not these somatic hybrid clones contain novel mitochondrial genomes is under analysis.

Board 12

ANALYSIS OF SOY BEAN MITOCHONDRIAL DNA HOMOLOGOUS TO A YEAST CYTOCHROME b GENE. Frank J. Turano,[1] Albert J. Wilson,[2] Kenneth G. Wilson[1] and Ronald W. Treick,[3] [1]Department of Botany, Miami University, Oxford, Ohio [2]Microbiological Research Corp., Bountiful, Utah, and [3]Department of Microbiology, Miami University, Oxford, Ohio.

The mitochondrial genome of several fungi and mammals has been extensively characterized. The organization and structure of the mitochondrial genome of vascular plants is an expanding area of research. The isolation and restriction map of a plant mitochondrial cytochrome b gene was first reported by this group in 1983. In that study a 3.7 kilobase pair Eco RI fragment showing homology with a cytochrome b yeast probe was isolated and cloned. The Eco RI fragment contained restriction endonuclease site for Bam HI, Hinc II and Sma I.

In the present study a 950 base pair Eco RI-Bam HI fragment was subcloned into M13 mp18 and mp19 and sequenced by the Sanger chain termination and Maxim and Gilbert methods. The sequence of the first 290 bps from the Eco RI site has been determined. Attempts to sequence the Bam HI end by the Sanger method reveal an area where the enzyme (Klenow fragment) can not read, suggesting secondary structure. This area is under investigation and is being sequenced by the Maxim and Gilbert method. Analysis and conclusions of these results will be presented at these meetings.

Board 13

THE PETUNIA MITOCHONDRIAL GENE FOR THE PROTON-TRANSLOCATING SUBUNIT OF ATPASE: Ellora Young and Maureen Hanson, Department of Biology, Gilmer Hall, Univ. of Virginia, Charlottesville, VA 22903

We have isolated a DNA segment of the mt genome of Petunia hybrida line 3704 that contains the entire coding sequence of subunit 9 of the F_0 ATPase. The coding region DNA sequence is 59% homologous with the mt ATPase proteolipid-like gene of Neurospora crassa. The predicted polypeptide sequences of these two genes are 62% homologous. The Petunia protein is highly hydrophobic and contains a glutamic acid at position 50 that has been shown to be involved in DCCD-binding and proton translocation in other organisms. S1 nuclease protection studies indicate that this gene is transcribed in leaves and in suspension cultures. Initiation of transcription begins approximately 135 nucleotides upstream of the ATG start codon. More than one ATPase subunit 9 gene, or fragments of the gene, exists per Petunia mitochondrial genome, according to Southern hybridizations of coding and flanking region probes.

Board 14

ORGANIZATION OF THE MITOCHONDRIAL RIBOSOMAL RNA GENES OF CARROT:
Lorin R. DeBonte and Benjamin F. Matthews, Tissue Culture and Molecular Genetics Lab, PPHI, ARS, USDA, Beltsville, MD 20705

We have localized the mitochondrial 26S, 18S and 5S ribosomal RNA genes to specific DNA fragments of the carrot mitochondrial genome. The entire ribosomal DNA (rDNA) region is found on two Hind III fragments of 9.17 and 3.64 kb. The 9.7 kb fragment contains the entire 26S and part of the 18S rDNA genes. The 3' end of the 18S gene, located by hybridization with the 3' end of the 17S Neurospora rDNA gene, lies within the 3.64 kb region. From PstI and SalI digests the 18S and 5S rDNA gene are found closely linked and physically distinct from the 26S. The arrangement of the rDNA genes is 26S-spacer-18S-5S. This arrangement is different from that of maize where the 18S-5S rDNA order is reversed. The distance between the 26S and 18S-5S genes is 2kb considerably less than in maize (15 kb). The 26S and 18S genes do not appear to be repeated in multiple copies. This is now being investigated using a mitochondrial DNA-Charon 35 library.

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