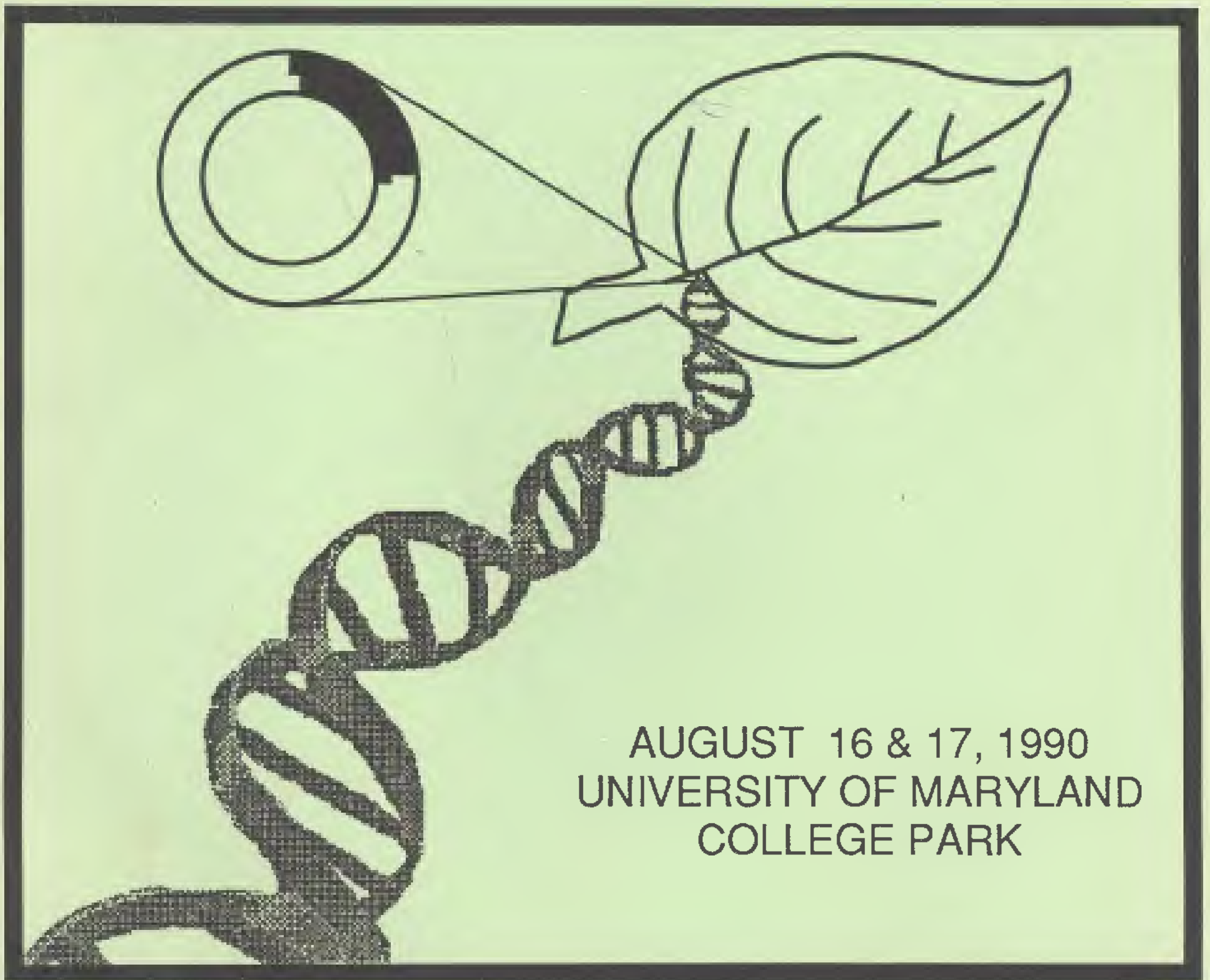
THE SEVENTH ANNUAL MEETING OF THE

MID-ATLANTIC

PLANT MOLECULAR BIOLOGY

SOCIETY



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INTRODUCTION

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

Since its inception and first meeting in 1984, the attendance and participation at the annual meeting has increased, as has the geographical representation. The original idea that led to the formation of MAPMBS is clearly an excellent one. It is our hope that the plant molecular biology community will continue to support the MAPMBS as it has done in the past.

We are pleased to host this year's meeting at the University of Maryland at College Park. No scientific conference organizes itself, and we want to express our sincere thanks to those people listed on next page for their hard work on committees. We also thank Tom Flynn of Campus Guest Services for his expertise and effort.

John Hammond
John C. Watson
Co-Organizers

1990 MAPMBS Organizing Committees

	PHONE	FAX	SITE
Program Committee			
Rob Griesbach	344-3574	344-3096	USDA
John Hammond	344-3313	344-3096	USDA
Ramon Jordan	344-1646	344-3096	USDA
Ben Matthews	344-2730		USDA
Janet Slovin	344-3632		USDA
David Straney	454-1966	454-0204	UMCP
Frank Turano	344-3145	344-4521	USDA
John Watson	454-0242	454-0204	UMCP
Publicity and Mailing			
John Hammond	344-3313	344-3096	USDA
Rose Hammond	344-3203	344-5435	USDA
Registration			
Kathy Kamo	344-3350	344-3096	USDA
Ben Matthews	344-2730		USDA
Jeannie Rowland	344-4654		USDA
Peter Ueng	344-3308		USDA
Funding			
Janet Slovin	344-3632		USDA
Local Arrangements			
John Watson	454-0242	454-0204	UMCP
Abstracts			
John Hammond	344-3313	344-3096	USDA
Ramon Jordan	344-1646	344-3096	USDA
David Straney	454-1966	454-0204	UMCP
Frank Turano	344-3145	344-4521	USDA
Greg Wadsworth	344-3304		USDA
Social Arrangements			
John Hammond	344-3313	344-3096	USDA
John Watson	454-0242	454-0204	UMCP

GENERAL INFORMATION

UMCP: The University of Maryland at College Park is the largest campus in the University of Maryland System, with approximately 38,000 students and over 100 departments and programs. The campus is located just 4 miles from the Washington D.C. border and 8 miles from the White House, and about 45 minutes south of Baltimore and west of Annapolis. If you have post-meeting free time and desire some non-college town night life, Washington, Baltimore, and Annapolis offer many delightful experiences.

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

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

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

Sponsors and Vendors for the Seventh Annual Meeting of the Mid-Atlantic Plant Molecular Biology Society

The following have made very generous contributions, which has enabled us to have these meetings and to keep the registration fees low enough so that students and post-docs will be able to attend. We are very grateful for their help.

Maryland Agricultural Experiment Station
University of Maryland
College Park, MD 20742

Center for Agricultural Biotechnology
Maryland Biotechnology Institute
University of Maryland
College Park, MD 20742

USDA/ARS
Area Office, Beltsville Area
Beltsville, MD 20705

EniMont Inc.
Monmouth Junction, NJ 08854

E.I. duPont de Nemours & Co.
Plant Science Division
Wilmington, DE 19880-0402

The following companies will be exhibiting their products during the meetings. In order to do this they each have made a generous contribution toward the expenses of running this meeting. We are very grateful for their interest in our meeting and hope they will continue to exhibit with us next year. Please spend some time with them and let them show you their latest in products and equipment.

American Type Culture Collection

Applied Biosystems

Bio-Rad

Eppendorf

Fisher/Promega

GIBCO/BRL

Hydro Service and Supplies, Inc.

International Biotechnologies, Inc., (IBI)

Research Products International Corp.

Carl Zeiss Instruments, Inc.

SCHEDULE OF PRESENTATIONS

Thursday, August 16th:

8:00 AM Registration - Prince Georges Room

9:00 AM Opening Remarks - John Hammond

PLANT/MICROBE INTERACTIONS convenor: John Hammond

9:10 AM Stephen C. Winans - Transcription of an *Agrobacterium tumefaciens* regulatory gene from natural and artificial promoters.

9:40 AM Anne Simon, Clifford D. Carpenter, Pamela J. Cascone, Xiao Hua Li, and Chunxia Zhang -
RNA recombination and defective interfering RNAs in the Turnip Crinkle Virus system.

10:10 AM Thomas A. LaRue and N.F. Weeden - A cluster of sym (symbiosis) genes on chromosome 1 of *Pisum sativum*.

10:30-10:50 AM *BREAK + EXHIBITORS*

10:50 AM David Straney - Regulation of Pisatin demethylase, a fungal virulence gene.

11:20 AM Jocelyn Malamy, John P. Carr, Daniel Klessig, and Ilya Raskin - Salicylic acid - a likely endogenous signal in the resistance response of tobacco mosaic virus infection.

11:40 AM David Dixon, John R. Cutt, and Daniel F. Klessig -
Intracellular and extracellular accumulation of the PR1 pathogenesis-related proteins of *Nicotiana tabacum*.

12:00-1:00 PM *LUNCH*

CELL CYCLE convenor: Kathy Kamo

1:00 PM Stephen Wolniak - The regulation of mitotic progression.

1:30 PM Berl R. Oakley, C. Elizabeth Oakley, M. Katherine Jung, Yisang Yoon, Hiromi Tanaka, and Yixian Zheng -
Gamma-tubulin and the regulation of microtubule assembly.

2:00 PM Shoupeng Lai, John Watson, Norman Hanson and Heven Sze -
Molecular cloning and sequencing of a cDNA clone encoding the proteolipid subunit of vacuolar H^+-ATPase from oat.

2:20 PM Cheol Ho Hwang and J. Lynn Zimmerman - Regulation and function of low molecular weight heat shock proteins from carrot.

2:40 PM Liqun Wang and J. Lynn Zimmerman - Regulated expression of a heat shock fusion gene in transgenic carrot.

3:00 PM *BREAK + EXHIBITORS + POSTERS (authors in attendance)*

Poster 1: Ann Smigocki and Scott Harding - Effects of two reconstructed cytokinin genes on development in transgenic plants.

Poster 2: Carol Preisig and Hans VanEtten - Biosynthesis of the phytoalexin pisatin: purification and characterization of the terminal enzyme and antisera.

Poster 3: John Hammond, Ramon L. Jordan, and Kathy K. Kamo - Use of deleted and chimeric coat protein constructs to examine potyvirus coat protein structure and coat protein mediated resistance.

Poster 4: Mary Polacco, Xiao-Hua Li, and Anne Simon - Turnip crinkle virus (TCV)/Arabidopsis: A pathogen-plant model interaction. Isolation of a resistant ecotype.

Poster 5: Chunxia Zhang, Xiao-Hua Li, and Anne Simon - Analysis of a *de novo* generated defective interfering RNA of Turnip Crinkle virus.

Poster 6: Xiao Hua Li and Anne E. Simon - Turnip Crinkle virus (TCV) and its small sub-viral RNAs.

Poster 7: Pamela J. Cascone, Clifford D. Carpenter, Xiao Hua Li, and Anne Simon - Recombination between satellite RNAs of Turnip Crinkle virus.

Poster 8: Peter McGarvey, J.M. Kaper, M.J. Avila-Rincon, L. Pena, and J.R. Diaz-Ruiz - Transformed Tomato plants express a satellite RNA of Cucumber Mosaic virus and produce lethal necrosis upon infection with viral RNA.

Poster 9: Tzion Fahima, Nadine Pobjecky, and Gail Dinter-Gottlieb - Self-splicing of the *Tetrahymena* group I intron in Tobacco plants transformed by *Agrobacterium tumefaciens*: evidence for DNA to DNA information transfer.

4:30 PM *KEYNOTE ADDRESS*

Peter Quail
Phytochrome genes and their expression

5:30 PM *SOCIAL HOUR - Ballroom Lounge*

6:30 PM *DINNER - Ballroom*

Friday, August 17th:

RFLP/LARGE DNA convenor: Ben Matthews

8:30 AM Meredith Bonierbale, R.L. Plaisted and S.D. Tanksley - Development and Application of an RFLP map of Potato

9:00 AM Joseph Ecker - Development of large DNA methods for plants.

9:30 AM Richard Schneeberger and Christopher Cullis - Long range analysis of environmentally induced 5S DNA rearrangements in Flax, *Linum usitatissim* L.

9:50 AM Craig Coleman, Anuradha-Singh, and Teh-hui Kao - Comparison of genomic clones representing two self-incompatibility alleles in *Petunia inflata.*

10:00 AM ***BREAK + EXHIBITORS + POSTERS (authors in attendance)***

Poster 10: Michal Oren-Shamir, Miguel C. Cervantes, Lee A. Newman, Noureddine Hadjeb, and Carl A. Price - Nuclear genes affecting chromoplast proteins in *Capsicum annuum.*

Poster 11: Ruben Cohen, Ann Callahan, and Peter Morgens - Peach fruit molecular biology: comparison of specific RNA levels in 10 phenotypically/genotypically different fruit.

