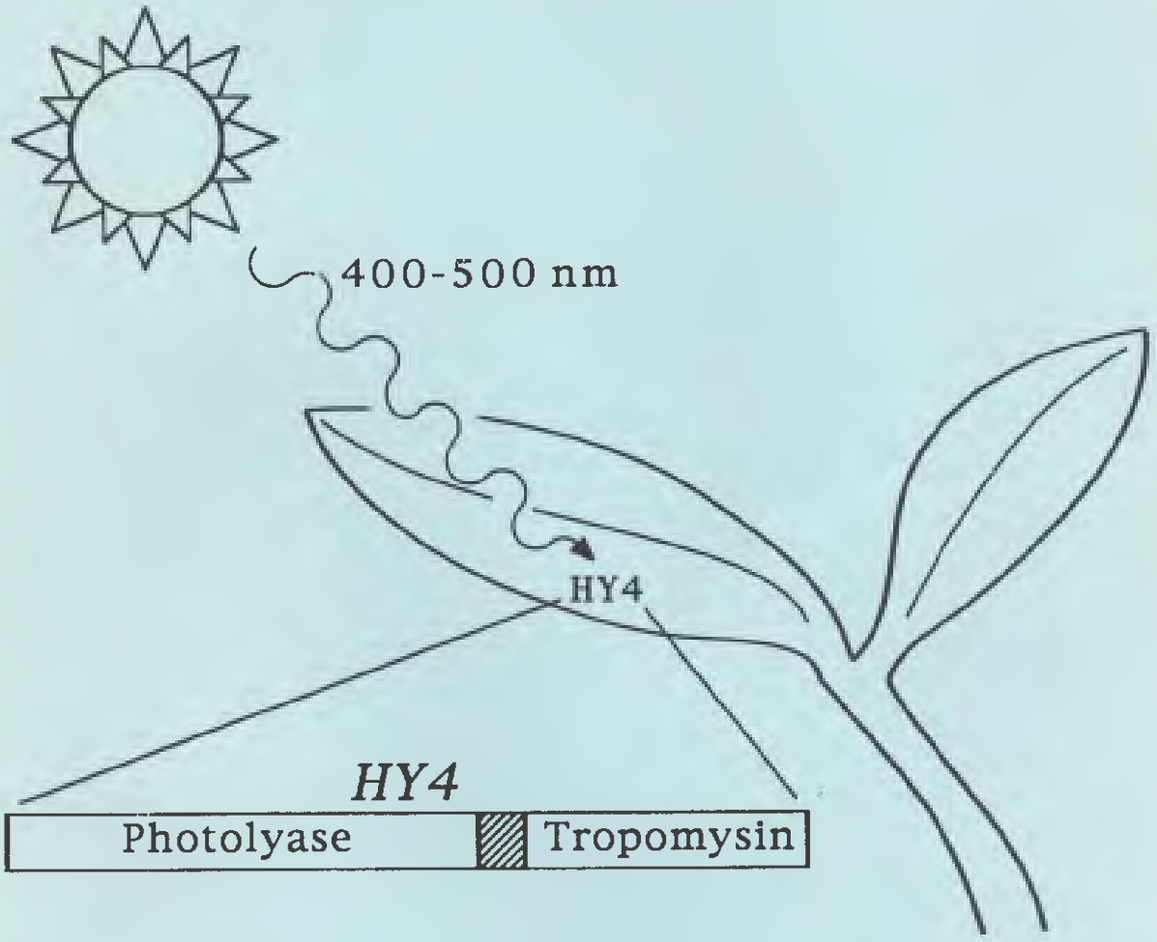
Sue Mischke

Mid-Atlantic Plant Molecular Biology Society



Eleventh Annual Meeting
Beltsville, Maryland
1994

CONTENTS

INTRODUCTION

Welcome to the eleventh annual meeting of the Mid Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. These meetings are designed to invite some of the best scientific minds and to introduce some of the most interesting advances in plant molecular biology to our area scientists at a reasonable price and an accessible location. We hope to entice a large number of students, postdocs and senior scientists to attend and actively participate in presentations and discussions. In addition, the meeting is designed to encourage mixing of scientists in an informal atmosphere during on-site lunches, breaks, socials, and banquet to provide each participant the opportunity to meet invited speakers and other members.

The meeting encompasses a large range of important research areas, which we hope will stimulate participants by informing them about advances outside of their own immediate interests. Please contact members of the organizing committee if you have thoughts or comments for consideration in the planning of future meetings or join next years organizing team and volunteer your services to improve upon what we did this year. All are welcome to help at every stage of planning and organizing each meeting.

Many people were involved in the organization and planning of this meeting and we give them our hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date product advances and help defray costs. **Please visit our exhibitors' displays located downstairs** with the posters during the opportunities afforded in the program. The level of interest you show in their products is a critical factor in their willingness to support future meetings. **Refreshments at breaks will be served in the exhibitors' room downstairs.**

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society. Enjoy the meeting!

Frank Turano
Benjamin Matthews
Co-Organizers

1994 MAPMBS EXHIBITORS

A. Daigger & Co.
Sharon Thompson

Fisher Scientific
Wib Pumpaly

FMC Corporation
Kathleen A. Nicholson

PGC Scientifics Corp.
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Promega Corp.
Marianne Zugel

USDA/ARS
Plant Genome Data and Information Center
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MAPMBS MEETING SCHEDULE

Thursday, July 21, 1994

8:00 AM Registration

9:00 AM Opening Remarks - Frank Turano

REGULATION

(Moderator: John Watson, University of Maryland, College Park)

9:05 AM Lynn Zimmerman (University of Maryland, Baltimore County) "Molecular and Genetic Analysis of Early Embryogenesis"

9:35 AM Brian W. Tague and Howard M. Goodman (Harvard Medical School) "Analysis of an *Arabidopsis* C_2H_2 Zinc Finger Protein Gene Family"

9:55 AM Andrew D. Lloyd, Ewa J. Mellerowicz, Cynthia H. Chow, Richard T. Riding and C. H. Anthony Little (Delaware State University) "Fluctuations in Ribosomal RNA Gene Content and Nucleolar Activity in the Cambial Region of Abies Balsamea (Pinaceae) Shoots During Reactivation"

10:15-11:00 AM **COFFEE BREAK, POSTER SETUP & EXHIBITORS**

11:00 AM Garrett Lee, Katherine Osteryoung and Elizabeth Vierling (University of Arizona) "Structural and Functional Studies of Heat Shock Proteins"

11:30 AM Andre Nantel and Ralph S. Quatrano (University of North Carolina) "Isolation of Rice bZIP Factors that Heterodimerize with EmBP-1"

11:50 AM Business meeting

12:00-1:20 PM **LUNCH**

TRANSFORMATION

(Moderator: Dennis Schaff, University of Delaware)

1:20 PM Kathy Kamo, A. Blowers, F. Smith and J. Van Eck (USDA/ARS/FNCL) "Transformation of *Gladiolus*"

1:50 PM Tomoko Fukasawa-Akada, Paul J. Bottino and John C. Watson (University of Maryland, College Park) "Regulation of Phenylalanine Ammonia-Lyase Gene Expression in Tobacco"

2:10 PM Jhy-Jhu Lin, Nacyra Assad-Garcia, and Jonathan Kuo (Life Technologies Inc., GIBCO/BRL) "Effect Of *Agrobacterium* Cell Concentration on The Transformation Efficiency of Tobacco and *Arabidopsis thaliana* Ecotypes Columbia and Landsburg"

2:30 PM Jeffrey A. Townsend and Laurie A. Thomas (Pioneer Hi-Bred International, Inc.) "Improving the Quality of Seed Proteins in Soybean"

3:00 PM Gregg W. Silk and Benjamin F. Matthews (USDA/ARS/PMBL) "Cloning and Expression of the Soybean Gene Encoding Dihydrodipicolinate Synthase (DS)"

3:20-4:20 PM **COFFEE BREAK, POSTER SESSION & EXHIBITORS**

KEYNOTE ADDRESS

(Introduction: Benjamin Matthews)

4:20 PM Chentao Lin, Margaret Ahmad, John W.Y. Chan, and Anthony R Cashmore (University of Pennsylvania) "The Cryptochrome Family of Blue/UV-A Photoreceptors"

5:20-6:20 PM **SOCIAL HOUR**

6:20 PM **DINNER**

Friday, July 22, 1994

8:00 AM Open Viewing of Posters

REGULATION

(Moderator: Joan Gebhardt, USDA/ARS/PMBL)

9:00 AM Andrew Dancis, D. S. Yuan and R. D. Klausner (NICHD/NIH) "Linkage of Copper and Iron Uptake in *Saccharomyces cerevisiae*"

9:30 AM Francis X. Cunningham, Jr., Zairen Sun, Daniel Chamovitz, Joseph Hirschberg and Elisabeth Gantt (Univ. of Maryland, College Park) "Molecular Structure and Enzymatic Function of Lycopene Cyclase from the Cyanobacterium *Synechococcus sp.* Strain Pcc7942"

9:50-10:50 AM **COFFEE BREAK, POSTER SESSION & EXHIBITORS**

10:50 AM Choirul Muslim and Asim Esen (Virginia Polytechnic Institute and State University) "Identifying Rice β-glucosidase Substrates and Their Physiological Role During Germination"

11:10 AM Mohammad Shahid and Asim Esen (Virginia Polytechnic Institute and State University) "Maize β-glucosidase Gene Contains Plastid Transit Peptide Sequence and Lacks Introns"

11:30 AM Elena del Campillo (University of Maryland, College Park) "Expression of Multiple Cellulase Genes During Abscission of Tomato Flowers: Relationship to Breakstrength"

11:50-1:00 PM **LUNCH**

PLANT/MICROBE INTERACTIONS

(Moderator: Susan Mischke, USDA/ARS/SBML)

1:00 PM Rosemarie W. Hammond and Shanta Rishi (USDA/ARS/MPPL) "Viroid Induction of a Host Plant Protein Kinase: Response to Infection and/or Role in Symptom Expression?"

