PLANT GENE TRANSFER

Organizers: Christopher Lamb and Roger Beachy

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Plenary Sessions Page

Poster Session

Crop Transformation

M 001 APPROACH TO CEREAL TRANSFORMATION VIA MICROINJECTION INTO MICROSPORE-DERIVED AND INTO ZYGOTIC PROEMBRYOS,

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There is considerable interest in the development of a routine and generally applicable method for gene transfer which also functions for cereals and other important crop plants. And there has been a great variety of different approaches to this goal including e.g. biolistics, electroporation, laser, agroinfection, bacterial and viral vectors, macroinjection, pollen transformation, pollen tube pathway, microinjection, and direct gene transfer. To date the only reliable transformation in cereals is based on direct gene transfer. Protoplast-based protocols, however, will probably be too complicated for general and routine future application.

Microinjection of a selectable marker gene into microspore-derived proembryos of oilseed rape has produced transgenic chimeras (Neuhaus et al.,TAG 75: 30-36, 1987). To test whether an analogous approach would work with cereals we established plant regeneration from microspores in wheat (Triticum aestivum), rice (Oryza sativa), maize (Zea mays, collaboration Academia Sinica, Beijing) and barley (Hordeum vulgare, collaboration Carlsberg Laboratory, Copenhagen), microinjected marker genes, regenerated plants and analysed the sexual offspring for their transgenic nature. This was, so far, negative. Although, if positive, this method would enable gene transfer to cereals, it still would suffer from similar draw backs as protoplast-based methods.

However, a system of real general applicability could be envisaged, if it were possible to work on the basis of zygotic proembryos. To this end we established isolation of and plant regeneration from zygotic proembryos as small as ca. 25 cells for wheat, rice, maize, and barley, microinjected marker genes, regenerated fertile plants, harvested sexual offspring, and subjected seedlings to the appropriate selective conditions. A molecular analysis of resistant plants will have to show, whether or not this method yields transgenic chimeras and whether some of those yield solid transgenic offspring.

M 002 TRANSFORMATION AND REGENERATION OF TRANSGENIC RICE AND MAIZE PLANTS,

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Efficient methods for gene transfer and plant regeneration are required for genetic manipulation of the plant genome. In this abstract, I will describe the protoplast method for obtaining transgenic rice plants and the biolistic process for obtaining transgenic rice and maize plants.

Recently, three laboratories have reported the regeneration of transgenic rice plants from protoplasts. Toriyama et al. (1) applied electroporation to protoplasts in the presence of a plasmid carrying an aminoglycoside phosphotransferase (NPTII) gene. After selection with the antibiotic, G418, five plants were regenerated. Cocking and Davey (2) used polyethylene glycol (PEG), or electroporation, in the presence of a plasmid carrying the NPTII gene, and regenerated several transgenic plants. Neither of these groups reported the production of rice seeds from the transgenic plants. Zhang and Wu transformed rice protoplasts in the presence of PEG and a plasmid carrying a β–glucuronidase (GUS) gene, and regenerated 86 transgenic plants. Many of these plants set seeds (3). Recently, seeds from several transgenic plant were germinated and DNA was isolated from the resulting plantlets. DNA from 73% of the plantlets gave positive hybridization signals using the GUS gene as the probe. Thus, the segregation ratio was close to the expected 3:1 ratio for a single dominant allele according to Mendel's law.

In the biolistic process, also known as the microprojectile bombardment method (4), mature rice and maize embryo sections were bombarded with DNA–coated tungsten particles. A plasmid carrying the GUS gene was used for the transformation of rice embryo sections. More than ten transgenic rice plants were generated. A plasmid carrying the GUS gene was also used for the maize experiment. Three transgenic maize plants were generated. Successful integration was shown by DNA dot–blot and genomic–blot hybridization using the GUS gene as the probe.

(1) Toriyama, K., Arimoto, Y., Uchimiya, H., Hinata, K. (1988) Bio/Technology 6: 1072–1074.
(2) Cocking, E.C. and Davey, M.R. (1988) Rice Genetics Newsletter 5: in press.
(3) Zhang, W.G. and Wu, R. (1988) Theor. Appl. Genetics, in press.
(4) Klein, T.M., Wolf, E., Wu, R. and Sanford, J.C. (1987) Nature 327: 70–73.

M 003 MECHANISM OF AGROBACTERIUM MEDIATED NATURAL GENE TRANSFER. Patricia Zambryski, Vitaly Citovsky, Elizabeth Howard*, Guido De Vos, Barbara Winsor. Division of Molecular Plant Biology, Hilgard Hall, University of California, Berkeley CA 94720. *PGEC-USDA, 800 Buchanan Street, Albany CA 94720.
Agrobacterium genetically transforms plant cells by transferring a particular DNA segment, T-DNA, from its large tumor inducing (Ti) plasmid into the plant nuclear genome. T-DNA transfer is under tight control, and is regulated to occur only following induction of the expression of another segment of the Ti plasmid, the virulence (vir) region. Our recent work focuses on the analysis of the structure of the T-DNA transfer copy, and on the vir specific products that directly interact with the T-DNA copy during its transit. The T-DNA intermediate, designated the T-strand, has been shown to be a linear single stranded (ss) copy of the bottom strand of the T-DNA region of the Ti plasmid, and its 5' and 3' ends map to the right and left T-DNA border repeats. The ss nature of the T-strand makes it a prime target for nucleases both within the bacterial cell soon after its synthesis, and later in the plant cell during its passage to the nucleus. Thus, it was predicted that one of the vir loci might encode a ssDNA binding protein. Indeed, the virE locus encodes such an activity. VirE protein binds tightly and cooperatively to ssDNA in a nonsequence specific fashion, VirE-ssDNA complexes are highly resistant to nucleases in vitro, andVirE-ssDNA complexes exhibit a unique structure when viewed under the electron microscope (1). VirE protein unfolds and extends ssDNA to an unusually thin complex, at the limit of resolution using direct platinum shadowing (20 A). These thin structure may reflect the size required for transfer of the T-strand-protein complex through the bacterial envelope into the plant cell. A second protein, VirD2, is found tightly associated with the 5' end of the T-strand. VirD2 is part of the VirD1/VirD2 endonuclease responsible for producing nicks at the T-DNA borders used in T-strand synthesis. The tight assocation of VirD2 with T-strands may serve at least 4 possible functions: unwinding the T-strand during its synthesis, protecting the 5' end from exonucleases, "piloting" the T-strand complex to the bacterial and plant cell membranes, and facilitating integration of the T-strand into plant DNA.

1) V.Citovsky, M.L.Wong, and P. Zambryski. 1989. PNAS, in press.

Discussion: Novel Transformation Technologies

M 004 **Stable nuclear transformation of *Chlamydomonas reinhardtii* using a *C. reinhardtii* gene as the selectable marker,** Stephen P. Mayfield[1] and Karen L. Kindle[2], [1]Dept. of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, Calif., 92037, [2]Biochemistry Dept., Cornell University, Ithaca, New York, 14853

We have development a DNA mediated transformation system for the nuclear genome of *Chlamydomonas reinhardtii*. A cloned *C. reinhardtii* gene was introduced into *C. reinhardtii* cells by bombardment with DNA-coated tungsten particles. The recipient strain was a non-photosynthetic mutant which recovered photosynthetic competence following transformation. Analysis of transformants shows that the introduced gene has integrated into the *C. reinhardtii* nuclear genome. Genetic analysis indicates that in some cases the introduced gene has integrated very close to the endogenous mutant gene, perhaps by homologous recombination. In other cases the introduced genes have integrated into the genome at positions unlinked to the endogenous gene. In the later case the introduced genes segregate as expected for single copy genes. We suggest that the success of the present system is largely due to using a homologous *C. reinhardtii* gene, leading to stable maintenance and expression of the gene, which may be precluded with heterologous genes due to an unusual codon bias in *C. reinhardtii*.

Viral Vectors, Organellar Transformation and Targeting

M 005 MECHANISMS OF VIRAL RNA REPLICATION AND GENE EXPRESSION, Paul Ahlquist, Philip Kroner, Radiya Pacha, Patricia Traynor and Richard Allison, Institute for Molecular Virology and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Though replicating without DNA, plant RNA viruses induce dramatically high levels of gene amplification and expression, and possess a variety of mechanisms for independently regulated expression of many genes from a single virus. The natural success of these viruses suggests that RNA-dependent replication and mRNA synthesis could also contribute to genetic engineering, including possible use of these pathways to further amplify or regulate expression of primary transcripts from chromosomally-integrated genes. Significant conservation of fundamental RNA replication genes across a wide range of plant and animal viruses suggests that understanding these mechanisms could also be useful for developing broad-spectrum antiviral strategies.

We have used biologically active viral cDNA clones to study RNA replication, gene expression, host specificity, and other aspects of the molecular biology of plant bromoviruses, including brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV). The genomes of these viruses are divided among messenger-sense RNAs 1, 2 and 3 (3.2, 2.9, and 2.1 kb). RNA3 encodes the coat protein and a noncapsid gene required for systemic infection. RNAs 1 and 2 encode proteins 1a (104 kD) and 2a (94 kD), which are trans-acting factors in viral RNA replication. One of two conserved domains in the 1a protein contains sequence similarities to known ATP-dependent helicases, while the 2a protein contains extended amino acid similarity to known RNA-dependent polymerase subunits. Site-directed mutagenesis is being used to study the functions of proteins 1a and 2a in RNA elongation and in regulating the levels of (+) and (-) strand genomic RNA and subgenomic mRNA produced in infection.

Considerable progress has been made in mapping and characterizing the cis-acting sequences which direct the replication of viral genomic RNAs and the internal initiation of subgenomic mRNAs. Using such characterized elements, a wide variety of novel RNA replicons can be constructed which, e.g., replicate and express foreign genes in transfected plant cells. Many of the replication elements contain sequence features related to tRNAs or their genes.

A large number of recombinant viruses exchanging selected genes, gene segments and regulatory sequences between BMV and CCMV have been constructed to exploit differences between the two viruses in studies of both replication and host specificity. Systemic infection of at least some hosts appears to require adaptation of multiple virus genes.

M 006 IDENTIFICATION OF THE TARGETING DOMAIN OF THE VACUOLAR GLYCOPROTEIN PHYTOHEMAGGLUTININ IN TRANSGENIC YEAST AND TOBACCO, Brian W. Tague and Maarten J. Chrispeels, Department of Biology, University of California, San Diego, La Jolla, CA 92093.

Many different proteins (acid hydrolases, storage proteins, lectins, enzyme inhibitors) accumulate in the vacuoles of plant cells. We are trying to identify the information (domain) that allows these proteins to be correctly targeted to the vacuoles. Vacuolar proteins, like secreted proteins, are synthesized on the rough endoplasmic reticulum, and their transport is mediated by the Golgi apparatus. They undergo a number of cotranslational and posttranslational processing steps in the ER, Golgi and vacuoles. Our model system is phytohemagglutinin (PHA), the tetrameric glycoprotein lectin of the common bean, *Phaseolus vulgaris*, that accumulates in the protein storage vacuoles (protein bodies) of the cotyledons. Expression in transgenic tobacco of a mutant PHA gene that has altered glycosylation sites results in the proper targeting of unglycosylated PHA to the protein bodies indicating that the targeting information is not in the glycan domains (Voelker, T.A., Herman, E.M., Chrispeels, M.J. 1989. The Plant Cell *1*, in press). When the PHA gene is expressed in yeast under control of the phosphatase (PHO 5) promoter, PHA is efficiently targeted to the yeast vacuole (Tague, B.W. and Chrispeels, M.J. 1987. J. Cell Biol. *105*:1971-1979). We have now generated fusions between the gene for PHA and the gene for the yeast secretory protein invertase to identify the polypeptide domain of PHA involved in vacuolar targeting in yeast and tobacco. The in-frame fusions of PHA and invertase were screened in yeast using an invertase filter assay. Our data indicate that an amino-terminal portion of PHA between amino acids 14 and 33 is sufficient to target invertase to the yeast vacuole. Examination of the crystal structure of homologous lectins (e.g., pea lectin) shows that this region contains an external loop flanked by β-sheets. Sequence comparisons of a large number of vacuolar proteins from plants and the yeast vacuolar protein, carboxypeptidase Y, show the presence of a relatively conserved tetrapeptide which in PHA has the sequence 18LQRD21. We are now examining the requirement for one or both of these for vacuolar targeting in yeast. Experiments to demonstrate that the same domain is of critical importance for targeting to vacuoles in transgenic tobacco are now in progress.

Supported by grants from the National Science Foundation, the U.S. Department of Energy, and the U.C. Biotechnology program.

M 007 PLANT MITOCHONDRIAL TRANFORMATION: DIRECT AND INDIRECT TECHNOLOGIES, David M. Lonsdale, Tony P. Hodge and Udo K. Schmitz, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trupington, Cambridge CB2 2JB, U.K.
In lower eukaryotes, in particular yeast, mitochondrial transformation has been achieved by two routes, 1. the import and targetting of foreign proteins which have been synthesized on cytosolic ribosomes and more recently 2. the direct introduction of DNA effecting mitochondrial transformation has been reported. The "transformation" of plant mitochondria can be achieved by importing foreign proteins using either yeast or plant signal peptides. The requirement for mitochondrial targetting in plants will be discussed. In addition, a consideration of the feasibility of introducing foreign DNA directly into plant mitochondria by particle ballistics will be discussed.

M 008 MECHANISM OF IMPORT OF PROTEINS INTO CHLOROPLASTS AND MITOCHONDRIA. D. Pain, H. Murakami, D. J. Schnell and G. Blobel. The Rockefeller University, Laboratory of Cell Biology, 1230 York Avenue, New York 10021, USA.
Using in vitro translation/translocation systems and an anti-idiotypic antibody approach we have identified and characterized proteins associated with the machineries involved in post-translational translocation of nuclear-encoded proteins into chloroplasts and mitochondria. Similar to protein translocation across the endoplasmic reticulum or inverted E.coli vesicles, our data suggest dual recognition of signal sequences for efficient import into mitochondria or chloroplasts. The first recognition system involves soluble cytoplasmic proteins whereas the second one is mediated by membrane-bound proteins. The latter has been localized to contact zones, distinct regions where the inner and outer membranes of these organelles are closely apposed to each other.

1. Pain, D., Kanwar, Y. S. & Blobel, G (1988) Nature 331, 232-237.
2. Murakami, H., Pain, D. & Blobel, G (1988) J.Cell.Biol. 107, 2051-2057.
3. Pain, D., Murakami, H. & Blobel, G. In preparation.

Gene Identification/Functional Analysis

M 009 DEVELOPMENT OF LARGE DNA METHODS FOR GENE ISOLATION FROM ARABIDOPSIS, Joseph R. Ecker and Plinio A. Guzmán, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

The recent development of several "large DNA" methods should dramatically increase the ease of gene isolation by chromosome walking. Primary amongst these is the development of pulsed-field gel electrophoresis (PFGE) (1, 2). This technique has allowed the separation of DNA molecules of up to 12 Mb; an improvement upon conventional electrophoresis techniques of over two orders of magnitude. A second important development is the demonstration that segments of DNA as large as 500 kb can be cloned as yeast artificial chromosomes (YACs) (3). This is an improvement of at least an order of magnitude over previous cloning procedures. We have begun to develop and apply these techniques to the analysis of the *Arabidopsis* genome.

Procedures for the preparation, cloning and analysis of large DNA molecules from plant cells have been developed (4). *Arabidopsis* and carrot cells were used for the preparation of large DNA molecules in agarose "plugs" or in solution. PFGE analysis of large plant DNA preparations using a contour-clamped homogeneous electric-field (CHEF) apparatus (5) indicated that the size of the DNA was at least 12 Mb. Large plant DNA preparations were shown to be useful for restriction enzyme analysis of the *Arabidopsis* genome using both frequent and infrequent cutting enzymes. A high-capacity yeast cloning system (3), which is based on the *in vitro* construction and subsequent propagation in yeast of large linear DNA molecules as yeast artificial chromosomes, was used to construct libraries of the *Arabidopsis* and carrot genomes. Analysis of plant DNA-containing YACs indicated that: 1) yeast transformants contained artificial chromosomes large enough to be directly visualized by ethidium bromide staining of CHEF gels; the average size of insert was about 150 kb; 2) the number of transformants with inserts was well over that needed to obtain complete genomic libraries; 3) the absence of minor bands in Southern blots of YAC clones suggests that both unique and repetitive DNA inserts are stable; 4) the inclusion of a DNA size-selection step prior to transformation has allowed the construction of YAC libraries with significantly larger size inserts and 5) low copy *Arabidopsis* genes can be isolated from YAC libraries using yeast colony hybridization techniques.

The ability to clone and stably propagate large segments of plant DNA in a simple genetic background has several several important implications; YACs should be useful for gene isolation via chromosome walking, physical mapping of the *Arabidopsis* genome and for the direct transfer of large DNA segments into plants.

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M 010 STRATEGIES FOR PHYSICAL MAPPING OF COMPLEX GENOMES BY COSMID MULTIPLEX ANALYSIS, Glen A. Evans, The Salk Institute for Biological Studies, La Jolla, California 92037

A strategy for linking individual clones of a cosmid library and the assembly of a large physical map is presented which depends on the simultaneous analysis of many cosmid clones for overlapping regions. This method uses cosmid vectors that contain endogenous bacteriophage T3 and T7 promoters to allow for the identification of overlapping clones through the synthesis of an "end-specific" RNA probes. A genomic library is constructed and organized as an ordered matrix such that each clone is assigned an identifying coordinate. DNA from mixtures of cosmid clones are pooled such that each pool contains only one member in common with any other pool. RNA probes are prepared from mixtures of cosmid clones, and groups of clones overlapping with the constituents of the mixtures determined by hybridization. Pooled probes are most simply prepared by grouping clones according to the rows and columns of the library matrix. The pairwise comparison of data generated by the hybridization of mixed probes can be decoded using simple algorithms which predict the order and linkage of all clones in the collection and organize them into predicted "contigs". To demonstrate the feasibility of multiplexed analysis of cosmids, a genomic library was prepared from a mouse-human somatic cell hybrid which contains a portion of the long arm of human chromosome 11. Preparation, arrangement on a matrix, and analysis of pooled cosmid clones from this collection resulted in the detection of 1099 linked pairs of cosmids which could be assembled into 315 contigs. Thus, with a minimal amount of effort, over 60% of this genomic region has been assembled in groups of overlapping cosmids. This method may have practical applications in the large scale mapping and sequencing of mammalian and plant genomes.

M 011 ISOLATION OF DEVELOPMENTALLY IMPORTANT GENES FROM ARABIDOPSIS, Howard M. Goodman, Brian Hauge, Bart den Boer, Jerome Giraudat and Hong-Gil Nam, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114. We are interested in elucidating mechanisms of gene regulatory control during early development and embryogenesis in higher plants. The short life cycle, availability of mapping strains, and the small genome size of Arabidopsis thaliana, offer the potential for the identification and cloning of those genes which, based on their mutant phenotype, appear likely to be involved in developmental regulation. As a first major step toward cloning such genes we are constructing physical and RFLP maps of the Arabidopsis thaliana genome. The physical mapping of individual clones is being carried out using the methodology described by Coulson, A., et. al. *Proc. Natl. Acad. Sci. USA*, 83, 7821 (1986). At present, ~15,000 clones have been fingerprinted and ~10,000 carefully analyzed. The clones in the database fall into 876 contigs and represent approximately 90-95% of the Arabidopsis genome. To be useful this overlapping cosmid set ("the physical map") must be correlated with the genetic map of Arabidopsis. To accomplish this, a RFLP map has been constructed and currently contains 90 new randomly distributed markers (10 identified Arabidopsis genes and 80 genomic cosmid clones) residing on all five chromosomes. When completed, the combined RFLP/physical map will allow ready access to any region of the genome of known genetic map location.

M 012 GENETIC PROPERTIES OF AC IN TOBACCO, Jonathan D.G. Jones*, Francine Carland and Hugo Dooner, Advanced Genetic Sciences, 6701 San Pablo Ave, Oakland, CA 94608, *Present address; The Sainsbury Laboratory, John Innes Inst., Colney Lane, Norwich, NR4 7UH, U.K.

It has been amply demonstrated that the corn transposable element *Activator* (*Ac*) can transpose autonomously in tobacco and other heterologous species. We have been conducting a research programme to compare in detail the genetic properties of *Ac* in corn and tobacco. In corn, increasing *Ac* dosage exerts a negative effect on somatic transposition frequency. Also, *Ac* preferentially transposes to linked sites. Germinal transposition frequencies in corn can range from 1% to 10%. When individual corn seedlings are picked which exhibit *Ac* excision from a specific locus, only about 50% of them show evidence of reinsertion of the element elsewhere in the genome.