Poster 12: Andrew D. Lloyd, Ann Callahan, and Peter Morgens - Isolation and Analysis of a green-fruit specific gene in tomato.

Poster 13: Xia Lin, Xinhua Feng, and John C. Watson - Differential accumulation of transcripts encoding protein kinase homologs in *Pisum sativum* is light-regulated.

Poster 14: John C. Wallace - High level of expression of gamma zein in modified *opaque-2* maize.

Poster 15 Anuradha Singh and Teh-hui Kao - S-protein associated ribonuclease activity in *Petunia inflata.*

Poster 16: Gregory J. Wadsworth, Frank J. Turano, and Benjamin F. Matthews - Characterization of the aspartate aminotransferase isozyme-gene system of Soybean.

Poster 17: Jane M. Weisemann and Benjamin F. Matthews - Cloning of a gene for homoserine dehydrogenase from Carrot and Soybean.

Poster 18: Susan Koehler and David Ho - Hormonal regulation, processing, and secretion of cysteine proteinases in Barley aleurone.

Poster 19: Yuen Yee Tam, Janet P. Slovin, and Jerry D. Cohen - Selection of mutant lines of *Lemna gibba* for studying developmental biochemistry.

Poster 20: Anthony J. Spano, Katherine F. Boese, Zhenghui He, Doris J. Morris, and Micheal P. Timpko - Genes encoding chlorophyll biosynthetic enzymes in angiosperms and gymnosperms.

Poster 21: Jhang Ho Pak and Michael S. Dobres - Sequence analysis of transcripts encoding lectin-like proteins in *Pisum sativum*

GENE REGULATION convenor: Frank Turano

11:20 AM Philip Benfey, Eric Lam, Fumiaki Katagiri, Philip Gilmartin, and Nam-Hai Chua - Combinatorial properties of CAMV 35S enhancer subdomains.

11:40 AM Gregg Silk and Madeline Wu, Detailed analysis of transcription of the tufA region of the chloroplast genome of *Chlamydomonas reinhardtii.*

12:00 noon: Business Meeting

LUNCH

GENE REGULATION convenor: Greg Wadsworth

1:20 PM June Medford and Harry Klee - Manipulation of development in transgenic plants.

1:50 PM Russell L. Malmberg - Genetic Analysis of polyamine synthesis.

2:20 PM Wenpei Su and Stephen H. Howell - Isolation and characterization of cytokinin response mutants in *Arabidopsis thaliana.*

2:40 PM: T.M. Spencer, W.J. Gordan-Kamm, M.L. Mangano, T.R. Adams, R.J. Daines, W.G. Start, J.V. OBrien, S.A. Chambers, W.R. Adams, N.G. Willetts, T.B. Rice, C.J. Mackay, R.W. Kreuger, A.P. Kausch, and P.G. Lemaux - Fertile transgenic maize.

3:20 PM: Closing remarks

TRANSCRIPTION OF AN AGROBACTERIUM TUMEFACIENS REGULATORY GENE FROM NATURAL AND ARTIFICIAL PROMOTERS.

Stephen C. Winans
Section Of Microbiology
Cornell University
Ithaca, New York 14853

The transcriptional activation of a set of *Agrobactium tumefaciens* virulence genes by wound-released phenolic compounds is controlled by the VirA and VirG proteins, two members of the family of two-component regulatory genes. Both the *virA* and *virG* structural genes are activated by this regulatory system, resulting in positive autoregulation. The *virG* gene is also expressed by a second promoter which shows sequence similarity to the family of *E. coli* heat shock genes. This promoter is induced at the transcriptional level by a variety of stress stimuli, including extremes of pH, heat, ethanol, cadmium, and mitomycin C. These treatments each of induce the synthesis of a set of genes similar to the *E. coli* heat shock genes. We propose that environmental stresses act through a regulatory system similar to the *E. coli* heat shock response to increase the pool size of VirG, and thereby potentiate *vir* gene induction. We have also replaced the natural *virG* promoters with the *lac* promoter to artificially control the VirG pool size. *vir* gene expression in response to phenolic compounds can indeed be dramatically influenced by altering the concentration of VirG. Finally, uncontrolled, high-level expression of VirG was found to lead to higher levels of *vir* gene expression than is found with the natural *virG* promoters.

RNA RECOMBINATION AND DEFECTIVE INTERFERING RNAS IN THE TURNIP CRINKLE VIRUS SYSTEM. Anne E. Simon, Clifford D. Carpenter, Pamela J. Cascone, Xiao Hua Li, and Chunxia Zhang. Department of Plant Pathology and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

Turnip crinkle virus, unlike other animal or plant viruses, supports the replication of a variety of linear subviral RNAs: (i) satellite (sat) RNAs which require a helper virus for infectivity and share little or no sequence similarity with the helper virus; (ii) defective interfering (DI) RNAs, derived almost exclusively from helper virus sequence; (iii) chimeric RNAs, composed of both sat-RNA and viral genomic RNA sequences (Simon and Howell, 1986; Li et al., 1989). Several different recombination events have been found in the TCV system: between a sat-RNA (sat-RNA D) and the chimeric RNA (sat-RNA C; Cascone et al., 1990) and between sat-RNA D and TCV genomic RNA. A replicase driven copy choice model combined with recognition by the replicase of internal initiation signals during plus strand synthesis of TCV or subviral RNAs is proposed to explain the formation of both recombinant RNAs and DI RNAs in the TCV system. This model is based on the finding that these discontinuous RNAs accumulate with right side junction sequences which are non-random and resemble either the 5′ end of TCV genomic RNA or the 5′ ends of the sat-RNAs. Preliminary results indicate that single point mutations in the internal replicase recognition motif in sat-RNA C eliminate recombination between sat-RNAs in vivo.

TCV subviral RNAs are present in infected leaf tissue in both monomeric and multimeric forms. Analysis of the junction sequences between monomeric units reveals substantial heterogeneity, including both deletions and insertions of nucleotides not present in the monomeric sequence. The junctions of the subviral multimers resemble junctions found between sat-RNA recombinants where additional nucleotides (mainly uracil residues) were also found. Based on these results and the cloning of sat-RNA heteromultimers (between sat-RNA D and sat-RNA C), we propose that formation of multimers of these linear subviral RNAs does not involve a rolling circle type mechanism thought to be important for the replication of circular sat-RNAs and viroids. Rather, we believe that multimers are a manifestation of the same process which results in the formation of recombinant RNAs and DI-RNAs; reinitiation of replication by the replicase before release of the nascent strand.

1. Simon, A.E. and Howell, S.H. (1986) The virulent satellite RNA of turnip crinkle virus has a major domain homologous to the 3′-end of the helper virus genome. *EMBO J.* 5, 3423-3428.

2. Li, X.H., Heaton, L., Morris, T.J. and Simon, A.E. (1989) Defective interfering RNAs of turnip crinkle virus intensify viral symptoms and are generated *de novo*. *Proc. Natl. Acad. Sci. (USA)* 86, 9173-9177.

3. Cascone, P.J., Carpenter, D.C., Li, X.H. and Simon, A.E. (1990) Recombination between satellite RNAs of turnip crinkle virus. *EMBO J.* 9, 1709-1715.

A CLUSTER OF SYM (SYMBIOSIS) GENES ON CHROMOSOME 1 OF PISUM SATIVUM: T.A. LaRue* and N.F. Weeden, Boyce Thompson Institute, Ithaca, NY and Department of Horticultural Sciences, Cornell University, Geneva, NY.

Pea is the species of choice to study the genetic contribution of the host legume, because it is easy to induce stable symbiosis (sym) mutants and because the genome is well mapped. We have identified sym genes by treating the cultivar 'Sparkle' with mutagens and screening progeny for abnormal nodulation. These sym genes are being mapped using a combination of allozyme markers and RFLP. Sym genes are found on all seven chromosomes and with one exception seem randomly distributed. On chromosome 1 there is a cluster of four sym genes near the leghemoglobin gene cluster. The phenotypes of these mutants (sym 2, sym 5, sym 18, and sym 19) will be described.

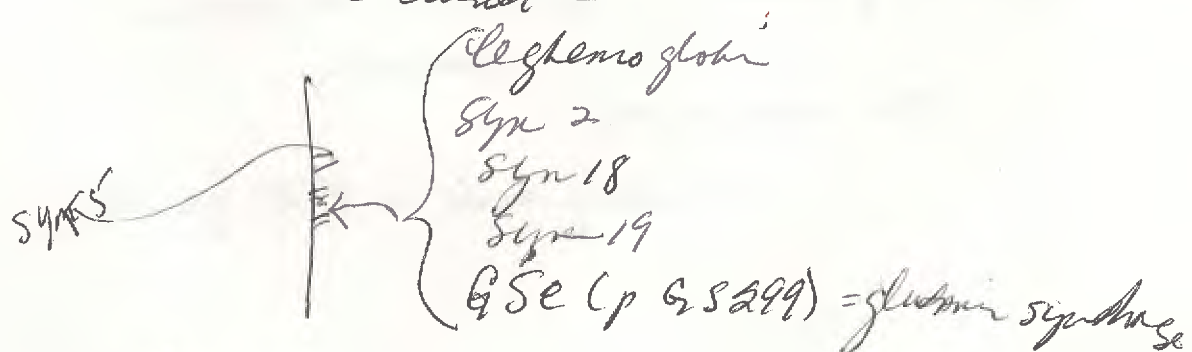
~30 non-allelic genes in pea are involved in nodulation

leghemoglobin – an important gene for nodulation. ~8 of them,

Glutamine synthase – 2 of them; on diff. chromo

Most sym genes are not closely linked to each other.

Except – chromosome 1 – a cluster –



Phenotype – sym 5 = mutational hot spot 7 isolates.

(C14 leu for in vivo protein labeling)
2D gel & autorad.

REGULATION OF PISATIN DEMETHYLASE, A FUNGAL VIRULENCE GENE:
David C. Straney; Dept. of Botany, University of Maryland, College Park, Maryland 20708.

Signal transduction between plants and their fungal pathogens determine the expression of multiple virulence genes in the pathogen. We are defining the molecular mechanisms by which the plant pathogenic fungus *Nectria haematococca* (imp. *Fusarium solani* fsp. *pisi*) uses the pea phytoalexin, pisatin, to induce its pisatin detoxification gene to allow it to be more virulent on pea. We have found the detoxification gene, that coding for pisatin demethylase, to be regulated by pisatin at the transcriptional level. Also, using the gel-retardation assay, we have defined a factor which is unique to pisatin induced mycelial extracts that specifically binds to DNA regions upstream of the pisatin demethylase promoter, acting as expected for a pisatin-responsive activator. The binding site has been mapped with DNAase I. The functional role of this putative activator binding site has been tested by transforming the fungus with multiple copies of the binding site and observing a depressed and delayed induction of the pisatin demethylase. Nutrient repression of this promoter has also been similarly studied and we have identified a possible amino acid repressor which seems to share a binding site with the pisatin-responsive activator. Our goal is to determine the signal pathways that control these putative regulatory proteins.

from plant / in fungus
Phytoalexin turns on phytoalexin detox (or tolerance)

Nectria haematococca (Fusarium solani — root-rot pathogen)

phytoalexin
→ Pisatin —demethylated→ DMP (a P450 mono-oxygenase)

Pisatin added to PDA inhibits growth of mutants which lack demethylase

(Demethylase is virulence gene)

How does pisatin get into cell & induce demethylase — Pisatin induces demethylase but + glucose, or aa ~~or NH4NO3~~ — don't get induction

+NH4NO3 get induction so not just N

Salicylic acid - a likely endogenous signal in the resistance response of tobacco to tobacco mosaic virus infection

Jocelyn Malamy#*, John P. Carr#[1], Daniel F. Klessig#, and Ilya Raskin+

Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855
+ Center for Agricultural Molecular Biology, Rutgers, The State University of New Jersey, Cook College, P.O. Box 231, New Brunswick, New Jersey, 08903-231

Some cultivars of tobacco are resistant to TMV infection, while others are susceptible[1]. Resistant cultivars synthesize new pathogenesis-related (PR) proteins upon TMV infection, although the role of these proteins is still unclear[2,3]. Little is known about plant signals that induce resistance and/or PR genes. However, salicylic acid (SA) has been shown to induce PR genes and enhance resistance to TMV when applied exogenously to tobacco plants[4,5]. Here we show that endogenous SA levels in resistant, but not susceptible cultivars increase at least 20-fold in infected leaves and 5-fold in uninfected leaves after inoculation with TMV. Induction of PR1 genes parallels the rise in SA levels. These findings suggest that SA functions as a natural signal for PR gene induction and disease resistance. The recent demonstration that SA is also a natural regulator of thermogenesis in lily[6] indicates that SA may be an important signal molecule in plants.