1:30 PM Y. Hu, P.A. Feldstein, R.W. Hammond, P.J. Bottino and R. A. Owens (University of Maryland, College Park) "The Influence of Mutations in the Variable Domain on PSTVd Replication and Pathogenicity"

1:50 PM Ralph Dean (Clemson University) "Cell Surface Communication in Appressorium Development by *Magnaporthe grisea*"

2:20 PM Yijun Ruan and David C. Straney (University of Maryland, College Park) "Spore Germination of the Pea Pathogen *Nectria haematoccoca* is Stimulated by the Host-specific Isoflavonoid Phytoalexin Pisatin through a cAMP Mediated Signal Transduction Pathway"

2:40 PM Closing Remarks - Benjamin Matthews

Poster Sessions
(Thursday 3:20-4:20 PM & Friday 9:50-10:50 AM)

Poster	(page)	
1	(29)	Kenneth A. Chapman and N. Kent Peters (The Ohio State University) "Biosynthesis of Rhizobitoxine by *Bradyrhizobium elkanii*, a Role for Sulfur Containing Amino Acids"
2	(30)	Benjamin F. Matthews, Greg Wadsworth, Joan S. Gebhardt and Barbara Wilson (USDA/ARS/PMBL) "Cloning and Expression of Genes Encoding Aspartate Aminotransferase in Soybean"
3	(31)	Joan S. Gebhardt and Benjamin F. Matthews (USDA/ARS/PMBL) "Characterization of the Aspartate Kinase-Homoserine Dehydrogenase Gene Family in Soybean"
4	(32)	Camellia Moses Okpodu, Janet L. Donahue, John L. Hess, Elizabeth A. Grabau, Carole L. Cramer and Ruth G. Alscher (Virginia Polytechnic Institute and State University) "The Effect of Oxidative Stress on Protoplasts Isolated from Two Cultivars of Pea *(Pisum sativum* L.)"
5	(33)	Michelle L. Kneissl, Margaret Ulrich and Jill Deikman, (Pennsylvania State University) "Investigation of the Function of E8 - an Ethylene-Induced Gene"
6	(34)	P. Christopher LaRosa and Ann C. Smigocki (USDA/ARS/PMBL) "Identification of Cytokinin-Inducible Genes in *Nicotiana plumbaginifolia*"
7	(35)	Jennifer Normanly, Joel Kreps and Debbie Pinto (University of Massachusetts) "Auxin Biosynthesis in *Arabidopsis thaliana*"
8	(36)	Rajnish Khanna, Xia Lin and John C. Watson (University of Maryland, College Park) "Photoregulated Expression of Protein Kinase Genes"
9	(37)	Jianming Li and Michael P. Timko (University of Virginia) "Isolation and Characterization of the Nuclear Gene Encoding the Light Dependent Protochlorophyllide Reductase of *Chlamydomonas reinhardtii*"
10	(38)	Jeffrey S. Skinner and Michael P. Timko (University of Virginia) "Expression Analysis of NADH-Protochlorophyllide Oxidoreductase in Loblolly Pine"
11	(39)	Gregory G. Tall, Abbey L. Remaley, and John C. Wallace (Bucknell University) "Changes in Gene Expression in the Moss, *Physcomitrella patens*"
12	(40)	Andrew. J. Bobb, Hans G. Eiben and Mauricio M. Bustos (University of Maryland, Baltimore County) "*Pvalf,* A Bean (*P. vulgaris)* Gene Belonging to the *vp1/abi3* Family: Cloning and Characterization"
13	(41)	Alison Hill, Andre Nantel and Ralph S. Quatrano (University of North Carolina) "The Product of the *viviparous-1* Locus Enhances the DNA Binding Activity of EmBP-1"

Poster (page)

MOLECULAR AND GENETIC ANALYSIS OF EARLY EMBRYOGENESIS

J. Lynn Zimmerman
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21228

The process of higher plant embryogenesis defines the beginning of each new generation of plant life. It is here that the basic body plan of the plant is established and the meristematic cells that support all subsequent growth are specified. However, **essentially nothing is known about the molecular mechanisms regulating differentiation and morphogenesis during early embryogenesis, nor of the genes which are essential to this process.** Our laboratory is taking a combined genetic and molecular approach to understanding one of the earliest stages of embryogenesis, **the globular stage**. We have chosen this stage because it is during the globular stage that all of the basic tissue systems of the mature plant are initiated and the primary meristems are established.

We have identified three *globular defective* mutants of *Arabidopsis* that show interesting defects in development during the globular stage. One of these mutants, *stout*, shows a defect in axis elongation and possibly cell wall deposition, another, *mora*, may exhibit a transformation of one embryo cell type to another, and the third, *banjo*, shows defects in tissue organization and the transition to polarized growth. Each of these mutations appears to be caused by T-DNA insertion, and hence the genes of interest should be tagged; indeed, we have already used T-DNA as a probe to begin the molecular characterization of two of the mutants.

A critical aspect of characterizing these mutants is an analysis of the specific identity of various cells within the developing embryo, and how this compares to wild type embryos. This analysis depends upon the availability of a collection of molecular markers for key cell types and/or differentiation events. Using carrot somatic embryos, our lab has been successful in isolating approximately 40 different genes that are specifically up-regulated in developing embryos. The identity, developmental expression and regulation of these clones will be discussed.

The combined molecular and genetic approaches to investigating gene expression and regulation during globular embryo development should provide new insights into some of the earliest and most critical events in higher plant development, which, until now have been virtually unexplored.

ANALYSIS OF AN *ARABIDOPSIS* C_2H_2 ZINC FINGER PROTEIN GENE FAMILY. Brian W. Tague and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston MA 02114.

A family of zinc finger protein genes from *Arabidopsis* (AtZFPs), each containing a single copy of a TFIIIA-like C_2H_2 finger motif, has been characterized. The predicted amino acid sequences share identity predominantly in the finger region with little similarity outside the nucleic acid binding domain. RNA blot analysis demonstrates that the genes are expressed in different but overlapping sets of organs in *Arabidopsis*. The expression pattern of one of these genes (AtZFP1) has been examined in more detail by histochemical analysis of *Arabidopsis* lines transformed with an AtZFP1 promoter:β-glucuronidase construct. AtZFP1 is expressed predominantly in tissues involved in the structural support of the plant. Evidence from transformation experiments and analysis of etiolated seedlings of the promoter:β-glucuronidase lines is also presented indicating a role for AtZFP1 in the development of leaves.

C_2H_2 Zinc Finger Proteins – Cysteine$_2$ Histidine$_2$ = C_2H_2 – found in many organisms, such as:
S. cerevisiae: ~~ADR1~~ ADR1 Activates ADH2 (Blumberg et al 1987)

FLUCTUATIONS IN RIBOSOMAL RNA GENE CONTENT AND NUCLEOLAR ACTIVITY IN THE CAMBIAL REGION OF ABIES BALSAMEA (PINACEAE) SHOOTS DURING REACTIVATION

ANDREW D. LLOYD,[1,2] EWA J. MELLEROWICZ,[2] CYNTHIA H. CHOW,[2] RICHARD T. RIDING[2] AND C. H. ANTHONY LITTLE[3]
[1]Department of Biology, Delaware State University, Dover, DE (Current Address)
[2]Department of Biology, University of New Brunswick and;
[3]Department of Natural Resources Canada, Fredericton, New Brunswick, Canada.

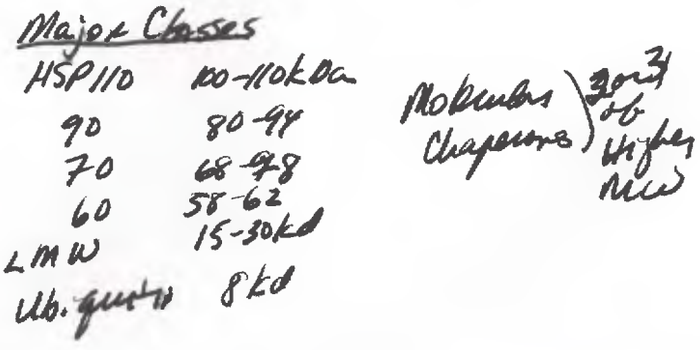
Tissue was collected from the vascular cambial region of 1-year-old balsam fir shoots from ten trees over an 11-week period in the spring, during which cambial reactivation occurred. The amount of rDNA (ribosomal RNA genes) relative to total genomic DNA was determined by slot blots for three trees, one of which showed a 3-week delay in reactivation. In addition, the nucleolar activity of these three trees was estimated by measuring the total nucleolar volume per cell. Relative rRNA gene content increased transiently prior to the onset of cambial cell division. Nucleolar volume also increased transiently, but 1-2 weeks prior to the maximal relative rDNA value. The increases in relative rDNA and nucleolar activity were delayed in the tree in which reactivation was late. We interpret these changes as reflecting the amplification of the genes encoding rRNA, possibly due to an increased demand for ribosomes during cambial cell reactivation.

STRUCTURAL AND FUNCTIONAL STUDIES OF HEAT SHOCK PROTEINS.

Garrett Lee, Katherine Osteryoung and Elizabeth Vierling. Department of Biochemistry, University of Arizona, Tucson, AZ 85721 .

Plants respond to high temperature and certain other stresses by synthesizing a discrete set of proteins known as heat shock proteins (HSPs). Our laboratory is interested in understanding the function of the small HSPs (15-30 kDa) (smHSPs) which are unusually diverse and abundant in plants as compared to other eukaryotes. We hypothesize that the smHSPs are required for high temperature tolerance, and that they represent a new type of molecular chaperone. There are four major nuclear gene families of smHSPs which have been conserved through the evolution of higher plants. Two encode cytosolic proteins (class I and II), one encodes an ER-localized protein, and the fourth encodes a protein targeted to the chloroplast. The evolutionary conservation of the smHSPs and their presence in three intracellular compartments are evidence for a critical function for these proteins in plants.

All smHSP genes tested show a similar pattern of expression in vegetative tissues. The proteins are undetectable in the absence of heat stress, but are expressed at high levels at elevated temperatures, accumulating up to 1.0% of total cell protein in the case of the class I cytosolic proteins. The cytosolic smHSPs are also expressed during seed maturation in the absence of stress. Biochemical studies indicate that *in vivo* the smHSPs assemble into large homo-oligomeric particles with an estimated twelve subunits. To investigate further the function of these proteins we have begun both transgenic plant analysis (antisense and over-expression) and *in vitro* activity assays. Although we have produced transgenic plants which constitutively express the chloroplast-localized smHSP, and plants which show 70% reduced expression of this protein, we have not detected any difference in whole plant temperature tolerance associated with the changes in smHSP expression. We are continuing to test these transgenic plants for other phenotypes and to produce transgenic plants altered in expression of other smHSPs. To test for chaperone activity, purified recombinant cytosolic smHSPs have been prepared. The purified proteins exhibit significant molecular chaperone activity *in vitro* in several assays. They prevent heat-induced aggregation of model substrates, facilitate refolding and activation of proteins diluted from denaturants, and most importantly can prevent heat-induced inactivation of enzymes at physiologically relevant temperatures. We are further characterizing these chaperone activities and attempting to identify critical *in vivo* substrates of the smHSPs.



Isolation of rice bZIP factors that heterodimerize with EmBP-1.

André Nantel & Ralph S. Quatrano. Dept. of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

Our lab is studying how the wheat and rice Em genes are induced by abscisic acid (ABA) during the mid-maturation phase of embryogenesis. Previous work identified an ABA-response element that contains two G-box-like sequences, Em1a and Em1b, both of which are necessary for the hormone response. G-boxes and other ACGT elements have been implicated in gene regulation by light, anaerobic stress, cell cycle as well as by other phytohormones. One of the major question in plant gene regulation is how the same elements can specifically mediate all of these stimuli. Our lab had already isolated EmBP-1, a wheat bZIP factor with a high affinity for Em1a. Binding of EmBP-1 to Em1b as well as two other G-box-like elements in the distal region of the Em promoter is much weaker, which led to the hypothesis that it may interact with these elements as a heterodimer with an unidentified bZIP factor.