We have made constructs in which *Ac* is cloned into the 5' leader of an engineered nuclear streptomycin resistance gene in order to monitor *Ac* excision in tobacco seedlings. Our data show that the genetic properties of *Ac* in tobacco are very similar to those of *Ac* in corn, except that in tobacco there is no apparent negative effect of *Ac* dosage on transposition frequency. These data are being incorporated into an approach to using engineered transposons for the isolation of disease resistance genes from tomato.

M 013 MANIPULATION OF FLOWER PIGMENTATION BY THE ANTISENSE STRATEGY.

Joseph N.M. Mol, Alexander R. van der Krol, Anton G.M. Gerats and Antoine R. Stuitje. Department of Genetics, Section Biosynthesis Secondary Metabolites, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Flowerpigments of higher plants are provided by the flavonoid biosynthesis pathway. We have explored the possibility to inhibit floral pigmentation by an antisense strategy. Therefore, cDNA encoding chalcone synthase (CHS) of Petunia hybrida in inverse orientation under the CaMV35S promoter was expressed in transgenic petunia and tobacco plants. [van der Krol et al., (1988) Nature 333, 866-869]. Some transformants show a reduction in floral pigmentation either evenly distributed over the flower or exhibit discrete patterns (rings, stars). Half of all transformants show no phenotypic effect at all. We find no correlation between the steady-state level of antisense CHS RNA in leaf tissue and flower phenotype. Attempts to detect duplex RNA were unsuccessful, suggesting rapid degradation of such molecules. In line with this, levels of antisense RNA in flower tissue were found to be extremely low. Some phenotypic patterns are uniform throughout a single plant whereas others are not. Backcross experiments show that pattern formation is also dependent on the genetic background of the plant. For some transformants it was shown that the variable degree of pigmentation in flowers of one plant might be caused by variable light conditions or internal hormone balances during plant growth. In this respect antisense patterns respond similar to the one's exhibited by the mutant Red Star [Mol et al., 1983 Mol. Gen. Genet. 192, 424-429]. In order to understand the molecular basis of antisense effectiveness, we have begun defining minimal requirements using antisense gene fragments and different promoters. Furthermore we investigate if pattern formation is an intrinsic property of antisense genes under the CaMV promoter and have therefore transformed petunia with antisense genes for chalcone flavanone isomerase (CHI) and dihydroflavonol reductase (DFR) respectively. The results will be discussed in the light of naturally occurring variability in plant gene expression.

M 014 STUDIES OF ANTISENSE INHIBITION OF GENE EXPRESSION IN TOBACCO: NOPALINE SYNTHASE AND PEROXIDASE, Steven Rothstein, Douglas Rice and L. Mark Lagrimini, Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada N16 2W1

The silencing of gene expression through the synthesis of antisense RNA has great potential for determining the function of isolated genes. This type of analysis should be particularly fruitful in studying multi-gene traits, where it is difficult to isolate genetic variants only differing at a single allele. As a model system, a study involving the expression of antisense RNA to decrease the synthesis of nopaline synthase in transformed tobacco was undertaken. The nopaline synthase enzyme activity was decreased eight to forty-fold, depending on the tissue analyzed, in plants expressing antisense RNA. This decreased expression was stable and heritable.

The use of antisense RNA was then applied to the tobacco peroxidase genes. Perixodases are important in secondary cell wall biosynthesis, catalyzing the last step in lignin biosynthesis and forming rigid cross-links between hydroxyproline-rich glycoproteins, cellulose, pectin and lignin. They are also essential for wound-healing and may have a role in auxin catabolism and defence of the plant against pathogen attack. Tobacco plants have been transformed either with a chimeric gene to overexpress peroxidase or to silence endogenous peroxidase activity through the synthesis of antisense RNA. The phenotypes of these plants are quite different than wild-type tobacco, with the magnitude of these changes being dependent on the quantitative variability of peroxidase expression in different transformed plants.

M 015 GENETIC AND PHYSICAL MAPPING OF TOMATO AND RICE CHROMOSOMES, S. Tanksley, M. Bonierbale, T. Fulton, M. Ganal, N. Lapitan, S. McCouch, R. Messeguer, A. Paterson, J. Prince, C. Vicente, Z. Wang, N. Young and Z. Yu, Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853

Our laboratory has been engaged in the development of high density restriction fragment length polymorphism (RFLP) maps of tomato and rice chromosomes. Currently, more than 500 cloned sequences have been located on the tomato genetic map and more than 250 on the rice map. Near-isogenic lines and segregating populations have been used to locate genes on these maps, both for quantitatively inherited characters (QTLs), as well as major genes for disease resistance. In tomato, genes for resistance to tobacco mosaic virus (TMV), root knot nematodes, Fusarium and Pseudomonas have been located near the cloned RFLP markers. Pulsed field gel electrophoresis has been employed to construct long-range restriction maps around the Tm-2a locus (resistance to TMV). In rice, genes for resistance to bacterial blight, rice blast, and insect resistance are being mapped. Long term goals include: 1) the development of RFLP-based selection procedures for these genes and others 2) the ultimate cloning of these genes through chromosome walking procedures.

Gene Regulation

M 016 THE MOLECULAR GENETICS OF FLORAL PATTERNS IN ANTIRRHINUM MAJUS, Enrico S. Coen, Jorge A.M.P. Almeida, Timothy P. Robbins, Joachim Ballmann, Da Luo, Sandra Doyle, Justin Goodrich and Rosemary Carpenter, John Innes Institute and AFRC Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.

We describe the molecular and genetic analysis of mutations at the pallida and nivea loci which encode enzymes required for flower pigment biosynthesis in A. majus. Many of the mutations change the spatial pattern of gene expression and are due to imprecise excision of transposable elements from the promoter regions of these genes. We propose that many of these patterns result from changes in regions of the promoter which interact with transcription factors having various spatial distributions in the flower. This is supported by data showing that some of the pallida mutations alter a sequence in the promoter region which is essential for the regulation of the pallida $^{+}$ gene by a trans-acting factor encoded by an unlinked locus, delila. In Pallida^{+} genotypes the Delila^{+} product is required for most pallida expression in flower tubes. However, pallida mutants lacking one part of the promoter region are uncoupled from regulation by the delila gene. To understand further the genetic control of floral patterns we are trying to isolate genes such as delila, by transposon-tagging. We are also using this technique to isolate other genes involved in flower development.

References:

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M 017 IN VITRO AND IN VIVO CHARACTERIZATION OF TRANS-ACTING FACTORS WHICH BIND TO THE CaMV 35S PROMOTER, Eric Lam, Philip N. Benfey, Philip M. Gilmartin, Fumiaki Katagiri and Nam-Hai Chua, Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021.

Several cellular protein binding sites on the 35S promoter from cauliflower mosaic virus (CaMV) have been mapped by DNaseI footprinting with tobacco nuclei extracts. Two of these sites are in the region between -107 and -59 upstream from the transcription start site. The factors that bind to these sites from tobacco leaf nuclei extract are designated ASF-1 and ASF-2 for the proximal and distal factor, respectively. ASF-1 binding site is found to contain two tandem TGACG motifs which are important for protein-DNA interaction. This motif is found in two other factor binding sites located in the promoter region of the plant histone H3 genes and the nopaline synthase gene found in T-DNA of agrobacterium tumafaciens. The binding sites located in these promoters are compared with the one for ASF-1 by in vitro competition studies with DNaseI footprinting and gel retardation analyses. Also, their in vivo function are compared by functional analyses of various promoter constructs in transgenic tobacco. Mutant binding sites defective in protein-DNA recognition are used as control for these studies. The functional role of ASF-2 will also be addressed by similar methods. The role of these and other factors in the expression of the 35S promoter in vivo will be discussed. *This work is supported by Monsanto.*

M 018 ISOLATION AND CHARACTERIZATION OF NUCLEAR PROTEINS BINDING TO A SILENCER REGION IN THE PROMOTER OF BEAN CHALCONE SYNTHASE GENE. M.J. Harrison, M.A Lawton*, C.J. Lamb* and R.A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. *Plant Biology Laboratory, Salk Institute for Biological Studies, P. O. Box 85800, San Diego, CA 92138.

Transient expression assays have defined a putative silencer element between positions -173 and -326 in the promoter of an elicitor-inducible bean chalcone synthase gene [1]. DNase 1 footprinting revealed 3 discrete binding sites within the element [2]. A 33 bp oligonucleotide complementary to the strongest of the 3 binding sites was retarded in gel-retardation assays by neclear proteins from bean and alfalfa; the binding efficiency of the oligonucleotide was much reduced if the 4 bp overhangs at its ends were unfilled. Specificity of binding was confirmed by competition gel-retardation assays, and an estimation of the Mr of the putative repressor protein obtained by UV-crosslinking to bromo-deoxyuridine-substituted oligo. The binding protein was partially purified by heparin agarose and DNA-affinity chromatography. A λgtll expression library from bean mRNA was screened with ligated oligo; we describe the selection and characterization of positive clones. Possible roles for the putative repressor are discussed in relation to the specificity and flexibility of the elicitation response.

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M 019 MOLECULAR ANALYSES OF OPAQUE-2; A ZEIN GENE REGULATORY LOCUS, Robert J. Schmidt*, Frances A. Burr†, and Benjamin Burr†, *Biology Department, University of California, San Diego, La Jolla, CA 92093, and †Biology Department, Brookhaven National Laboratory, Upton NY 11973.

Molecular and genetic data suggest that the regulatory locus opaque-2 (o2) controls the level of zein gene transcription. In order to begin investigating the manner by which the o2 locus promotes transcription of zein genes we cloned o2 through transposon tagging using the maize transposable element Spm (1). Hybridization of an o2 specific probe to maize mRNAs isolated from different organs indicates that the endosperm is the only tissue where the o2 gene is expressed. In this tissue the increase in zein gene transcription during endosperm development correlates with the level of o2 gene expression. O2 cDNA clones were obtained and subcloned into appropriate expression vectors that allowed for the generation of large amounts of o2 transcript in vitro. The translation of this o2 mRNA in a rabbit reticulocyte lysate system generated a polypeptide that migrated with an apparent molecular weight of 60,000 on SDS polyacrylamide gels. The sequence of the 5' end of the cDNA revealed that the o2 mRNA has a long leader containing three AUG codons upstream from the functional initiation codon. Each upstream AUG codon is followed by a short open reading frame which terminates with an inframe stop codon. This unusual feature is similar to that which has been found in the yeast regulatory gene, GCN4, and in several mammalian oncogenes, and may reflect an interesting mode of translational regulation. The analysis of zein proteins in an o2 mutant with undetectable levels of o2 transcript reveals the presence of a small number of zein proteins. This suggests that the expression of a small subset of zein genes is completely independent of o2, indicating that another zein regulatory locus is required for the expression of this group of zein genes.

1. Schmidt, Robert J., Frances A. Burr, Benjamin Burr. 1987. Science 238: 960-963.

Signals and Transduction Mechanisms

M 020 GENETIC ANALYSIS OF PHOTORECEPTOR ACTION PATHWAYS IN *ARABIDOPSIS THALIANA*, Joanne Chory, Plant Biology Laboratory, The Salk Institute, San Diego, CA 92138.

The process of greening, or differentiation of the chloroplast, involves the coordinate regulation of many nuclear and chloroplast genes. The cues for the initiation of the developmental program are both extrinsic (e.g., light) and intrinsic (cell-type and plastid signals). Several regulatory photoreceptors are involved in the perception of light signals; however, the exact mechanisms by which light and other signals are perceived by plant cells and converted into molecular genetic information are not understood. We have identified *Arabidopsis thaliana* mutants in both signal perception and transduction elements of these pathways.

The first class of mutants develop true leaves in the absence of light, and are therefore designated *det*, for de-etiolated. The eight mutant lines fall into at least 2 complementation groups, one of which has been analyzed in some detail and is called *det*1. Homozygous recessive *det*1 plants develop light-grown characteristics even when grown in darkness (leaf and chloroplast development, anthocyanin accumulation, and constitutive dark expression of several nuclear and chloroplast light-regulated genes). Because the *det*1 mutation affects a wide variety of traits known to be regulated by light, we hypothesize that *det*1 is a regulatory gene and that its action is early in the transduction pathway. Since it is likely that the *det*1 mutation causes a loss-of-function of a component in the light regulation pathway, we propose that the wild-type *Det*1 gene product functions as a repressor of light-regulated genes.

Long hypocotyl (*hy*) mutants initially have an extended hypocotyl when germinated in the light, a phenotype normally associated with dark-grown plants. Of over 55 mutant alleles analyzed by M. Koornneef (1) and myself, we have identified 7 complementation groups; 3 of these show deficiencies in photoreversible phytochrome activity, and are presumably signal perception mutants. The defects in the other 4 classes are not known. The mutations which affect phytochrome photoreversibility are likely to reside in chromophore functions (synthesis, attachment, photoconversion, etc.) and not in the phytochrome apoprotein itself, since we have shown that the apoprotein is present in each case. The phytochrome-deficient mutants accumulated wild-type levels of *cab* mRNAs and other mRNAs thought to be regulated by phytochrome when grown for 3 weeks in light-dark diurnal cycles; however, a marked reduction in the accumulation of proteins encoded by these genes was observed. Thus, if the etiolated-plant phytochrome plays a role in the accumulation of photosynthesis proteins in light-grown *Arabidopsis*, it is at a post-transcriptional level.

Further molecular and genetic analysis of the *det* and *hy* loci will aid in the construction of the pathways of sensory perception associated with chloroplast development in plants.

(1) Koornneef, M., E. Rolff, and C.J.P. Spruit. 1980. Z. Pflanzenphysiol. 100:147.

M 021 PROTEIN-TYROSINE KINASES AND THEIR TARGETS. Tony Hunter, Bill Boyle, Ellen Freed, Kathy Gould, Detlev Jähner, Rick Lindberg, Jill Meisenhelder, and John Pines. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

We have begun to identify novel protein-tyrosine kinases (PTK) by screening cDNA libraries with oligonucleotide probes to consensus sequences, and with anti-P.Tyr antibodies. Among the candidate PTK cDNAs we have isolated is a novel receptor-like PTK, *eck*, predominantly expressed in tissues containing cells of epithelial origin, which is closely related to *eph*, a newly described receptor-like PTK. We are currently attempting to prove that the *eck* protein has PTK activity. A second putative PTK, is closely related but distinct from the *trk* receptor-like PTK. This gene, which may encode another receptor-like PTK, is most highly expressed in the brain. Using anti-P.Tyr screening we have identified a cDNA clone for the *lyn* PTK, and at least two other putative PKs that are being characterized.

We have continued to analyse a number of PK substrates. p81, a substrate for both the EGF receptor and $pp60^{v\text{-}src}$ PTKs, is localized to the core of surface microvilli. With Tony Bretscher (Cornell), we have isolated a full length p81 cDNA clone. The predicted sequence of p81 is being used to elucidate its function and phosphorylation sites. In collaboration with Sue Goo Rhee (NIH) we have recently identified phospholipase C II (PLC-II) as a substrate for the PDGF and EGF receptors. PLC-II is rapidly (within 30 sec) phosphorylated on Tyr and Ser to a high stoichiometry when quiescent NIH 3T3 cells are treated with PDGF. The same Tyr sites are phosphorylated in vitro by purifed PDGF receptor. We are testing whether these phosphorylations stimulate PLC-II activity and thus account for increased PI turnover in PDGF-treated cells. Cyclin, is a highly conserved cell cycle regulated protein that is required in embryogenesis for entry into mitosis, and which may associate with the cdc2 protein-serine kinase. We have isolated a human cyclin B cDNA, and we are testing whether cyclin B associates with cdc2 and whether this association is regulated by phosphorylation.

To determine how PTKs activated at the cell surface induce nuclear events, we have started to examine the phosphorylation of nuclear regulatory proteins, which could be the target for a surface-initiated PK cascade. $p48^{v\text{-}myb}$, the AMV oncogene product, and its cellular counterpart, $p75^{c\text{-}myb}$, are phosphorylated at 5 clustered Ser near its C-terminus, and among several PKs tested only glycogen synthase kinase 3 (GSK3) can phosphorylate these sites in vitro. The transcription factor AP-1 and Fos, which form a tight complex, are both phosphoproteins and we are studying whether phosphorylation affects the ability of this complex to promote transcription. We have isolated 10 cDNAs for mRNAs whose synthesis is rapidly increased following shift of ts $pp60^{v\text{-}src}$ infected NRK cells. These include sequences shown by others to be mitogen-inducible (c-*fos*, KC, ODC, *Egr*-1) as well as some novel cDNAs.

M 022 MOLECULAR CLONING OF PLANT TRANSCRIPTS HOMOLOGOUS TO EUKARYOTIC PROTEIN KINASES. Michael A. Lawton, Robert T. Yamamoto, Shaohui Zhang, Steven K. Hanks*, and Christopher J. Lamb. Plant Biology Laboratory and *Molecular Biology Laboratory, Salk Institute for Biological Studies, P.O. Box 85800, San Diego, California 92138-9216, USA

Partially degenerate, synthetic oligonucleotides, corresponding to conserved regions of the catalytic domains of animal protein-serine/threonine kinases, have been used to isolate two related but distinct partial cDNAs encoding plant protein kinase homologs in the dicot French bean (*Phaseolus vulgaris* L.) and the monocot rice (*Oryzae sativa*). The carboxyl-terminal regions of the deduced polypeptides encoded by the bean (PVPK-1) and rice (G11A) cDNAs contain sequence motifs characteristic of the catalytic domains of known eukaryotic protein-serine/threonine kinases, strongly suggesting that these plant cDNAs encode protein kinases. The putative catalytic domains are most closely related to the cyclic nucleotide-dependent protein kinase and the protein kinase C families, and this strong conservation suggests that the plant homologs may have a similar function in the cellular transduction of external signals. However, outside the putative catalytic domains, PVPK-1 and G11A exhibit no homology either to each other or to regulatory domains of animal and yeast protein kinases. Hence, these plant cDNAs may encode novel eukaryotic protein kinases. PVPK-1 is homologous to a transcript of 2.5 kb that is present at low levels in suspension cultured bean cells. Southern blot hybridization of genomic DNA indicates that PVPK-1 and G11A correspond to single copy genes that form part of a family of related plant sequences. The existence of a gene family encoding plant protein kinases is supported by the isolation of several related sequences from *Arabidopsis thaliana* genomic DNA.

M 023 THE PARASITIC PLANTS: INSIGHTS INTO THE MECHANISMS FOR THE ACTIVATION OF CELLULAR DIFFERENTIATION,

David G. Lynn, Christopher E. Smith, Gwendolyn D. Fate and Tom Ruttledge, Department of Chemistry, 5735 Ellis Avenue, The University of Chicago, Chicago, IL 60637

While for many years phenolic compounds have been known to possess general allelopathic activity and thereby to exhibit marked effects on plant growth, only recently have such compounds been highlighted as specific signals controlling precise gene expression. The pathogenic bacteria *Agrobacterium tumefaciens* have been found to initiate gene transfer into plant cells in response to phenolic signals, and the symbiotic *Rhizobium spp.* to initiate responses to legume hosts on exposure to related compounds. Our work has shown that higher plant cells respond to similar signal molecules. The nature of these molecular signals makes them able to provide the required spacial and temporal information and to be detected with sufficient resolution for the control of specific cellular developmental and mitotic events. Our efforts have now focused on deciphering the mechanisms for the establishment of the spacial resolution and the mechanisms for cellular activation. Evidence will be presented that is consistent with electron transfer processes being of central importance in cellular activation. Since such widespread responses to phenolic signaling events have now been characterized, it may be that parasitic plants can provide some insights into the evolutionary process and thereby new strategies for plant gene transfer.

Receptors

M 024 CHARACTERIZATION OF HORMONE-RESISTANT MUTANTS OF *ARABIDOPSIS*. James H. Britton, Cynthia Lincoln, F. Bryan Pickett, Jocelyn C.Turner, Allison K. Wilson and Mark A. Estelle. Dept. of Biology, Indiana University, Bloomington IN. 47405

We are interested in the role of the plant hormones IAA and ethylene during plant growth and development. In order to understand the function of these regulatory molecules at the tissue or whole plant level it will be necessary to understand the mode of hormone action as well as the mechanisms which regulate hormone levels throughout the plant. Our approach to these problems has been to identify genes involved in hormone action by isolating mutants of *Arabidopsis* which are resistant to exogenous application of either auxin or ethylene. Because disruption of any of a number of functions may confer resistance (uptake or transport proteins, receptors, signal transduction components), our initial goal has been to use screens for hormone-resistance to identify as many interesting genes as possible. M2 seedlings were screened for resistance to auxin on agar media containing inhibiting concentrations of either IAA, 2,4-D or 1-NAA. Ethylene-resistant mutants were isolated in a similar way using the ethylene precursor ACC as a selective agent. A number of mutants which confer resistance to either auxin or ethylene have now been recovered. Genetic analysis has demonstrated that we have identified at least 3 genes which confer resistance to auxin and at least 2 which result in ethylene resistance. In addition to hormone resistance, mutations at each gene confer a distinctive morphological phenotype. As a prelude to cloning we have positioned two of the auxin-resistant loci (*axr1* and *axr2*) on an RFLP map generated by Chang et al. (1). Both genes appear to lie within approximately 2 map units of the closest RFLP . The results of our genetic studies as well as the characterization of the mutant phenotypes will be presented.