Nature Ilya (RASKIN)

* To whom correspondence should be addressed
[1] Present address: Department of Plant Pathology, Cornell University, Ithaca, New York 14853

Systemic responses (HSR)
protease inhibitor
hydrolases
PR protein
HRGP = glycoproteins (for cell construction)

Intracellular and Extracellular Accumulation of the PR1 Pathogenesis-Related Proteins in *Nicotiana tabacum.*

David C. Dixon, John R. Cutt and Daniel F. Klessig
Waksman Institute at Rutgers University,
P.O. Box 759 Piscataway, New Jersey 08854

Pathogenesis-related (PR) proteins are synthesized by many plants in response to stresses from pathogen infection, flowering, senescence, etc. and are thought to play a role in limiting the effects of stress on the plant. We are studying the localization of the PR1 family of PR proteins in tobacco plants. Initial immunolocalization experiments identified PR1 proteins in the extracellular spaces and xylem elements of TMV-infected leaves and supported the observation that mesophyll protoplasts synthesize and secrete these proteins. However, in recent experiments we have detected these proteins within the central vacuoles of crystal idioblasts. These cells are morphologically distinct and contain crystals of calcium oxalate within their vacuoles. Preliminary *in situ* hybridization studies indicate that PR1 genes are expressed within crystal idioblasts. This suggests that the vacuolar localization of PR1 proteins within these cells results from synthesis and targeting to the vacuole rather than uptake of PR1 proteins secreted by surrounding cells. Transgenic plants which constitutively express an introduced chimeric PR1b gene accumulate the PR1b protein in the extracellular spaces and the vacuoles of crystal idioblasts and further confirm the identity of the PR1b gene product in this intracellular location. Other, more preliminary, work with floral structures in healthy plants indicates that these proteins are also accumulating intracellularly within the cells of developing ovules. Thus the same proteins appear to be differentially sorted within different cell types.

idioblast cells - assoc w/ Ca^{++} regulation
Can release Ca^{++} if Ca^{++} deficit
take it up if Ca^{++} surplus.

Antibody specific for PR proteins
reacts w/ this.

THE REGULATION OF MITOTIC PROGRESSION. Stephen M. Wolniak. Department of Botany and Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742.

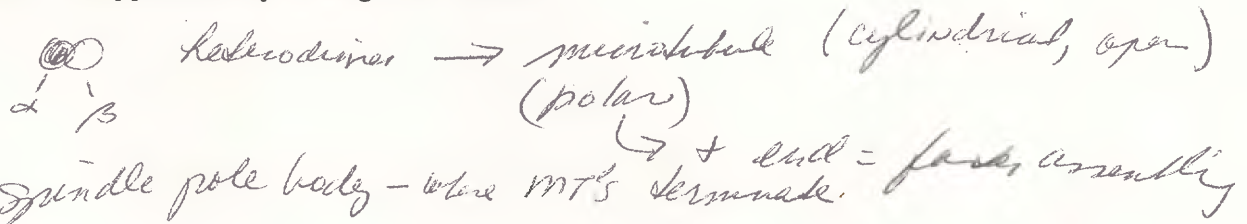
The process of mitosis involves a series of complex biochemical and physiological processes that are manifested morphologically as major changes in the distribution of cellular constituents. Of these morphological changes, nuclear envelope breakdown and anaphase chromosome separation are prominent morphological transitions that are readily visible with the light microscope. In my laboratory, we have focused on the signaling pathways responsible for these morphological transitions in living stamen hair cells from the spiderwort plant *Tradescantia virginiana*. My working hypothesis is that the same types of elicitors and signals are recurrent at different points during the cell cycle and mitosis. I suspect that the signals are components in both an independent activation cascade, involving a cell-cycle specific protein kinase known as $p34^{cdc2}$, and a dependent modulation cascade, involving oscillations in the cytosolic activities of calcium and calcium-dependent kinases. These elicitors, generated at different times, act on a variety of substrates and thereby promote progression through a series of regulatory switch points.

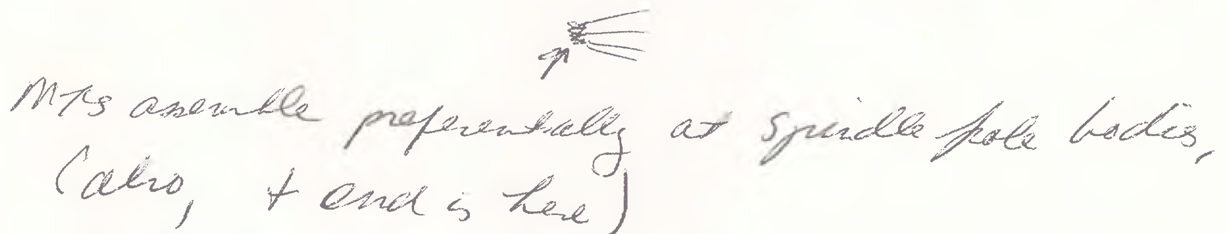
In a series of pharmacological studies, we have taken advantage of the remarkably predictable rate of progression through metaphase and anaphase exhibited by stamen hair cells, using the kinetics of reversible arrest in metaphase as a bioassay for our experimental treatments. Among the more likely regulatory signals for the modulation of mitosis are fluctuations in the cytosolic calcium activity and shifts in the rate or extent of phosphorylation of mitotically-related substrates. A reduction in extracellular calcium activity or in calcium conductance across the plasma membrane results in metaphase arrest. Conversely, an increase in calcium conductance or a suppression of calcium extrusion and sequestration from the cytosolic compartment results in precocious entry into anaphase. The regulation of calcium itself, appears to be mediated by the biochemical cycling of inositol lipids, and herein lies a direct connection with protein phosphorylation. While it is clear that a rise in cytosolic calcium activity appears to be necessary for anaphase onset, the cation may not be sufficient for sister chromatid separation. We have been able to supplant the usually necessary rise in calcium by treatment of cells with a 1,2-diacylglycerol, a potent activator of protein kinase C. We have observed a time-dependent response from the cells; treatments commencing up to 16 min after nuclear envelope breakdown result in precocious entry into anaphase, while treatments commencing 17-25 min after nuclear envelope breakdown result in metaphase arrest. We believe that the interval beginning 17 min after nuclear envelope breakdown, signals passage through a regulatory switch point, or transition point, after which, the physiological status of the cell is irreversibly altered, in a step that is necessary for progression into anaphase. We are presently examining the basis for this change.

nifedipine – UV labile (breaks down)
Ruthenium red – prevents Ca^{++} extrusion
Bay K 8644 – like nifedipine but
maintains channel activity (if open, keeps open)

GAMMA-TUBULIN AND THE REGULATION OF MICROTUBULE ASSEMBLY: Berl R. Oakley, C. Elizabeth Oakley, M. Katherine Jung, Yisang Yoon, Hiromi Tanaka and Yixian Zheng; Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210, USA.

We have discovered that the _mipA_ gene of _Aspergillus nidulans_ encodes gamma tubulin, a new member of the tubulin superfamily (Nature 338,662). We have used integrative transformation by homologous recombination to disrupt the _mipA_ gene _in vivo_. We maintained nuclei carrying the disruption in a heterokaryon and determined the phenotype caused by the disruption in uninucleate spores produced by the heterokaryon. The disruption (_mipAd1_) is a recessive lethal mutation, demonstrating that gamma tubulin is essential for growth in _A. nidulans_. _mipAd1_ strongly inhibits nuclear division, less strongly inhibits nuclear migration and causes a reduction in the numbers and lengths of cytoplasmic microtubules and a virtually complete loss of mitotic spindles. Gamma tubulin is, thus, essential for microtubule function in _A. nidulans_. We purified antibodies against gamma tubulin and immunofluorescence microscopy with these antibodies indicates that gamma tubulin is a component of the spindle-pole-body. We hypothesize that gamma tubulin nucleates the assembly of microtubules from, and attaches microtubules to, the spindle-pole-body. Finally, we have used low stringency hybridizations to identify gamma tubulin cDNAs from _Drosophila melanogaster_ and _Homo sapiens_ and we have used polymerase chain reaction procedures to identify a putative second _D. melanogaster_ gamma-tubulin sequence and three putative gamma-tubulin sequence from _Arabidopsis thaliana_. We conclude that gamma tubulin is almost ceratinly present in all eukaryotic cells and that, in many organisms, there are probably small families of gamma tubulin genes. Supported by NIH grant 31837.





Aspergillus 25-42 good growth
(best at 37)
44 inhibits
But 42 inhibits mutant γ

MOLECULAR CLONING AND SEQUENCING OF A cDNA ENCODING THE PROTEOLIPID SUBUNIT OF VACUOLAR H^+-ATPase FROM OAT

Shoupeng Lai[1], John C Watson[+1], Norman J Hanson[2], and Heven Sze[1]
[1]Botany & [2]Biochemistry Depts, [+]Center of Agricultural Biotechnology, Univ. Maryland, College Park, MD 20742

A cDNA library in the expression vector \gt11 was constructed from total cellular poly(A)$^+$ RNA from oat roots (*Avena sativa L.* var. Lang). A synthetic oligonucleotide probe, corresponding to a conserved region in the proteolipid gene from bovine brain chromaffin granule H^+-ATPase, was used to screen the cDNA library. Seventeen positive cDNA clones were obtained. A fusion protein, expressed in *E. coli* containing one of the recombinant phages, cross-reacted with affinity-purified antibody to the 16 kDa proteolipid of oat vacuolar H^+-ATPase. The deduced amino acid sequences of the cDNA clones revealed extensive homology with bovine and yeast V-type H^+-ATPase proteolipids. The nucleotide sequences of the cDNA clones suggest the presence of a gene family in oat genome.

REGULATION AND FUNCTION OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN CARROT

Cheol Ho Hwang and J. Lynn Zimmerman
Department of Biological Science
University of Maryland Baltimore County
Catonsville, MD 21228

We have analyzed the heat shock response of cultured cells of carrot (*Daucus carota L.*) and found variability in the heat shock (HS) proteins synthesized by different cell lines. These differences are especially evident in the low molecular weight (LMW) HS protein group. Moreover, some of the lines which showed loss of some LMW hsps also exhibit temperature sensitive blocks in somatic embryogenesis, which may suggest some roles of hsps in thermo-protection in general or in early development of somatic embryos specifically.

Analysis of LMW HS gene expression through early development showed an unusual pattern of regulation. This is characterized by the lack of transcriptional induction of the heat shock genes (at certain embryo stages; most dramatic in globular embryos) resulting in a reduced level of the messages encoding the LMW HS proteins, while the same or even enhanced level of corresponding HS proteins are synthesized at the globular stage. We have identified another example analogous to the globular stage (where there is little HS mRNA but significant HS protein) in the expression of LMW HS genes at 30°C. These data lead us to consider that there may be some type of translational compensation mechanism functioning in the plant cells, whereby equal and high levels of protein can be synthesized from unequal and low levels of mRNA. In support of this idea, we have also found some distinct differences between *in vivo* and *in vitro* translation of 30°C messages which may suggest the existence of such a translation enhancing machinery in living cells.

As a next step toward understanding the role of LMW HS proteins in plants, we have initiated experiments designed to block HS message flow with antisense message. We are targeting our initial analysis on early embryos and cells at 30°C to take advantage of the naturally lower level of LMW HS messages we have observed in these cells.

Regulated Expression of a Heat Shock Fusion Gene in Transgenic Carrot

Liqun Wang and J. Lynn Zimmerman
Dept. of Biological Sciences, UMBC
Baltimore, MD 21228

ABSTRACT

Plants, like all other organisms, respond to elevated temperature by inducing the expression of a set of heat shock genes and repressing the gene activities encoding non-heat shock proteins. We have studied the regulation of the heat shock gene expression in cultured carrot cells by constructing a chimeric gene consisting of a putative HS promoter and the firefly luciferase gene as a reporter gene. The functional integrity of a 1 kb promoter fragment, from a cloned carrot gene encoding an abundant low molecular weight heat shock protein, was demonstrated by its ability to confer thermoinducibility on the adjacent luciferase gene in transgenic carrot cells. The transcriptional activation appears to be the primary induction leading to the accumulation of the luciferase mRNA exclusively under heat shock condition, probably through the interaction between the heat shock transcription factor and one or more of the five putative heat shock elements present in this promoter fragment. On the other hand, unlike most non-heat shock mRNA which are translationally sequestered, the non-heat shock luciferase mRNA is not excluded from translation during a heat shock when it is highly transcribed by the HS promoter. However, in contrast to the endogenous heat shock mRNA, the luciferase mRNA does show less selective association with polyribosomes, which supports the putative function of the 5' untranslated heat shock leader sequence (absent in this construct) in signaling the translational machinery to selectively translate HS mRNA during HS. The overall induction of the chimeric gene in the transgenic line is about 50 fold, as assayed by the luciferase enzyme activity. Thus, it seems that there are multiple levels of control exerted after the transcriptional activation; apparently there is a mechanism which tags a mRNA for translation by virtue of its transcription during heat shock and a mechanism which utilizes signal sequences in the 5' end of the untranslated leader of a HS mRNA for the translational preference.