Large amount of bacterially expressed EmBP-1 was purified as a fusion with the maltose-binding protein and biotinylated with NHS-LC-Biotin. We then used this biotinylated probe to screen an expression library prepared from embryonic rice suspension cells that had been treated with ABA. We have isolated 3 cDNAs encoding the rice bZIP factors osZIP-1a, osZIP-2a and osZIP-2b.

The rice osZIP-1a is homologous to EmBP-1 and other G-box-binding factors. It efficiently dimerizes with EmBP-1 and can bind the Em promoter as a homo or heterodimer. Interestingly, it carries a P-loop domain in its amino-terminal half. Similar elements act as GTP/ATP-binding sites in several proteins. Using UV-crosslinking, we have indeed demonstrated that osZIP-1a is capable of binding GTP. When used as a probe, the osZIP-1a cDNA will hybridize to a single band on a genomic Southern blot and to a 1.8 kb transcript on a northern blot. This transcript is repressed about 3-fold in rice suspension cells after incubation with 100μM ABA.

The osZIP-2 gene family contains from 3-5 members with high degree of sequence homology. The DNA-binding basic domains of the osZIP-2a and osZIP-2b factors are similar but they have only a weak homology to other bZIP factors. The binding affinity of these members for the Em promoter is very weak and we are currently working on defining their DNA-binding specificity.

Clone - in plasmid w/ Maltose-Binding Protein

TRANSFORMATION OF *GLADIOLUS*

[1]K. Kamo, [2]A. Blowers, [2]F. Smith, [2]J. Van Eck, [1]Floral & Nursery Plants, USDA, Beltsville, MD 20705-2350, [2]Sanford Scientific, 877 Marshall Rd., Waterloo, NY 13165.

We have developed an efficient transformation system for *Gladiolus* which relies upon particle bombardment of regenerable suspension cells, callus, or cormel slices. Transgenic phosphinothricin-(PPT) resistant *Gladiolus* plants which express GUS have been obtained following co-bombardment with the *pat* and *gusA* genes. We have examined the efficacy of various gene promoters and the effect of monocot introns on transient GUS gene expression in *Gladiolus*. Addition of osmoticum to the medium during bombardment of suspension cells increased the rates of both transient GUS expression and stable transformation. Transgenic plants which express bean yellow mosaic virus (BYMV) coat protein or BYMV antisense genes have been recovered. Transformation has been verified by DNA gel blot analysis, and by detection of BYMV coat protein by ELISA and Western blot analysis.

REGULATION OF PHENYLALANINE AMMONIA-LYASE GENE EXPRESSION IN TOBACCO

Tomoko Fukasawa-Akada[1,2], Paul J. Bottino[1] & John C. Watson[1,3]; [1]Department of Botany, [2]Center for Agricultural Biotechnology, and [3]Maryland Agricultural Experiment Station, University of Maryland, College Park, MD 20742

Phenylalanine ammonia-lyase (PAL) catalyzes the first reaction in the general phenylpropanoid pathway leading to the production of various phenolic compounds with a significant range of biological functions. We previously reported the presence of four PAL genes (designated *pal1* through *pal4*) in the tobacco genome, based on genomic Southern blot analyses and sequence analyses of cDNA and genomic clones. The PAL gene family was divided into two distinct subfamilies, subfamily I consisting of *pal1* and *pal2*, and subfamily II consisting of *pal3* and *pal4*. Transcript accumulation profiles were examined using subfamily -specific probes for RNA gel-blot hybridizations. The levels of PAL transcript from both subfamilies were significantly higher in flowers and roots than in leaves and stems, suggesting that the two subfamilies exhibit similar expression profiles in mature organs of tobacco. To study spatial and temporal regulation of PAL gene expression with higher resolution, a 1.7 kb fragment containing the *pal1* promoter was fused transcriptionally to β-glucuronidase (GUS) gene, and introduced into tobacco by *Agrobacterium* mediated-leaf disk transformation. In mature transgenic plants, the *pal1* promoter directs GUS expression in all major organ systems, but to differing extents and with distinct tissue and cell type specificities. Both light- and dark-grown seedlings show very high GUS activities in tips and maturation zones of roots. In hypocotyls of 7 day-old seedlings, high GUS activity was observed in vascular cylinder. The expression in cotyledons was observed only in light-grown seedlings.

Effect Of *Agrobacterium* Cell Concentration on The Transformation Efficiency of Tobacco and *Arabidopsis thaliana* Ecotypes Columbia and Landsburg.

Jhy-Jhu Lin, Nacyra Assad-Garcia*, Jonathan Kuo Agriculture Biotechnology and Molecular Biology R&D, Life Technologies Inc., GIBCO/BRL, Gaithersburg, Maryland 20884 and *Centro De Investigacion Y De Estudios Avanzados Del I. P. N. Aparatado Postal 629, 36500 Irapuato,Gto. Mexico

The effect of the *Agrobacterium* cell concentration on the transformation efficiency of tobacco and *Arabidopsis thaliana* was examined using *Agrobacterium tumefaciens* LBA4404 cells containing a shutter vector with the GUS gene [pBI12l) or a shutter vector with the CAT gene (pBICAT). Both transient expression, detected either by an antibody based ELISA/CAT assay or by a fluorometric MUG assay, and stable transformation, determined by the formation of green callus and shoot, were significantly increased as the total cell number of *Agrobacterium* increased from 10^6 to 10^{10}. For the *Agrobacterium* - mediated transformation of *Arabidopsis thaliana*, using the addition of higher concentration of *A. tumefaciens* LBA4404 cells containing pBI12l or pBICAT and different explants of 10 days-old seedlings significantly increased the regeneration of green calli of the ecotype Landsburg as well as the ecotype Columbia. Moreover, the regeneration efficiency of transgenic plants from hypocotyl or cotyledon explants was shown to be higher than those from root explants of both ecotypes Landsburg and Columbia, as the amount of *Agrobacterium* cells increased from 10^6 to 10^9. The existence of the CAT proteins and the CAT DNA sequences in the regenerated transgenic plants from these *Agrobacterium* -mediated transformation was determined by an ELISA/CAT assay and a PCR reaction.

IMPROVING THE QUALITY OF SEED PROTEINS IN SOYBEAN

Jeffrey A. Townsend and Laurie A. Thomas, Pioneer Hi-Bred International, Inc., Johnston, IOWA 50131

One approach for enhancing the amino acid composition of seed proteins is to express in seeds of a target plant a gene encoding a heterologous protein which is rich in the limiting amino acids. We have developed a method for delivering such genes into elite soybean germlines by cocultivation of cotyledon explants with *Agrobacterium tumefaciens*. Factors have been identified which affect the process. Among the most important of these appears to be the induction of the *Agrobacterium* virulence genes using signal molecules in cocultivations conducted at low temperature and pH. Transformation efficiency is bacteria concentration dependent. Inoculation conditions have been defined which ensure consistently high frequencies of transformation. Under the improved coculture conditions soybean varieties show little variation in susceptibility to transformation. Selection on kanamycin containing media has allowed the recovery of transformed plants from one percent of treated explants. A chimeric gene encoding a Brazil nut methionine-rich seed protein (BNP) was introduced into the soybean chromosome. The mature Brazil nut protein is comprised of over 19% methionine residues. The protein accumulates in transgenic seed. T2 seed homozygous for the introduced gene contained BNP levels between 4 and 8% of the total salt-soluble protein. The accumulation results in a significant increase (26%) in the level of methionine in transgenic seed. The improved soybean varieties have the same mean yield (bushel/acre) as the progenitor variety and are not compromised in any of several other characters analyzed in field tests.

Acetosyringone increased transf. with increased dose to (Must have some present for good efficient transformation).
↳ Signal molecule

Temperature affects signalling;
above 24°, dramatic decrease in # transformants.
22, 24 – Lots
26 – few
28 – 0

pH dependence –
Reduced above 5.75
10 mM MES, 5.5

Conc. of agrobacteria: 10^9 better than 10^7
More in susceptible soybean (or double) than in resistant.
Similar frequencies with diff. Agrobact. strains.

CLONING AND EXPRESSION OF THE SOYBEAN GENE ENCODING DIHYRDODIPICOLINATE SYNTHASE (DS)

Gregg W. Silk and Benjamin F. Matthews, USDA Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD, 20705-2350

In plants, the first committed step in the pathway to lysine synthesis is the condensation of aspartate B-semialdehyde and pyruvate, catalyzed by the enzyme dihydrodipicolinate synthase (DS), which is encoded by the nuclear DapA gene. DS activity undergoes feedback inhibition by lysine, and this inhibition limits lysine synthesis. Cloning of the soybean DapA gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the DapA gene may be a way of increasing lysine synthesis in soybeans and other crop plants. We have previously cloned the DapA gene of soybean (G. max var. Century) using PCR, and expressed the cloned soybean cDNA as a lacZ fusion protein in an E. coli auxotroph. The cloned DS enzyme was feedback inhibited by lysine like the wild type soybean DS enzyme. Three mutants of the cloned DapA gene were produced by site specific mutagenesis. The mutant DS enzymes were expressed in E. coli, and characterized using in vitro assays of E. coli lysates. The DS activities of all three mutants were insensitive to lysine at concentrations up to 1 molar.

AEC = toxic lysine analogue. Mutants for DS will grow on this.

Can get dapA⁻ E.coli auxotrophs isolated DDPA by complementing them.

THE CRYPTOCHROME FAMILY OF BLUE/UV-A PHOTORECEPTORS:
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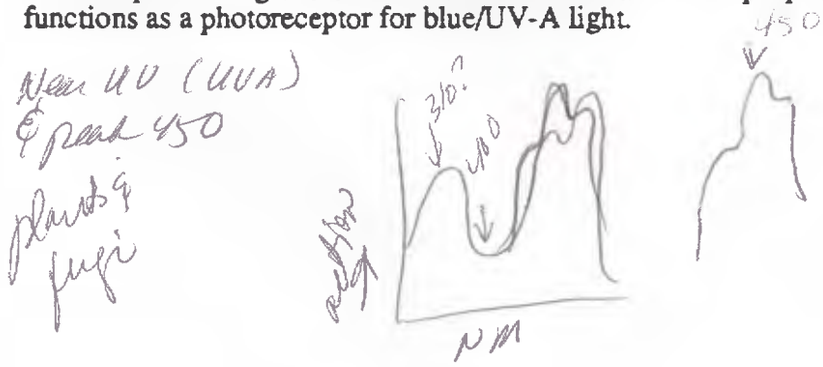
Mutants of *Arabidopsis thaliana* containing lesions at the *HY4* locus fail to respond to blue light in the inhibition of hypocotyl elongation response. The gene corresponding to the *HY4* locus has been isolated and shown to contain an open reading frame encoding a protein with significant homology to microbial photolyases. Photolyases are flavoproteins which function in the repair of UV-damaged DNA. Since flavoproteins have long been suggested as candidates for plant blue-light photoreceptors, and since photolyases are a unique class of flavoproteins which act in response to the absorption of blue/UV-A light, we have proposed that the protein encoded by *HY4* is the photoreceptor responsible for blue-light mediated inhibition of hypocotyl growth [Ahmad, M. and Cashmore, A. R. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366, 162-166].