1) Chang et al. Proc. Natl. Acad. Sci. 85:6856-6860

M 025 THE SENSITIVITY OF PLANT CELLS TO AUXINS : MODULATION OF RECEPTORS AT THE PLASMALEMMA. Jean Guern, Hélène Barbier-Brygoo, Geneviève Ephritikhine, Wen-Hui Shen and Christophe Maurel., C.N.R.S.-I.N.R.A., Physiologie Cellulaire Végétale, bat. 15, 91198 Gif-sur-Yvette Cedex, France.

Large variations in the sensitivity of plant cells to auxins can be induced by mutagenesis in tobacco or by *Agrobacterium rhizogenes* transformation in different plants (1). These modifications of sensitivity can be characterized by measuring the auxin-induced modifications of the transmembrane electrical potential difference (ΔEm) of isolated protoplasts. On tobacco mesophyll protoplasts, the response to auxin is blocked by antibodies directed against an auxin-binding protein from maize coleoptile membranes (2) or the plasmamembrane ATPase from yeast (3). The auxin-induced ΔEm thus involves the occupancy of a membrane receptor which in turns activates the proton-pump ATPase at the plasmalemma (4). The fusicoccin-induced ΔEm is not blocked by the antibody raised to the auxin receptor and the sensitivity to fusicoccin is not modified by *A. rhizogenes* transformation. This strongly suggests that auxin sensitivity is modified either at the reception step or at one of the early steps specifically transducing the auxin signal.

Immunotitrations of functional receptors at the external surface of tobacco protoplasts indicated that transformed protoplasts have more receptors than protoplasts from the normal genotype, whereas protoplasts isolated from the auxin-tolerant mutant have less receptors than their wild-type counterpart. Furthermore, preliminary results suggest that manipulating the number of functional receptors at the surface of protoplasts markedly influences their response to auxin, thus showing that the density of receptors at the plasmalemma is an essential factor of plant cell sensitivity to auxin.

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2- Löbler M. and Klämbt D. (1985), J. Biol. Chem., 260, 9848
3- Clément J.D., Ghislain M., Dufour J.P. and Scalla R.(1986), Plant Sci. , 5, 43
4- Barbier-Brygoo H., Ephritikhine G., Klämbt D., Ghislain M. and Guern J. (1988) Proc Natl Acad Sci USA, in press

M 026 PHYTOCHROME MEDIATED SIGNAL TRANSDUCTION: AN IN VITRO APPROACH*, J. Clark Lagarias, Tom R. Berkelman, Tedd D. Elich, Daniel G. Kidd and Robert W. McMichael, Jr., Department of Biochemistry and Biophysics, University of California, Davis CA 95616

A major goal of our research is the development of an in vitro assay for the study of early events in phytochrome signal transduction pathway. Much of our work has involved characterization of the light-dependent changes in the conformation of purified Avena phytochrome. Regions of the phytochrome photoreceptor which undergo light-dependent conformational changes are likely sites for interactions with as yet unidentified cellular component(s) or receptor(s). Progress in the in vitro synthesis and assembly of spectrally active phytochrome holoprotein using a full length Avena cDNA clone will be presented. This approach will enable us to introduce structural changes into the phytochrome polypeptide and ultimately to examine their effect on photoreceptor function. Owing to the complexity of higher plant systems, we have focussed recent attention on the green alga Mesotaenium caldariorum. In this alga, phytochrome mediates chloroplast orientation to optimize photosynthetic light capture. As a unicellular organism, Mesotaenium has obvious advantages to higher plant experimental systems. We will describe our progress in two major areas: 1) purification of Mesotaenium phytochrome and 2) fractionation and characterization of the Ca^{2+} transport systems in this alga. We have chosen to study Ca^{2+} transport in this alga because changes in intracellular Ca^{2+} levels have been strongly implicated in the phytochrome mediated orientation of algal chloroplasts.

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M 027 THE SENSITIVITY OF PLANT CELLS TO AUXINS: HAIRY ROOT T-DNA MODIFY THE HORMONE RESPONSE, J. Brevet, D. Clerot, C. David, E. Davioud, G. Hansen, M. Larribe, C. Maurel, A. Petit, W.H. Shen, J. Tempe, CNRS-INRA Institut de Microbiologie, Batiment 409, Universite de Paris Sud, 91405 Orsay, France

In contrast with crown gall tumors for which the main determinants for cell proliferation are diffusible plant hormones, auxin and cytokinin, hairy root does not require continued production of auxin for proliferation. Yet the phenotype of hairy root transformants is suggestive of a response to high auxin. However, this response is not due to an increase in auxin concentration but to an increase in auxin sensitivity. This increase is correlated with the lack of geotropic response of transformed roots. Different sets of T-DNA genes are necessary for increased auxin sensitivity in different plant species.

M 028 CLONING THE mRNA OF ACC SYNTHASE: THE KEY REGULATORY ENZYME IN THE ETHYLENE BIOSYNTHETIC PATHWAY IN PLANTS, Athanasios Theologis, Takahide Sato, Pung-Ling Huang, William H. Rottmann and Gary P. Peter, Plant Gene Expression Center, ARS, USDA, 800 Buchanan Street, Albany, California 94710 and Department of Molecular Plant Biology, University of California at Berkeley, Berkeley, California 94720.

Ethylene, the simplest ofefin, is the plant hormone that controls plant senescence. A variety of plant hormones and environmental stimuli enhance ethylene production. The enhancement is due to the induction of ACC synthase by a mechanism which is currently poorly understood. As a first step towards understanding the complex regulation of ACC synthase we have isolated a complementary DNA sequence (pACC1) encoded this enzyme in Cucurbita fruits by a novel experimental approach. The cloning strategy is based on immmunoscreening gt11 cDNA libraries with polyclonal antiserum obtained with partially purified enzyme preparations and subsequently purified by affinity column chromatography with total proteins from uninduced tissue. The authenticity of the cDNA clone has been confirmed a) by immunoblotting analysis with antiserum released from the putative gt11 cDNA alone, and most importantly by b) expressing it and recovering ACC synthase activity in E. coli and yeast. RNA hybridization analysis reveals that the pACCI clone hybridizes to an mRNA 1900 nucleotide long which is greatly induced by auxin and Li ions. Cytokinins stabilize the ACC synthase mRNA. Southern analysis shows the ACC synthase gene is not represented by a multigene family in Cucurbita. Isolation of genomic clones confirms this conclusion. Low stringency hybridizations with the Cucurbita cDNA clone has led to the isolation of cDNA and genomic clones to tomato ACC synthase. The authenticity of the clone has also been confirmed by successfully expressing it and recovering enzyme activity in E. coli. Structural analysis of the ACC synthase genes is currently in progress. This work was supported by grants from NSF (DCB-2157), NIH (GM-35447) and USDA (5835-23410-D002) to AT.

M 029 THE FUSICOCCIN-RECEPTOR OF HIGHER PLANTS

Elmar W. Weiler, Martin Feyerabend, Christiane Meyer and Axel Mithöfer, Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D-4630 Bochum, West Germany. The *Fusicoccum amygdali* toxin, fusicoccin (FC), profoundly alters plasma membrane traffic in that it stimulates proton extrusion, hyperpolarizes the membrane potential and thus increases the proton motive force (pmf). FC represents a unique tool to elucidate the regulatory mechanisms underlying many transport processes at the plasma membrane [1]. Higher plants posess high-affinity ($K_a = 0.2 - 0.7 \times 10^9\ M^{-1}$) FC binding sites located presumably at the plasmalemma [2, 3]. These sites are glycoproteins and FC binding is both trypsin and periodate labile. The FC binding domain is exposed to the apoplastic side of the membrane [2]. FC-binding is rapid ($t_{1/2} \approx 6$ min) and optimum at apoplastic pH values (5.5 - 6.0). The specificity of the binding of FC analogues to the FC binding protein (FCBP) is correlated with their biological activities, suggesting a physiological role of the FCBP [2, 3]. Two independent approaches - (*i*) purification, by FPLC, of the FCBP-radioligand complexes and (*ii*) photoaffinity labelling with an azido analogue of FC - have resulted in the identification of a 34-35 kDa toxin-binding polypeptide in *Vicia faba* [4] and *Arabidopsis thaliana* [3]. The solubilized FCBP-radioligand complex of *V. faba* exhibits an apparent molecular weight of 80 ± 20 kDa in gel permeation chromatography [4]. Thus, it is possible that the FCBP occurs as an oligomeric complex in the plasma membrane, part of which is the 34-35kDa toxin-binding polypeptide.

[1] Marré, E. (1979) Ann. Rev. Plant Physiol. 30: 273
[2] Feyerabend, M., Weiler, E. W. (1988) Planta 174: 115
[3] Meyer, C., Weiler, E. W. (1989) Plant Physiol. (in press)
[4] Feyerabend, M., Weiler, E. W. (1988) Planta (in press)

Protein Engineering

M 030 CREATION OF ALTERED OXYGEN-EVOLVING PHOTOSYSTEMS THROUGH PROTEIN ENGINEERING,

Lee McIntosh*#, Idah Sithole*, Bridgette Barry*, Richard Debus*, Mary Kakefuda*, Neal Bowlby, and G.T. Babcock+, *D.O.E. Plant Research Laboratory, #Biochemistry Department, +Chemistry Department, Michigan State University, East Lansing, MI, 48824.

Structural information garnered from bacterial reaction centers that have been resolved at the atomic level (1,2) and reaction center gene sequences from plants and cyanobacteria have been combined with genetic approaches to dissect the assembly and function of Photosystem II. In cyanobacteria such as Synechocystis 6803, it is now possible to alter any characterized "photosynthesis" gene and replace the native gene with a modified form, thus allowing restructuring of the protein architecture for photosynthesis. Recently, we have shown that the intermediate electron acceptors "D" and "Z"--previously thought to have been quinones--are, in fact, tyrosine radicals on the cyanobacterial Photosystem II reaction center subunits D2 and D1, respectively (3,4). These tyrosines appear to reside in similar alpha-helicies on the D1 and D2 reaction center core polypeptides. Interestingly, both are next to prolines that are conserved from photosynthetic bacterial reaction centers. It should be noted that, as opposed to bacterial photosynthesis, oxidizing equivalents generated by photooxidation of P680 in Photosystem II are accumulated in a cluster of four manganese atoms where water is oxidized to molecular oxygen; the molecular link being the tyrosine radical "Z". With our ultimate goal as the understanding of oxygenic photosynthesis, we have made a series of "nested" mutations surrounding these tyrosines. A "model" structural analysis of these mutated regions of the Photosystem II reaction center, as compared with the bacterial reaction centers, will be presented along with a biophysical characterization of the mutants.

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4. Debus, R.J., et al. (1988) Biochemistry 27, 9071-9074.

M 031 ENKEPHALINS PRODUCED IN PLANT SEEDS USING ENGINEERED 2S ALBUMINS, J Vandekerckhove, J Van Damme (1), M Van Lijsebettens, V Gossele (1), J Leemans (1), J Botterman (1), and E Krebbers (1), Laboratory for Genetics, Rijkuniversiteit Gent, Ledeganckstraat 35, B-9000 Gent and (1) Plant Genetic Systems, J. Plateaustraat 22, B-9000 Gent, Belgium.

Modified 2S albumins can be used to produce biologically active peptides in the seeds of plants. 2S albumins constitute up to 60% of total seed protein in dicots. They are synthesized as a prepropeptide which undergoes several proteolytic cleavage steps. The mature form found in the protein bodies consists of 2 subunits of approximately 30 and 75 amino acids interconnected by disulfide bridges. The most abundantly expressed 2S albumin gene of Arabidopsis thaliana was modified by substituting a nucleotide sequence encoding the 5 amino acid peptide Leu-enkephalin flanked by two lysine residues for a sequence of equivalent length in the large subunit. The substitution was carried out in a region displaying high amino acid sequence variability in comparisons of 2S albumins from different species. The spacing of the cysteine residues was unaltered.

The modified gene, with its own promoter, was transformed into Arabidopsis and plants regenerated using published procedures. Protein analysis showed that in some plants the modified protein was expressed at levels similar to that of endogenous 2S albumins. Enkephalin was recovered from the seeds of transgenic plants by high salt extraction followed by either gel filtration or dialysis, digestion with trypsin to separate the peptide from the 2S albumin, treatment with carboxypeptidase B to remove the extra lysine remaining at the carboxyl end, and finally reversed phase HPLC. The identity of the recovered peptide was verified by amino acid analysis and sequence determination. As much as 200 nanomol was recovered from 1 gr of seeds. The simple purification procedures outlined here can easily be scaled up without fundamental modification and may therefore open new perspectives for large scale production of biologically active peptides from plant seeds.

M 032 EFFECT OF LYSINE SUBSTITUTIONS ON ZEIN PROTEIN SYNTHESIS AND PROTEIN BODY FORMATION, John C. Wallace, Takeshi Ohtani, Craig R. Lending, Gad Galili, and Brian A. Larkins, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, and Dept. of Plant Sciences, University of Arizona, Tuscon, AZ 85721.

Zeins, the storage proteins of maize, are almost completely lacking in the essential amino acids lysine and tryptophan. We have introduced lysine and tryptophan codons into a cloned zein cDNA and assayed the resultant effect on the protein's ability to aggregate into protein bodies in a heterologous (amphibian) system. Surprisingly, introduction of several lysines or of lysine- and tryptophan-rich oligopeptides into the zein does not significantly alter its ability to aggregate into dense, membrane-bound vesicles similar to maize protein bodies. The position of the alteration within the molecule makes no difference; only insertion of an unrelated 17 kD peptide near the amino terminus abolished aggregation. The modified zein constructs have also been introduced into transgenic tobacco plants. Since zein promoters do not function efficiently in dicots, a promoter for the bean storage protein phaseolin was utilized to effect the expression of the zein genes. Northern and dotblot analyses showed that the mRNAs for the zeins are expressed at high levels. The zein proteins, however, are present only in very low amounts. Immunocytochemical localization of the normal and modified zeins in tobacco seeds will be presented. Work will also be described on the contributions of the different types of zeins to protein body formation and structure.

Herbicide Resistance

M 033 HERBICIDE RESISTANT PLANTS CARRYING MUTATED ACETOLACTATE SYNTHASE GENES, Barbara J. Mazur, Agricultural Products Department, E. I. du Pont de Nemours & Co., Inc., Experimental Station 402, Wilmington, DE 19880-0402.

Acetolactate synthase (ALS) is the target enzyme for multiple classes of herbicides, including the sulfonylureas, the imidazolinones, and the triazolopyrimidines. We have cloned the genes which specify ALS from a variety of wild type plants. The genes from most plant species are highly conserved; within the species we have studied, the number of ALS genes ranges from one to three. Studies on the expression of the two native tobacco genes have indicated that transcription from one of the two genes always predominates, and that the ratio of the two transcripts from these genes remains constant in all tissue types, despite the variation found in overall expression of the ALS genes in different tissue types. We have also cloned ALS genes from mutant plants which are resistant to sulfonylurea herbicides. The herbicide resistance mutations in these plants have been deduced by comparing the nucleotide sequences of cloned sensitive and resistant ALS genes. Additional genes which confer resistance have been created by in vitro mutagenesis of plant ALS genes, using as guides for the location of these mutations seven different sites identified in mutant yeast ALS genes. The mutated plant genes have been assayed by determining the resistance levels that they confer either in E. coli or in transgenic plants. Single and double mutations have been effective in conferring resistance. When a native tobacco promoter was used with these mutant genes, the level of herbicide resistant enzyme found in transgenic tobacco and tomato plants approached 50% of total activity. Although the tobacco genes also confer resistance in heterologous species, promoter modifications have sometimes been required to obtain agronomically useful levels of resistance. The total ALS activity in these plants appears to be regulated, in that the use of promoters which lead to overexpression of ALS transcripts does not lead to a proportionally large increase in ALS enzyme levels.

Viral Resistance

M 034 *THE MOLECULAR BIOLOGY OF CMV SATELLITE RNA.* David Baulcombe, Martine Jaegle*, Martine Devic*. Sainsbury Laboratory, John Innes Institute, Colney Lane, Norwich, Norfolk, NR4 7UH, UK and *Institute for Plant Science Research, Maris Lane, Cambridge CB2 2LQ, UK.

Satellite RNA of cucumber mosaic virus (CMV) exists in several different forms. Most of these forms have the capability to attenuate the effects of the helper virus, but others, superimposed on the attenuation, cause the induction of completely novel symptoms. The attenuation capability has been used to engineer plants which resist the effects of CMV infection as a result of the expression of satellite RNA sequences by transcription from the nuclear genome. However the exploitation of that effect is complicated by the potential of CMV satellite RNA to induce symptoms. Recently we have analyzed domains within the satellite RNA which are involved in symptom production so that the appropriate regions can be eliminated from the satellite RNA sequence and the potential risk reduced. It is hoped in addition that the information will lead to an understanding of the mechanisms by which satellite RNA of CMV can cause disease. Using the satellite RNA of CMV strain Y we have identified two domains of symptom induction in the satellite RNA. One is located centrally within the sequence and is associated with the presence of a region of sequence which is unique to the Y satellite. This domain affects the formation of a yellow mosaic disease on tobacco.

The second domain is located close to the 3' end of the molecule and affects the induction of a lethal necrotic disease on tomato. The induction of this disease is a positive action by the satellite RNA. Single nucleotide changes within the necrotic domain destroy the ability of the satellite to induce the symptoms.

Experiments combining satellite RNA molecules with different helper strains of CMV indicate that the disease induction involves an interaction between the satellite RNA and a viral component. It is clear however that in this interaction the satellite RNA effect does not involve the production of satellite RNA-encoded peptides.

M 035 COAT PROTEIN PROTECTION IN TRANSGENIC PLANTS: MORE FACTS AND A BETTER UNDERSTANDING OF MECHANISM(S), Roger N. Beachy, James C. Register, David A. Stark, Ali Nejidat, W. Gregg Clark, Department of Biology, Washington University, St. Louis, MO 63130

The expression of chimeric viral coat protein (CP) genes leading to the accumulation of high levels of CP in transgenic plants provides protection against the virus from which the CP gene was obtained and against related viruses. For example, accumulation of the CP of the U_1 (common) strain of TMV provides protection against tomato mosaic virus (-20% a.a. sequence divergence) and tobacco mild green mild mosaic virus (U_2; -31% a.a. sequence divergence) but not against sunn hemp mosaic virus (-58% a.a. sequence divergence). Furthermore, in a transient protoplast assay, we found that greater degrees of CP-protection resulted when polymerized forms of TMV-CP were introduced into protoplasts than when free capsid proteins were introduced prior to infection by TMV. These data support the hypothesis that protection is dependent upon the form of the CP as well as its sequence. This hypothesis is corroborated by experiments with potyviruses where expression of one potyvirus CP gene provides protection against heterologous potyviruses as well as against the homologous potyvirus. To determine the role that specific amino acid sequences play on protein stability, directed deletions and point mutants were made in genes encoding the CP of TMV and tobacco etch virus (a potyvirus). The stability of mutant proteins and effects on CP-protection will be discussed.

M 036 MOLECULAR FUNCTIONS FROM SATELLITE RNA, Wayne L. Gerlach, J Haseloff, R. Perriman and L. Graf, CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

From mutational studies of the self cleavage region of the plus strand of tobacco ringspot virus, together with a consideration of related self cleavage regions of other RNAs, we have deduced design rules for the construction of RNA segments with endoribonuclease activities ("ribozymes"). The structural model consists of a catalytic domain flanked by sequencesa which base pair with the target RNA (Haseloff and Gerlach, 1988, Nature 334:585-591).