DEVELOPMENT AND APPLICATION OF AN RFLP MAP OF POTATO

M.W. Bonierbale, R.L. Plaisted and S.D. Tanksley, Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853.

A genetic map of potato, based on restriction fragment length polymorphisms (RFLPs) of single copy DNA probes from tomato, was constructed in a segregating cross among diploid *Solanum* species. The employment of tomato clones was suggested by the facts that potato and tomato are closely related, and have nearly identical karyotypes with significant nucleotide sequence homology. The high degree of DNA homology and the use of tomato clones allowed the development of comparative maps between potato and tomato. This research revealed that chromosome contents/ gene order are very highly conserved between these two species. At this level of resolution the two species were found to be differentiated by four intrachromosomal inversions.

Applications of RFLP maps to crop improvement include germplasm evaluation, and locating genes of agronomic importance in the plant genome. RFLP markers closely linked to genes of interest can be used as selection tools or as starting points for gene cloning. Linkage analysis with these markers also permits the mapping of cloned genes to chromosomal loci.

Genetic variability within and among 90 *Solanum* accessions representing 18 species of agronomic interest have been assessed with mapped RFLPs, by converting similarities among restriction patterns to estimates of genetic distance. Quantitative information about the variation within and among species will be valuable in directing efforts toward the utilization of related species as sources of desirable genes for potato breeding. In a similar study, a collection of cultivars has been fingerprinted with RFLPs. Several simply inherited traits have been located with RFLPs in other crops, and the methods used are being adapted for tetraploid potatoes. We are currently evaluating progenies segregating for virus and nematode resistance with RFLPs in efforts to locate the single genes controlling these traits. Diploid progenies involving a wild species are being used to study quantitatively inherited insect resistance with RFLPs. With these techniques, loci governing both desirable and undesirable traits from wild species can be identified and monitored during introgression, and recombination between foreign genomes can be observed.

DEVELOPMENT OF LARGE DNA METHODS FOR PLANTS

Joseph R. Ecker, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018

The development of several methods for the manipulation and cloning of large DNA molecules will dramatically increase the ease of gene isolation by chromosome walking. Primary amongst these is the development of pulsed-field gel electrophoresis (PFGE) (1, 2). This technique has allowed the separation of DNA molecules of up to 12 million basepairs (Mbp) (3); an improvement upon conventional electrophoresis techniques of over two orders of magnitude. The second important development is the demonstration that segments of DNA as large as 1 Mbp can be cloned as yeast artificial chromosomes (YACs) (3). This is an improvement of over an order of magnitude over previous cloning procedures. We have begun to apply these techniques to the analysis of the *Arabidopsis* and rice genomes.

Procedures for the preparation and cloning of large DNA molecules from plant cells have been developed (5). Plant cell protoplasts are used for the preparation of large DNA molecules in agarose plugs or in liquid. PFGE analysis of *Arabidopsis* or rice large DNA preparations using a contour-clamped homogeneous electric-field (CHEF) apparatus (6) indicated that the size was at least 9 Mbp. Large DNA prepared in plugs or as liquid is useful for the construction of long range restriction enzyme maps using infrequent cutting enzymes. Previously, carrot DNA-containing YACs as large as 0.5 Mbp have been constructed via transformation of yeast with PFGE-sized ligation products (7). Using similar methods, large DNA from *Arabidopsis* and rice has been used to construct YACs; analysis of these YAC clones is in progress.

The ability to clone and stably propagate large segments of DNA in a simple genetic background has several important implications for plant research; YACs will be useful for gene isolation via chromosome walking, physical mapping of plant genomes and for the direct transfer of large DNA segments back into the host organism.

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(This work is supported by the NCHGR - HG00322 and the Rockefeller Foundation).

250 mM EDTA works better than 25 mM EDTA (keeps it from being degraded)
gets solid lanes due to degradation

LONG RANGE ANALYSIS OF ENVIRONMENTALLY INDUCED 5S DNA REARRANGEMENTS IN FLAX, LINUM USITATISSIMUM, L: Richard G. Schneeberger and Christopher A. Cullis; Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106.

The induction and inheritance of environmentally induced variation in flax involves changes in the copy numbers of most of the highly and intermediately repetitive DNA fractions of the genome. As a model for studying rapid sequence alterations in flax we have described a detailed molecular characterization of a highly repetitive gene class, the 5S RNA gene family. One group of 5S DNA sequences has been shown to detect RFLPs between the progenitor inbred line Pl and environmentally induced genotrophs. Identical RFLP patterns were detected in four small phenotype genotrophs produced in separate environmental induction experiments, indicating that similar DNA alterations had occurred in association with the induction of the small phenotype. Segregation analysis indicates that the RFLPs are linked to a single chromosomal locus. Long range analysis of this chromosomal region has been employed in order to further characterize the rearranged locus.

Flax — Genome 1.5pg/2C, 7×10^8 bp/~~chromosome~~ 1C

(small for angiosperms, but 8-10x arabidopsis)

2N=30

44% single copy; 56% repetitive sequence

700 MB total (1 copy)

[illegible] in LMP agarose: Use .5M EDTA, pH9.3, 1% sarkosyl, 1mg/ml Prot K 20 24hr. Store at 4° C (1yr ok)

Wash in TE, 10mM PMSF

Wash out PMSF in TE

Digest in 250-500 µl w/ 50-150 units of enzyme, overnight

(Do FIGE w/ DNAStar program, affordable) → Hoefer vertical unit.

Minimum pulse time = 50msec

COMPARISON OF GENOMIC CLONES REPRESENTING TWO SELF-INCOMPATIBILITY ALLELES IN *PETUNIA INFLATA*

Craig E. Coleman, Anuradha Singh and Teh-hui Kao, Dept. of Molecular and Cell Biology, Penn State University, University Park, PA 16802.

The solanaceous species, *Petunia inflata,* displays monofactacorial, gametophytic self-incompatibility. In the pistil, a single 24-25 kDa glycoprotein has been shown to be associated with self-incompatibility, and cDNAs for three alleles of the gene encoding this protein have been isolated and sequenced (*Sexual Plant Rep.* **3**:130-138). These cDNAs were used to probe a multi-allelic genomic library from *P. inflata* resulting in the identification of two clones representing the S_1 and S_3 alleles. Sequence data from these clones shows the presence of a single intron in both alleles. Although there is 75% homology between the coding regions of these S-alleles, comparison of the flanking regions reveals a striking disimilarity, with virtually no homolgy detectable. A 2.5 kb fragment of the S_1 clone, located about 7 kb downstream from the S-gene coding region, has been shown to hybridize to genomic DNA from plants containing the S_3 allele while intervening sequences do not hybridize. This fragment also cross-hybridized to genomic DNA from the related, self-incompatible species, *Solanum chacoense.* We are currently examining the flanking regions for sequences important to the developmental control of S-gene expression. Also, we are attempting to transfer the S_3 allele into an S_1 genetic background through *Agrobacterium* -mediated transformation.

COMBINATORIAL PROPERTIES OF CAMV 35S ENHANCER SUBDOMAINS: Philip N. Benfey, Eric Lam, Fumiaki Katagiri, Philip Gilmartin and Nam-Hai Chua; Laboratory of Plant Molecular Biology, Rockefeller University, 1230 York Ave., New York, N.Y. 10021

The 35S enhancer from cauliflower mosaic virus (35S) is able to confer high level constitutive expression in transgenic plants. We have used this enhancer as a model system to understand how constitutive expression arises. In our initial studies we were able to show that we could divide the enhancer into two domains each of which is able to confer a different tissue specific and developmentally regulated expression pattern when placed upstream of the 35S TATA region. We have now further divided the upstream domain (domain B) into five subdomains. Each of the subdomains is able to confer a different tissue specific expression pattern when fused to the minimal promoter. In addition, when each subdomain is combined with the downstream domain (domain A) we detect new expression patterns. This apparent synergism is also observed with a combination of subdomains from domain B. In an attempt to understand the molecular basis of these synergistic interactions we have identified a cDNA that encodes a factor that specifically binds to a sequence within domain A. The level of the RNA homologous to this sequence is five to ten fold higher in root than in leaf which is similar to the expression pattern of domain A. The protein encoded by this cDNA is able to activate expression from the A domain promoter in vitro and in vivo. Our analysis of this viral promoter suggests that constitutive expression can arise from the accumulation of modules that are capable of independently driving tissue-specific expression. In addition, combination of these modules results in expression in new tissue types which suggests that there may be a combinatorial code that controls expression throughout development.

Detailed Analysis of Transcription of the *tufA* Region of the Chloroplast Genome of *Chlamydomonas reinhardtii*

Gregg W. Silk and Madeline Wu
Department of Biological Sciences
UMBC, Baltimore MD 21228

The *tufA* gene of the *C. reinhardtii* chloroplast genome encodes elongation factor EF-Tu, a 50 kD G protein essential for peptide bond formation during protein translation. We have previously shown that the *tufA* transcript accumulates during the greening of dark-grown cells. This region of the chloroplast genome was studied to identify possible regulatory sequences of the *tufA* gene as well as flanking genes. The 5′ and 3′ ends of the *tufA* transcript were mapped, and the transcript extends from approximately -255 to +200 relative to the *tufA* open reading frame. It was also determined that *tufA* RNA bears the same 5′ and 3′ ends during the accumulation of this transcript. DNA sequences flanking the *tufA* gene were cloned. Subclones of these flanking regions were used to probe Northern blots to determine the approximate location of neighboring transcripts. The flanking regions were sequenced and the flanking genes were identified by DNA sequence analysis. The *psbK* gene is located 5′ and a tRNA gene is located 3′ to the *tufA* gene. The *tufA*-proximal ends of the flanking transcripts were also mapped to precisely define the untranscribed intergenic regions. Putative regulatory sequences were identified.

MANIPULATION OF DEVELOPMENT IN TRANSGENIC PLANTS. June Medford and Harry Klee*. Dept. of Biology, Pennsylvania State University, University Park PA 16802 and *Monsanto Co. 700 Chesterfield Village Parkway, St. Louis MO 63198.

In plant development, the body plan is gradually unfolded through continuous events. The shoot system develops from continuous activities of a small localized group of cells known as the shoot apical meristem (SAM). All events related to organ formation, tissue differentiation and totipotency can be found in the shoot apical meristem (SAM). We have used the fortuitous amplification of SAMs found in *Brassica oleracea* var. botrytis (cauliflower) as a system to isolated SAMs in quantity. cDNA clones preferentially expressed in SAMs have been isolated from cauliflower using differential hybridization. We designated these clones 'meri' for their expression in meristems. Since, cauliflower is closely related to *Arabidopsis*, isolation of corresponding cDNA and genomic clones from *Arabidopsis* was greatly facilitated. The transcriptional promoters have been defined for the meri genes and fused to GUS reporter genes. Analysis of the promoter fusions in transgenic plants have shown that the promoters direct expression in vegetative and reproductive SAMs.

Besides features which act in localized regions, the development of a plant must include features that act across cells and organs. The plant hormones, auxin and cytokinins have been well documented to alter developmental signals throughout the plant. We used auxin and cytokinin biosynthesis genes from *Agrobacterium tumefaciens* to alter hormone levels in transgenic plants. Auxin and cytokinin levels have been altered in transgenic plants in a temporal manner by fusing the biosynthesis genes to a heat inducible promoter. These studies have shown there are specific aspects of plant morphology that can be easily altered. Alteration of organ morphology is easily done when the organ is in an expansive state.

Since the shoot apex has often been described as a source of a plant hormones and hormonal gradients, we directed auxin and cytokinin biosynthesis in the meristem by fusing the biosynthesis genes to the meri promoters. We will describe the preliminary results of these chimeric genes in transgenic plants.

Molecular events → → → phenotype
regulated in space & time

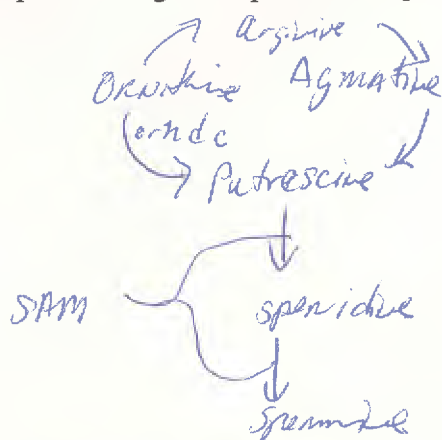
GENETIC ANALYSIS OF POLYAMINE SYNTHESIS

Russell L. Malmberg
Botany Department, University of Georgia, Athens, Georgia, 30602

Polyamines are small positively charged compounds that have been hypothesized to be involved in a wide variety of plant physiological and developmental functions. They are essential for cell survival and cell division, and they have been correlated with phenomena as diverse as senescence, floral development, hormone response, somatic embryogenesis, and certain stress responses. Polyamine synthesis is also unusual in that there are two pathways to putrescine, from ornithine via ornithine decarboxylase, and from arginine via arginine decarboxylase through agmatine. Two basic questions about polyamines in plants are thus what are their functions, and how are the pathways regulated?