In continuing our studies of this blue photoreceptor we have expressed HY4 in baculovirus-infected insect cells and obtained relatively large amounts of purified protein. By spectroscopic analysis we have demonstrated that HY4 is indeed a flavoprotein and by thin layer chromatography we have identified the flavin as FAD. Using a pyrimidine dimer repair assay we have also demonstrated that HY4 has no detectable photolyase activity -- this is consistent with our belief that HY4 is a photoreceptor with biochemical functions distinct from the photolyases.

Overexpression of HY4 in both tobacco and Arabidopsis plants confers hypersensitivity to blue light resulting in seedlings with abnormally short hypocotyls. These results are reminiscent of the hypersensitivity to red light observed by others for transgenic plants overexpressing phytochrome.

HY4 is a member of a small gene family. The protein encoded by a second member of this family, CRY2, contains the photolyase domain like HY4 but contrasts with HY4 in possessing a distinct C-terminal domain. We propose that CRY2 also functions as a photoreceptor for blue/UV-A light.



LINKAGE OF COPPER AND IRON UPTAKE IN SACCHAROMYCES CEREVISIAE

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In our studies of the single-cell eukaryote Saccharomyces cerevisiae, we have identified a set of mutants with defects in copper uptake and iron uptake. The mutants were initially isolated by their inability to repress transcription of FRE1, a gene required for ferric iron uptake and negatively regulated by iron uptake. Although the mutants were found to be deficient in both copper and iron uptake, the iron uptake defect was found to be secondary since it could be corrected by growth of the cells in high copper concentrations. Further analysis revealed that the mutated gene in this set of mutants, CTR1, encoded a protein specifically required for high-affinity (Km1-4mM) copper uptake. The abundance of the CTR1 transcript was regulated by copper, with levels induced by copper deprivation and repressed by subtoxic concentrations of copper. Localization of an epitope-tagged protein by immunofluorescence techniques indicated that it resides in the plasma membrane. Sequence analysis indicated the presence of at least three transmembrane domains as well as an unusual methionine- and serine- rich domain containing 11 examples of a motif, M-X-X-M, also noted in bacterial proteins involved in copper metabolism. Together these results indicate that CTR1 most likely is the transport protein mediating uptake of copper from the environment to the cell interior of S. cerevisiae, and that defective copper transport leads to defective iron transport.

required
Copper ~~uptake~~ in presence of O_2
not in Absence

MOLECULAR STRUCTURE AND ENZYMATIC FUNCTION OF LYCOPENE CYCLASE FROM THE CYANOBACTERIUM *SYNECHOCOCCUS* SP STRAIN PCC7942

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A gene encoding the enzyme lycopene cyclase in the cyanobacterium *Synechococcus* sp strain PCC7942 was mapped by genetic complementation, cloned, and sequenced. This gene, which we have named *crtL*, was expressed in strains of *Escherichia coli* that were genetically engineered to accumulate the carotenoid precursors lycopene, neurosporene, and ζ-carotene. The *crtL* gene product converts the acyclic hydrocarbon lycopene into the bicyclic ß-carotene, an essential component of the photosynthetic apparatus in oxygen-evolving organisms and a source of vitamin A in human and animal nutrition. The enzyme also converts neurosporene to the monocyclic ß-zeacarotene but does not cyclize ζ-carotene, indicating that desaturation of the 7-8 or 7'-8' carbon-carbon bond is required for cyclization. The bleaching herbicide 2-(4-methylphenoxy)triethyl-amine hydrochloride (MPTA) effectively inhibits both cyclization reactions. A mutation that confers resistance to MPTA in *Synechococcus* sp PCC7942 was identified as a point mutation in the promoter region of *crtL*. The deduced amino acid sequence of lycopene cyclase specifies a polypeptide of 411 amino acids with a molecular weight of 46,125 and a pI of 6.0. An amino acid sequence motif indicative of FAD utilization is located at the N terminus of the polypeptide. DNA gel blot hybridization analysis indicates a single copy of *crtL* in *Synechococcus* sp PCC7942. Other than the FAD binding motif, the predicted amino acid sequence of the cyanobacterial lycopene cyclase bears little resemblance to the two known lycopene cyclase enzymes from nonphotosynthetic bacteria. Preliminary experiments indicate that, like the genes encoding phytoene synthase and phytoene desaturase, two earlier enzymes in the carotenoid biosynthetic pathway, the *Synechococcus* gene encoding lycopene cyclase will enable the identification and cloning of homologous genes encoding this enzyme in plants and algae.

IDENTIFYING RICE β-GLUCOSIDASE SUBSTRATES AND THEIR PHYSIOLOGICAL ROLE DURING GERMINATION

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Partially purified rice β-glucosidase hydrolyzed in vitro several known glucosides such as pyridoxine-glucoside, gibberellin-glucoside, and some cyanogenic-glucosides. Methanol extracts of rice seed, rice bran, and rice seedlings contained several hydrolyzable compounds that were visualized by paper and thin layer chromatography stained for glucose. These compounds were purified partially using Sephadex G-10 and Sephadex LH-20. Some fractions inhibited enzymatic cleavage of paranitrophenyl β-D-glucopyranoside. β-glucosidase inhibitors were used to evaluate the physiological roles of rice β-glucosidase and its substrates during seed germination. The inhibitor δ-gluconolactone inhibited rice germination at activation stage and this inhibition corresponded to the low α-amylase activity. Collectively our data and data from others (Schliemann 1984) suggest that rice β-glucosidase significantly contributes to the availability of rice gibberellin before germination, and promotes the expression of several hydrolytic enzymes during germination.

MAIZE β-GLUCOSIDASE GENE CONTAINS PLASTID TRANSIT PEPTIDE SEQUENCE AND LACKS INTRONS

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Maize β-glucosidase plays and important role in the defence of a young plant against insects, fungi, and bacteria. It catalyzes the hydrolysis of DIMBOA-glc, releasing DIMBOA (a hydroxamic acid) which is toxic to the attacking organisms. β-glucosidase in maize has been localized in plastids. We isolated a near full length cDNA of β-glucosidase from the cDNA library of a maize inbred K55. The subsequent sequencing of the clone revealed that it contains 54 amino acids long transit peptide sequence. Comparison with the other transit peptide sequences pointed out that it is a typical transit peptide found in plastid targeted proteins. Based on the cDNA sequence of the enzyme, different sets of primers were synthesized. These primers were used to amplify different parts of β-glucosidase using the cDNA clone and K55 genomic DNA as templates. The comparison of the PCR products of the two templates indicates that there is no intron in the maize β-glucosidase gene.

hydrolyze alkyl & aryl β-glucosides
In cell wall of dicots; in plastid of monocots.
lysozyme of animals

The most polymorphic ~~of~~ enzymes among all organisms. (31 alleles for Maize)
(Bozyme)
At least 100 alleles at protein level ?

1931 bp → 566 aa preprotein
531(?) protein
transit peptide 54 aa

EXPRESSION OF MULTIPLE CELLULASE GENES DURING ABSCISSION OF TOMATO FLOWERS : RELATIONSHIP TO BREAKSTRENGTH. Elena del Campillo. Department of Botany, University of Maryland at College Park. MD 21047.

Tomato flowers abscise naturally if pollination fails and no fruit is set. In addition, excised flowers abscise rapidly when exposed to ethylene. Breakstrength (BKS) distribution was used to classify naturally abscising flowers and flower explants induced to abscise with ethylene into several categories. The correlation of BKS with cellulase gene expression was then analyzed. To isolate cellulase genes responsible for flower abscission in tomato, total RNA isolated from flower abscission zones (FAZ) treated with ethylene, was reverse transcribed and amplified by polymerase chain reaction (PCR) using short degenerate primers to conserved amino acid sequences from avocado fruit, ripe tomato fruit and bean abscission zone cellulases. These primers amplify a region of approximately 500 bp in length in all plant cellulases. Six cellulase gene fragments were identified, four of the six genes are homologous to fruit pericarp cellulases and the other two represent new cellulase genes, referred to as *cel5* and *cel6*. Expression studies in natural abscising flowers and flower explants induced to abscise in air or ethylene demonstrated that both new cellulases are correlated with flower shedding. While the *cel5* message increases in abscising FAZ, (BKS < 200 g), the *cel6* message increases in FAZ, (BKS > 200 g) and then decreases (BKS < 200 g). The presence of IAA in the ethylene treatment reduced by 99% the levels of expression of *cel5*, consistent with the low levels of abscission (3%). There was also some increase in *cel6* message when IAA was added in addition to ethylene. Studies of spatial distribution in abscised flowers showed that expression of *cel5* was confined to the FAZ, while *cel6* expression was high outside the FAZ and the stem before shedding and low across the pedicel after shedding. Interestingly, expression of *cel6* remained high in stem explants treated with ethylene. These results suggest that abscission may develop as a multi-step process that involves several types of cellulase genes which are up and down regulated during the process. *cel6* may loosen the cell-wall which precedes either shedding or cell-wall hardening and expression would not have to be confined to the abscission zone. Expression of one of the four cellulases sharing strong homology with a fruit cellulase (*cel1*) was also strongly correlated with abscission in FAZ but not in naturally abscising flowers.

VIROID INDUCTION OF A HOST PLANT PROTEIN KINASE: RESPONSE TO INFECTION AND/OR ROLE IN SYMPTOM EXPRESSION?

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Viroids are the smallest known agents of infectious disease and a number of diseases in crop plants are known to be caused by them. Viroids are characterized as covalently closed, circular RNA molecules, in the size range of 250-450 nucleotides, which replicate without a helper virus and are not encapsidated. Viroids do not apparently encode proteins, therefore they rely upon their host for replication machinery and it is the RNA itself which may trigger induction of symptoms. In many cases, viroid infection results in symptoms of stunting, epinasty, and vein clearing and discoloration in the host, the severity of which depends upon both the host and viroid genomes. We are studying the response of the tomato host to infection by different strains of potato spindle tuber viroid. The responses of plants to environmental stresses, and in particular, the defense responses of plants to infection by pathogens include a number of inducible reactions. It is known that protein phosphorylation plays a role in response of animal cells to viral infection. Phosphorylation of specific proteins may also be one mechanism used in plants for the regulation and activation of the defense response. Degenerate oligonucleotides corresponding to the conserved catalytic domains of known protein-serine/threonine kinases were used as primers to amplify cDNA synthesized from either total RNA or poly (A)+ RNA purified from tomato leaves of plants mock-inoculated or inoculated with a mild, intermediate, or severe strain of potato spindle tuber viroid. A ladder of PCR products were obtained from all treatments and sequence analysis of several of the products revealed homologies with more than one type of serine/threonine protein kinase. One product in particular, although present in mock-inoculated tissue at very low levels, was induced in extracts of plants inoculated with the intermediate and severe strains of the viroid, and this fragment has significant sequence homology to cyclic nucleotide-dependent protein kinases and protein kinase C, suggesting that these sequence homologs may be involved in signal transduction and in the disease response. We are examining the accumulation of the transcript in two cultivars of tomato, one which exhibits typical stunting symptoms upon infection with the severe strain of PSTVd and one which does not. We are also attempting to obtain the full-length cDNA in order to examine the regulatory domain of the enzyme.