Early characterization of RNA enzymes designed according to this model showed that, at temperatures similar to those expected under physiological conditions, the annealing of RNA enzyme and substrate was the rate-limiting step in the cleavage reaction. However, if the extent of complementarity between the the ribozyme and substrate was extended, rates of cleavage at lower temperatures were markedly increased. To capitalize on this effect, a modified *in vitro* mutagenesis technique was used to introduce multiple catalytic domains into a test anti-sense RNA. This offers the dual benefits of an arithmetic increase in rates of cleavage due to the number of catalytic domains, and extended complementarity between enzyme and substrate sequences. Modification of the catalytic domain of a ribozyme can also result in a marked increase in catalytic activity.

Such ribozymes offer the possibility of an increase in the efficacy of the anti-sense approach to gene inactivation and are being tested *in vivo*.

Insect Resistance

M 037 ENGINEERING ENHANCED NATURAL RESISTANCE TO INSECT PESTS - A CASE STUDY, Donald Boulter, Angharad M.R. Gatehouse and Vaughan Hilder, Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, England.
Flowering plants and insects have co-evolved for more than one hundred million years. Insects are both beneficial, as pollinators necessary to complete the sexual reproduction cycle of many flowering plants, and harmful as pests causing losses of about 15% world-wide in crop productivity, in spite of the use of large amounts of chemical protectants. During the course of this close interaction between insects and plants, many mechanisms have arisen which confer on plants field resistance to a range of insect pests. Any one mechanism is not 100% effective and normally several methods occur in a single plant. Our strategy has been to attempt to identify the basis of these natural resistance mechanisms and to exploit them, particularly if they involve the products of one or a few genes. In this research a defensive mechanism based on antimetabolic proteins evolved by one particular plant, is transferred by plant genetic engineering to a crop susceptible to insect attack. In this way plants resistant to insects, and thus requiring much reduced or no insecticide application, can be produced. One example of this approach is the transfer of a trypsin inhibitor gene, CpTI of cowpea. These inhibitors are small proteins belonging to the Bowman-Birk type of double-headed serine protease inhibitors, and are the product of a small gene family. A gene encoding a cowpea trypsin inhibitor which had been shown to give some measure of field resistance to insect pests, was transferred to tobacco by using an Agrobacterium tumefaciens Ti plasmid binary vector. The transgenic plants were shown to have enhanced resistance to the tobacco's own herbivorous insect pests. Several other genes are now becoming available. For example, several lectins have been shown by in vitro bioassay to afford protection against insect pests and transgenic crop plants expressing this gene have been produced.

M 038 PROTEINASE INHIBITOR GENES: THE SEARCH FOR WOUND-INDUCIBLE SIGNALLING MECHANISMS IN NORMAL AND TRANSGENIC PLANTS. Clarence A. Ryan, Gynheung An, Curtis J. Palm, Edward E. Farmer, Thomas Moloshok, Russell Johnson and Gerald Hall, Institute of Biological Chemistry, Program in Biochemistry and Biophysics and Program in Plant Physiology, Washington State University, Pullman, WA. 99164-6340.

The synthesis of two proteinase inhibitors in tomato and potato leaves, called Inhibitor I, (Mr 8100) and Inhibitor II, (Mr 12,300) is systemically regulated by wounding. In excised leaves, the expression of the genes coding for these proteins can be regulated by oligouronides derived from the plant cell walls, suggesting that the carbohydrates are a part of the early signals produced by wounding. Recent results in our laboratory have shown that oligouronides produce enhanced phosphorylation of specific proteins in isolated plasma membranes from potato and tomato leaves, indicating that protein kinase activity may be part of the signalling mechanism for regulating proteinase inhibitor synthesis. Proteins have also been identified from wounded tomato nuclei that bind strongly in gel retardation assays to fragments from the 5' regions of the wound-inducible Inhibitor II gene. In nightshade plants (*S. nigrum*) transformed with a wound-inducible tomato Inhibitor I gene, tomato Inhibitor I protein is constitutively expressed, but is induced to higher levels (2% of the soluble leaf proteins) by wounding. Thus, in nightshade, the signalling mechanism for regulating wound-inducible Inhibitor I expression is in place. (Supported in part by grants from NSF and EniChem Americas, Inc.)

Microbial Resistance

M 039 FUNCTIONAL ANALYSIS OF DNA SEQUENCES RESPONSIBLE FOR ETHYLENE AND ELICITOR REGULATION OF THE DEFENSE RELATED GENE, CHITINASE, Richard Broglie, Karen Broglie, Dominique Roby and John Gaynor*, E.I. du Pont de Nemours & Co. (Inc.), Agricultural Products Department, Wilmington, DE 19880-0402 and *Rutgers University, Department of Biology, Newark, N.J. 07102

Chitinase is a lytic enzyme found in most higher plants; this enzyme catalyzes the hydrolysis of chitin, a ß-1,4-linked homopolymer of N-acetyl-D-glucosamine. The production of chitinase by higher plants is thought to be part of a natural defense mechanism against chitinous pathogens. In bean, this enzyme is encoded by a small multigene family and its synthesis is strongly regulated at the level of gene transcription by a number of inducers, including oligosaccharide elicitors and the phytohormone, ethylene. To define the DNA sequence elements that are involved in mediating this response and to understand better the role of ethylene in controlling the expression of specific defense-related proteins during pathogen attack, we have introduced a 4.7 kb fragment of bean genomic DNA containing the CH5B gene into tobacco, using Ti-plasmid vectors. Northern blot analyses indicate that the expression of the foreign gene in tobacco is dependent upon treatment with exogenous ethylene or fungal elicitor. Expression of a chimeric gene consisting of 1.6 kb of 5' flanking DNA derived from the CH5B gene fused to the coding sequence of ß-glucuronidase, both in transgenic tobacco and in bean protoplasts, indicates that this region of CH5B is sufficient for ethylene and elicitor-regulated expression. Deletion analysis of the promoter region has allowed us to localize the ethylene-responsive element to within a 228 bp region situated upstream of the transcription start site. Preliminary evidence suggests that this same region is involved in mediating the response of this gene to fungal elicitors. DNA sequence comparisons of the 5' flanking regions of two ethylene-regulated chitinase genes revealed two short DNA sequence motifs which are exactly concerved between the two bean genes. Both conserved regions are characterized by a high GC content.

M 040 DEFENSE GENE TRANSCRIPTION FACTORS. Rick Dixon, Maria Harrison, Mike Lawton*, Sue Jenkins*, and Chris Lamb*. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. *Plant Biology Laboratory, Salk Institute for Biological Studies, P. O. Box 85800, San Diego, CA 92138.

Exposure of cultured bean cells to elicitor molecules results in the rapid transcriptional activation of a number of genes encoding (a) enzymes involved in the biosynthesis of antimicrobial, phenylpropanoid-derived phytoalexins and lignin (b) cell wall hydroxyproline-rich glycoproteins and (c) antifungal hydrolases. Functional analysis of the promoter of an elicitor-inducible gene encoding a member of the chalcone synthase (CHS) multigene family has revealed putative cis-acting silencer and activator regions. Regions of homology are found in the promoter of the coordinately induced phenylalanine ammonia-lyase 2 gene. The CHS silencer region contains binding sites for a putative repressor protein(s) as determined by DNase 1 footprinting and gel retardation analysis using nuclear extracts from suspension cultured cells. We will describe characterization of the repressor and its binding site(s) by gel retardation competition, UV-cross-linking, sequence-specific DNA affinity chromatography and oligonucleotide screening of a cDNA expression library. The preliminary characterization of proteins which bind to other areas of the promoter will also be outlined.

These studies suggest potential strategies for manipulating enhanced, regulated expression of co-ordinately induced plant phenylpropanoid genes.

M 041 ELICITOR-BINDING PROTEINS AND SIGNAL TRANSDUCTION IN ELICITOR ACTION, Jürgen Ebel, Institut für Biologie II der Universität, D-7800 Freiburg, Federal Republic of Germany

One inducible defense mechanism of soybean against potential pathogens is the production of phytoalexins. To investigate the mechanisms underlying signal perception and transduction in the activation of the defense response, we are studying both the inoculation of soybean roots with the fungus, Phytophthora megasperma f. sp. glycinea, and the interaction of soybean cell cultures with a fungus-derived ß-glucan elicitor. We have identified binding sites in soybean membranes for the fungal ß-glucan fraction. The characteristics of ligand binding suggest that the sites may play a role in the elicitor-mediated activation of genes associated with phytoalexin synthesis in soybean. Addition of the ß-glucan elicitor to soybean cell cultures, which is known to stimulate phytoalexin accumulation, also results in rapid changes in the phosphate turnover of several phosphoproteins. This indicates that posttranslational protein modification may be involved in elicitor action. Extracellular Ca^{2+} ions enhance phytoalexin accumulation in cell cultures, which further supports the hypothesis that signal transduction in elicitor action includes a membrane-mediated step. (Supported by the Deutsche Forschungsgemeinschaft, SFB 206)

Physical Stresses

M 042 ENZYMES AND GENES OF THE BETAINE PATHWAY IN PLANTS, Andrew D. Hanson, Renaud Brouquisse, Pierre Weigel, Claudia Lerma and Elizabeth A. Weretilnyk, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Betaine accumulation is a precisely controlled response to salinity or water deficit found in some but not all higher plant families, and in many bacteria and cyanobacteria. There is now good evidence that betaine acts as a non-toxic cytoplasmic osmolyte, thereby permitting metabolic function at low solute potential. Betaine is synthesized from choline by a simple two-step oxidation:

$$\underset{\text{Choline}}{(CH_3)_3{}^{+}N\text{-}CH_2\text{-}CH_2OH} \longrightarrow \underset{\text{Betaine aldehyde}}{(CH_3)_3{}^{+}N\text{-}CH_2\text{-}CHO} \longrightarrow \underset{\text{Betaine}}{(CH_3)_3{}^{+}N\text{-}CH_2\text{-}COOH}$$

Because plants have little or no capacity for betaine degradation, the betaine level is probably determined solely by the rate of biosynthesis in relation to growth. We have therefore sought to identify the enzymes and genes for the two steps of betaine synthesis in plants, with a view to future manipulation of the betaine pathway.

Both enzymes are chloroplastic in spinach and other chenopods (1). The enzyme which oxidizes choline to betaine aldehyde is not yet well characterized, but appears to be a stromal monooxygenase which converts choline to the hydrate form of betaine aldehyde by a hydroxylation reaction, resulting in the incorporation of an atom of ^{18}O from $^{18}O_2$ into betaine (2). Reducing power for the reaction comes from reduced ferredoxin, so that the reaction is promoted by light in intact chloroplasts (3) and in vivo (2). The choline oxygenase activity increases three-fold in salt-stressed plants. The enzyme catalyzing oxidation of betaine aldehyde to betaine is a stromal NAD-linked betaine aldehyde dehydrogenase (BADH); the native enzyme is a dimer with subunits encoded by a single nuclear gene (4,5). BADH enzyme and protein levels increase about three-fold after salt stress, and levels of translatable BADH mRNA increase more than three-fold.

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2. Lerma, C. et al. (1988) Plant Physiol (in press)
3. Weigel, P. et al. (1988) Plant Physiol 86:54
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5. Weretilnyk, E.A. & A.D. Hanson (1988) Biochem Genet 26:143

M 043 **DROUGHT STRESS, ABSCISIC ACID AND GENE EXPRESSION.** John E. Mullet, Hugh S. Mason, Felix Guerrero, Jennifer T. Jones, Robert Creelman, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2128.

Plants exposed to water limiting conditions exhibit growth inhibition, stomatal closure, inhibition of photosynthesis, and increased root/shoot ratios. Many of these responses result from low turgor or changes in plant growth regulators. Decreased leaf turgor results in a 50-fold increase in ABA. The increase in ABA requires transcription and cytoplasmic translation suggesting a change in gene expression couples turgor loss and ABA induction (1,2). Analysis of poly(A)RNA populations showed that a small group of RNAs were rapidly induced in wilted leaves (3). These poly(A)RNAs were not induced by ABA or heat shock. Four cDNAs corresponding to genes which are rapidly induced in wilted leaves have been isolated. Run-on transcription assays show that loss of cell turgor causes an increase in transcription of these genes. The function of the turgor-responsive genes is being investigated.

Soybean seedlings exposed to low water potential vermiculite (ψ^M = -0.3 MPa) show inhibition of stem elongation but little change in root growth. ABA accumulates rapidly in the stem and root growing zones (4) even though turgor does not decrease. Several changes in gene expression in the soybean stem were noted. In particular, proteins of 28 and 31 kDa accumulate in hooks of water deficient seedlings. The genes for these proteins were isolated (5) and were found to be homologous to soybean vegetative storage proteins. Analysis of the genes and the implications of this finding will be discussed.

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Metabolic and Developmental Engineering

M 044 STRAGEGIES FOR MODIFYING PLANT LIPID COMPOSITION.

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Modifying the fatty acid composition of both plant membranes and the triacylglycerols of seed oils are attractive goals in plant biotechnology to investigate the significance of membrane structure in plant function or to produce improved vegetable oils for food and manufacturing industries. Conventional and mutation breeding have been used to produce some desirable alterations in the fatty acid composition of several oilseed crops. However, it will only be possible to achieve more sophisticated modifications by using genetic engineering. Unfortunately, as in many other aspects of plant biology, the lack of specific information about the biochemistry and regulation of lipid metabolism makes it difficult to predict how the introduction of one or a few genes might usefully alter lipid biosynthesis. An additional problem arises from the fact that many of the key enzymes of lipid metabolism are membrane-bound, and attempts to solubilize and purify them from plant sources have not been successful.

We have isolated a series of mutants of Arabidopsis with specific defects in fatty acid and lipid metabolism. These lines have been used to help understand the biochemistry and regulation of the pathways involved. More importantly, the development of Arabidopsis as a model for molecular genetics means that these genetically-mapped mutations can be the basis for identifying and cloning the corresponding wild-type genes, either by chromosome walking from RFLP sites, or by transposon tagging. Potentially useful genes from non-plant sources include fatty acid synthesis genes from E. coli and genes encoding animal short chain fatty acid hydrolases. As the number of genes available increases, it is becoming more important to predict which ones hold the most promise for usefully modifying the composition of plant lipids.

M 045 **MOLECULAR ASPECTS OF SELF-INCOMPATIBILITY,** M.A. Anderson, A. Atkinson, A. Bacic, A.E. Clarke, M. Lush, W. Jahnen, S-L. Mau, G. McFadden, Plant Cell Biology Research Centre, School of Botany, The University of Melbourne, Parkville, Victoria 3052, Australia

The interacting partners during fertilization in higher plants are pollen grains and the female pistil. If mating is compatible, pollen produces a tube which grows through the pistil to the embryo sac. In many plant families inbreeding is prevented by rejection of pollen tubes after they grow some distance down the style. Rejection is controlled by the product of the *S*-gene, which has multiple alleles. Our group is investigating several aspects of self-incompatibility.

We have isolated cDNA and genomic clones encoding several alleles. Overall, the sequences are approximately 70% homologous at the nucleic acid level and 60% homologous at the amino acid level. The sequences are punctuated with highly variable regions which encode hydrophilic amino acids which are predicted to be on the surface of the protein. Southern analysis of *N. alata* genomic DNA using the S_2,S_3 and S_6 cDNAs as probes indicates that the gene is restricted to a single locus and is present in low copy number. Characteristic restriction fragment length polymorphisms have been demonstrated for the different *S*-alleles.

The *S*-allele products, *S*-glycoproteins, can be isolated by FPLC and reversed phase HPLC. The *S*-glycoproteins have, in most cases, four conserved glycosylation sites, although the number of N-glycosyl chains may vary. Different *S*-glycoproteins partially inhibit *in vitro* pollen tube growth.

The style also contains products of a number of genes related to defense, for example a $(1\rightarrow3)$-β-glucan hydrolase, chitinase, proteinase inhibitor and hydroxyproline-rich glycoproteins. These products may be related to a requirement for protection of the female sexual tissue from infection.

An S_2-specific antibody has been raised using a synthetic peptide that corresponds to one of the variable hydrophilic regions in the *S*-associated molecules. Using electron-immunocytochemical techniques the antibody has been shown to bind specifically to the intercellular fluid of the transmitting tissue of the S_2 styles, that is, the site of the incompatibility reaction.

Anderson, M.A., Cornish, E.C., Mau, S-L., Williams, E.G., Hoggart, R., Atkinson, A., Bonig, I., Grego, B., Simpson, R., Roche, P.J., Haley, J.D., Penschow, J.D., Niall, H.D., Tregear, G.W., Coghlan, J.P., Crawford, R.J. and Clarke, A.E. (1986) *Nature* **321**: 38-44.

Cornish, E.C., Pettitt, J.M., Bonig, I. and Clarke, A.E. (1987) *Nature* **326**, 99-102.

Cornish, E.C., Anderson, M.A. and Clarke, A.E. (1988) *Annual Review of Cell Biology* **4**: 209-228.

M 046 *CMS AND DISEASE SUSCEPTIBILITY IN THE MAIZE TEXAS CYTOPLASM,* Charles S. Levings, III, Carl J. Braun and James N. Siedow*, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614; *Department of Botany, Duke University, Durham, NC 27706.

Texas cytoplasm (*cms-T*) of maize carries two traits, cytoplasmic male sterility (cms) and disease susceptibility. A mitochondrial gene, designated *urf13* is implicated with the two traits in *cms-T*, because *urf13* mutations abolish cms and disease susceptibility. *urf13* encodes a 13 kDa polypeptide that is localized in the inner mitochondrial membrane. *cms-T* plants are susceptible to the fungal pathogen, *Bipolaris maydis*, race T, which produces a pathotoxin, T-toxin, that inhibits mitochondrial respiration, uncouples oxidative phosphorylation and causes massive ion leakage. We have transformed and expressed the *urf13* gene in *E. coli* to study its activity. *E. coli* cells expressing the URF13 polypeptide are sensitive to T-toxin, while cells not expressing URF13 are insensitive. The effects of toxin on *E. coli* are analogous to those exhibited by mitochondria. These results indicate that the *urf13*-encoded polypeptide is responsible for conferring toxin susceptibility to *cms-T* mitochondria. The effect of toxin on ion uptake has been monitored in *E. coli* using the isotope, [86Rb]. Cells expressing the URF13 polypeptide exhibit massive ion leakage when exposed to toxin, whereas cells not expressing URF13 are unaffected. The toxin-URF13 interaction thus permeabilizes the membrane to cause massive ion leakage and the concomitant loss of membrane potential. Binding studies reveal that toxin binds specifically to URF13 in a cooperative fashion. Amino acid substitutions and deletions have been introduced in URF13 by site-directed mutation of the *urf13* gene. Analyses of these mutants have identified amino acid residues and domains involved in toxin binding and conferring toxin sensitivity. Finally, we will consider the relationship between cms and disease susceptibility and the role URF13 plays.

M 047 ENGINEERING OF A NEW FLOWER COLOR VARIETY OF PETUNIA
Peter Meyer, Felicitas Linn and Heinz Saedler, Max-Planck-Institut für Züchtungsforschung, Egelspfad, 5000 Köln 30, FRG
The A1-gene of Zea mays codes for Dihydroflavanolreductase (DFR), an enzyme involved in anthocyaninbiosynthesis. A cDNA of the A1-gene was inserted between the 35S-promotor from CaMV and its corresponding terminator and was cloned on a plant expression vector. The plasmid was introduced by direct transfer into protoplasts of a petunia mutant, RL01, which accumulates Dihydrokaempferol (DK). While the DFR of petunia does not accept DK as a substrate, the maize DFR shows a broader substrate specificity and converts DK into leucopelargonidin, which can be further processed into pelargonidin-derivatives. Therefore transgenic petunia plants which express the A1-construct show a brick red flower pigmentation representing a new variety of petunia. Among different transformants three types were observed, which either showed no flower pigmentation, pigmentation only in some cells of the flower or coloration on the whole flower. These three types of expression, termed 'white', 'variegated' and 'red', were analysed. We observe a correlation of A1-expression with the number of integrated copies, as 75% of the red plants contain one copy of the A1-construct, while 80% of the variegated transformants and 89% of the white transformants contain two or more A1-copies, which are mostly inserted at different positions in the genome. Integration of multiple copies also correlates in more than 90% of the analysed plants with the methylation of a HpaII-site within the 35S-promotor. These data could be explained in two different ways: 1) If the A1-gene integrates seperately from the selectable marker gene, most integration loci will be silent and the gene is not expressed, while coupling to the selectable marker gene preferentially leads to an expression of the A1-gene. 2) Integration of multiple A1-copies stimulates methylation mechanisms or leads to a depletion of binding factors necessary for expression. At present both theories are under investigation.