For a number of years our experimental approach was to isolate mutants with altered polyamine synthesis, using tobacco cell cultures. Since potent and/or specific inhibitors exist for many of the enzymes in the pathway, it was possible to select cell culture lines resistant to these inhibitors. Our collection of tobacco mutants includes DFMO resistance and MGBG resistant lines. Biochemical and developmental analysis of these mutants suggested a possible role for polyamines in tobacco floral development. The mutants generally had such distorted floral morphologies that they were sterile, ruling out a genetic analysis.

Recently, we purified arginine decarboxylase from oat leaves, obtained an amino terminus amino acid sequence, then used this information to isolate a cDNA encoding oat arginine decarboxylase. Comparison of the derived amino acid sequence with that of the arginine decarboxylase gene from *E. coli* reveals several regions of sequence similarity that may play a role in enzyme function. The open reading frame in the oat cDNA encodes a 66 kDa protein, but the arginine decarboxylase polypeptide we purified has an apparent molecular weight of 21 kDa and is encoded in the carboxyl terminal region of the open reading frame. A portion of the cDNA encoding this region was expressed in *E. coli*, and a polyclonal antibody was developed against the expressed polypeptide. The antibody detects 31 kDa and 21 kDa polypeptides on western blots of oat leaf samples. Maturation of arginine decarboxylase in oats appears to include processing of a precursor protein.



Abstract

Isolation and characterization of cytokinin response mutants in *Arabidopsis thaliana* Wenpei Su and Stephen H. Howell, Boyce Thompson Institute, Cornell University, Ithaca NY 14853.

Cytokinin (benzyladenine, BA) inhibits primary root growth and induces root hair elongation in *Arabidopsis*, while a cytokinin antagonist, tubercidin (7-deaza-adenosine) counteracts the effect of cytokinin on root growth. We used BA and tubercidin to screen *Arabidopsis* M2 populations for cytokinin mutants. Three types of mutants were sought: Cytokinin insensitive mutants, cytokinin "overproduction" mutants, and cytokinin"receptor" mutants. Several representatives of each class were obtained. In cytokinin insensitive mutants, primary roots grew significantly longer than those of wildtype in micromolar concentrations of BA. In addition, these mutants had few, short root hairs in the absence of BA. Low concentrations of BA stimulated more root hair formation in the mutants, but higher concentrations of BA failed to induce root hair elongation as it did in the wild type. Leaves in the insensitive mutants were also altered in color, shape and/or size in the absence of cytokinin. Both roots and leaves in the "overproduction" mutants, in the absence of exogenous cytokinin, have a phenotype similar to wild-type plants grown in high levels of cytokinin. The "receptor" mutants were selected on the basis of resistance to tubercidin, but normal response to their endogenous hormone. Preliminary genetic data indicate that the cytokinin insensitive determinants may be dominant in some mutants and recessive in others. The two "overproducer" mutants appear to be recessive. In summary, primary root and root hair growth can be used as phenotypic indicators for *Arabidopsis* mutants with altered responses to cytokinin. Several classes of mutants were selected and are being analyzed at the genetic and physiological levels.

FERTILE TRANSGENIC MAIZE

T.M. Spencer, W.J. Gordon-Kamm, M.L. Mangano, T.R. Adams, R.J. Daines, W.G. Start, J.V. O'Brien, S.A. Chambers, W.R. Adams, N.G. Willetts, T.B. Rice, C.J. Mackey, R.W. Krueger, A.P. Kausch, P.G. Lemaux

Discovery Research, DeKalb Plant Genetics, Eastern Point Road, Groton, CT 06340

A reproducible system for the generation of fertile, transgenic maize plants has been developed. Cells from embryogenic maize suspension cultures were transformed with the bacterial gene, *bar*, using microprojectile bombardment. Transformed calli were selected using the herbicide bialaphos. Integration of *bar*, and activity of the enzyme phosphinothricin acetyl transferase (PAT) encoded by *bar*, were confirmed in all bialaphos-resistant callus lines. Fertile transformed maize plants (R_o) were regenerated and progeny (R_1) were recovered from R_o plants representing several transformation events. All PAT-positive progeny analyzed contained *bar*. Localized application of herbicide to leaves of *bar*-transformed R_o and R_1 plants resulted in no necrosis, confirming functional activity of PAT in the transgenic plants. Cotransformation experiments were performed using a mixture of two plasmids, one encoding PAT and one containing the nonselected gene encoding β-glucuronidase. R_o plants regenerated from cotransformed callus expressed both genes. These results describe and confirm the development of a system for introduction of DNA into maize.

Poster #1

EFFECTS OF TWO RECONSTRUCTED CYTOKININ GENES ON DEVELOPMENT IN TRANSGENIC PLANTS: Ann C. Smigocki and Scott A. Harding; USDA/ARS, Plant Molecular Biology Laboratory, Beltsville, Md. 20705.

Expression of the cytokinin gene, isopentenyl transferase (ipt) from *Agrobacterium tumefaciens*, was examined in transgenic plants to gain a better understanding of its function in controlling growth and differentiation. The gene was placed under the control of heat (hs-*ipt*) and wound (IIk-*ipt*) inducible promoters and introduced into *Nicotiana plumbaginifolia* by cocultivation of leaf discs with *Agrobacterium tumefaciens*. Plants transformed with the hs-*ipt* gene exhibited phenotypes associated with excess cytokinin even when thermally not induced. They had less developed root systems, reduced leaf size, increased growth of axillary buds, and in general were shorter and greener than control plants. Following heat treatment, R1 plants showed increased transcription of the *ipt* gene in leaves and stems but not roots. Maximum transcript levels were detected 2 hours after heat treatment and declined over the next 46 hours to 6% in leaves and to uninduced levels in stems. Increases in transcription were directly correlated with accumulation of zeatin and zeatinriboside. In contrast to the hs-*ipt* plants, those transformed with the IIk-*ipt* gene were characterized by extreme apical dominance with little release of axillary buds. The plants were taller than either the hs-*ipt* transgenic or normal plants and had larger, light green leaves at the base of the stem. Maximum *ipt* messages were detected 12 to 24 hours after wounding of the leaves. Transient accumulation of endogenous cytokinins did not negatively affect the growth and development of transgenic plants. However, it is the promoters of chimeric cytokinin gene constructs which appear to affect the resulting phenotypes of transgenic plants suggesting that cytokinin production may need to occur at an appropriate stage of cell development or be localized in specific cells, tissues, or organs to affect preprogrammed patterns of differentiation.

Poster #2

BIOSYNTHESIS OF THE PHYTOALEXIN PISATIN: PURIFICATION AND CHARACTERIZATION OF THE TERMINAL ENZYME AND ANTISERA

Carol L. Preisig[1] and Hans D. VanEtten[2]
[1]USDA Eastern Regional Research Center, Philadelphia, PA 19118
[2]Department of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721

The isoflavonoid phytoalexin pisatin is synthesized by *Pisum sativum* in response to microbial infection and certain other forms of stress. An enzyme which synthesizes pisatin by methylating the 3-hydroxyl of (+)6a-hydroxymaackiain (HMK) was extracted from $CuCl_2$-stressed pea seedlings. The enzyme has been enriched 250-fold by standard purification methods. A silver nitrate-staining band of 43 kd by SDS-PAGE was most intense in chromatographic fractions containing peak enzyme activity throughout purification; this protein was the only one in a partially purified preparation which was photoaffinity labelled with the substrate [^{3}H]*S*-adenosyl-L-methionine. The 43 kd band was cut from SDS gels and used to raise antisera. The antisera specifically recognized a 43 kd protein that was induced by $CuCl_2$ or wounding, and specifically inhibited HMK methyltransferase activity.

The purified enzyme was quite specific for HMK as substrate, and strongly preferred the (+) stereoisomer of HMK and other pterocarpans. K_m values were relatively low, 2.3 µM for (+)HMK and 35 µM for the methyl donor, *S*-adenosyl-L-methionine. Its substrate specificity and its induction when pisatin synthesis is elicited suggest that this enzyme plays a specific role as part of the phytoalexin response of pea.

Western blot analysis suggests that the enzyme is newly synthesized by garden pea in response to stress. Further studies of the induction are in progress.

Poster #3

USE OF DELETED AND CHIMERIC COAT PROTEIN CONSTRUCTS TO EXAMINE POTYVIRUS COAT PROTEIN STRUCTURE AND COAT PROTEIN MEDIATED RESISTANCE.

John Hammond, Ramon L. Jordan, and Kathy K. Kamo, USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, MD 20705.

A number of monoclonal antibodies (MAbs) [prepared to a mixture of potyviruses] reacted with a fusion protein, expressed in *E. coli* from a cDNA clone which contains the complete coat protein (CP) gene of bean yellow mosaic virus (BYMV). Several of these MAbs also react with the CPs of other potyviruses. A series of carboxyterminal deletions from the BYMV CP gene were provided termination codons in all reading frames from a synthetic oligonucleotide, thus minimizing the non-viral amino acids in the fusion protein. Chimeric constructs were prepared between BYMV and the pepper mottle virus or the zucchini yellow mosaic virus CP genes. The truncated and chimeric CP genes were expressed in *E. coli* and analyzed by ELISA and Western blotting with the MAbs. Patterns of MAb reactivity were compared to amino acid sequences and structural predictions for the CP. Selected constructs are being expressed in *Nicotiana benthamiana* by *Agrobacterium*-mediated transformation to examine the contribution of different domains to the protective effect reported in plants expressing viral CP; transformed plants have been identified by PCR.

Poster #4

TURNIP CRINKLE VIRUS (TCV) - ARABIDOPSIS: A PATHOGEN - PLANT MODEL INTERACTION. Isolation of a resistant ecotype.

Mary Polacco[1], Xiao-Hua Li[2], Anne Simon[2]
[1]Dept. Biochemistry, University of Missouri-Columbia 65211; [2] Dept. Plant Pathology, University of Massachusetts- Amherst 01003.

Turnip crinkle virus (TCV), with its virulent satellite RNA C (sat RNA C), is the only known viral pathogen to kill Arabidopsis thaliana [1, 2]. The virus and sat RNA C replicate rapidly, becoming major RNA species 3 days post-inoculation. Virus induced proteins are prominent in whole leaf extracts 1 week post-inoculation. In a search for genetic resistance to TCV, we screened: **(a)** 6000 individuals of a second generation (M2) following ethyl methyl sulfonate mutagenesis, **(b)** 17 ecotypes and **(c)** 8 mapping stocks. We have identified an ecotype, Dijon-0, that confers resistance to TVC + sat RNA C, inoculated mechanically as naked RNA's. Dijon-0 does not support systemic infection of TCV and at 1 week post-inoculation, does not produce novel leaf proteins. Resistance is partially dominant: the Dijon-0/ Columbia, glabrous hybrid has less severe symptoms than Columbia,glabrous. Analysis of F2 progeny from the cross Dijon x Columbia, glabrous is underway. Preliminary results, based on backcross and F2 progenies, indicate that 1-2 loci confer resistance.

[1] Li, XH, AE Simon (1990) Phytopathology 80: 238-242.

[2] Abstracts, Oct. 1989 Arabidopsis Meetings, Bloomington,ID

Poster #5

ANALYSIS OF A DE NOVO GENERATED DEFECTIVE INTERFERING RNA OF TURNIP CRINKLE VIRUS. Chunxia Zhang, Xiao Hua Li and Anne E. Simon. Department of Plant Pathology and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

Defective interfering (DI) RNAs are defective versions of viral RNAs that have lost essential coding sequences required for independent replication, maturation, or packaging. DI RNAs are thought to play an important role in virus replication and yields. A DI RNA has been generated de novo after inoculation of TCV-B in vitro synthesized transcripts and was called DI1 RNA (Li et al, 1989) . DI1 RNA (383 bases), which contains the exact 5′ and 3′ ends of TCV as well as an internal virus segment, has been cloned and sequenced (Li et al., 1989). A full length cDNA clone has now been constructed in a vector containing a T7 bacteriophage promoter. In vitro synthesized transcripts of DI1 RNA, which have two extra guanosine residues at the 5′ end and no extra bases at the 3′ end, were inoculated on turnip and mustard along with TCV helper virus inoculum which contains the small satellite (sat-) RNA D. This experiment confirmed that the in vitro transcripts of DI1 RNA were infectious and these transcripts intensified the symptoms of the helper virus.