If viroids induce responses (such as kinase activity), could they be engineered, or could strains be found to induce defense responses,

PR proteins induced – realistic to think can induce disease resistance.

The influence of mutations in the variable domain on PSTVd replication and pathogenicity

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We are attempting to isolate temperature-sensitive PSTVd mutants in order to better understand the relationship between viroid replication and pathogenicity. A series of seven mutations in "premelting loop 3" within the variable domain have been constructed and characterized. These mutations may affect the ability of PSTVd to form alternative structural interactions during the breakdown of the rod-like native structure. Infectivity studies using PSTVd RNA transcripts revealed that all seven mutants were viable and caused disease. The *in vivo* stability of the mutations were monitored through sequencing of progeny populations. Symptom development and progeny accumulation were also compared quantitatively for selected mutants . Six of the seven mutations underwent *in vivo* changes which seem to maintain the overall stability of premelting region 3 at near wild-type levels. The structural properties of the mutant RNA transcripts are being compared with those of their respective progeny using temperature gradient gel electrophoresis, the results of which may be useful in predicting mutant behavior *in vivo*. We will attempt to identify mutants expressing a temperature-sensitive phenotype by monitoring their sequence stabilities after growth at different temperature.

~~Have you ever gotten mutants, (or are there strains) that don't cause disease, but are still viable,~~

~~Have had a chance to put mutant into a plant yet?~~

~~Pictures of tomato grown~~ At Difference in symptoms that is temp-dependent?

Cell Surface Communication in Appressorium Development by *Magnaporthe grisea.*

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Differentiation of a dome shaped, highly melanized infection cell, the appressorium, from germinating spores of *Magnaporthe grisea* is essential for successful infection of plant tissues. The induction of appressorium formation is in response to environmental stimuli. In part, the fate of a germ tube tip depends on the hydrophobicity of the surface on which it is growing. On hydrophobic surfaces the germ tube ceases polar extension and the tip swells to form an appressorium. By contrast, on hydrophilic surfaces polar growth continues and the hypha branch to form vegetative mycelium. Host factors in addition to hydrophobicity also appear to regulate appressorium formation. The exogenous and endogenous signaling mechanisms involved in surface recognition and the transfer of this information into the cell leading to infection-related morphogenic events remain to be elucidated. Evidence suggests that fibronectin-like and vitronectin-like proteins are involved in spore adhesion and surface recognition. These proteins are detected on Western blots of protein extracted from *M. grisea* and are immunolocalized to the spore tip and occasionally to the appressorium. The addition of purified anti-sera prevents spore adhesion and appressorium formation. We have recently shown that cyclicAMP, a second messenger involved in signal transduction systems, regulates appressorium formation. The primary known target for cAMP is cAMP-dependent protein kinase. This kinase initiates a phosphorylation / dephosphorylation cascade which results in specific developmental changes. We have isolated and sequenced a gene, *cpk*A, encoding the catalytic subunit of this kinase from *M. grisea*. To evaluate the role of this gene product in the infection process, *cpk*A was replaced by a hygromycin resistance gene cassette. To date, two independent deletion / replacement transformants have been verified by Southern analysis. Preliminary characterization reveals that both are unable to produce normal appressoria, the addition of cAMP or IBMX does not restore the ability to form appressoria, and they are unable to cause disease when inoculated onto rice cultivar S201. Other aspects of growth and development, including growth rate, sporulation and sexual competence, appear to be unaffected. This is the first direct evidence that a cAMP dependent phosphorylation pathway mediates morphogenic events required for fungal infection of plants.

Will appressorium adhere to hydrophilic surface?
Is there any structure at cell top where adhesive is released.
What host factors are needed for appressoria?

Spore Germination of the Pea Pathogen *Nectria haematoccoca* is Stimulated by the Host-specific Isoflavonoid Phytoalexin Pisatin through a cAMP Mediated Signal Transduction Pathway

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Spores of soil-borne fungal plant pathogens generally lie dormant until stimulated to germinate by the presence of a potential host plant. Even though many efforts to identify compounds from plant exudates which stimulate fungal spore germination have been made, still little is known of what host specific compounds are responsible. We present here that low concentration of the pea-specific isoflavonoid phytoalexin pisatin, which like all other phytoalexins are known to inhibite the growth of most fungi, stimulates spore germination of the pea pathogen *Nectria haematoccoca* in minimal liquid medium. This stimulation of spore germination can be mimicked by the addition of cyclic AMP, or its analogous such as 8-Bromo-cAMP, and interfered with by specific inhibitors of cAMP-dependent protein kinase. However the germination in nutritionally rich medium is insensitive to these inhibitors. This strongly indicates that a pisatin-induced signal is transduced through cAMP to initiate spore germination, while the nutrition directed germination is not. By using these inhibitors, we were able to determine that the spore germination of this soil-borne fungus on root surface and in rhizosphere of pea is cAMP mediated, implicating an *in vivo* role for this isoflavonoid response. Furthermore, several leguminous plant flavonoids were tested for stimulation of fungal spore germination. Compounds such as naringenin and apigenin that are known to induce expression of nodulation genes of *Rhizobium* spp. stimulate the spore germination, whereas biochanin A that does not induce nodulation genes has no effect. The finding of stimulative effect on fungus by a phytoalexin provides new clues to the understanding of early host-specific recognition by plant pathogenic fungi. Stimulation of fungal spore germination by flavonoids which also induce *nod* gene expression in *Rhizobium* suggests that both eukaryotic and prokaryotic microbes in soil may have evolved to recognize similar plant signals.

Where did he get isoflavonoids?

Biosynthesis of Rhizobitoxine by *Bradyrhizobiium elkanii*, a role for sulfur containing amino acids

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The Gram-negative soil bacterium *Bradyrhizobium elkanii* is able to form a nitrogen fixing symbiosis with ureide transporting legumes including soybeans. This species also synthesizes rhizobitoxine (RTX), a phytotoxin that causes chlorosis on several cultivars of soybeans. The role of RTX in the establishment of symbiosis or in other aspects of the life cycle of *B. elkanii* is unclear. RTX has been previously shown to inhibit both ethylene biosynthesis and β-cystathionase, which is involved in methionine biosynthesis. We describe experiments aimed at determining the role of sulfur containing amino acids and sulfur limitation on the regulation of RTX biosynthesis.

RTX biosynthesis by *B. elkanii in vitro* is inhibited by methionine, cysteine, and cystathionine. Western blot experiments, and amino acid homology between the *B. elkanii* rtxA/B gene product and yeast O-acetyl homoserine sulfhydrolase, indicate that the inhibition of RTX biosynthesis by methionine operates post-translationally. *In vitro* growth experiments in sulfate free media indicate that *B. elkanii* can utilize methionine, cysteine or cystathionine as the sole sulfur source.

We suggest that RTX may play a role in the establishment of a symbiosis with some legumes by inhibiting methionine biosynthesis in order to increase the local concentration of cystathionine so that the bacterium can utilize it as a sulfur source.

CLONING AND EXPRESSION OF GENES ENCODING ASPARTATE AMINOTRANSFERASE IN SOYBEAN

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In plants aspartate aminotransferase (AAT; EC. 2.6.1.1) plays an important role in hydrogen shuttles, carbon shuttles and nitrogen distribution. AAT reversibly transfers an amino group from glutamate to oxaloacetate to form aspartate and α-ketoglutarate. We identified a family of at least five isoforms of AAT in soybean which can be separated and visualized on agarose gels. These isoforms are named AAT-1 through AAT-5 according to their migration on agarose electrophoretic gels from slowest to fastest. Soybean AATs are differentially expressed in different organs during soybean development. We localized three AAT isoforms to cellular compartments. AAT-1 was localized to glyoxysomes, AAT-4 to the mitochondria, AAT-5 to the chloroplast. cDNA clones encoding AAT-4, AAT-5 and a cytoplasmic form were identified and sequenced. The cDNA encoding AAT-4 and AAT-5 contain sequences for putative transit polypeptides, whereas the third cDNA clone does not appear to encode a transit polypeptide sequence. The coding regions encoding AAT-4 and AAT-5, representing the mature AAT protein were functionally expressed in *E. coli*. They migrate on agrose gels identically to the native AAT-4 and AAT-5 isofomrs. Antibodies were made to AAT-4 and to AAT-5 purified from *E. coli*; each antibody is specific for the proper soybean AAT isoform as indicated in immunoprecipitation reactions and confirms the identity of the clones.

CHARACTERIZATION OF THE ASPARTATE KINASE-HOMOSERINE DEHYDROGENASE GENE FAMILY IN SOYBEAN

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Aspartate kinase (AK) controls the first step common to the biosynthesis of the amino acids lysine, threonine, isoleucine, and methionine. As the first enzyme in the pathway, AK potentially plays a key role in regulating the entry of aspartate into the pathway. The ATP-dependent phosphorylation of aspartate to ß-aspartyl phosphate is catalyzed by AK. ß-aspartyl phosphate is reduced to aspartate semi-aldehyde which may be used as a substrate by either dihydrodipicolinate synthase (DS), leading to lysine biosynthesis, or homoserine dehydrogenase (HSDH), leading to threonine, isoleucine, and methionine biosynthesis. Higher plants commonly contain at least two forms of AK whose activities are differentially feedback inhibited by lysine or threonine. HSDH activity is feedback inhibited by threonine.

The cloning and sequencing of the plant genes that code for the enzymes of the aspartate pathway have begun only recently. Two partial cDNAs encoding one bifunctional protein possessing both AK and HSDH activities have been isolated from soybean cDNA libraries. The 5' end of the cDNA encodes a chloroplast transit peptide consistent with the biochemical localization of these enzymatic activities. Differential screening of genomic libraries and Southern blots suggested the presence of additional genes encoding AK or HSDH activities. Genomic clones representing the cDNA and a second bifunctional AK-HSDH have been identified. Sequence analysis of these clones has demonstrated a high amount of similarity between the two gene copies within the AK and HSDH coding regions and the chloroplast transit peptides. This similarity includes the positions of introns within the AK and HSDH coding sequences and the sequences encoding the transit peptides. The introns are of different sizes in the two gene copies but share some sequence similarity, particularly at the intron/exon borders. Southern blot hybridizations suggested the presence of a third AK gene in the soybean genome. A portion of this third tentative AK gene was amplified from size-fractionated restricted genomic DNA. Sequence analysis of this fragment has demonstrated the presence of AK coding sequences similar to the two bifunctional AK-HSDH genes. Similar PCR reactions employing HSDH-specific primers indicate that this third AK gene also contains HSDH coding sequences.

THE EFFECT OF OXIDATIVE STRESS ON PROTOPLASTS ISOLATED FROM TWO CULTIVARS OF PEA (*Pisum sativum* L.).