M 048 GENES FOR BIOSYNTHESIS OF STILBENE-TYPE PHYTOALEXINS,
Joachim Schröder, Thomas Lanz and Gudrun Schröder, Institut für Biologie II, Universität Freiburg, Schänzlestr. 1, D-7800 Freiburg, FRG.
Stilbenes are secondary plant products which occur constitutively in the wood of trees; in other cases they are induced by stress. They may be considered as phytoalexins since they belong to the general defense mechanisms against pathogen attack. Biosynthetic key enzymes are the synthases which synthesize the stilbene backbone. The best known is resveratrol synthase (RS) which synthesizes this stilbene from three malonyl-CoA and 4-coumaroyl-CoA. These substrates are also used by chalcone synthase (CHS) which forms naringenin chalcone in the pathway for flavonoid biosynthesis. cDNAs and genomic sequences for RS from peanut (Arachis hypogaea) (1) show that RS and CHS are related. CHS sequences have been reported for at least nine different plants, including two monocots. The proteins are highly conserved, and a consensus sequence was established (2). A comparison shows that all RS share 85% of the CHS consensus sequence and that they are different in 15% . It appears that the two proteins share a common scaffold necessary for binding of the substrates and the type of enzyme reaction, and that the differences are responsible for formation of the different products.
Peanuts contain several RS genes, and gene-specific oligonucleotides were used to investigate expression in cell cultures and seedlings under various stress conditions. Induction is gene- and stress-specific, and one the genes was not induced under any of the conditions tested.
RS genes appear to be absent in most crop plants, and therefore it is of interest whether transferred RS genes confer increased resistance against pathogen attack in other plants. These experiments are in progress in collaboration with R. Hain and P. Schreier (Bayer AG).

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Transformation, Vectors and Targeting

M 100 GENE TRANSFER TO NEW ZEALAND HORTICULTURAL CROPS, Ross G. Atkinson[1,2] and Richard C. Gardner[1,2] [1]Division of Horticulture and Processing, DSIR, Auckland, New Zealand, [2]Dept. of Cellular and Molecular Biology, University of Auckland, New Zealand

We have initiated a programme for the application of gene transfer techniques to New Zealand grown cultivars of apple and three subtropical fruits: pepino (*Solanum muricatum*), tamarillo (*Cyphomandra betacea*) and feijoa (*Feijoa sellowiana*). Initial research has concentrated on *Agrobacterium*-mediated gene transfer. Some *Agrobacterium* strains have been shown to cause galls on cultivars of pepino, tamarillo and apple. Tissue culture systems for the efficient regeneration of plantlets from tissue explants are being developed for these species. Expression of the GUS reporter gene has been demonstrated after co-cultivation of leaf discs of pepino and tamarillo. After 1-2 weeks, callus growing on kanamycin media has also been obtained for these two species. Initial goals are to transform tamarillo with DNA coding for the coat protein of tamarillo mosaic virus, to confer resitance to this serious disease and to transfer the Bt toxin gene into apple to confer resistance to leafroller pests.

M 101 TRANSFORMATION OF GUAYULE USING AGROBACTERIUM-MEDIATED DNA TRANSFER, Ralph A. Backhaus and Jingkun Ho, Department of Botany, Arizona State University, Tempe, AZ 85287

Guayule (Parthenium argentatum) is an industrial, rubber-producing crop which is being developed for arid regions. In vitro-derived shoot explants of a high yielding line (11591) where inoculated with Agrobacterium tumefaciens LBA 4404 harboring pBI121 (a binary vector conferring kanamycin resistance and beta-glucoronidase "GUS" activity) and cultured on shoot induction medium containing kanamycin. Shoots obtained from kanamycin-selected tissues showed variations in expression for GUS activity compared to non-transformed shoots, which had no GUS activity. Rooted, transgenic guayule plants are now being attempted. A system for transforming guayule could have significant impact on crop improvement leading to commercial development.

M 102 TRANSGENIC RICE PLANTS FROM PROTOPLASTS AFTER ELECTROPORATION MEDIATED DNA UPTAKE: CO-TRANSFORMATION WITH SELECTABLE AND NON-SELECTABLE GENES, Michael J. Battraw and Timothy C. Hall, Department of Biology, Texas A&M University, College Station, TX 77843-3258.

A method for transferring non-selectable genes into rice is presented, which utilizes co-transformation with a selectable marker gene. A plasmid containing a selectable chimeric gene (CaMV 35S promoter-neomycin phosphotransferase II coding region-nopaline synthase 3' non-coding region) and a non-selectable gene (CaMV 35S promoter-ß-glucuronidase coding region-nopaline synthase 3' non-coding region) was used to transform rice protoplasts. Protoplasts were isolated from embryogenic suspension cultures, incubated with the plasmid DNA and electroporated. Nurse culture treatment of the electroporated protoplasts produced the highest frequency of callus formation. When the antibiotic kanamycin was included in the culture medium, resistant calli developed from protoplasts electroporated with the plasmid DNA; these calli also express npt-II activity. Control calli from protoplasts electroporated without DNA and cultured on medium without kanamycin do not express npt-II activity. Some of the npt-II positive calli also express GUS activity, demonstrating expression of a non-selectable gene in addition to a selectable marker gene. Kanamycin-resistant calli transferred to regeneration medium produced green plants expressing npt-II activity. The GUS gene was chosen for the non-selectable gene in these experiments because its expression is easily detected; presumably other non-selectable genes may be used in place of the GUS gene.

M 103 PROTEIN TARGETING TO PLANT MITOCHONDRIA, Marc Boutry and François Chaumont, Unité de Biochimie Physiologique,University of Louvain, B-1348 Louvain-la-Neuve, Belgium.
The beta subunit of the mitochondrial ATPase from Nicotiana plumbaginifolia is synthesized as a precursor with an N-terminal presequence involved in mitochondrial targeting. In vitro import experiments indicated that the precursor (Mr=59,000) was taken up by isolated mitochondria in an energy-dependent process to give a mature product (Mr=51,000) corresponding to the beta subunit. We previously showed that the beta presequence was able to target in vivo the bacterial chloramphenicol acetyl transferase (CAT) to plant mitochondria in a specific way (Boutry et al. (1987) Nature 328, 340-342). To define the amino acid residues involved both in the specific addressing and in the presequence cleavage, we prepared a series of deletions in the presequence, fused them to cat and introduced the chimeric genes into plant. Analysis of transgenic plants is under way. In addition we have cloned the second N. plumbaginifolia gene (atp2-2) encoding the ATPase beta subunit in order to reveal conserved residues in the presequence.

M 104 CORRELATION BETWEEN GENE COPY NUMBER AND EXPRESSION OF CHLORAMPHENICOL ACETYL TRANSFERASE IN TRANSGENIC TOBACCO, Ben A. Bowen[1], Elie H. Gendloff[2] and Wally G. Buchholz[2]; 1)Pioneer Hi-Bred International, Inc., 7300 N.W. 62nd Avenue, Johnston, Iowa 50131-38; 2)Botany Department, University of Wisconsin, Madison, WI 53704.
Differences in the level of gene expression between transgenic plants have often been ascribed to position effects, because no clear correlation with gene copy number has been observed. We developed a quantitative ELISA for chloramphenicol acetyl transferase (CAT) to assay the levels of this protein in 18 independently transformed tobacco plants containing the CAT gene fused to the CaMV 35S promoter. We also determined the CAT gene copy number and the number of independently segregating loci in each transformant. We found that out of ten plants with a single CAT gene, only one expressed a significantly different level of CAT from the others. We also found that there was a significant correlation between CAT gene copy number and the level of CAT expressed in each plant. In this population, therefore, copy number contributed more to the variation in CAT gene expression than did position effects. We have also confirmed by progeny testing that our assay is sufficiently precise to distinguish F_1 plants homozygous for a single CAT locus from those which are hemizygous. Using this assay, it may also be possible to select seedling plants which are homozygous for more than one CAT locus.

M 105 TRANSFORMATION OF QUINOA (CHENOPODIUM QUINOA) WITH A SELECTABLE MARKER GENE USING AGROBACTERIUM TUMEFACIENS. Chris Brinegar, James M. Gilmore and Margaret Ryan, Department of Biological Sciences, San Jose State University, San Jose, CA 95192.
Quinoa (Chenopodium quinoa) is a dicotyledonous grain plant indigenous to the Andes Mountains of South America. Having been a major crop of the Incas for hundreds of years, quinoa is still a staple food in many areas of Chile, Bolivia and Peru. The seed protein quality is excellent (nearly equivalent to the FAO standard), the leaves are edible, the dry stalks can be used for fuel or fodder, and the saponins coating the seeds contain pharmacologically active triterpenes. The major drawback to the introduction of quinoa into American agriculture is its susceptibility to water stress (high transpiration rate and shallow root system) and heat stress (pollen inviability). Our long term goal is to improve the ability of quinoa, via genetic manipulation, to better cope with these stresses. Our short term goal is to demonstrate the feasibility of gene transfer into quinoa. Preliminary experiments indicate that explants of hypocotyls (but not cotyledons) of six-day-old quinoa seedlings infected with Agrobacterium tumefaciens (containing the gene for neomycin phosphotransferase II) can form callus on kanamycin-containing media with a 10-12% efficiency. Current efforts are aimed at improving the efficiency of transformation and at developing a regeneration protocol.

M 106 **HIGHLY CONSERVED RESIDUES IN LHC II: ROLES IN TARGETING TO CHLOROPLASTS, PROCESSING AND PHOSPHORYLATION.** Wilson E. Buvinger[1], Hanspeter Michel[1], Donald F. Hunt[2], and John Bennett[1]. (1) Biology Department, Brookhaven National Laboratory, Upton, NY 11973. (2) Department of Chemistry, University of Virginia, Charlottesville, VA 22903. We have utilized synthetic peptides to reveal the functions of highly conserved regions of amino acid residues at the N-termini of mature light harvesting chlorophyll a/b proteins (LHC II) and in the transit peptides of their precursors. We found that an analog of the first 20 residues of an Arabidopsis thaliana transit peptide inhibited in vitro import of precursor proteins into pea chloroplasts. Synthetic analogs of the N-termini of mature LHC II were used to reveal those residues which are specifically phosphorylatable by the thylakoid-bound LHC II kinase of pea and spinach. Current research is aimed at elucidating which conserved amino acid residues are recognized in the final cleavage step that creates the mature LHC II apo-proteins and that delineates the N-termini of the mature proteins. At the juncture of the transit peptide and the mature N-terminus is a highly conserved motif [GR**MR(K/R)(S/T/A)(A/V/I)(T/G/A)K]. Site-directed mutagenesis of a cloned LHC II protein (type II) from petunia was used to generate 10 amino acid changes in this region in order to study their effects on in vitro import into chloroplasts and specifically on processing. In another current investigation, microsequencing analysis of phosphorylated N-terminal peptides isolated from spinach LHC II reveals that the cleavage step appears to occur between the fifth and sixth residues (M-R) of the motif and is followed by acetylation of the arginyl residue. Along with results from the present experiments, we will present a map of residues as related to their roles in import, final cleavage, and phosphorylation.

M 107 ELECTROPORATION OF POTATO (SOLANUM TUBEROSUM L. CV. RUSSET BURBANK), Ming-Mei Chang and Wayne H. Loescher, Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414. Procedures were established for electroporation of potato (Solanum tuberosum L. cv. Russet Burbank) protoplasts. The plasmid, pCaMVCAT, was introduced into potato via electroporation. Two electroporation systems generating either square wave (Progenitor I) or exponentially decaying pulses, were used in this study. In the square wave system, when a single pulse was applied at 100 µs, increasing voltage increased and then decreased CAT activity. Increasing pulse number, however, did not increase CAT activity. Highest CAT activity was obtained at 300 volts with a 100 µs pulse. In the exponentially decaying system, no significant differences in protoplast survival were observed when protoplasts were electroporated in media containing different salt concentrations, but higher CAT expression was generally observed at 150 mM NaCl. Actively dividing protoplasts gave higher CAT activity regardless of salt concentration. CAT activity was correlated with the potential for cell division in the protoplasts. Higher CAT activities were observed in those protoplast preparations actively dividing 42 hrs after electroporation. Of the two electroporation systems tested, although a square wave pulse often resulted in higher CAT activity, exponentially decaying pulses gave more reproducible results.

M 108 HOMOLOGOUS RECOMBINATION IN *ARABIDOPSIS THALIANA*, Abdul M. Chaudhury and Ethan R. Signer. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Understanding the mechanism of homologous genetic recombination in higher plants will not only illuminate an important biological process but will also provide ways to make targeted changes in the plant genome. As a first step of studying homologous recombination in higher plants we have introduced into *Arabidopsis thaliana* a construct designed to create a one step deletion of the *ADH* gene. Homologous events are being detected by the polymerase chain reaction as well as phenotypic selection. Strategies to detect only homologous and not nonhomologous events are also being developed. Results and implications of these experiments will be presented.

M 109 *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF *CITRUS*, Gloria A. Moore, Maria G. DeWald, and Kenneth Cline, Fruit Crops Department, IFAS, University of Florida, Gainesville, FL 32611
The immediate goals of this project are to transform *Citrus* cells with an antibiotic resistance gene using the *Agrobacterium tumefaciens* vector system. This will provide a selectable marker that can be used in citrus breeding to distinguish hybrids from nucellar seedlings, something that cannot be easily done using conventional methods. Internodal stem segments are incubated with vector bacteria harboring a T-DNA borne NPT-II gene. Shoots are subsequently regenerated in the presence of kanamycin, established in soil, and analyzed for the presence of T-DNA by Southern analysis. One established plant has been verified as transformed by this technique. We are currently testing the efficiency of different *A. tumefaciens* vectors for citrus transformation using T-DNA that also contains a gene for β-glucuronidase (GUS), a scorable marker. With certain vectors, up to 40% of regenerated shoots were positive for GUS activity. Regenerated shoots are presently being established in soil. The results of Southern analysis of these plants will be presented.

M 110 AGROBACTERIUM-MEDIATED TRANSFORMATION OF VITIS VINIFERA LEAVES AND REGENERATION OF TRANSFORMANTS EXPRESSING B-GLUCURONIDASE. Sheila M. Colby, James A. Stamp, and Carole P. Meredith, Department of Viticulture and Enology, University of California, Davis, California 95616
We are developing an Agrobacterium-mediated genetic transformation system for grapevine (Vitis vinifera). The long generation time, large plant size, and genetic heterozygosity of grape limit conventional approaches to genetic improvement and so make transformation an attractive approach for the directed improvement of existing grape cultivars. Leaf explants from in vitro-grown plants were co-cultivated with a disarmed strain of A. tumefaciens containing a plasmid carrying both the neomycin phosphotransferase II selectable marker gene for kanamycin resistance and a scorable marker gene encoding ß-glucuronidase (GUS). Adventitious buds and shoots were regenerated on levels of kanamycin which prevented regeneration in control explants not treated with Agrobacterium. Histochemical analysis of GUS expression in adventitious buds after six weeks revealed transformed buds. Additional evidence for transformation, including Southern analysis and further histochemical analysis of GUS expression in kanamycin-resistant shoots, will be presented.

M 111 TARGETING CELLS OF POPULUS DELTOIDES EXPLANTS FOR TRANSFORMATION BY AGROBACTERIUM TUMEFACIENS, Gary D. Coleman and Stephen G. Ernst, Department of Forestry, Fisheries & Wildlife, University of Nebraska, Lincoln, NE 68583.
To maximize the number of transformed shoots produced per explant of Populus deltoides, and percent explants producing adventitious shoots, experiments have been directed towards: (1) manipulating the competence state of the explant to maximize the number of adventitious shoots produced, (2) determining which cell types and tissues in the explant give rise to the adventitious shoots, and (3) determining the optimal cocultivation and competence induction periods to maximize transformation of those cells. Competence "windows", the amount of time an explant must be on callus induction medium before transfer to shoot induction medium, are genotype specific in P. deltoides, and have been defined for 14 genotypes of P. deltoides. Histological analysis shows that the wedge of cells which gives rise to the adventitious shoots on internodal stem explants are derived from cambial/phloem tissues. Using an Agrobacterium oncogenic binary system (A281:pTiBo542, having introduced pBI121, which has GUS under control of the CaMV 35S promoter), results are presented showing how histochemical staining for GUS activity can be used to identify the cocultivation and competence treatment combinations which best target those cells that give rise to the transformed adventitious shoots.

M 112 CULTURE CONDITIONS INFLUENCING PLANT REGENERATION FROM MAIZE PROTOPLASTS. V.C. Courreges, R.J. Daines, T.R. Adams, M.L. Mangano, R.W. Krueger, A.P.Kausch and C.J. Mackey. Pfizer Central Research, Plant Genetics Department, Eastern Point Road, Groton, Ct 06340.

Protoplasts were isolated from suspension cultures derived from Type II embryogenic callus of several F_1 hybrids of *Zea mays*. Culture parameters specifically influencing the recovery of embryogenic callus from protoplasts were optimized by comparing plating efficiencies from media and feeder culture variations. Similarly, a variety of media progressions have been evaluated for efficiency of plant regeneration from protoplast-derived somatic embryos. The optimized protoplast regeneration protocol has been used to investigate selection of cells transformed with recombinant DNA via electroporation.

M 113 MORPHOGENESIS AND SECONDARY METABOLISM IN TAGETES TISSUES TRANSFORMED BY ONCOGENES OF AGROBACTERIUM, Anton F. Croes and George J. Wullems, Department of Experimental Botany, Nijmegen University, 6525 ED Nijmegen, NETHERLANDS

Roots of *Tagetes* plants accumulate thiophenes, heterocyclic secondary metabolites with biocidal activity. Neither cell suspensions nor calli produce thiophenes. We are studying the relation between differentiation and secondary metabolite production using *Agrobacterium* strains. Infection of hypocotyls with wild-type *A. tumefaciens* or with shooter strains mutated in one of the tms loci, led to the formation of callus with a low thiophene level. However, when a mutant defective in the tmr locus was used as the pathogen, stable root cultures were established which contained 6 times more thiophene than roots on the plant. Highly productive roots were also obtained through transformation with *A. rhizogenes*. Mannopine strains which possess the rol loci as the only oncogenes, induce formation of roots with a low degree of branching. Agropine strains which have additional aux genes on the T-DNA, induce roots with large numbers of laterals. An inverse relationship was found between the extent of branching and thiophene content. Potentials for further manipulation of the *Tagetes* system are offered by the observation that *A. rhizogenes* is an effective vehicle for the introduction of foreign genes into *Tagetes*. A nos gene located on a Bin 19 vector in *A. rhizogenes* was transferred to 50% of the individual hairy roots obtained.

M 114 STABLE TRANSFORMATION OF ARABIDOPSIS THALIANA USING DIRECT GENE TRANSFER TO PROTOPLASTS, Brigitte Damm and Lothar Willmitzer, Institut für Genbiologische Forschung Berlin GmbH, 1000 Berlin 33, Ihnestraße 63, Federal Republic of Germany

Arabidopsis thaliana has gained increasing attention as a model system for plant molecular biologists because of its small genome size, its short generation time, the presence of an extensive genetic map and the availability of many mutants. Transformation via Agrobacterium tumefaciens is a well established method in this species however direct DNA transformation has not yet been achieved due to the fact that so far no protoplast-plant regeneration system was available. We have recently established a high efficiency protoplast-plant regeneration system (B. Damm and L. Willmitzer, MGG 213, 15-20). Presently we are using this protocol to set up conditions for direct gene transfer to Arabidopsis protoplasts.

Freshly isolated mesophyll protoplasts were incubated with polyethylene glycol (PEG) and plasmid DNA containing the coding region for the hygromycin phosphotransferase gene (hpt) under the control of the Cauliflower Mosaic Virus 35S promoter. Hygromycin resistant calli and plants have been regenerated. Transformation of regenerated plants was confirmed by Southern blot analysis showing that the foreign gene is integrated with a different copy number into the Arabidopsis genome. Genetic analysis of the hygromycin resistant plants show that the hpt-gene is transmitted to the progeny. Currently factors affecting the efficiency of transformation are studied.

M 115 CHLOROPLAST GENE TRANSFER: EXPRESSION OF AUTONOMOUSLY REPLICATING CHLOROPLAST VECTORS IN TOBACCO CELLS DELIVERED BY GENE GUN,
H. Daniell[1], J.C. Sanford [2], K.K. Tewari[3], and L. Bogorad[4] [1] University of Idaho, Moscow, ID 83843, [2] Cornell University, Geneva, NY 14456; [3]University of California, Irvine CA 92717 and Harvard University, Cambridge MA 02138.