Several deletions were constructed in DI1 RNA cDNA and plants were inoculated with in vitro transcripts of these deleted DI1 RNAs along with the helper virus inoculum. DI1 RNA with deletions of bases 91 to 107 or bases 77 to 96 (from 5′ to 3′) were not infectious. During the course of these experiments, an RNA species of approximately 400 bases accumulated in some plants. This RNA was cloned and sequenced and was found to be composed of sat-RNA D at 5′ end and TCV sequence at the 3′ end. In previous experiments recombination had been found to occur between sat-RNA D and sat-RNA C. In these recombinants, different lengths of sat-RNA D molecules were found to be joined to sat-RNA C; the left side of the recombination junctions varied (sat-RNA D sequence) while the right side of the junctions all began with one of the three consecutive bases of sat-RNA C. A region of 20 bases at the right side of the sat-RNA recombinant junctions was nearly identical to a 20 base sequence located very near the 5′ end of TCV as well as the right side junctions of other discontinuous RNAs and may signify a replicase recognition motif (Cascone et al., 1990) . However, no similar motif was found at the junction of sat-RNA D and TCV sequence in this new chimeric RNA.

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2. Cascone, P.J., Carpenter, D.C., Li, X.H. and Simon, A.E. (1990) Recombination between satellite RNAs of turnip crinkle virus. EMBO J. 9, 1709-1715.

Poster #6

TURNIP CRINKLE VIRUS (TCV) AND ITS SMALL SUB-VIRAL RNAs. Xiao Hua Li and Anne E. Simon. Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

Naturally occurring turnip crinkle virus (TCV) isolate, TCV-M, supports a family of small satellite RNAs (sat-RNAs) (194-355 b). Sat-RNA C (355 b), the only virulent sat-RNA, was previously found to intensify viral symptoms on turnip cultivar Just Right. We have analyzed the effect of sat-RNA C on symptom intensification using other cruciferous plants. Sat-RNA C exacerbates symptoms on all hosts where TCV produced visible symptoms including cultivars of *Brassica rapa* and *Arabidopsis thaliana*. However, sat-RNA C does not have any effect on four cultivars of *B. rapa* that are tolerant (symptomless) to TCV infection. Lack of symptoms is not accompanied by lower accumulation of TCV sat-RNA C.

A second isolate of TCV, TCV-B, was also thought to support a "sat-RNA C-like" molecule. However, instead of producing severe symptoms resembling sat-RNA C, this molecule produces milder symptoms than sat-RNA C. Using Northern hybridization with specific radioactively labeled oligonucleotides, we have found that this RNA shares almost no sequence homology with sat-RNA C at the 5′ end. cDNA cloning and sequencing demonstrated that the RNA is actually a defective interfering RNA (DI-RNA), denoted DI-RNA G. DI-RNA G is a mosaic molecule with 5′ and 3′ viral segments and a repeat of 36 nucleotides at the beginning of the 3′ segment. The 5′ terminal 21 nucleotides of DI-RNA G are not similar to genomic TCV sequences but resemble sequences found at the 5′ end of other small RNAs associated with TCV (sat-RNAs). DI-RNA G interferes with the accumulation of TCV genomic RNA and, unlike other DI-RNAs, intensifies the symptoms of one isolate of TCV, TCV-m. To understand DI-RNA replication and generation, we constructed a series of deletions in a full length cDNA copy of DI-RNA G at base 98, at which a *Apa*I linker had been inserted. Transcripts harboring a deletion of bases 72-98 are not infectious. However, deletion of bases 84-98 or 98-116 are infectious and wild type levels of DI-RNA accumulate in plants.

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Poster #7

RECOMBINATION BETWEEN SATELLITE RNAS OF TURNIP CRINKLE VIRUS

Pamela J. Cascone[1], Clifford D. Carpenter[2], Xiao Hua Li[2] and Anne E. Simon[1,2]. [1]Program in Molecular and Cellular Biology and [2] Department of Plant Pathology, University of Massachusetts, Amherst, MA 10003

Turnip crinkle virus (TCV) is a positive sense RNA virus associated with a variety of small, linear, single-stranded RNAs: 1) satellite RNAs (sat-RNAs D and F) which share little sequence homology with TCV, 2) defective interfering RNAs (DI RNA G and DI1 RNA), which are derived exclusively or nearly exclusively from TCV sequences and 3) a chimeric RNA (sat-RNA C) composed of sat-RNA sequences at the 5′ portion and viral sequence at the 3′ end.

When plants were inoculated with TCV, sat-RNA D and *in vitro* transcripts of sat-RNA C containing non-viable mutations in the 5′ domain, recombinant sat-RNAs were recovered. These recombinant molecules were composed of sat-RNA D at the 5′ end and sat-RNA C at the 3′ end; the crossover events occurred *in planta* in a region which is related but not identical in the two RNAs. Analysis of 20 independent recombination junctions revealed that varying lengths of sat-RNA D were joined at one of three consecutive nucleotides in sat-RNA C. Approximately one-third of the recombinants also had additional nucleotides inserted at the junctions which did not correspond to sequence from either parental satellite. Comparisons of the sequences around the sat-RNA C\D crossover points with junctions in other discontinous RNAs associated with TCV revealed that two sequence motifs were common to the right hand side of these junction points; these motifs were also found at the 5′ ends of TCV and associated small RNAs.

Based upon this sequence information, we propose a replicase-driven copy-choice mechanism for RNA recombination as well as DI RNA formation. The replicase, while replicating viral or subviral minus strands, can dissociate from the template along with the nascent plus strand and reinitiate synthesis at one of the two recognition motifs on the same or different template thus generating recombinant RNAs or DI RNAs.

Two approaches are being used to test this model: 1) changing the orientation, location and/or copy number of a motif (MOTIF 1) and 2) generating point mutations in the recognition signal in sat-RNA C molecules containing non-viable mutations in the 5′ domain. To date, we have found that certain single base changes in MOTIF 1 do not allow recombination to occur between sat-RNA D and altered sat-RNA C molecules.

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Poster #8

TRANSFORMED TOMATO PLANTS EXPRESS A SATELLITE RNA OF CUCUMBER MOSAIC VIRUS AND PRODUCE LETHAL NECROSIS UPON INFECTION WITH VIRAL RNA: P.B. McGarvey,* J.M. Kaper,* M.J. Avila-Rincon,‡ L. Peña,‡ and J.R. Diaz-Ruiz‡

*Microbiology and Plant Pathology Laboratory, P.S.I., A.R.S., U.S. Department of Agriculture, Beltsville, Maryland 20705, U.S.A. ‡U.E.I. de Fitopatologia, Centro de Investigaciones Biologicas, C.S.I.C., Madrid, Spain

Cucumber Mosaic Virus (CMV) is a widespread pathogen that infects numerous horticultural crops and has been estimated to be one of the most economically damaging viruses to agriculture worldwide. It sometimes carries along a small satellite RNA called CARNA 5 for (CMV Associated RNA 5) which multiplies in the host plant but only when CMV infection has been established. The presence of CARNA 5 can increase or decrease the severity of disease symptoms depending on the particular CARNA 5 and host plant involved. Some CARNA 5s can aggravate disease symptoms to the point that the plant dies while others can reduce symptoms to the extant that they have proved useful as control agents to prevent more severe infections.

Tomato plants transformed with a single copy of a tomato necrosis causing satellite RNA of cucumber mosaic virus (CMV) express the satellite sequence, but the plants show no disease symptoms and have a normal appearance. Upon challenge infection of the F1 progeny with a CMV strain free of any detectable encapsidated satellite the plants accumulated single and double-stranded forms of satellite RNA and developed lethal necrosis.

Poster #9

SELF-SPLICING OF THE *TETRAHYMENA* GROUP I INTRON IN TOBACCO PLANTS TRANSFORMED BY *AGROBACTERIUM TUMEFACIENS*: EVIDENCE FOR DNA TO DNA INFORMATION TRANSFER

Tzion Fahima, Nadine Pobjecky and Gail Dinter-Gottlieb
Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104

A unique experiment was designed to determine if the self-splicing *Tetrahymena* intron can be transferred via a Ti-plasmid into transgenic plants, with a view to testing for an RNA intermediate in Ti-plasmid transformation.

A plasmid containing the GUS reporter gene was obtained from Dr. R.Jefferson, and the intron fragment was cloned in both orientations between the CaMV promoter and the GUS gene. The resultant plasmids were introduced into *Agrobacterium tumefacians*, and tobacco leaf disks were transformed and regenerated, with selection for kanamycin resistance and GUS expression.

Assay of the plant DNA, using the polymerase chain reaction, showed the presence of the GUS marker gene and the intron, in both orientations, in the transformed tobacco plants. This would indicate that no RNA intermediate was involved in the transformation. Analysis of the mRNA product for GUS in the plant revealed that efficient and complete splicing of the intron had occurred in the transcripts in which the RNA polarity would allow self-splicing. In the reverse orientation, the intron was still present. This supports the model of DNA to DNA information transfer, since an RNA intermediate, if present, should have been capable of such splicing. The experiment also demonstrates the ability of a ribosomal RNA intron from a protozoan to be efficiently spliced in a plant milieu.

Poster #10

NUCLEAR GENES AFFECTING CHROMOPLAST PROTEINS IN *CAPSICUM ANNUUM*: Michal Oren-Shamir, Miguel Cervantes C., Lee A. Newman, Noureddine Hadjeb, and C. A. Price; Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759

The color of mature pepper fruits is controlled by four genes showing Mendelian segregation: *y*, c_1, c_2, and *cl*. We examined seven varieties of *Capsicum annuum* whose genotypes differ in these four genes for possible correlations between specific proteins and one or more of the genes. The principal method employed was SDS-polyacrylamide gel electrophoresis. ChrA, a chromoplast-specific protein of 58 kDa, was detected only in the varieties with a y^+ genotype and its accumulation was unaffected by allelic differences in the three other genes. The association of ChrA with y^+ was confirmed by probing western blots with antiserum to ChrA.

We infer from the data that ChrA is either the gene product of y^+ or that y^+ is pleiotropic. Does ChrA play a role in controlling the color of mature fruit? One possibility is that ChrA is responsible for formation of the keto groups characteristic of the carotenoids of mature red and orange peppers (Davis et al., 1970). Since ChrA is a carotenoid-binding protein (Cervantes-Cervantes et al., 1990), an alternative role for ChrA may be in the stabilization of ketocarotenoids.

The accumulation of ChrB, a chromoplast-specific protein of 35 kDa, is independent of all four genes. We detected no proteins correlating with c_1, c_2, or *cl*.

Supported in part by the New Jersey Commission of Science and Technology and the Busch Memorial Fund.

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Poster #11

PEACH FRUIT MOLECULAR BIOLOGY: COMPARISON OF SPECIFIC RNA LEVELS IN 10 PHENOTYPICALLY/GENOTYPICALLY DIFFERENT FRUIT

Reuben Cohen, Ann Callahan and Peter Morgens, USDA/ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

ABSTRACT

Nine cDNAs representing genes involved in peach fruit development were isolated. Homologous RNA accumulation during fruit development and softening in ten phenotypically different cultivars was characterized. For eight of the genes a similar pattern of RNA accumulation is detected for all ten cultivars, the ninth gene reveals a cultivar-dependent pattern. The amount of gene-specific RNA was compared at distinct points in development and softening among the 10 cultivars. For 5 of the 6 genes tested the peach-almond hybrid shows significantly lower levels of RNA accumulation compared to the peaches. Among the nine peach cultivars there is at most a five-fold difference in the amount of gene specific RNA.

Poster #12

ISOLATION AND ANALYSIS OF A GREEN-FRUIT SPECIFIC GENE IN TOMATO

Andrew D. Lloyd, Ann Callahan and Peter Morgens. Appalachian Fruit Research Station, USDA-ARS, Kearneysville, WV 25430

Green and ripe fruit specific cDNA clones were isolated from a tomato fruit library by differential hybridization. One of these clones, 204, was found by northern analysis of fruit mRNA to correspond to an approximately 800 bp message that was expressed primarily in green fruit, with message levels dropping as the fruit ripened. This partial cDNA clone has been sequenced and no significant homologies to known genes were found. A corresponding genomic clone has been isolated and partially sequenced. The levels of 204 message were also analyzed after wounding and exposure to the ethylene-releasing compound Ethephon. Results of these experiments demonstrate a decrease in 204 message after exposure to ethylene as well as a drop after wounding that was coincident with the initial peak of wound ethylene evolution.

Poster #13

DIFFERENTIAL ACCUMULATION OF TRANSCRIPTS ENCODING PROTEIN KINASE HOMOLOGS IN *PISUM SATIVUM* IS LIGHT-REGULATED: **Xia Lin, Xinhua Feng, and John C. Watson**
Department of Botany and Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742-5815

Protein kinases frequently play a regulatory role in developmental cascades. As a first step in determining whether protein kinases are involved in signal transduction during photoregulated leaf development, we have examined transcripts encoding protein kinase homologs. Mixed oligonucleotides, corresponding to conserved regions of known protein kinase sequences, were used as primers for the polymerase chain reaction (PCR). As template for the PCR, we used cDNA synthesized from poly(A)$^+$ RNA from 7 day-old pea seedlings grown in darkness or in continuous white light. We detect five PCR products, called PsPK1 - PsPK5 in order of decreasing length. The length of PsPK1, the longest and most prominent product, is that predicted from the sequence of a bean cDNA clone encoding a kinase-like protein [Lawton *et al.* (1989) *PNAS* 86: 3140]. Of the 4 shorter PCR products, PsPK3 and PsPK5 are considerably more abundant in samples from dark-grown plants, while PsPK4 is much more abundant in samples from light-grown plants. The PCR results have been confirmed by hybridizing RNA gel blots of total RNA with PsPK probes. For example, PsPK5 hybridizes to a transcript that decreases in abundance as dark-grown seedlings are exposed to continuous white light. Hybridization of PsPK5 with DNA gel blots of restricted pea genomic DNA suggests that it is encoded by a small multigene family. All five of the PCR products have been cloned and their DNA sequences determined. The deduced amino acid sequences show that they encode protein kinase-like proteins. The differential changes in transcript abundance we observe may reflect alterations in the protein kinase forms present during photoregulated leaf development in the garden pea. (Supported by C.A.B. Fellowships to X.L. and X.F. and a competitive grant from the Maryland Agricultural Experiment Station to J.C.W.)