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The effects of exposure to paraquat and sodium sulfite were examined in protoplasts isolated from two cultivars of pea, Nugget and Progress. Three enzymes of the ascorbate/glutathione/NADPH scavenging cycle, glutathione reductase (GR), ascorbate peroxidase (AP) and superoxide dismutase (SOD), were studied for their responsiveness to these forms of oxidative stress. GR and AP activities declined precipitously during a 12 hour exposure to 10 mM paraquat, while SOD activities increased in both cultivars. Three forms of SOD were observed on activity gels: Cu/Zn-plastidic, Cu/Zn-cytosolic and Mn-mitochondrial. After 6 hours of exposure to paraquat, the plastidic SOD decreased; however, the cytosolic and mitochondrial SODs increased in activities relative to the controls in both cultivars. By 12 hours, the total specific activity had increased to 30 % in Nugget and 80 % in Progress.

Differential sensitivity was also observed in protoplasts isolated from the two cultivars using 10 mM sodium sulfite as a stress. CO_2-dependent O_2 evolution was inhibited by 50 % in Progress after 1 hour and continued to decline after 2 hours of exposure to sulfite (90 % inhibition). In Nugget protoplasts, exposure to 10 mM sulfite for 1 hour decreased the CO_2-dependent O_2 evolution by 60 % which recovered to control levels by 1.5 hours. The GR activity was unchanged in protoplasts from Progress over the entire 2 hour time course. In Nugget, the GR activity increased by 30 % after 1 hour, followed by a recovery to pre-exposure rates. In addition, antibodies to the plastidic GR cross-reacted with both a 56 and 50 kDa polypeptide in Nugget, however, only the 56 kDa polypeptide was apparent in Progress. An additional band of 36 kDa was observed in both cultivars.

No detectable transcripts were observed by Northern analysis using a GR cDNA probe to RNA isolated from paraquat- or sulfite-treated protoplasts. In addition, probes to both the plastidic and cytosolic SOD showed no hybridization in paraquat-treated protoplasts. However, all three probes (GR, cytosolic-SOD and plastidic-SOD) did hybridize with RNA of the correct sizes when freshly isolated leaf RNA and freshly isolated protoplast RNA were used. Collectively, these data suggest the existence of a stress-mediated, post-transcriptional mechanism of regulation involving SOD and GR in pea protoplasts.

INVESTIGATION OF THE FUNCTION OF E8 - AN ETHYLENE-INDUCED GENE

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Ethylene plays an important role in virtually every stage of plant development, from seed germination and seedling growth to flowering, fruit ripening and senescence. Results from antisense experiments suggested that E8 acts to negatively regulate ethylene biosynthesis. We have transformed tomato plants with an overexpression vector composed of the transcribed regions of the E8 gene driven by the CaMV 35S promoter. Because a large number of the transgenic plants exhibit low fertility compared to plants transformed with a control vector, we began to investigate E8 expression in the flowers of wild-type plants. Although E8 expression had previously been thought to be fruit specific, we have found moderately high levels of E8 mRNA in anthers including mature pollen. It has been shown that a progression of ethylene synthesis from the stigma and style to the ovary, receptacle, and petals follows pollination. The pollen of many species has been shown to contain high concentrations of ACC. Given these facts, it is reasonable to hypothesize that E8 may have a role in pollination. We are now in the process of analyzing the level of ACC in the pollen of the transgenic plants. We are also determining the effect of E8 overproduction on ethylene evolution from fruit.

IDENTIFICATION OF CYTOKININ-INDUCIBLE GENES IN *NICOTIANA PLUMBAGINIFOLIA*

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Transient accumulations of endogenous cytokinins in plants of *N. plumbaginifolia* transformed with a cytokinin biosynthesis gene are associated with induction of generalized plant stress response genes (Harding and Smigocki, Physiol. Plant. 90: 327-333) and increased resistance to insect predation (Smigocki et al., 1993, Plant Mol. Biol . 23: 325-335). Alterations in the phenotypes of the plants containing an inducible cytokinin biosynthesis gene suggest that increases in endogenous cytokinin levels are affecting other cytokinin modulated processes including cell proliferation, delay of senescence, chloroplast development and plant morphogenesis. Sixty-one hybridization classes of cDNA clones were isolated from a population of cDNAs derived from mRNA in plants 1, 2, 3 and 4 h after an induced endogenous cytokinin pulse. The mRNAs corresponding to eight of the cDNA hybridization groups were found to accumulate by Northern blot analyses of total RNA in response to endogenous cytokinin. One putative cytokinin responsive cDNA contained a truncated ORF with sequence homology to cytochrome P450 genes associated with, but not proved to have a role in indole alkaloid synthesis. Complementary DNAs with strong sequence similarities to nuclear-encoded chloroplast genes occurred most frequently. These include cDNAs similar to the the small subunit of ribulose bisphosphate carboxylase/oxygenase, the 10 kDa precursor polypeptide associated with the water oxidizing photosystem II, ferrodoxin I, and an extrinsic membrane photosystem I protein (psaF gene). An apparently full length gene with an ORF encoding a protein with strong amino acid sequence similarity to metallothioneins, including the cys-X-cys motifs, was also isolated. These results are consistant with cytokinin's role in induction of chloroplast development and plant defense responses.

Auxin Biosynthesis in Arabidopsis thaliana

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In Arabidopsis thaliana seedlings biosynthesis of the primary plant auxin indole-3-acetic acid (IAA) occurs by way of a novel biosynthetic pathway that does not utilize tryptophan as a precursor. This tryptophan-independent biosynthetic pathway is not yet characterized, although our data is consistent with indole or indole-3-glycerol phosphate being precursors. We are currently attempting to isolate the genes involved in this pathway.

In both maize and Arabidopsis a tryptophan-independent IAA biosynthetic pathway appears to predominate, however in other plant species it is clear that tryptophan acts as a precursor. In the case of cultured carrot cells two pathways exist, one requiring tryptophan and one that is independent of tryptophan. We are investigating the possibility that a tryptophan-requiring IAA biosynthetic pathway also exists in Arabidopsis. Our strategy is to isolate Arabidopsis homologues of genes postulated to catalyze the conversion of tryptophan to IAA in other plant species. If these genes are present in Arabidopsis as well, we can test their function by reverse genetics.

PHOTOREGULATED EXPRESSION OF PROTEIN KINASE GENES:
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We are studying a set of genes encoding putative protein kinases in the garden pea to understand their role in photoregulated development. We previously isolated a suite of partial cDNA clones that encode distinct forms of protein kinase homologs. These partial cDNAs, called PsPK1 through PsPK5, correspond to rare class mRNAs that are differentially expressed during de-etiolation in continuous white light. We sequenced a full-length cDNA clone for PsPK3 that encodes a predicted polypeptide of 479 amino acids. The catalytic domain of the PsPK3 polypeptide is closely related to second messenger-dependent protein kinases and possesses all the invariant amino acids charactistic of protein serine/threonine kinases. The PsPK3 polypeptide has interesting features in that the N-terminal domain is very rich in serines and threonines, whereas the C-terminal domain is rich in basic amino acids (as in many nucleic acid binding proteins) and contains a potential, bipartite nuclear localization signal sequence. Exposure of etiolated seedlings to continuous white light causes PsPK5 mRNA to decline to its minimum level within one hour while PsPK3 mRNA declines more slowly. To determine what photoreceptor regulates PsPK3 and PsPK5 expression, we tested the effects of brief pulses or continuous light. Neither gene responds to pulses of red, far-red or blue light. Continuous red and continuous blue light trigger the decline in mRNA levels whereas continuous far-red light is ineffective. This pattern is consistent with a high irradiance response regulated by type II phytochrome. The rapid light-regulated expression of the PsPK3 and 5 genes and the similarity of their polypeptides with known second messenger-dependent kinases suggests to us that they may be involved in signal transduction during the early phases of photoregulated development.
(This work was supported by NSF grant IBN-9118226.)

Isolation and characterization of the nuclear gene encoding the light-dependent protochlorophyllide reductase of *Chlamydomonas reinhardtii*.

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The reduction of protochlorophyllide to chlorophyllide is one of the key regulatory steps of chlorophyll biosynthesis. In most green algae and gymnosperms, this reaction is catalyzed by two distinctive enzymes: a nuclear-encoded light-dependent and a chloroplast-encoded light-independent protochlorophyllide reductase. We have isolated the lpcr (light-dependent protochlorophyllide reductase) structural gene from a *Chlamydomonas reinhardtii* λEMBL3 genomic library by using a lpcr cDNA probe from pine. Sequence analysis of the NheI-SalI 8 kb DNA restriction fragment containing the entire lpcr gene from one of the positive genomic clone demonstrated a high degree sequence similarity at the amino acid level with the light-dependent protochlorophyllide reductases of angiosperms and gymnosperms. Southern blot analysis of the genomic DNA indicated that the *C. reinhardtii* genome contained only one copy of the lpcr gene. The identity of the cloned gene as the light-dependent protochlorophyllide reductase was confirmed by its ability to complement by transformation a *pc-1* mutation of *C. reinhardtii* which was defective in the light-dependent protochlorophyllide reduction. Northern analysis of the steady-state mRNA level showed that the lpcr transcript accumulated to high levels in the dark-grown cells of wild-type and the *pc-1* mutant, but was undetectable in cells grown in the light. We are currently attempting to map the 5' and 3' ends of the lpcr transcript by RACE analysis.

EXPRESSION ANALYSIS OF NADPH-PROTOCHLOROPHYLLIDE OXIDOREDUCTASE IN LOBLOLLY PINE

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Chlorophyll synthesis in gymnosperms occurs via a light-independent and light-dependent mechanism. We are examining the gene family size and expression of the light-dependent chlorophyll biosynthetic enzyme NADPH-protochlorophyllide oxidoreductase (pchlide reductase) during light induced development of loblolly pine (*Pinus taeda*). Two separately expressed pchlide reductase forms have been isolated from dark grown loblolly cotyledon tissue utilizing 3'-RACE analysis. Northern analysis of greening pine seedlings with a pchlide reductase coding region probe demonstrated a constitutive level of expression in cotyledon tissue while induction occurred in stem tissue. We are in the process of using gene specific regions of the two 3'-RACE products to analyze expression of these two pchlide reductase family members. Using a putative promoter fragment of a loblolly pchlide reductase genomic clone, we have demonstrated luciferase reporter gene expression in tobacco leaves using the biolistic process. Work is under way to determine the number of pchlide reductase family members and assign them to specific gene products by Southern analysis.

CHANGES IN GENE EXPRESSION IN THE MOSS, *Physcomitrella patens*: Gregory G. Tall, Abbey L. Remaley, and John C. Wallace, Dept. of Biology, Bucknell University, Lewisburg, PA 17837.

The moss *Physcomitrella patens* represents, in many ways, an excellent model system for the study of the fundamental processes of plant development. Initial stages of moss growth are filamentous; at later stages more complex two- and three-dimensional growth ensues. During the filamentous (protonemal) stage, the first developmental transition in *Physcomitrella* is the change from a slow-growing, chloroplast-rich cell type, the chloronema, to a faster-growing more invasive type, the caulonema. In an effort to elucidate the molecular events behind this switch, we have employed an RT-PCR approach using an arbitrary ten-mer as second primer to amplify subsets of mRNAs expressed in the two cell types[1]. Analysis of the amplification products on a sequencing gel revealed several differences between the two cell types. The differentially expressed PCR products were further amplified and cloned. Dot blot analysis of their expression will be presented.