Expression of chloramphenicol acetyltransferase (cat) by autonomously replicating vectors in chloroplasts of cultured tobacco cells, delivered by high velocity microprojectiles, is reported here. For construction of chloroplast expression vectors, restriction fragments of chloroplast DNA containing the entire promoter region and 5'-untranslated region of the psbA gene from spinach, pea or rbcL and atpB promoter region from maize have been individually inserted into the multiple cloning site 5'-proximal to a promoterless cat gene. Several pea chloroplast DNA fragments containing replication origins, identified as displacement loops (D-loops), have been tested for in vitro DNA synthesis using a replication fraction containing RNA polymerase, DNA polymerase, DNA primase and topoisomerase I activities, isolated from pea chloroplasts. A fully characterized chloroplast replicon was finally inserted into chloroplast expression vector pHD312, which contains pea psbA promoter 5'-proximal to the cat gene. Cultured NT1 tobacco cells collected on filter papers were bombarded with tungsten particles coated with pUC9 (negative control), 35S-cat (nuclear expression vector) and pHD407 (chloroplast expression vector). Sonic extracts of cells bombarded with pUC19 showed no detectable cat activity in the autoradiograms. While nuclear expression of cat was maximal 72 hr after bombardment, those bombarded with chloroplast replicon construction showed low level of expression until 48 hr of incubation. As nuclear cat expression decreased, chloroplast cat expression was maintained at a high level up to the last time point tested.

M 116 EXPRESSION OF ß-GLUCURONIDASE ACTIVITY IN TRANSFORMED MAIZE CALLUS, Nancy Dwan and Ronald Lundquist, Molecular Genetics, Inc., Minnetonka, MN 55343.

The cauliflower mosaic virus 35S promoter is widely used to drive expression of genes introduced into plants. Recent reports indicate that this promoter may not be transcriptionally active in all cell types or at all stages of the cell cycle (Nagata, et al., MGG 207:242-244, 1987: Jefferson, et al., EMBO J. 6:3901-3907, 1987). We have electroporated maize Black Mexican Sweet (BMS) suspension culture cells with pBI221, a plasmid carrying a chimeric gene consisting of the CaMV35S promoter and E. coli ß-glucuronidase (GUS), and pCHN1-1, which carries a hygromycin resistance selectable marker. The BMS culture consists of undifferentiated nonregenerable cells. After selection on hygromycin containing media, resistant calli were screened for GUS expression using 5-bromo-4-bromo-3-indolyl ß-D-glucuronide. Hygromycin resistant lines have retained the unselected GUS marker after more than 7 months of culture. However, all such lines have exhibited a non-uniform distribution of GUS activity, which does not appear to be due to chimeric cell populations. These observations are consistent with the possibility that the CaMV 35S promoter is not always active in BMS cells. Experiments to determine the basis for the non-uniform expression of GUS activity are in progress.

M 117 THE INTRODUCTION OF THE MAIZE AC - DS ELEMENT FAMILY INTO ARABIDOPSIS THALIANA : TOWARDS THE ESTABLISHMENT AND APPLICATION OF A TRANSPOSON TAGGING SYSTEM. **David J. Earp, Mary Honma and Barbara Baker,** Plant GeneExpression Center, 800, Buchanan Street, Albany, California 94710. Fax. 415-559-5777

In parallel with the characterisation of the maize *Activator* and *Dissociation* transposable elements in transgenic tobacco and tomato plants in our laboratory we are currently constructing vectors based on these elements with features specifically designed to facilitate gene tagging. The well documented characteristics of *Arabidopsis thaliana* as a model plant system (small genome size, paucity of highly repetitive DNA, short generation time etc..) make this organism an attractive candidate in which to establish and apply a gene tagging system.An initial and necessary step will be to characterize the extent and nature of the transposition of the elements in this plant and to assess the viability of the system for tagging genes of interest via insertional inactivation and cloning out of flanking regions (i.e. the inactivated genes). Towards the latter goals we are currently investigating systems for rapid and easy cloning of inserted elements. If the system proves to be a succesful approach to gene isolation, many classes of genes should be amenable to this approach. Initial experiments will involve the cloning and mapping of stable, marked Ds elements which may provide starting lines suitable for the tagging of closely located genes.

M 118 INJECTION OF ENOGENOUS DNA INTO YOUNG FLORAL TILLERS OF WHEAT, Y.L Fan, J. Du, B.H Liu*, D.X Xie, X.J Hua, Laboratory of Molecular Biology, Biotechnology Research Center, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
The archesporial cells of wheat (Triticum aestivum) before the first meiotic metaphase, were used for purified exogenous DNA injection in order to know if archesporiae cells of wheat are a competent stage for genetic transformation. Total six hundreds plants at this stage were injected with plasmid DNA consisting of vector pGA492, soybean 11S seed storage protein gene and nos-nptII gene by tubeline syringe. Concentration of plasmid DNA to be injected was 200ug/ml, Injected volume per each plant was 100ul. After injection the floral tillers were allowed to grow to maturity. Ten thousands seeds were obtained from treated plants The sterilized seeds were germinated at 30°C in an aqueous solution containing 150ug/ml Kanamycin for 48hrs, then grown on sterilized sand watering by Kanamycin-containing solutions.
From six thousands seeds, twenty seedling remained green, others were abino, as well as control seedling. DNA isolated from green plants leavies were hybridized with 11S legumin gene by dot blotting and shown that.seven DNAs positively hybridized with probe. Southern blotting experiments are under way.
Evaluation of the injecting method for cereal transformation developed by Dr. Lörz group will be discussed.

* Crop Breeding Institute, C.A.A.S.

M 119 **SHOTGUN CLONING BY DIRECT GENE TRANSFER: OPTIMIZATION OF DNA TRANSFER CONDITIONS.**
P. Gallois, K. Lindsey, M.G.K. Jones, M. Kreis.
AFRC Institute of Arable Crops Research, Dept. of Biochemistry, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, U.K.

The application of shotgun cloning by direct gene transfer for the isolation of plant genes is limited by a) the proportion of the protoplast population which i) takes up and ii) stably integrates foreign DNA and b) the amount of DNA integrated per transformed protoplast . In order to reduce the number of transformants required to recover a gene of interest by shotgun cloning, we are 1) optimizing the electroporation conditions for maximizing DNA uptake and 2) optimizing post-electroporation conditions to maximize plating efficiency in protoplasts of tobacco leaf tissue and of sugar beet suspension culture cells. DNA uptake conditions were optimized with respect to field strength, conductivity (salt concentration in the electroporation medium) and capacitance. Highest transient expression was obtained for tobacco with 225 V/cm, 80mM KCl, 100μF and for sugar beet with 280-300V/cm , 80 mM KCl, 200μF. Optimum culture conditions gave a plating efficiency of 60-70% for tobacco and 40-60% for sugar beet. A NPT-II gene was introduced by electroporation followed by culture and selection on kanamycin. Absolute transformation frequencies of approximately 10^{-3} for tobacco and 5. 10^{-5} for sugar beet were obtained. A comparison of the pattern and the copy number of plasmid DNA integrated into tobacco and sugar beet transformants under different electroporation conditions will be discussed in relation to shotgun cloning.

M 120 PROTOPLAST CULTURE, PLANT REGENERATION IN PHASEOUS ANGULARIS,WIGHT AND GENE TRANSFER BY ELECTROPORATION, K. L. Ge, Y. Z. Wang, X. M. Yuan, P. M. Huang, J. S. Yang and Z. P. Nie, Institute of Genetics, Fudan University, Post Box 200433, Shanghai, China; D. Testa and N. Lee, Interferon Sciences Inc. New Brunswick, N. J., 08901, USA. Mesophyll protoplast were isolated from leaves taken from 11-16 days old seedlings grown up from seeds in MS medium. Enzyme mixture was composed of 0.7% celluluse 0.7% hemicellulase in a plasmalying solution of CPW-13M, PH5.8 in which protoplasts could be released. The media used for plant regeneration were MS with different concentration of auxin or/and cytokinin. Callus from mesophyll protoplast still developed into yellow embryo-like structure and increased obviously in size on transfer from amplicating medium 1-S-2 to some of redifferentiating medium. After 96 days on subsequent transfer of calluses to the same redifferentiating medium, green spot appeared and a total of 13 from them continuously developed into intact plantlets in 2 kinds of medium only, R102 and R108. On the other hand, using a electroporation technique, the bacterial chloramphenicol acetyltransferase (CAT), gene via plasmid $_{p}$NOSCAT DNA was introduced into freshly isolated mesophyll protoplast. After one month, calli were formed from the pulse-treated protoplasts. When the calli were put onto some suitable selected media containing Gmp 100 ug/l, some calli grew slowly and showed obvious resistance to Gmp. The effect of various voltage, pulse length and other factors to the protoplasts' viability has been measured.

M 121 TRANSIENT EXPRESSION OF MICROPROJECTILE-INTRODUCED DNA IN DOUGLAS-FIR, Barry Goldfarb, Steven H. Strauss, Libby M. Bailey, and Joe B. Zaerr, Department of Forest Science, Oregon State University, Corvallis, OR 97331, and Dennis E. McCabe, Agracetus, Middleton, WI 53562.

Gold particles coated with a plasmid containing the beta-glucuronidase (GUS) gene were introduced into cultured Douglas-fir (Pseudotsuga menziesii) cotyledons by particle acceleration. Transient expression of the GUS enzyme was monitored histochemically 1-2 days after particle introduction.

Several parameters were investigated for their effect on the level of transient expression, as measured by the number of discrete areas (spots) of GUS activity. Individual seedlings varied considerably, ranging from 1.6 to 6.6 spots/cotyledon. The discharge voltage affected the average penetration depth of particles but had no significant effect on the total number of spots/cotyledon. Multiple blasts increased expression substantially over single blasts. Current work is focusing on obtaining stable expression and selecting transgenic tissues and plantlets.

M 122 STABLE TRANSFORMATION OF EMBRYOGENIC MAIZE CULTURES BY MICROPROJECTILE BOMBARDMENT. W.J. Gordon-Kamm, T.R. Adams, W.R. Adams, S.A. Chambers, V.C. Courreges, R.J. Daines, M.L. Mangano, J.V. O'Brien, T.M. Spencer, W.G. Start, N.G. Willetts, A.P. Kausch, R.W. Krueger, P.G. Lemaux and C.J. Mackey. Plant Genetics Research, DeKalb-Pfizer Genetics. Pfizer Central Research, Groton, CT 06340

Stable genetic transformants of embryogenic maize cultures were obtained via microprojectile bombardment. A188xB84 Type II callus was used to initiate embryogenic suspension cultures, which were bombarded with a mixture of two plasmids, one encoding neomycin phosphotransferase, APH(3')-II, and the other encoding beta-glucuronidase (GUS). A histochemical assay for transient GUS activity was used to assess the number of cells expressing exogenous DNA. Plates of bombarded suspension containing relatively high numbers of GUS-expressing cells were placed under selection with the aminoglycoside G418. Callus was recovered that stably expresses the neo gene and efforts are underway to regenerate this tissue.

M 123 POLLEN GAMMA-IRRADIATION AND GENE TRANSFER IN PLANTS. Dmitry M. Grodzinsky and Sergey V.Andreichenko,Department of Radiobiology, Institute of Botany, Kiev,U.S.S.R.

We have managed to inject foreign chromatin fragments into embryo sac of Petunia hybrida during the process of double fertilization.This operation resulted in so called "egg transformation" phenomenon,which is responsible for integration of some injected foreign genes in zygote chromosomes.In our case the integration of An4 and S_6 genes was stable.The inheritance of acquired traits has shown Mendelian character.Thus the using of pollen tube of gamma-irradiated pollen for gene transfer in female sex cells provides plant genetic engineering with method analogous to that of DNA microinjections in animals.However the former one has essential advantage because of natural way of exogenic DNA introduction in cell.Besides,we have established that gamma-irradiation may be a reliable instrument in regulation of DNA and membrane repair system activities in pollen and for control of pollen tube growth speed in pistil consequently.In other words, the correct dose selection for pollen irradiation determines the efficacy of gene transfer by the technique in question.

M 124 TRANSFORMATION OF *SOLANUM TUBEROSUM* CVS. RUSSET BURBANK AND ATLANTIC USING BINARY VECTORS. P.S. Hogan, S.E. Ruzin, C.J. Boyes and R.G. Hadley, Plant Genetics, Inc., Davis, CA 95616

Commercial cultivars of potato were transformed using a vector with an RK2 replicon pGA472 (constructed by Dr. G. An) and pPGI475, a vector containing the wide host range replicon of RSF1010. The non-oncogenic *Agrobacterium tumefaciens* C58 host contained a 17 kb deletion within its T-DNA. After co-cultivation of pGA472 with Atlantic and Russet Burbank internodes, regeneration occurred at 17% and less than 1%, respectively. After co-cultivation of pPGI475 with Atlantic and Russet Burbank internodes, regeneration occurred at 6% and 3%, respectively. For 17 transgenic plants there was a 63 fold difference in NPT-II expression. Up to 8 independent insertion events were observed in a single plant and 3 plants had approximately 5-10 copies of the transferred DNA per site. Co-transformation of the partially deleted T-DNA in the Ti plasmid was seen in 8 of 14 plants tested. For the RSF1010-derived vector, 1 of 8 plants examined received DNA sequences from the 2.5 kb *ori-mob* region present in the *NcoI/PvuII* fragment. The size of each potato DNA restriction endonuclease fragment that hybridized to the NPT-II gene was approximately as large or larger than the distance between the T-DNA borders on the binary vectors, suggesting that truncated copies were not being transferred.

M 125 T-DNA PRESENCE AND OPINE PRODUCTION IN AGROBACTERIUM TUMEFACIENS A281 INDUCED TUMORS ON NORWAY SPRUCE. Elizabeth E. Hood[1], Inger Ekberg[2], Thomas Johannson[2], and David H. Clapham[2], [1]Department of Biology, Utah State University, Logan, Utah 84322, [2]Department of Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The hypervirulent Agrobacterium tumefaciens strain A281 formed frequent tumors (31%) on Picea abies (Norway spruce), an economically important tree species in Swedish forests. Three month old seedlings were inoculated and tumors were established that grew hormone independently in culture. Tumors contained agropine and mannopine/mannopinic acid as determined by acid pH paper electrophoresis. In addition, DNA hybridization studies showed that the DNA from these tumor lines contained sequences homologous to Ti plasmid T-DNA, whereas wild type spruce seedling DNA did not. These results suggest that Agrobacterium vectors can be used for gene transfer into this important forest species.

M 126 LIPOSOME-MEDIATED TRANSFORMATION, Karen Woodbury Hughes, Zhu Zhen and Leaf Huang, Depts. of Botany and Biochemistry, University of Tennessee, Knoxville, TN 37996. pH-sensitive liposomes composed of dioleoylphosphatidylethanolamine: cholesterol: oleic acid (4:4:2 molar ratio) and pH-insensitive liposomes composed of dioleoylphosphatidylcholine: cholesterol: oleic acid (4:4:2 molar ratio) were compared for ability to deliver DNA to plant protoplasts. Treatment of plant protoplasts with pH-sensitive liposomes resulted in a transformation frequency of 2×10^{-4}, approximately 300 fold greater than that for pH-insensitive liposomes. Genetic segregations with plants regenerated from transformed protoplasts indicated either one or two active gene sites. In some cases regenerated plants were polyploid. When 2 different selectable markers (Neomycin phosphotransferase and hygromycin resistance) were encapsulated in liposomes, 20% of the transformants had functional copies of both genes. Callus regenerated from protoplasts transformed with NPT-II lost detectable NPT-II activity; However, plants regenerated from NPT negative callus were NPT positive. Shoot cuttings from NPT positive plants were occasionally found to be NPT negative. Treatment of NPT negative callus or plants with 5'-azacytidine resulted in restoration of detectable gene activity indicating that methylation is responsible for the observed suppression of NPT-II gene activity.

M 127 TRANSFORMATION OF CHRYSANTHEMUM MORIFOLIUM BASED ON AGROBACTERIUM GENE TRANSFER, James F. Hutchinson, R. Miller, V. Kaul, T. Stevenson[a], D. Richards, Horticultural Research Institute, P.O. Box 174, Ferntree Gully 3156, Victoria, AUSTRALIA. [a]Calgene Pacific Pty. Ltd., 16 Gipps Street, Collingwood 3066, Victoria, AUSTRALIA.

Agrobacterium mediated gene transfer systems are being developed for a range of cultivars of the economically important cut flower Chrysanthemum morifolium. Adventitious shoot regeneration from both stem and leaf explants occurs on MS medium supplemented with 5 μM BAP and 5 μM NAA but the response is cultivar dependent. Regeneration is a single cell event and occurs from cortical cells and to a lesser extent from epidermal cells. Plantlets produced are true-to-type as shown by morphological and biochemical analysis. Co-cultivation with cointegrate and binary vectors containing GUS and NPT II under the control of the 35S promoter is being evaluated. Kanamycin response curves have shown an extreme, cultivar related sensitivity. Shoots have been produced from co-cultivation experiments and root initiation found in the presence of kanamycin. Screening for GUS, NPT II and nopaline has commenced. Supported by the National Biotechnology Programme.

M 128 ENDOPLASMIC RETICULUM TRANSPORT AND GLYCOSYLATION OF PLANT-BACTERIAL FUSION PROTEINS IN TRANSGENIC TOBACCO. Gabriel Iturriaga, Richard A. Jefferson and Michael W. Bevan. Department of Molecular Genetics, I.P.S.R., Maris Lane, Trumpington, Cambridge CB2 2JB, UK

We have developed a system to target heterologous proteins to the endoplasmic reticulum (E.R.) of transgenic plants using the signal peptide (S.P.) of the major storage protein of potato tuber (patatin) fused to the bacterial gene, β-glucoronidase (GUS). Analysis of Ti-transformed tobacco plants shows that GUS fusions comprising the patatin S.P. of 23 amino acids(aa) plus part of the mature protein (+1,+3,+8,+14,+25 or +33 aa) exhibit about 100 fold less enzymatic activity than a GUS fusion to only the first 2 aa of patatin preprotein. When cell suspension cultures from our transgenic plants were incubated in the presence of tunicamycin we recovered more than 100 fold GUS activity for the fusions containing S.P. whereas the fusion lacking it is not affected. These results strongly suggest that : 1) GUS fusions containing patatin S.P. have been transported to the lumen of the E.R. 2) GUS is N-glycosylated - it has two cryptic N-glycosylation sites (Asn-X-Ser/Thr). Western gel experiments corroborate this result. 3) GUS is apparently more stable inside the E.R. than in the citosol. Patatin S.P.-GUS fusion protein (+1) is also translocated and glycosylated in vitro. We are currently trying to localize the intracellular storage site of our hybrid proteins using immuno-electron microscopy.

M 129 GENETIC TRANSFORMATION OF PISUM SATIVUM BY AGROBACTERIUM TUMEFACIENS, Jennie Jackson, Shaun Hobbs and John Mahon, National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, Sask. S7N 0W9

An efficient method for transferring cloned genes into Pisum sativum is necessary for improvement of the agronomic performance of this important crop species. The aim of this work is to investigate means of achieving stable transformation of peas using Agrobacterium tumefaciens as a vector. A selection of genotypes was screened for susceptibility to wild-type strains of A. tumefaciens by infecting stems of whole plants in vivo or immature leaf explants in vitro. Some genotype/strain interaction was found. Axenic growth of galls on hormone-free medium, the production of Ti-specific opines and Southern blot analyses were used to confirm transformation. Genotypes of P. sativum were also screened for their ability to regenerate plants from immature leaflet explants in vitro. Genotypes were thus identified which had maximal potential for transformation and regeneration.

Using disarmed strains of A. tumefaciens containing plasmids with the genes coding for kanamycin resistance and GUS, attempts were made to infect immature leaflets to regenerate transformed plants.

M 130 GENETIC MANIPULATION OF ARABLE CROP PLANTS BY PROTOPLAST FUSION AND DIRECT GENE TRANSFER. Michael G.K. Jones, Heddwyn Jones, Bruce T. Lee, Keith Lindsey, Robert Potter, Angela Karp and Eija Pehu. Institute of Arable Crops Research, Department of Biochemistry, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ. U.K.

Somatic hybrid plants, produced by fusing together protoplasts of dihaploid *Solanum tuberosuum* (potato) and the diploid wild species *S. brevidens*, have been analysed at the molecular, cytological and morphological levels, and in field experiments (under Advisory Committee for Genetic Manipulation guidelines). Hybrids express resistance to potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX) from the wild parent. Six combinations of genetic material (nuclear and chloroplastic genomes) have been obtained in euploid plants (tetraploids and hexaploids). Direct gene transfer of a range of gene constructs (both tissue specific and constitutive) has been examined after their introduction by electroporation into protoplasts of a range of species (potato, *S. brevidens*, sugarbeet, wheat, barley) and different protoplast types (e.g. mesophyll, tuber, cell suspension, aleurone, endosperm). Gene expression has been studied both by transient expression and after stable integration.