Poster #14

HIGH LEVEL OF EXPRESSION OF GAMMA ZEIN IN MODIFIED *opaque-2* MAIZE: John C. Wallace, Dept. of Biology, Bucknell University, Lewisburg, PA 17837.

The *opaque-2* mutant of maize has generated considerable interest due to its increased nutritional value. In order to overcome the deleterious effects associated with the *opaque-2* phenotype, "modifier" genes have been found which increase kernel hardness, vitreousness, and protein content, while maintaining the desired nutritional aspects. Analyses of several modified *opaque-2* (QPM) lines by SDS-PAGE and quantitative ELISA have shown that a common feature of the modified state is a marked increase in the level of Mr 27 kD gamma zein, while amounts of the other zeins remain low (JC Wallace et al., 1990, Plant Phys. *92*, 191-196). Dot blot analysis of RNA from developing QPM material shows that the increase in gamma zein protein is due to levels of its mRNA two to four times that found in other varieties. Southern blots reveal differences in the restriction maps of the gamma zein genes in modified maize when compared to normal genotypes, but do not indicate any change in gene copy number. The factors causing this increased expression and the means by which it alters the *opaque-2* phenotype are being examined.

Poster #15

S-PROTEIN ASSOCIATED RIBONUCLEASE ACTIVITY IN *PETUNIA INFLATA*

Anuradha Singh and Teh-hui Kao

The Pennsylvania State University

403 Althouse Lab

University Park PA 16802

We have cloned and sequenced cDNAs encoding three S-allele associated proteins (S-proteins) from *Petunia inflata*, a species with gametophytic self-incompatibility. All three S-proteins share sequence homology with ribonuclease T2 from *Aspergillus*. S-proteins (S1, S2 and S3) were purified from pistil extracts using gel filteration chromatography and FPLC. Each S-protein peak was associated with high levels of ribonuclease activity, and the ribonuclease activity was found to comigrate with the S-protein bands on activity staining polyacrylamide gels. Here we report on the biochemical characterization of the S-protein associated ribonuclease activity and consider its relevance to the biological function of S-proteins.

Poster #16

Characterization of the Aspartate Aminotransferase Isozyme-Gene System of Soybean.

Gregory J Wadsworth, Frank J Turano, and Benjamin F Matthews
USDA-ARS
Plant Molecular Biology Laboratory
Beltsville, MD 20705

Aspartate aminotransferase (AAT) plays multiple metabolic roles in plants including hydrogen shuttles, carbon/nitrogen metabolism, and amino acid biosynthesis. Plants are characterized by having multiple isozymes of aspartate aminotransferase encoded by multiple genes. In soybean we can distinguish five different isoforms electrophoretically. Two of the isozymes are spatially and temporally limited in their expression. AAT-1 is detected only in the post-germination cotyledon and AAT-3 is found only in green tissues such as green leaves. Organelle fractionation on sucrose gradients indicate the AAT isozymes are localized in different subcellular compartments. AAT-1 is localized in the glyoxysome; AAT-4 is localized in the mitochondria; and AAT-5 is localized in the plastids. AAT-2 and AAT-3 do not sediment in sucrose gradients and may be cytosolic isozymes. A soybean leaf cDNA library has been screened with a carrot AAT cDNA probe. Five soybean cDNA clones have been isolated which hybridize to the carrot cDNA. Restriction and southern analysis indicates that these clones represent at least 3 different AAT genes.

Poster #17

CLONING OF A GENE FOR HOMOSERINE DEHYDROGENASE FROM CARROT AND SOYBEAN

Jane M. Weisemann and Benjamin F. Matthews, PMBL, USDA/ARS, Beltsville, MD

Homoserine dehydrogenase (HSDH) catalyzes an intermediary step in the conversion of aspartate to methionine and threonine. In order to study the regulation of HSDH in plants we have purified and characterized the protein from carrot cell suspension cultures. Recently we have used information from the purified protein to construct a probe for the gene(s) for HSDH from carrot (*Daucus carota*) and soybean (*Glycine max*). HSDH protein was subjected to proteolysis and two peptides were sequenced. From the amino acid sequences of these peptides DNA coding sequences were deduced and two oligonucleotides were synthesized. These were used to prime a polymerase chain reaction using as a template either first strand DNA synthesized from carrot cell culture poly $(A)^+$ RNA or double-stranded cDNA from carrot root poly $(A)^+$ RNA. The 1100bp PCR product was used as a probe of a λgt11 carrot root cDNA library and a soybean leaf library. From both libraries we isolated clones of about 2000bp in length which showed strong homology to the bacterial genes for HSDH. All the clones found appeared to be missing sequences at the 5' end of the gene. In order to obtain clones which included the 5' cDNA sequences we constructed a carrot cDNA library using an HSDH-specific oligonucleotide to prime first strand cDNA synthesis from a site in the middle of the gene. Two clones isolated from this library contain a possible start site for the HSDH protein. The total length of the carrot cDNA is about 3000bp, which is in agreement with the length of the RNA hybridizing to the PCR product on Northern blots. At the present time about 80% of the DNA sequence of both the full-length carrot gene and the partial soybean gene have been determined. Deduced amino acid sequences from these regions show 37% homology between the carrot HSDH gene and the *E. coli thrA* gene and 80% homology between the carrot and soybean clones.

Poster #18

HORMONAL REGULATION, PROCESSING, AND SECRETION OF CYSTEINE PROTEINASES IN BARLEY ALEURONE

S. Koehler[1] and D. Ho Biology Department, Washington University, St. Louis, Mo 63130

Two major cysteine proteinases designated EP-A (37,000 M_r) and EP-B (30,000 M_r) are known to be induced in barley aleurone layers in response to gibberellic acid (GA_3). Three cDNAs for EP-B have been cloned and genomic Southern blot analysis indicates that they are encoded by a small gene family with no more than 4 to 5 different genes. The proteins encoded by two of these clones, pHVEP1 and 4, are 98% similar to each other and are isozymes of EP-B. The proteins contain large preprosequences followed by the amino acid sequence described as the mature N terminus of purified EP-B, and the proteins are antigenic to EP-B antiserum. The results of pulse-chase experiments indicate that the post translational processing of large prosequences proceeds in a multistep fashion to produce the mature enzymes. Processing intermediates for EP-B are observed both in the aleurone layers and surrounding incubation medium, but only mature EP-A is secreted. The regulation of synthesis of EP-A and EP-B and other aleurone cysteine proteinases was compared at the protein and mRNA level. We conclude that barley aleurone cysteine proteinases are differentially regulated with respect to their temporal and hormonally induced expression.

[1]Current address: USDA-ARS, PSI, Bldg. 001, Rm 328, BARC-West, Beltsville, MD 20705

Poster #19

Selection of Mutant Lines of *Lemna gibba* for Studying Developmental Biochemistry.

Yuen Yee Tam, Janet P. Slovin, and Jerry D. Cohen*, Botany Department, University of Maryland, College Park, MD 20742, and *Plant Hormone Laboratory, USDA/ARS, Beltsville, MD 20705

Lemna gibba G-3 is an aquatic monocot that has many advantages over other higher plants for studying biochemical pathways or processes important for plant growth and development. The metabolism of the plant hormone indole-3-acetic acid (IAA) is thought to play an important role in such plant processes, from seedling growth to fruit ripening. Evidence for its involvement in these processes is primarily correlative. One of the problems that makes IAA studies difficult is that we do not have a complete understanding of the metabolic origin, regulation and fate of IAA in plants. Two lines of research in our laboratory, the selection of mutant plants and development of precise methods to biochemically characterize such plants, should help resolve many of these questions, as suitable plant materials become available. We are therefore using these approaches to investigate the biochemical pathway for indole biogenesis leading to tryptophan, IAA, and other indole compounds. We will then be in a better position to determine which part(s) in that pathway should be investigated at the molecular level.

Two highly inbred plant lines of *Lemna* were treated with 5-10 nM nitrosomethyl urea for up to 30 min. Mutagen treated plants were allowed to grow vegetatively until abnormal pigment sectors occurred, then screened for resistance to 10 μM α-methyl-tryptophan (αMT). Four mutant lines of *Lemna* (MTR1, MTR2, MTR3,and MTR4) which are resistant to growth inhibition by αMT also show cross resistance to 5-methyl-tryptophan. These four mutants are probably dominant mutations as they were derived by somatic mutagenesis.

During these studies we have accumulated a large number of mutants that have pigment abnormalities. One of these, a chlorophyll b deficient mutant, has been previously described at these meetings (1988). We report here on some properties of a different line (NMU-45) which is totally devoid of chlorophyll but has a normal complement of carotenoids. Both of these mutants flower and are potentially useful for doing genetics with *Lemna.*

As expected, NMU 45 does not accumulate the light harvesting chlorophyll a/b binding proteins and *in vivo* pulse labeling shows that they are not being synthesized either. We are currently measuring the levels of a/b binding proteins mRNA in NMU 45 to determine whether the lack of accumulation of the protein is due to rapid turnover of the peptides because there is no chlorophyll to bind with, or whether accumulation is being controlled transcriptionally of translationally. This mutant is easily grown in large amounts on sucrose containing medium and is therefore unique among the known chlorophyll mutants in higher plants.

Poster #20

Genes Encoding Chlorophyll Biosynthetic Enzymes In Angiosperms and Gymnosperms Anthony J. Spano, Kathrine F. Boese, Zhenghui He, Doris J. Morris, and Michael P. Timko. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Chlorophylls and their derivatives play a fundamental role in the energy absorbing and transducing activities of all photosynthetic organisms. Light is known to regulated chlorophyll synthesis at to points in the biosynthetic pathway. The first point is at the formation of ALA the first committed precursor to all plant tetrapyrroles; the second is at the level of protochlorophyllide reduction. This latter activity is mediated by the enzyme NADPH-protochlorophyllide oxidoreductase (Pchlide reductase). In angiosperm species, Pchlide reductase is a light-dependent enzyme requiring reduced pyridine nucleotides and stoichiometric amounts of light quanta for activity. In contrast, gymnosperms are able to synthesize chlorophyll in the dark as well as in the light by virtue of a light-independent Pchlide reductase activity. We have isolated and characterized cDNAs from pea (Pisum sativum) and white pine (Pinus strobus) encoding Pchlide reductase. The structure and expression characteristics of the encoded gene products has been examined. We have also isolated and characterized cDNA sequences encoding intermediate enzymes of the chlorophyll biosynthetic pathway. The results of this analysis will be presented.

Poster #21

<u>SEQUENCE ANALYSIS OF TRANSCRIPTS ENCODING LECTIN-LIKE PROTEINS IN *PISUM SATIVUM*</u> Jhang Ho Pak and Michael S. Dobres, Department of Bioscience, Drexel University, Philadelphia, PA 19104

The shoot-specific transcript for the partial pea cDNA pEA207 encodes a protein with significant sequence similarity to the pea seed lectin. This includes the highly conserved $Ca^{2+}$and Mn^{2+} binding sites and those residues known to be involved in ß-sheet formation in concanavalin A. The transcript accumulates in the actively growing bud of the pea plant and is either absent or present at very low levels in the expanded leaves below the bud (Dobres and Thompson, 1989, Plant Physiology **89**: 833-838). We are interested in further characterizing the pEA207 protein. As a first step we have used pEA207 to isolate several almost-full-length cDNA clones. At least two members of the pEA207 gene family are transcribed: the deduced amino acid sequence of pEA207-I and pEA207-II differ only by three amino-acid substitutions. One of these substitutions results in an additional N-glycosylation site in pEA207-II as compared to PEA207-I. The physiological significance of these glycosylation-site differences remains to be determined. Antibodies generated against the pEA207 protein will be used to further characterize this gene product.