1. P. Liang and A.B. Pardee, Differential display of eukaryotic mRNA by means of the polymerase chain reaction. Science *257*, 967-971 (1992).

***Pvalf*, A BEAN (*P. vulgaris*) GENE BELONGING TO THE *vp1/abi3* FAMILY: CLONING AND CHARACTERIZATION**, Andrew. J. Bobb, Hans G. Eiben & Mauricio M. Bustos, Department of Biological Sciences, UMBC, 5401 Wilkens Ave., Baltimore, MD 21228-5398, USA.

In maize, *Arabidopsis*, and many other plant species, mutations in members of the *vp1/abi3* gene family increase the rate of premature germination and reduce the expression of maturation-specific genes, such as those coding for 7S globulin storage proteins. Depending on the stage of development and culture conditions (e.g. presence or absence of ABA or GA), premature germination can also be brought about in wild-types by culturing excised embryos *ex planta*. Conditions leading to premature germination in culture also cause maturation-specific gene transcription to be repressed. As a step towards studying the mechanism by which phaseolin gene transcription is either induced during maturation or repressed during germination, we have cloned a *Phaseolus* gene homologous to the *vp1/abi3* family. Nested oligo-nucleotide primers were used for 3'-RACE (Rapid Amplification of cDNA Ends). In order to assemble a complete ORF a similar approach was used to amplify the 5'-end of the corresponding mRNAs. We are calling this gene *Pvalf* (*P. vulgaris* ABI3-like factor). The deduced amino acid sequence of the PvAlf protein shows high similarity (up to 98 % over domain IV) to maize and rice Vp1 and *Arabidopsis* ABI3 proteins. When fused to a Gal4 DNA binding domain, the amino terminal 121 aa of PvAlf activated transcription from a β-galactosidase reporter gene in yeast. RNA blot hybridization analysis has shown embryo-specific expression of PvAlf mRNA in bean. From these data we conclude that PvAlf belongs to the Vp1/ABI3 family of maturation-regulating transcription factors.

The product of the *viviparous-1* locus enhances the DNA-binding activity of EmBP-1.

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Plant embryos homozygous for a mutation in the *viviparous-1* (*Vp1*) locus suffer from several enzymatic deficiencies including a reduced sensitivity to abscisic acid (ABA). As a consequence, they precociously germinate while still on the mother plant without accumulating storage protein and without acquiring dessication tolerance. Expression of the ABA-induced Em gene during embryogenesis is dependent on a functional *Vp1* gene. The *Vp1* gene was cloned by transposon tagging and shown to encode a transcriptional activator (McCarty *et al.*, 1991). When overexpressed in maize or rice protoplasts, Vp1 has been shown to transactivate the Em promoter. Overexpressed Vp1 can also act synergystically with exogenous ABA to activate the Em promoter. More detailed work has demonstrated that Vp1 trans-activation acts through two G-boxes in the ABA-response element, Em1a and Em1b, as well as elements in more distal regions of the promoter. Previous work demonstrated that these elements are recognized by the wheat bZIP factor EmBP-1.

EmBP-1 and a partial (a.a. 190-692) Vp1 were purified from *E. coli* as fusions with the maltose-binding protein (MBP). In this report, we demonstrate that Vp1, although incapable of binding DNA on its own, can greatly enhance the DNA-binding activity of EmBP-1 in a gel shift assay. The enhancement by Vp1 is most pronounced at low concentration of the bZIP factor. By selecting from a pool of random oligos, we have observed that Vp1 reduced EmBP-1 DNA-binding specificity and allows it to interact with any ACGT elements. We received from Dr. Don McCarty two Vp1 mutants, with deletions in small basic regions, which show reduced transactivating activity. One of the two basic regions, the BR2 domain, is both necessary and sufficient for enhancing the DNA-binding activity of EmBP-1. This domain is perfectly conserved among Vp1 homologs in maize, rice and *Arabidopsis thaliana*.

The capacity of Vp1 to enhance DNA-binding activity has also been observed on transcription factors as diverse as opaque-2, max, NF-kB and Sp1. This lack of specificity might explain why a mutation in the *Vp1* locus has pleiotropic effects. Attempts at demonstrating a physical interaction between Vp1 and EmBP-1 have not yielded convincing results.

TWO bZIP PROTEINS FROM THE COMMON BEAN (*P. vulgaris*) BIND TO THE VICILIN-BOX OF A β-PHASEOLIN PROMOTER, Maw-Sheng Chern and Mauricio M. Bustos. Department of Biological Sciences, University of Maryland, Baltimore County. 5401 Wilkens Ave., Catonsville, MD 21228

The UAS1 enhancer (-295/-106) of a β-phaseolin promoter directs transcription during seed maturation. UAS1 contains two ACGT-centered DNA elements similar to G-box sequences, which are known to interact with basic leucine-zipper (bZIP) proteins in plants. These two DNA elements interact with bean cotyledon nuclear proteins *in vitro* and are required for full promoter activity *in vivo*. In order to study the transcription factors that interact with these *cis*-elements, we have used the method of Rapid Amplification of cDNA Ends (RACE) with degenerate primers specific to the basic domain of plant bZIP proteins to clone cDNA from immature bean embryos. Two cDNA clones, *PvbZIP1* and *PvbZIP2*, containing a bZIP domain were obtained. Northern analysis showed that *PvbZIP2* is preferentially expressed during seed maturation. The bZIP domains of these proteins were expressed in *E. coli*, purified, and used for *in vitro* protein-DNA interaction assays. Binding competition and DNase I footprinting assays demonstrated specific binding of these PvbZIP proteins to two ACCT- and ACGT-centered DNA elements on the "vicilin box" of β-phaseolin promoter. PvbZIP2 apparently has higher affinity than PvbZIP1 in this binding. These DNA *cis*-elements were widely found on the promoters of 7S (vicilin) and 11S (legumin) genes, as well as lectin genes, from *Vicia*, *Glycine*, *Pisum* and *Phaseolus*. Therefore, PvbZIP2 is very likely to play a role in regulating the transcription of seed-expressed genes in beans.

UAS-1 REGULATED GENE TRANSCRIPTION DURING SEED MATURATION INVOLVES MULTIPLE *cis*-ACTING DNA SIGNALS, Mauricio M. Bustos, Steven J. Gagliardi and Helen Murphy, Department of Biological Sciences UMBC, 5401 Wilkens Ave., Baltimore, MD 21228-5398.

The promoters of seed storage proteins are good markers for the period of embryogeny known as maturation. This feature has been exploited in order to investigate the molecular basis of maturation-specific gene control. The proximal β-phaseolin promoter comprises a maturation regulatory element, UAS1 (-295 to -103), and a developmentally neutral TATA region. A dual criterion, based on *in vitro* protein:DNA binding and transient gene expression by particle bombardment of bean cotyledon tissues, was used to evaluate the effects of linker replacement and site-specific mutations engineered in UAS1. This approach yielded three main DNA determinants of gene expression. In a 5' to 3' order they are CACGTG (Vb3-G), TTTTCT (Vb2-AG) and CACGTC (Vb1-B). These motifs are located within different sub-domains of the UAS1 enhancer, namely Vb1 (-134/-83), Vb2 (-200/-156) and Vb3 (-302/-216). The mutational analysis provided information concerning specific sites; however, experiments conducted in our own laboratory and others, suggest that the context of each site plays an important role in the overall function of UAS1. Sub-domain Vb1 is highly conserved in 7S promoters of *Vicia*, *Glycine*, *Phaseolus* and *Pisum* functioning as an endosperm-specific element in tobacco and can bind the maize endosperm transcription factor Opaque2. Sub-domain Vb2 contains several protein:DNA binding sites in addition to Vb2-AG. Deletion of sub-domain Vb3 reduces phaseolin expression in stable tobacco transformants (Burow et al., 1992, *The Plant J.*, **2**, 537-548). The information presented here supports a tripartite structure for the 7S maturation-specific enhancer UAS1. Such a complex structure is consistent with the effects of multiple mutations on the activity of 7S promoters in *A. thaliana*.

AGROBACTERIUM-MEDIATED TRANSFORMATION OF SUGARBEET

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Sugarbeet (*Beta vulgaris* L.) is an important economic crop grown in many of the temperate regions of the world, and accounts for approximately 60% of the domestic production of sugar. Genetic improvement of sugarbeet is hampered by the complexity of the genome, making it an ideal candidate for direct gene transfer. An *Agrobacterium tumefaciens* mediated method of genetic transformation has been developed for use with leaf disc callus from Rel-1 (Bentahar et al, 1991 Pat. #WO9113159), and currently the method is being tested on organogenic hypocotyl callus. Approximately 20 putative transgenic sugarbeets have been regenerated from a single experiment, with *β-glucuronidase* (GUS) activity ranging from 50-400 pMol min^{-1} mg^{-1} protein. Genes designed to increase the sucrose content of the beet will be transferred to sugarbeet. One gene is the maize sucrose phosphate synthase gene (SPS) which will have expression driven by a spinach rubisco activase promoter. The second gene will be a cell wall invertase under transcriptional control of a patatin promoter. Our goal is to increase the production of sucrose in the leaves with the SPS gene and enhance translocation of the sucrose to the storage taproot with the invertase gene.

OPTIMIZED TRANSIENT EXPRESSION IN SUGARBEET SUSPENSION CELLS FOR PROMOTER ANALYSIS

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A group of small cysteine-rich proteins from different plant species appears to possess anti-fungal activity. These proteins have a structural likeness to thaumatin, a sweet-tasting protein from *Thaumatococcuc danielli*. Included in this group are two pathogenesis-related (PR) proteins of tobacco, osmotin (acidic) and its basic counterpart PR-S. Both osmotin and PR-S are effective in inhibiting hyphal growth in *Cercospora beticola*, the causative agent of Cercospora leaf spot disease in sugarbeet (*Beta vulgaris*). Another family of small cysteine-rich proteins involved in pathogen resistance is the leaf-specific thionins of barley. To aid in designing efficiently expressed chimeric constructs of these genes for introduction into sugarbeet plants a transient assay to assess promoter strength was developed. Constructs of the osmotin, PR-S, and a potato proteinase inhibitor 2 (*pin2*) promoter fused to the β-glucuronidase (*gus*) target gene were prepared for analysis in sugarbeet suspension cells. Experiments using 35S-*gus* chimerics were performed to optimize transient expression. The optimized protocol consisted of layering suspension cells (150 mg over a 47 mm diameter) onto a 0.45 micron nylon filter followed by a four hour incubation on SIMM media (MS salts, MS vitamins, 0.1 mM adenine sulfate, 2.5 mM MES, 3% sucrose, NAA [0.1 mg/L], BA [0.3 mg/L], pH 5.8) augmented with equal proportions of sorbitol and mannitol (250 mOsM total). Gold microcarrier particles (1.6 micron in dia.) coated with DNA were helium-propelled (Biorad Particle Delivery System) 11 cm at 1300 psi into the sugarbeet cells. GUS activity determined by histochemical analysis resulted in the appearance of up to 2000 blue foci per assay. The promoter constructs were co-transformed with a 35S-luciferase (*luc*) reporter gene (internal standard) and the osmotin promoter was found to be expressed 2-4 fold higher than the CaMV 35S promoter in sugarbeet suspension cells.