M 131 TRANSFORMATION OF PLANT MITOCHONDRIA WITH MITOCHONDRIAL DNA PLASMIDS VIA PROTOPLAST FUSION, Roger J. Kemble, Tina L. Barsby*, and Stephen A. Yarrow, Allelix Inc., 6850 Goreway Drive, Mississauga, Ontario, L4V 1P1, Canada. Brassica napus cybrid plants which contain novel nucleus-mitochondria-chloroplast combinations have been constructed, via protoplast fusion. Such fusions resulted in mitochondrial DNA plasmids being transferred from mitochondria of one protoplast population to mitochondria of the other population (at a frequency of 6.1%). Mitochondria containing their 'new' DNA complement became the dominant organelle population in regenerated plants and were faithfully maternally inherited through successive sexual generations. Protoplast fusion can, therefore, be used to transform plant mitochondria with naturally occurring mitochondrial plasmids. The potential for mitochondrial transformation with recombinant vectors will be discussed.

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M 132 ASSEMBLY OF NUCLEAR ENCODED PROTEINS IN CHLOROPLAST MEMBRANES, Bruce D. Kohorn, David Murray, Andrea Auchinchloss, Elaine Walker, and Gerty C. Ward, Botany Dept., Duke University, Durham, NC 27706

We are studying the process by which a cytoplasmically synthesized protein is directed to an organelle and then inserted into a functional photosynthetic membrane. Both a genetic and a biochemical approach are being taken.

We wish isolate nuclear mutations that disrupt the normal transport of proteins to the chloroplast. DHFR, whose location in the cytoplasm is essential under selective conditions has been targeted to the chloroplast of a transformed Arabidopsis plant by the fusion of the LHCP transit peptide. Mutants surviving selection on methotrexate are hoped to be deficient in transport.

A study of the import of in vitro derived mutant LHCPs into isolated chloroplasts has shown that a putative alpha-helical membrane spanning domain (helix 3) near the carboxy-terminus is essential for stable insertion of LHCP into the thylakoid. Helix 3, when fused to a soluble protein, can target it to the thylakoid, yet the full insertion of helix 3, and of LHCP requires a complex interaction of different protein domains. In vitro studies also point to the involvement of 3 histidine residues within LHCP that are important for its association with the chlorophyll-protein complex LHCII.

M 133 A RAPID AND SIMPLE METHOD FOR TRANSFERRING GENE INTO GRAPEVINE cv. CABERNET SAUVIGNON, Pavel G. Kovalenko and Anatoly P. Galkin, Inst. of Bioorganical Chemistry, Acad. Sci. of the Ukrainian, Labor of Plant Gen. Eng., Murmaskaya str. 5, Kiev 94, 252660 USSR

For study any genetical manipulation of Grapevine with Ti- plasmid vector pGV 3850 as gene expression, most effectively to use a leaf disc transformation procedure. Seven week- old plants gave the best quality leaves. Leaf discs were punched a 5-mm paper punch. Discs were placed upside down on modified liquid medium with containing 1/2 MS salts, Nitsch/H/ vitamins, 6% sucrose, benzyladenine (2.0 mg/l), naphthalene acetic acid (4.5 mg/l), adjusted to pH6.1. After 3 days at 27C, the discs were selected for inoculation with Ti-plasmid vector pGV 385o. An overnight culture of A. Tumefaciens grown in YEB modified medium. Procedure of transformation: 1ml of a suspension A. tumefaciens was diluted in the ratio of 1/30 in a modified MS medium with addition of 12mM CaCl2, 6% glucose and DMSO. The leaf discs are placed in a test-tube and the A. tumefaciens culture is poured in. Then, the infected leaf discs were blotted dry on sterile filter paper and transferred upside down on to nurse culture plates. For the nurse culture plates was used modified 1/3 MS medium; it contains Nitsch vitamins, 6% sucrose, BA (2mg/l), 0.7% agar, at pH 6.1. After 2 days of coculture, the leaf discs are transferred to the same medium containing (500mg/17 Cefadroxil/ Bristol/France/ to kill A. tumefaciens and containing (150mg/l) kanamycin sulfate to select for growth of transformed plants. One month after inoculation was obtained antibiotic-resistant shoots.

M 134 CHARACTERIZATION OF BROME MOSAIC VIRUS REPLICATION GENE FUNCTIONS USING TARGETED SUBSTITUTION AND IN-FRAME INSERTION MUTATIONS, Philip Kroner, Patricia Traynor, Doug Richards and Paul Ahlquist, Institute for Molecular Virology and Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

The brome mosaic virus (BMV) genome is divided among three capped, single-stranded RNAs which serve as mRNAs for three nonstructural proteins (1a, 109 kd; 2a, 94 kd; and 3a, 32 kd). Several lines of evidence suggest that functions involved in directing BMV replication reside within the 1a and 2a proteins. BMV RNAs 1 and 2, which encode these proteins, are necessary and together sufficient to direct BMV replication in barley protoplasts. In addition, the 1a and 2a proteins share extensive amino acid homology with suspected replication proteins encoded by a wide variety of morphologically distinct plant viruses, as well as the animal alphaviruses. To define the functions of both highly conserved and divergent domains in the BMV replication proteins, we have constructed and tested a large set of substitution and in-frame insertion mutations in cloned cDNA copies of the BMV 1a and 2a genes. Northern blot analyses of mutant BMV replication in barley protoplasts reveal phenotypes ranging from apparently wild type (wt) to apparently lethal and include several mutants in both genes which show temperature-sensitive (ts) defects in BMV RNA accumulation. In addition, one ts 2a mutant, DR17, preferentially suppresses the synthesis of genomic RNA compared to subgenomic RNA at the permissive temperature. To identify the specific step affecting viral RNA accumulation in vivo we are analyzing viral RNA synthesis in vitro using BMV polymerase extracts isolated from barley systemically infected with mutant viral RNA.

M 135 STABLE CO-TRANSFORMATION OF MAIZE PROTOPLASTS WITH THE GENES FOR NPTII AND GUS, Leszek A. Lyznik, Randy Ryan and Thomas K. Hodges, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Maize protoplasts isolated from A188 x BMS suspension cells have been transformed with separate plasmid constructions containing the ß-glucuronidase gene (*uid A*) or neomycinphosphotransferase (*neo)* gene coding sequences, pPUR and pKAN respectively. Both genes were under control of the CaMV 35S promoter. DNA uptake was mediated by polyethylenglycol (PEG). Transformation efficiency, based on kanamycin selection, was $1x10^{-4}$; 90% of the resistant colonies contained the *neo* gene stably integrated into the maize genome. Among 52 kanamycin-resistant lines, 33% also showed high levels of GUS activity (above 2 units), and an additional 20% exhibited low levels of GUS. Southern blot hybridizations confirmed the presence of numerous *uid* A and *neo* coding regions in the maize genome, and specific cell lines exhibited similar frequencies of insertions of the *neo* and *uid A* genes. There was no correlation between GUS activity and *uid A* gene copy number. Use of different restriction enzymes revealed that several inserted sequences were organized in tandem head-to-tail repeats with one or several sites of insertion. Some inserted sequences appeared to be partially methylated.

M 136 AGROBACTERIUM MEDIATED TRANSFORMATION IN MANGO (MANGIFERA INDICA) Helena Mathews and Richard E. Litz, University of Florida, Tropical Research and Education Center, 18905 S.W. 280 Street, Homestead, FL 33031

Mango (Mangifera indica L.) cv. Parris, a poly embryonic cultivar, was used in transformation studies. Embryogenic suspensions derived from nucellar explants were incubated with Agrobacterium tumefaciens containing disarmed, cointegrate plasmid pGV3850::1103 conferring resistance to the antibiotic kanamycin. Eight cell aggregates and small proembryos were able to multiply in kanamycin selection medium. Our approach for obtaining transformed mango somatic embryos will be discussed.

M 137 EXPRESSION OF GUS ACTIVITY IN ELECTROPORATED POLLEN Benjamin F. Matthews[1], James A. Saunders[2] and Aref A. Abdul-Baki[1] USDA-ARS, [1]Plant Molecular Biology Laboratory and [2]Plant Germplasm Quality & Enhancement Laboratory, Beltsville, MD 20705.

Most current methods of gene transfer require the use of Agrobacterium tumefaciens or require the regeneration of protoplasts or cells if whole plants are to be obtained. Each method has certain inherent restrictions, thus are not applicable to many economically important crops. To extend the use of gene transfer to a broader range of crop species we are developing methods for the incorporation of foreign DNA into pollen grains which can be used for fertilization of flowers to obtain genetically modified seed. We have optimized major parameters for the uptake of [^{3}H]-DNA into pollen by electroporation. Up to 10% of the donor DNA has been successfully taken up by the pollen. Field strengths of up to 11 Kvolts/cm could be applied to the pollen without detrimentally affecting viability. Successful incorporation by pollen of the plasmid vector pBI 221, containing the CaMV 35S promoter and the gene encoding beta-glucuronidase (GUS), was indicated by transient expression of GUS activity in the pollen 24 to 48 hours after electroporation.

M 138 DNA Delivery by Microprojectiles Accelerated by a "Vaccination Gun". Paul D. Miller and James A. Lauritis. DNA Plant Technology Corporation 2611 Branch Pike, Cinnaminson, New Jersey 08077.

A novel mechanism for DNA transfer by microprojectile delivery has been developed. The microbeads, gold spheres (1.5 to 3.0μm) or colloidal gold (0.03μm), are propelled by a mechanical motive force, rather than chemical or arc discharge explosive forces. Transient expression of transferred plasmid DNA has been monitored in callus cells and embryos by β-glucuronidase assays. The major advantages to this apparatus are its reduced cost, complete mobility, lack of need for a partial vacuum and successful introduction into cells of submicron-sized particles.

M 139 DEVELOPMENT OF SATELLITE TOBACCO MOSAIC VIRUS FOR THE EXPRESSION OF HETEROLOGOUS GENES IN PLANTS, T. Erik Mirkov and Leona Fitzmaurice, SIBIA, P.O. Box 85200, San Diego, CA 92138

Satellite tobacco mosaic virus (STMV) is a small icosahedral RNA virus which depends on tobacco mosaic virus (TMV) for its replication. This is the first plant virus satellite that does not have a spherical virus as a helper, and it is one of the few satellite viruses (as opposed to satellite RNAs). It is systemically distributed in infected plants such as tobacco, along with its well characterized helper virus.

Our goal is to use STMV as an accessory to TMV for the expression of foreign genes in plants. The open reading frame (ORF) for the capsid protein of the satellite is a candidate for deletion and substitution of foreign gene sequences. Satellite capsid protein is expressed at very high levels in infected plants. High concentrations of foreign RNA transcripts and proteins, therefore, might result if the modified STMV genome were used as inoculum.

Progress has been made towards achievement of these goals. The sequence of the STMV genome has been determined, and full-length cDNA clones have been constructed. These full-length cDNA clones have been sub-cloned into appropriate transcription vectors such that genome sense RNA can be produced. Infectivity tests of these in vitro transcripts have been initiated.

M 140 *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF *CITRUS*, Gloria A. Moore, Maria G. DeWald, and Kenneth Cline, Fruit Crops Department, IFAS, University of Florida, Gainesville, FL 32611
The immediate goals of this project are to transform *Citrus* cells with an antibiotic resistance gene using the *Agrobacterium tumefaciens* vector system. This will provide a selectable marker that can be used in citrus breeding to distinguish hybrids from nucellar seedlings, something that cannot be easily done using conventional methods. Internodal stem segments are incubated with vector bacteria harboring a T-DNA borne NPT-II gene. Shoots are subsequently regenerated in the presence of kanamycin, established in soil, and analyzed for the presence of T-DNA by Southern analysis. One established plant has been verified as transformed by this technique. We are currently testing the efficiency of different *A. tumefaciens* vectors for citrus transformation using T-DNA that also contains a gene for β-glucuronidase (GUS), a scorable marker. With certain vectors, up to 40% of regenerated shoots were positive for GUS activity. Regenerated shoots are presently being established in soil. The results of Southern analysis of these plants will be presented.

M 141 EFFICIENT RICE PROTOPLAST REGENERATION USING GENERAL MEDIAM AND NURSE CULTURE, Zhijian Li and Norimoto Murai, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803 Efficient plant regeneration from protoplasts is critical for successful genetic transformation. To improve the suspension culture system and consequently to increase the frequency of protoplast regeneration different basal media were evaluated with the *japonica* rice cultivar Nipponbare (*Oryza sativa* L.). Of these media a General Medium developed for rice anther culture was found to be the best for initiating fast growing suspensions within five weeks from scutellum-derived calli. From these cultures a higher yield of protoplasts was obtained. Thus isolated protoplasts with dense cytoplasm grew actively and gave rise to high plating efficiencies ranging from 6.3% to 15.8% when cultivated in a nurse culture system. Different rice nurse cell lines and varied lengths of culture duration influenced the plating efficiency. From Protoplast-derived calli plant regeneration frequencies of up to 66.7% were obtained using N6 regeneration medium. Protoplast-derived plants grown to maturity were fertile. Self-pollinated progenies (F2) grew normally and exhibited no appearant variations.

M 142 INTRACELLULAR TRANSPORT OF NAPIN, A STORAGE PROTEIN IN BRASSICA NAPUS
Eva Murén, Mats Ericson, Anna-Stina Höglund and Lars Rask. Dept. of Cell Research, Swedish Univ. of Agriculture, Uppsala, Sweden.
Napin is one of the two major storage proteins of rapeseed (Brassica Napus). It consists of two subunits, 29 and 86 amino acid residues long, that are held together by two disulfide bridges. The comlete primary structure of napin was determined. A structural comparison between the napin chains and the deduced sequence from a napin cDNA clone revealed that napin is synthesized as a precursor of 178 amino acid residues. An amino terminal ER-signal sequence and two additional peptides are proteolytically cleaved off within 3 hours in vivo. The drug monensin blocks this processing, indicating that the Golgi apparatus is involved in the intracellular transport of napin. Napin is stored in specialized storage organelles, protein bodies, which was demonstrated by immunogold labeling of rapeseed embryo thin-sections. Peptide antisera directed against the processed parts of the precursor likewise stain the protein bodies specifically. This was interpreted as if napin is transported in the precursor form to the protein bodies. In order to study the storage protein signals, we are presently expressing the napin protein in yeast since a heterologous system could provide a screening system for genetically engineered protein variants.

M 143 HIGH VELOCITY MICROPROJECTILE DELIVERY OF FOREIGN GENES AND THEIR EXPRESSION IN APICES OF TRITICUM AESTIVUM.
Jim Oard[1], John Simmonds[2], Jan Dvorak[1], [1]Agronomy and Range Science, University of California, Davis, CA 95616; [2]Agriculture Canada, Plant Research Centre, Ottawa, Ontario, Canada.
Apices, microdissected to expose the apical meristematic dome, could be recultured giving rise to normal fertile plants. This system was used to develop methods of direct DNA uptake into cells within the meristem in order to facilitate transformation of wheat. The DNA vector used was PAT-13, which contained a maize ADH promotor-maize intro-GUS-NOS expression cassette. Tungsten particles (1.2 μ), coated with DNA, were propelled by an air-gun apparatus into microdissected apices. The apices were transferred to culture medium for 48 h and then the developing leaf primordia were excised to expose the apex which was assayed histochemically for GUS activity. Up to 40% of the apices showed GUS activity, 3% being recorded in the region of the apical meristematic dome.

M 144 EXPRESSION OF TOMATO POLYGALACTURONASE IN TRANSGENIC TOBACCO PLANTS.
K.W. Osteryoung, B. Hall, V. Winkler and A.B. Bennett. Dept. of Vegetable Crops, University of California, Davis, CA 95616.
Tomato polygalacturonase (PG) is a cell wall enzyme synthesized and secreted in large amounts during tomato fruit ripening. Three isoforms of PG, all derived from a single gene product, are present in ripe tomato fruit. The events giving rise to these isoforms are at present unknown. In order to investigate further the posttranslational processing and secretion of this enzyme, we have inititated studies of PG expression in transgenic tobacco plants. Agrobacterium-mediated transfection was used to introduce into tobacco leaf discs a full-length PG cDNA under control of the CMV 35S promoter. Regenerated plants selected for kanamycin resistance and expression of nopaline synthase were analyzed for various aspects of PG expression. Our results indicate that 1) immunologically detectable PG can be isolated from the cell walls of transgenic tobacco plants; 2) the enzyme is electrophoretically and immunologically indistinguishable from that isolated from ripe tomato fruit; 3) the protein is enzymatically active in in vitro assays; and 4) only two of the three PG isoforms found in ripe tomato fruit appear to be present in transgenic tobacco. Further analysis of the localization, processing and in vivo activity of PG in this transgenic system will be presented.

M 145 INFLUENCE OF A PLANT TRANSIT PEPTIDE ON BACTERIAL CHITINASE ACTIVITY IN TOBACCO
Joan E. Passiatore, Frank Cannon, Vicky Buchanan-Wollaston, BioTechnica International, Inc., 85 Bolton Street, Cambridge, MA 02140.

Proteins such as chitinase and ß-1,3 glucanase which are induced in plant tissue upon wounding are thought to play a role in the plant's defense response. Chitin is a major component of cell walls of many fungi and plant chitinases have been found to be active in inhibiting fungal growth, *in vitro*. These, and other defense related proteins have been shown to be secreted proteins. Such proteins are generally synthesized with an N-terminal transit peptide which functions to transport the protein across the ER where proteolytic processing , glycosylation and further suborganellar and extracellular sorting occur. Translocation of the protein across a membrane may also be required for correct folding which is necessary for protein stability and optimal activity.

The chitinase gene from *Streptomyces plicatus*, the product of which is a secreted protein, was cloned with and without extensin and proteinase inhibitor I (PI-I) plant transit peptides under the regulation of the CaMV 35S and PI-I promoters and assayed for expression and activity of the protein in *Agrobacterium*- transformed tobacco callus and greenhouse-grown plants. The transit peptide constructions were also analyzed in an *E. coli* expression vector under the regulation of the Tac promoter. Results from these studies will be presented.

M 146 TRANSFORMATION OF *PISUM SATIVUM* (L.), Johanna Puonti-Kaerlas and Tage Eriksson, Departement of Physiological Botany, University of Uppsala, BOX 540, S-751 21 Uppsala, Sweden

We have developed an efficient method for transforming pea and are now attempting to regenerate shoots from transformed callus. The relative efficiencies of different transformation methods using protoplasts, protoplast-derived minicolonies and different types of explants were compared using engineered strains of *Agrobacterium tumefaciens*. The most efficient method was cocultivation of explants of stem or leaf pieces with non-oncogenic strains of *A. tumefaciens*. Transformed calli could be selected on either 75 mg/l kanamycin sulphate or 15 mg/l hygromycin, and their transformed nature confirmed by opine or DNA analysis. Putative transformed shoots have been regenerated from the calli growing on hygromycin. As we have developed a method for shoot regeneration from protoplasts, cocultivation of protoplasts with *Agrobacterium* was tested, but this was lethal to the protoplasts. However, small protoplast-derived colonies could be transformed, albeit at a very low frequency. The results of *in planta* inoculation studies and regeneration experiments will be discussed.

M 147 INFECTIOUS IN VITRO TRANSCRIPTS FROM CLONED cDNA OF TOBACCO VEIN MOTTLING VIRUS, Robert E. Rhoads#, Leslie L. Domier*, Kathleen M. Franklin*, Arthur G. Hunt+ and John G. Shaw*, Departments of #Biochemistry, *Plant Pathology and +Agronomy, University of Kentucky, Lexington, KY 40536

Two methods have been employed to introduce genes from the potyvirus tobacco vein mottling virus (TVMV) into tobacco plants. In the first, full-length cDNA copies of TVMV RNA were constructed downstream from either bacteriophage T7 orT3 RNA polymerase promoters. The plasmids were designed to produce in vitro transcripts containing, respectively, one or two G residues at the 5' terminus not derived from the TVMV sequence and a single C residue at the 3' terminus following the poly(A) tail. Introduction of transcripts from either plasmid into tobacco mesophyll protoplasts resulted in the accumulation of TVMV coat protein (CP) and RNA. Neither CP nor viral RNA accumulated in protoplasts inoculated with linearized cDNA or with in vitro transcripts synthesized in the absence of m7GpppG. Tobacco seedlings inoculated with native TVMV RNA developed symptoms after four to five days while those inoculated with in vitro transcripts developed symptoms after seven to ten days. Symptoms produced by native viral RNA and in vitro transcripts were, however, indistinguishable three weeks after inoculation. In the second method, sequences encoding CP were introduced into tobacco plants using a Ti plasmid-associated binary vector. Expression of the CP was detected immunologically. The combination of these two technologies opens new possibilities for the investigation of potyviruses or vectors. Supported by Grant 4E021 from the University of Kentucky Tobacco and Health Research Institute and Grant 85-CRCR-1-1536 from the USDA Competitive Grants Program.