Allnutt, F.C. Thomas
Martek Corp.
Microbiology
6480 Dobbin Road
Columbia, MD 21045

Bandaranayake, Hema
University of Maryland
Botany
College Park, MD 20742

Barnett, Neal
University of Maryland
Botany
College Park, MD 20740

Bauchan, Gary R.
USDA-ARS, PSI
Soybean & Alfalfa Research Lab
B-001, Rm. 323, BARC-West
Beltsville, MD 20705

Benfey, Philip N.
Rockefeller University
Lab. of Plant Molecular Biology
1230 York Avenue
New York, NY 10021

Billings, Sharon
Rutgers University, Cook College
Horticulture Department
Blake Hall
New Brunswick, NJ 08903

Bonierbale, Meredith
Cornell University
Dept. of Plant Breeding & Biometry
252 Emerson Hall
Ithaca, NY 14853-1902

Bottino, Paul
University of Maryland
Botany
College Park, MD 20742

Budelier, Kim
EniMont America, Inc.
Molecular Genetics
2000 Cornwall Road
Monmouth Junction, NJ 08850

Butcher, Henry C.
Loyola College
Biology Department
4501 N. Charles Street
Baltimore, MD 21239

Carpenter, Clifford D.
Univ. of Massachusetts
Dept. of Plant Pathology
Fernald Hall
Amherst, MA 01003

Carson, Sandra
American Cyanamid Co.
Plant Biotech
P.O. Box 400
Princeton, NJ 08540

Cascone, Pamela
Univ. of Massachusetts
Dept. of Plant Pathology
Fernald Hall
Amherst, MA 01003

Chen, Qi
Rutgers University
Cook College
Dept. of Horticulture
New Brunswick, NJ 08903

Christensen, Alan H.
George Mason University
Biology Department
4400 University Drive
Fairfax, VA 22030

Cohen, Jerry
USDA-ARS
Plant Hormone Lab
B-050, HH 4, BARC-West
Beltsville, MD 20705

Cohen, Reuben
USDA-ARS
Appalachian Fruit Res. Sta.
45 Wiltshire Road
Kearneysville, WV 23540

Coleman, Craig E.
Penn State University
Molecular and Cell Biology
403 Althouse Lab
University Park, PA 16802

Crosslin, James M.
USDA-ARS
MPPL
Rm. 252, B-011A, BARC-West
Beltsville, MD 20705

Davis, R.E.
USDA-ARS
MPPL
Rm. 252, B-011A, BARC-West
Beltsville, MD 20705

Deitzer, Gerald
University of Maryland
Horticulture
College Park, MD 20742

Dinter-Gottlieb, Gail
Drexel University
Bioscience Dept.
32 & Chestnut
Philadelphia, PA 19104

Dixon, David
Rutgers University
Waksman Institute
Rm. 208, B-004, BARC-West
Piscataway, NJ 08854

Ecker, Joseph
Univ. of Pennsylvania
Dept. of Biology, Plant Science Inst.
38th and Hamilton Walk
Philadelphia, PA 19104

Feng, Xin-Hua
University of Maryland
Botany
3229 H.J. Patterson Hall
College Park, MD 20742

Fishel, Deanna
USDA-ARS
Appalachian Fruit Res. Sta.
45 Wiltshire Road
Kearneysville, WV 25430

Frett, John
University of Delaware
Plant and Soil Sciences Dept.
Newark, DE 19717

Frias, Clarisse
Rutgers University
Horticulture Department
Blake Hall, Cook Campus
New Brunswick, NJ 08903

Frick, Hugh
University of Delaware
Plant and Soil Sciences Dept.
Newark, DE 19716-1303

Fuqua, Clay
University of Maryland
Microbiology
College Park, MD 20742

Gorman, Mark
Baldwin-Wallace College
Biology-Life Sci. Bldg.
Berea, OH 44017

Gosfreda, Joe
Rutgers University
New Brunswick, NJ 08903

Griesbach, Rob
USDA-ARS, FNCL
Rm. 208, B-004, BARC-West
Beltsville, MD 20705

Gunning, Dorian
American Cyanamid Co.
Plant Biotechnology
Box 400
Princeton, NJ 08540

Hammond, John
USDA-ARS, FNCL
Rm. 208, Bldg. 004, BARC-West
Beltsville, MD 20705

Hammond, Rosemarie W.
USDA-ARS, MPPL
Rm. 252, B-011A, BARC-West
Beltsville, MD 20705

Hines, Pam
Science, AAAS-Editorial Dept.
1333 H Street, NW
Washington, DC 20005

Hsu, Hei-Ti
USDA-ARS
FNCL
Rm. 208, B-004, BARC-West
Beltsville, MD 20705

Hua, Shaobing
University of Maryland
Botany
College Park, MD 20742

Huang, Chen Ho
UMBC
Biological Science
5401 Wilkens Ave.
Catonsville, MD 21228

Hutcheson, Steve
University of Maryland
Botany
College Park, MD 20742

Inamine, Gordon
USDA-ARS, PHL
Rm. 130, B-050, HH-4
BARC-West
Beltsville, MD 20705

Jelenkovic, Gojko
Rutgers University
Horticulture Dept.
New Brunswick, NJ 08903

Jordan, Ramon
USDA-ARS
FNCL
Rm. 208, B-004, BARC-West
Beltsville, MD 20705

Kamo, Kathy
USDA-ARS
FNCL
Rm. 208, B-004, BARC-West
Beltsville, MD 20705

Kelley, Sarah
Microbiology
University of Maryland
3421 Tulane Drive, #31
Hyattsville, MD 20783

Kennedy, Robert
University of Maryland
Maryland Agric. Exp. Station
Symons Hall, Rm. 1116
College Park, MD 20742

Koehler, Susan
USDA-ARS
Soybean & Alfalfa
B-001, Rm. 328, BARC-West
Beltsville, MD 20705

Kubicek, Quentin B.
USDA-APHIS
Biotechnology Permits
6505 Belcrest Road
Hyattsville, MD 20770

Kung, Shain-dow
University of Maryland
Center for Agric. Biotechnology
H.J. Patterson Hall, Rm. 1206
College Park, MD 20742

Lai, Shoupeng
University of Maryland
Botany
College Park, MD 20740

LaRosa, Thomas
University of Maryland
Botany
College Park, MD 20740

LaRue, Thomas A.
Boyce Thompson Institute
Tower Road
Ithaca, NY 14853-1801

Lee, Ing-Ming
USDA-ARS
MPPL
Rm. 252, B-011A, BARC-West
Beltsville, MD 20705

Li, Xiao Hua
Univ. of Massachusetts
Dept. of Plant Pathology
Fernald Hall
Amherst, MA 01003

Lin, Xia
University of Maryland
Botany
College Park, MD 20742

Lloyd, Andrew D.
USDA-ARS
Appalachian Fruit Res. Sta.
45 Wiltshire Road
Kearneysville, WV 25430

Malamy, Jocelyn
Rutgers University
Waksman Institute
Piscataway, NJ 08854

Malmberg, Russell L.
Dept. of Botany
University of Georgia
2502 Plant Sciences
Athens, GA 30602

Matthews, Ben
USDA-ARS
PMBL
Rm. 118, B-006, BARC-West
Beltsville, MD 20705

McGarvey, Peter
USDA-ARS
MPPL
B-011A, Rm. 252, BARC-West
Beltsville, MD 20705

Medford, June
Pennsylvania State University
Department of Biology
415 Wantik Laboratory
University Park, PA 16802

Miller, Raymond
University of Maryland
Agriculture and Natural Resources
Symons Hall, Rm. 1110
College Park, MD 20742

Mischke, Sue
USDA-ARS
Biocontrol of Plant Diseases
B-011A, Rm. 275, BARC-West
Beltsville, MD 20705

Moynihan, Michael
Enimont America
Cell Biology
2000 Cornwall Road
Monmouth Jct, NJ 08852

Oakley, Berl
Ohio State University
Dept. of Molecular Genetics
484 W. 12th Avenue
Columbus, OH 43210

Olson, Lee C.
Christopher Newport College
Biology Department
50 Shoe Lane
Newport News, VA 23606

Oren-Shamir, Michal
Rutgers University
Waksman Institute
Piscataway, NJ 08855-0759

Osei, Joseph
Penn State University
Foods Science Department
215 Borland Lab
University Park, PA 16802

Polacco, Mary
Univ. of Missouri-Columbia
Biochemistry
11 Eames Place
Amherst, MA

Polayes, Deborah
BRL
Molecular Biology R & D
P.O. Box 6009
Gaithersburg, MD 20877

Preisio, Carol L.
USDA-ARS, ERRC
Plant Science
600 E. Mermaid Lane
Philadelphia, PA 19118

Quail, Peter
USDA-ARS
Plant Gene Expression Center
800 Buchanan Street
Albany, CA 94710

Radin, David N.
VPI & SU
Crop & Soils Environmental Science
Blacksburg, VA 24061

Reardon, Ellen M.
University of Arizona
Plant Sciences Dept.
2988 E. Windsor Street
Tucson, AZ 85716

Ribnicky, David
Botany
University of Maryland
H.J. Patterson Hall
College Park, MD 20742

Rowland, Lisa J.
USDA-ARS
Fruit Lab
B-004, BARC-West
Beltsville, MD 20705

Sadasivan, Lakshmi
321 Hampshire Court
Piscataway, NJ 08854

Schaff, Dennis
University of Delaware
Plant and Soil Sciences Dept.
Townsend Hall
Newark, DE 19717-1303

Schmitz, Sonja
Dupont
Agricultural Products
E 402/4256
Wilmington, DE 19805

Schneeberger, Richard
Case Western Reserve Univ.
Biology
2080 Adelbert Rd.
Cleveland, OH 44106

Schultz, David
University of Delaware
Plant and Soil Sciences Dept.
Townsend Hall
Newark, DE 19717-1303

Silk, Greg
UMBC
Biological Science
Baltimore, MD 21228

Simon, Anne
University of Massachusetts
Dept. of Plant Pathology
Fernald Hall
Amherst, MA 01003

Singh, Anuradha
Penn State University
Molecular and Cell Biology Dept.
403 Althouse Lab
University Park, PA 16802

Slovin, Janet
UMCP/USDA
Botany/Plant Hormone
B-050, BARC-West
Beltsville, MD 20705

Smigocki, Ann
USDA-ARS
PMBL
B-006, Rm. 2, BARC-West
Beltsville, MD 20705

Spencer, T. Michael
DeKalb Plant Genetics, Dis. Res.
Pfizer Central Research
Eastern Point Road
Groton, CT 06340

Straney, David
University of Maryland
Botany
College Park, MD 20708

Sturner, Stephen
American Cyanamid Co.
Plant Biotechnology
P.O. Box 400
Princeton, NJ 08540

Su, Wenpei
Boyce Thompson Institute
Cornell University
Rm. 311, Boyce Thompson Instit.
Ithaca, NU 14850

Tam, Yuen Yee
University of Maryland
Botany
Patterson Hall
College Park, MD 20742

Turano, Frank
USDA-ARS
Climate Stress
B-001, Rm. 206, BARC-West
Beltsville, MD 20705

Ueng, Peter
USDA-ARS
PMBL
B-006, BARC-West
Beltsville, MD 20705

Van Wert, Sally
USDA-ARS
Rm. 5, B-009, BARC-West
Beltsville, MD 20705

Verrett, Pamela C.
USDA-APHIS
BBEP/BP
6505 Belcrest Road
Hyattsville, MD 20782

Wadsworth, Gregory J.
USDA-ARS
PMBL
B-006, BARC-West
Beltsville, MD 20705

Wallace, John
Bucknell University
Biology Department
Lewisburg, PA 17837

Wang, Liqun
UMBC
Biological Science
Catonsville, MD 21228

Watson, John
University of Maryland
Botany
College Park, MD 20742

Weisemann, Jane
USDA-ARS
PMBL
B-006, BARC-West
Beltsville, MD 20705

Weissenborn, Deborah L.
VPI & SU
Plant Molecular Biology Dept.
Glade Road
Blacksburg, VA 24061

White, James L.
USDA-APHIS
Biotechnology Permits
6505 Belcrest Road, Rm. 850
Hyattsville, MD 20770

White, Richard
Temple Univ. School of Medicine
Biochemistry
3420 N. Broad Street
Philadelphia, PA 19140

Wilson, Barbara J.
USDA-ARS
PMBL
B-006, BARC-West
Beltsville, MD 20705

Winans, Stephen
Cornell University
Dept. of Microbiology
Ithaca, NY 14853

Wolniak, Stephen
Botany Department
University of Maryland
College Park, MD 20742

Yi, Hu
Botany
University of Maryland
College Park, MD 20742

Zhang, Chunxia
Univ. of Massachusetts
Dept. of Plant Pathology
Fernald Hall
Amherst, MA 01003

Zhang, Xiaorong
VPI & SU
Crop & Soils Environ. Science
Blacksburg, VA 24061

Zimmerman, Lynn
UMBC
Biological Science
Catonsville, MD 21228

Late Registrants

Mandaci, Sevnur
Drexel University
Bioscience Dept.
Philadelphia, PA 19104

Spano, Anthony
University of Virginia
Biology Department
Charlottsville, VA 22901