PRODUCTION OF FERTILE TRANSGENIC ASPARAGUS PLANTS USING PARTICLE GUN BOMBARDMENT

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Suspension cells from seedling-derived calli of G203 were used as the targets of particle gun bombardment. Tungsten particles, coated with pKGUS plasmid carrying genes encoding NPTII and GUS, were accelerated by the helium. Through histological staining, about 80 cells or cell clumps per plate showed positive GUS reactions. For production of resistant calli, the bombarded cells were incubated in either of two media: 1) MS medium plus 0.1mg L^{-1} 2.4-D, 25mg L^{-1} kanamycin and 3g L^{-1} gelrite and 2) MS medium plus 0.2mg L^{-1} NAA, 0.02mg L^{-1} kinetin, 25 mg L^{-1} kanamycin and 3 g L^{-1} gelrite. About 10 resistant calli per plate were recovered from each of the two media after 20 days. Some of the resistant calli were transferred to embryo induction medium to produce embryos and plantlets, while the rest were further subcultured to produce more resistant calli. Plantlets produced were transferred to rooting medium with 100 mg L^{-1} kanamycin to kill the escapes. The surviving plants were transferred to greenhouse. Positive GUS reaction was detected in resistant calli, embryos, plantlets, 20 greenhouse-grown plants and pollen from these plants, but not in control. Southern hybridization confirmed the integration of NPTII and GUS genes into asparagus genome.

TRANSFORMATION OF *PSEUDOMONAS* SPECIES WITH TABTOXIN PRODUCTION GENES USING ELECTROPORATION

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As part of a program to improve weed biocontrol organisms, genes for additional toxin production were added to three native pseudomonads (*Pseudomonas fluorescens* D7[PFD7], *Pseudomonas syringae* 3366[PS3366], and *Pseudomonas syringae* 2V19[PS2V19]) developed for the control of downy brome (*Bromus tectorum* L.) a major weed in winter wheat. The 34 kilobase cosmid PRTBL823 (a gift from D.K. Willis) containing the genes necessary for the production of tabtoxin were transferred by electroporation into the three pseudomonads. The potential transformants were selected on tetracycline. A total of forty-four possible transformants were grown in liquid culture. A tabtoxin sensitive *E. coli* strain was used to screen for activity using the supernatant from the cultures. All forty-four transformants showed some activity in the bioassay, and minipreps showed the plasmid present in every case. Transformants whose supernatants showed larger clear zones in the bioassay were grown in batch cultures for the production of tabtoxin. Semipurified toxins were compared to authentic dipeptide tabtoxin and the product of peptidase activity, tabtoxin-β-lactam (TBL) using TLC. Transformants that show high tabtoxin production will be evaluated for their ability to control downy brome in greenhouse studies.

Inhibits glutamine synthetase

MUTATIONAL ANALYSIS OF *GLIOCLADIUM VIRENS* TO DETERMINE THE ROLE OF GLIOTOXIN IN SUPPRESSION OF *PYTHIUM* DAMPING-OFF.

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Gliocladium virens is a fungal biocontrol agent which has been commercially developed as Gliogard™ for control of damping-off diseases. Certain strains of *G. virens*, including that used in Gliogard™, produce the antimicrobial metabolite gliotoxin. Seven UV-mutants of strain G20-4VIB (wild type) lacking gliotoxin production were isolated using selection-based enrichment and screening procedures. This gliotoxin non-producing phenotype was designated as Glx⁻. Colony appearance, sporulation, ability to grow on minimal medium, and soil colonization of these mutants were comparable to G20-4VIB. *In vitro* antagonism studies using these *G. virens* isolates demonstrated that the mutants had lost most of the apparent antibiosis displayed by the wild type against most soilborne plant pathogens tested. The *G. virens* isolates were further tested in disease suppression activity against *Pythium ultimum* damping-off. The Glx⁻ mutants displayed on average only 54% of the disease-suppressive activity of the wild type strain. This supports a major role for gliotoxin in *G. virens* suppression of *P. ultimum*, and represents strong genetic evidence that antibiosis acts in fungal-biocontrol of plant disease. The lack of gliotoxin in the mutants may be due to a change in regulatory function as certain mutants also lack resistance to gliotoxin and/or display an earlier expression of viridin, a sterol antibiotic.

ANALYSIS OF A FUNGAL PROMOTER WHICH IS RESPONSIVE TO A HOST-SPECIFIC ISOFLAVANOID, *IN VITRO*, IN CULTURE AND *IN PLANTA*.

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The pisatin demethylase gene (*PDA*1) of *Nectria haematococca* MP VI (anamorph *Fusarium solani*) encodes an enzyme which detoxifies pisatin, the isoflavanoid phytoalexin of pea. This system provides a unique fungal gene whose expression is induced by a well characterized host-derived compound. We are analyzing the molecular components responsible for the pisatin-responsiveness of the *PDA*1 gene.

In mycelial culture, addition of micromolar quantities of pisatin induces expression of pisatin demethylase enzyme, *PDA*1 mRNA, and *PDA*1 promoter activity in the presence of nutrient limitation. We have identified cis and trans-acting components producing this pisatin responsiveness of the *PDA*1 promoter using *in vitro* methods. Gel shift analysis has defined a specific pisatin-responsive DNA-binding factor (PRF). This factor binds to a 35 bp region approximately 500 bp upstream of the transcription start point. Mutation of nucleotide positions has defined regions critical for binding within one half of a partial direct repeat within this 35 bp region. Southwestern analysis and UV crosslinking have identified a 35 kDa protein which appears to be the PRF. In order to analyze function of these components, we have developed a homologous *in vitro* transcription system which reconstitutes Pol II transcription. Transcription *in vitro* accurately initiates at the *PDA*1 promoter and reflects pisatin-responsive regulation. Both deletion analysis on the PDA1 promoter-template, and oligonucleotide binding site competition analysis strongly indicate that the binding site of the PRF is necessary for a high level of transcription in extract from pisatin-treated mycelium. In order to study the effects of promoter alterations on *PDA*1 regulation during pathogenesis, stable transformants harboring a *PDA*1 promoter::*GUSA* fusion were inoculated onto pea epicotyl. GUS expression in the diseased tissue indicated a specific stimulation, presumably in response to pisatin. The combination of *in vitro* and *in vivo* techniques allow detailed analysis of the components which determine regulation of this fungal promoter by a signal compound from the host plant, and is an important step in characterizing how a fungal pathogen can perceive and respond to interaction with its host.

Differential display of tobacco root genes expressed during infection by Striga asiatica as detected by DD-PCR

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Striga asiatica (commonly known as witchweed) is a parasitic angiosperm which attacks the roots of many agriculturally important monocots. Infection of host roots is via a specialized structure called a haustorium. The haustorium penetrates through the epidermis and cortex of host roots to form a link with the host vascular system. Among species resistant to infection, Striga begins to penetrate the host root but growth is seized mid-cortex, presumably due to a pathogen related defense mechanism. We are attempting to find the factors involved in this response using differential display PCR (DD-PCR). We have found over 60 reproducible differentially amplified cDNA fragments between infected versus non-infected tobacco (Nicotiana tabacum) roots. We are currently screening these bands using northern analysis and DNA sequencing.

Characterization of mutant *apt* gene alleles from *Arabidopsis thaliana.*

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The *apt* gene codes for the enzyme adenine phosphoribosyltransferase (APRT). APRT is a purine salvage enzyme that catalyzes the formation of 5'-AMP from adenine and 5-phosphoribosyl-1-pyrophosphate. APRT deficient *Arabidopsis thaliana* plants were selected from a population of ethylmethane sulfonate (EMS) treated seeds. EMS is a methylation agent which can methylate guanine to change guanosine to adenosine in DNA molecules. Independent *apt* mutant lines, BM1, BM2, BM3, were isolated by the germination of seeds on a medium containing 0.1mM 2,6-diaminopurine. The APRT assay showed that BM1 had 10 to 15% of the APRT activity level detected in extracts prepared from wild-type plants; BM2 and BM3 had approximately 2 and 1% wild-type APRT activity levels, respectively. H(G)PRT assay was developed to help characterize the *Arabidopsis* APRT wild-type (+/+), heterozygous (+/-), and homozygous (-/-). H(G)PRT is a purine salvage enzyme which uses guanine and hypoxanthine, instead of adenine, to produce HMP and GMP. The H(G)PRT assay was used as a control for this characterization. APRT wild-type (+/+), heterozygous (+/-), homozygous (-/-) mutants were distinguished because they have different levels of APRT activity, but almost the same H(G)PRT activity level.

BM2 and BM3 have almost no APRT activity. Two probable causes for the BM2 and BM3 mutant phenotypes are: 1) a missense mutation (if the G of the UAG codon for Trp changed to UAA which is a stop codon); or 2) a splice site mutation (if the G of the splice site GT/AG changed). BM1 could have changes in important amino acids (e.g., a catalytic region) so that the APRT activity is reduced only to 10 to 15%. To understand mutations responsible for the BM2 and BM3 phenotypes, we isolated and sequenced *apt* genomic DNA and cDNA. There was a transition of G to A found in the splice site at the junction of intron 2 and exon 3 in BM2 and BM3 *apt* genomic DNA. We also found that BM3 cDNA did not have exon 3. The G to A mutation in BM2 and BM3 APRT genomic DNA alters the AG consensus sequence at the intron 2-exon 3 splicing junction to AA and can lead to aberrant splicing. Aberrant splicing at this site would cause the deletion of exon 3 of BM3 cDNA. The BM2 and BM3 mutants produced by using EMS are, therefore, mRNA splicing mutants. Because the mutants were generated by EMS, there may be other changes in the genomic DNA sequence between BM2 and BM3.

Gea8, A GENE EXPRESSED DURING CARROT SOMATIC EMBRYOGENESIS

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The early stages in plant embryogenesis involve a series of morphological and cellular changes, during which the zygote develops through the globular, heart, torpedo and cotyledon stages. As a result, the primary apical meristems and the embryo polarity axis are set up. Although plant embryogenesis has been well described morphologically, very little is known about the process at the molecular level. Using the carrot somatic embryo system, we previously isolated 50 clones which are preferentially expressed in globular embryos compared to vegetatively growing seedling tissues, and thus named Gea1-50 (Globular embryo abundant). The expression of these clones through embryo development fall into several different patterns. Here we report the characterization of Gea8, one of the 10 clones whose transcripts are very abundant in heart embryos. Several overlapping cDNA clones and a genomic clone were isolated and sequenced. This gene encodes a 53Kd peptide with homology to 7S globulins (seed storage proteins) from other plant species. The RNA accumulation in callus and globular embryos can be enhanced by ABA. Further experiments using transgenic plants are being done to study its expression and regulation in somatic as well as zygotic embryos.

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