M 148 **Study of telomere-related repetitive elements in *Arabidopsis thaliana*; candidates for centromeric DNAs.**
Eric J. Richards, and Frederick M. Ausubel. Department of Molceular Biology, Massachusetts General Hospital, Boston, MA. 02114.

We have previously described the isolation of an *A. thaliana* telomeric clone [Cell 53:127 (1988)]. Study of this clone indicated that telomeres in *A. thaliana* are composed of tandem repeats of the sequence 5'-[C(C/T)CTAAA]-3' organized into arrays of $\leq$ 2.5 kb per chromosomal end. The telomeric clone also hybridizes, under reduced stringency conditions, to the discrete Bal31-insensitive bands on Southern blots that represent similar sequences located at "internal" sites in the chromosome arm.

We have isolated several genomic clones corresponding to these internal telomere-related sequences. One clone, designated pAtT12, has been analyzed in some detail. The pAtT12 insert consists of a 12 kb region composed of highly repeated DNAs flanked by a 2.5 kb domain containing middle repetitive DNAs. The highly repeated domain is composed of tandem repeats of a ~ 500 bp unit. Sequencing of several 500 bp elements has revealed the presence of a ~200 bp simple-sequence domain of degenerate telomere-like repeats. Using probes from the middle repetitive domain we (in collaboration with the Goodman laboratory at Massachusetts General Hospital) have mapped this repetitve array to the middle of chromosome 1 in the vicinity of the centromere. We are currently investigating the possibility that these repetitive DNAs are part of the centromere or flanking heterochromatic domains. Further characterization of the pAtT12 insert and chromosomal mapping of the repeat elements will be presented.

M 149 TRANSIENT AND STABLE TRANSGENIC CELLS AND CALLI OF TOBACCO AND MAIZE FOLLOWING MICROPROJECTILE BOMBARDMENT, Margit C. Ross and Dwight T. Tomes, Department of Biotechnology Research, Pioneer Hi-Bred International, Inc., Johnston, IA 50131
Vectors containing Beta-glucuronidase (GUS) and either Neomycin Phosphotransferase (NPTII) or Acetolactate Synthase (ALS) were transferred into tobacco and BMS maize suspension cells, respectively, using the microprojectile bombardment technique. The NPTII genes used for this study are driven by either a NOS or 35S promoter. The ALS gene used is driven by a 35S promoter. Following microprojectile bombardment, cells were maintained on either 100mg/L kanamycin or 10ppb glean selection. The histochemical GUS assay was used on growing tissue to test for transient and stable transformants. Forty-eight hours post treatment, samples sacrificed for the assay showed single cells and cell clusters identified as transient transformants. Tissue was sampled weekly and stained with 400ul of stain containing the substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-glu). Stable transformants were identified four weeks post treatment. These colonies, growing under selection, showed positive GUS transformation in the histochemical assay and presence of either the NPTII, GUS, or ALS gene in southern blot analyses.

M 150 TRANSFORMATION OF PICKLING CUCUMBER (CUCUMIS SATIVUS L.) BY AGROBACTERIUM TUMEFACIENS AND EXPRESSION OF KANAMYCIN RESISTANCE IN REGENERATED TRANSGENIC PLANTS, Gerald G. Sarmento, Kevin B. Alpert, Zamir K. Punja and F. Archie Tang, Genetic Engineering Department, Campbell Soup Company, Davis, CA 95616. Genetic transformation of two inbred lines of Cucumis sativus has been achieved using Agrobacterium tumefaciens strain LBA 4404 (with disarmed Ti plasmid and containing various binary plasmids with different T-regions i.e. pBIN 19 or pCGN 783) carrying a chimeric NPT-II gene with either the 35S CaMV or mas promoter. The protocol used was as follows: explants were preincubated on a tobacco (N. tabacum 'Xanthi') feeder layer for 24 hr, exposed to $5x10^8$ cells/ml bacterial suspension for 10 min, reincubated on the feeder plates for 48 hr and plated onto MS medium with 75 mg/l kanamycin and NAA/BA or 2, 4-D/BA as the hormone source. Cultures were incubated in the dark for 3 wk, then placed in the light. Both shoots and somatic embryos were obtained from leaf and petiole explants 8 weeks after cocultivation and following selection on kanamycin containing media. Transformation was confirmed by dot-blot assays for NPT-II activity and by Southern analysis. Fertile plants were recovered which were pollinated using pollen from a nontransformed plant. NPT-II assays and Southern analysis were also performed on the progeny plants to determine segregation. Segregation for the NPT-II gene occurred at an expected 1:1 ratio. Twenty-one transformed plants have been recovered to date.

M 151 ANALYSIS OF THE TARGETING SEQUENCES OF THE β-CONGLYCININ α SUBUNIT GENE OF SOYBEAN IN HETEROLOGOUS SYSTEMS. Federico L. Sebastiani, Leigh B. Farrell and Roger N. Beachy. Department of Biology, Washington University, St. Louis, Mo 63130.
A modified α subunit cDNA clone was isolated and its nucleotide sequence determined. Leaf discs of Nicotiana tabacum were transformed via Agrobacterium tumefaciens with plasmids containing the α subunit mini-gene behind the 35S promoter of CaMV or the promoter from the α' subunit β-conglycinin gene on a Ti based vector. Western blots of seed extracts of transgenic tobacco plants containing the mini-gene behind both promoters show a protein with equal electrophoretic mobility as the α subunit of β-conglycinin of soybean. In the case of plants harboring the p35S:α cDNA the relative level of this protein in seeds was higher than both in leaves and callus tissue.
For Yeast expression studies, the α mini-gene was cloned behind the Gal-1 promoter carried on a 2um based yeast vector. Membrane fractionation studies showed that a protein, immuno-reactive with antibodies directed against the β-conglycinin complex, co-fractionated with the vacuolar marker enzyme α-mannosidase.
With the knowledge that the α subunit protein is correctly targeted to the yeast vacuole, the targetin signals of the leader will be delineated. The approach we have chosen is to fuse a number of C-terminal deletions of the α-subunit precursor to the GUS reporter gene and expressing the chimeric proteins in tobacco cells and yeast cells. Determination of subcellular localization of GUS activity in these systems should give insights into those sequence motifs that are important to the targeting of the α subunit.

M 153 ANALYSIS OF LEAKY VIRAL TERMINATION CODONS IN TRANSFECTED TOBACCO PROTOPLASTS, Jim Skuzeski, Lindy Nichols, John Atkins and Ray Gesteland, Department of Human Genetics, Howard Hughes Medical Institute, 743 Wintrobe, University of Utah, Salt Lake City, Utah 84132.
The synthesis of several proteins encoded by plant RNA viruses is achieved by the readthrough of stop codons. This process apparently acts to regulate the expression of specific viral polypeptides, e.g., tobacco mosaic virus (TMV) replicase, but is poorly understood. To investigate readthrough mechanisms in vivo, we have developed a system which utilizes transient expression of B-glucuronidase (GUS) reporter genes as a measure of the ability of ribosomes to bypass viral translational termination signals. Our results show that constructs containing the leaky amber codon and flanking codons of TMV direct 3-4% of the GUS activity produced from comparable plasmids lacking the stop codon. The level of readthrough appears to be influenced by flanking codons and an analysis of this context affect is now underway. Our ability to detect low level events has been made possible by the use of GUS expression vectors which direct ca. 40-fold higher levels of GUS than the commercially available plasmid, pBI221, and by the establishment of optimal electroporation conditions. Vector constructions and electroporation experiments will be presented.

M 154 **REGENERATION OF CAULIFLOWER TRANSFORMED BY AN ONCOGENIC AGROBACTERIUM AND EXPRESSION OF FOREIGN GENE.** VIBHA SRIVASTAVA, A.S.REDDY AND SIPRA GUHA-MUKHERJEE. SCHOOL OF LIFE SCIENCES, JAWAHARLAL NEHRU UNIVERSITY, NEW DELHI-110 067, INDIA. Brassica oleracea var.botrytis cv.synthetica (cauliflower) was transformed by an oncogenic Agrobacterium tumefaciens strain $C_{58}C_1Rif^R$ harboring pLGVTi23neo using leaf-disc transformation procedure. As a result of integration of T-DNA in plant genome, several morphological variants including a chlorophyll deficient mutant were obtained. It is significant to obtain high percent (50-60%) of regeneration in cauliflower, Since, oncogenic Agrobacterium is known to induce crown gall formation which does not differentiate. The expression of the chimaeric gene (Nos/NPTII) in regenerated shoots was ascertained using kanamycin phosphorylation assay and the presence of the foreign gene was determined by southern hybridization using Tn5 encoded NPTII gene as probe. Similar experiments were performed with the model system of tobacco. The transformation of tobacco using the same oncogenic strain of Agrobacterium, resulted in the formation of non-differentiating, slow growing, non-green callus. The regeneration of shoots from such type of callus was obtained on providing BAP exogeneously. The shoots were found to be NPTII positive and the integration of T-DNA was confirmed by southern analysis.

M 155 INDUCTION OF AGROBACTERIUM VIR E LOCUS BY EMBRYOGENIC CALLI OF ELITE INBRED LINES OF MAIZE, Janet Suttie, Nancy Torkewitz and Gary Pace, CIBA-GEIGY Corporation, Agricultural Biotechnology Research, POB 12257, Research Triangle Park, NC 27709

Induction of the Ti plasmid vir region by plant produced chemical inducers has been well documented. The hypothesis that Agrobacterium-mediated transformation of gramineae is restricted by the absence of such inducers is questionable in light of recent evidence for wheat, oats and maize. Using a vir::lacZ fusion plasmid in C58 we have demonstrated that embryogenic calli from elite inbred lines of maize induce the virE locus. Induction persisted for 3 days in the presence of embryogenic calli of maize. The addition of a known dicot inducer, acetosyringone, enhanced the endogenous inducing ability of a friable embryogenic maize callus but interfered with the endogenous induction by a non-friable embryogenic callus of a different maize genotype. A small amount of induction was detectable from the spent medium of suspension cells with a similar enhancement in the presence of acetosyringone.

M 156 GENETIC TRANSFORMATION OF SOYBEAN (GLYCINE MAX), William Swain, Paul Christou, Brian Martinell, and Dennis McCabe, Agracetus, 8520 University Green, Middleton, WI 53562.

Successful application of conventional transformation methods to major, agronomically important crop plants, like corn and soybean, has been limited by host-range restrictions of Agrobacterium-based systems and difficulty in regenerating fertile plants from transformed tissue cultures. To circumvent these obstacles we have employed high-velocity gold particles to deliver plasmid DNA, including a beta-glucuronidase expression cassette, into meristems of immature soybean seeds. Shoots derived from these meristems form fertile, zero-generation (G0) plants that typically produce normal quantities of self-pollinated (G1) seed. Enzyme assays of G0 plants and genetic analyses of self-pollinated G1 progeny, indicate that the original shoots are chimeric, comprising transformed and nontransformed cells. Transformants recovered in the G1 generation contain one to several copies of the beta-glucuronidase gene. The foreign DNA is incorporated into high molecular weight soybean DNA and is stably expressed in G1 plants. This transformation method appears to be generally applicable to any plant species or plant cell type provided the particle-acceleration parameters are adjusted to suit the tissue.

M 157 EXPRESSION OF FOREIGN DNA IN SEED DERIVED EMBRYOS OF IMPORTANT CROPS Reinhard Töpfer, Bruno Gronenborn, Jeff Schell and Hans-Henning Steinbiß, Max-Planck-Institut für Züchtungsforschung, Abteilung Genetische Grundlagen der Pflanzenzüchtung, D-5000 Köln 30, FRG. - A system for the transient expression of foreign DNA in embryos of a wide variety of cereal and legume plant species has been developed. The procedure is based on the uptake of DNA in dry and viable embryos by imbibition in DNA solution. The presence, in an active form, of a defined reporter enzyme coded for by the DNA offered for uptake, was used to monitor transfer of DNA. The constructs used carry the neomycin phosphotransferase II (NPT II) reporter gene fused to the 35S RNA promoter of cauliflower mosaic virus or represent NPT II replacement clones of the capsid protein gene of a cloned wheat dwarf virus, a monopartite Gemini virus. Reproducible and improved conditions were elaborated for wheat embryos using the 35S RNA promoter constuct. Possible artefacts could be excluded, when engineered viral vectors known to be capable of autonomous replication were shown to be effective in amplifying the transient expressed NPT II activity in embryos. The experimental protocol was easily applied to other cereals such as barley, maize, oats, rice, rye, and triticale as well as legumes like pea and bean. The system might also be regarded as a first step towards the goal of stable transformation of plants.

M 158 TRANSFORMATION OF CHRYSANTHEMUM MORIFOLIUM USING A SUPERVIRULENT AGROBACTERIUM TUMEFACIENS STRAIN, Monique F. van Wordragen, Jan de Jong, Hans J.M. Dons. Institute for Horticultural Plant Breeding (IVT), P.O.Box 16, 6700 AA Wageningen, The Netherlands

As part of our attempts to develop an Agrobacterium-mediated transformation system for the ornamental crop Chrysanthemum morifolium we infected leaf disks of sterile plantlets with two Agrobacterium strains: A.tumefaciens A281 (pTVK291) (1) and A.rhizogenes LBA9402. A281 is called a supervirulent strain because the transformation rates on several plant species are up to 10-fold higher compared with other Agrobacterium strains. A281 (pTVK291) induces agropine/mannopine producing tumours, LBA9402 induces agropine/mannopine producing hairy roots on a large number of plants.

On Chrysanthemum leaf disks however, LBA9402 did not induce hairy roots but callus and a few normal looking roots. The callus grew slowly on hormone-free medium, the roots did not. Neither the callus, nor the roots did produce agropine or mannopine. Yet some transformation event must have happened because an avirulent Agrobacterium strain, used as a control did not induce tumours or roots. On infection with A281 (pTVK291), Chrysanthemum leaf disks developed fast growing tumours that produced high amounts of agropine and mannopine. The tumours appeared sooner and were larger than tumours induced by A.tumefaciens Ach5, a commonly used wild type Agrobacterium that is also virulent on Chrysanthemum. A transformation system is being developed using the supervirulent strain in combination with binary vectors. ((1) Jin, S., et al., (1987) J.Bact. 169: 4417-4425)

M 159 CLONING AND STRUCTURAL ANALYSIS OF DIVERSE NOPALINE TYPE T-DNA OF TI-PLASMIDS FROM JAPAN, Hiroetsu Wabiko, Masako Kagaya, Hiroshi Sano, The Biotechnology Institute, Akita Prefectural College of Agriculture, Akita 010-04, Japan

A bacterium Agrobacterim tumefaciens transforms normal plant tissues to tumorous state upon infection and subsequent delivery of T-DNA of the Ti plasmid to the plant chromosome. To study host-bacterium specificity in detail, we isolated and classified nopaline type A. tumefaciens from Japan. Eighteen strains were divided into 6 groups based on tumorigenicity, biovars, and plasmid profiles. The Ti plasmids were identified by hybridization with the well known pTiT37 T-DNA, and T-DNA fragments from each representative 6 strains were cloned into the vector pUC19. From restriction enzyme cleavage and Southern blot analyses, T-DNA regions were found to be composed of two distinct loci. One locus contained the genes 1,2 and 4 that are required for phytohormone biosynthesis and was highly conserved in all isolates except one strain (AKE10). The other locus, which is functionally unknown, was higly variable among 6 strains. This difference was the result of insertions of DNA with various sizes, which were strain-specific. Five strains induced rapidly growing tumors on petunia, whereas AKE10 induced slow growing tumors, which correlated with its specitic structures of genes 1,2 and 4. Teratomas were formed on poplar in a strain-dependent manner. These results suggest that the structural diversity of T-DNA is related to the host-bacterium-specific tumor growth and teratoma formation.

M 160 GENE TRANSFER INTO HIGHER PLANTS WITH A UV-LASER MICROBEAM, Gerd Weber, Shamci Monajembashi, Karl-Otto Greulich, Jürgen Wolfrum, Max Planck Inst. Zellbiologie, Ladenburg and Phys. Chem. Institute, University Heidelberg, Fed. Rep. Germany.

Rigid plant cell walls can be perforated with a UV-laser microbeam (1). Genes can be introduced directly into plant cells and tissue avoiding protoplasts. Genetic manipulations of organelles as well as chromosomes is another application of this tool (2). A laser beam of a N_2-laser was focussed to its diffraction limits and coupled into a microscope. Holes of less than 1µm diameter can be cut by single pulses.

Individually selected cells or microspores of Brassica napus (L.) were placed in hypotonic buffer containing plasmid DNA with marker genes. At the laser focus one pulse opened the membrane for less than 5s. Buffer with DNA was taken up into more than 90% of the cells. They developed into colonies or embryos, respectively, with a frequency of at least 30% of untreated controls. Within the first few days after irradiation transient expression was monitored (3). Incorporation of genes into the genome was followed by using markers coding for resistance to antibiotics.

Incorporation of plasmids into chloroplasts inside of a cell was accomplished. DNA was introduced into the cytoplasm. Then individual chloroplasts were punctured with a laser pulse. The holes in the organelles closed within 1.2s (4).

A laser beam was also used to dissect chromosomes of cells allowing the establishment of segment-specific DNA libraries (5).

1. Weber, G., et al., Naturwissenschaften, 75, 36-36 (1988).
2. Weber, G., et al., Plant Cell Tissue and Organ Culture 12, 219-222 (1988).
3. Weber, G., et al., Ber. Bunsenges. Phys. Chem., in press (1988).
4. Weber, G., et al., submitted (1988).
5. Ponelis, N., et al., submitted (1988).

M 161 TRANSFORMATION OF SUGARBEET CELL SUSPENSION CULTURES MEDIATED BY Agrobacterium tumefaciens. Chris A. Wozniak and Lowell D. Owens, USDA-ARS, Plant Molecular Biology Laboratory, Beltsville, Maryland 20705.
Suspension culture cells of Beta vulgaris L. 'REL-1' were cocultivated with A. tumefaciens strain LBA4404 (disarmed) harboring pAL4404 and pBI121. T-DNA included neomycin phosphotransferase (NPTII) and beta-glucuronidase (GUS) coding regions directed by nopaline synthase and CaMV35S promoters, respectively. Histochemical examination of cells following incubation in X-glu (GUS substrate) yielded positively stained cells at a low frequency (0.1 - 1.0%). Cell wall degrading enzymes were used to reduce interference from GUS expressing LBA4404 cells bound to sugarbeet cell surfaces prior to assay. Current aims are directed at enhancement of transformation frequency through the use of other B. vulgaris/A. tumefaciens genotype interactions, chemical induction of virulence regions and improvement of tissue culture methods. This cell line has breeding utility and is highly morphogenic in vitro; cells are being selected for resistance to geneticin prior to further analysis and regeneration.

M 162 EXPRESSION OF HOP STUNT VIROID FROM ITS cDNA IN TRANSGENIC TOBACCO PLANTS: IDENTIFICATION OF TOBACCO AS A HOST PLANT. Jun Yamaya, Masaharu Yoshioka, Teruo Sano[1], Eishiro Shikata[1] and Yoshimi Okada[2], Plant Laboratory, Kirin Brewery, Kitsuregawa-machi, Tochigi 329-14, Japan. 1: Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo 060, and 2: Department of Biophysics and Biochemistry, Faculty of Science, Tokyo University, Tokyo 113.
Hop stunt viroid (HSV) cDNAs were introduced into tobacco plants by the Ti plasmid mediated transformation. The replication of infectious HSV was detected in the transgenic tobacco plants carrying two tandemly repeated HSV cDNA sequences and also in their progeny plants. This is the first report of transgenic plants expressing viroids and offers a novel strategy for the study of viroid-host interactions. Previously, tobacco has been reported to be not susceptible to HSV infection. However, here we show that tobacco can be readily agroinfected with HSV, and also can be mechanically infected, although at a greatly reduced level of efficiency.

M 163 PLANT MOLECULAR BREEDING IN CHINA. Guang Yu Zhou, Lab., of Plant Molecular Genetics, Shanghai Institute of Biochemistry, Academia Sinica. 320 Yue-yang Rd., Shanghai 200031, People's Republic of China.
The technique of introducing exogenous DNA has been applied to cotton, rice, wheat, soya bean, cabbage, forage, oil tree and etc. New varieties have been breeded. Some of them passed their 5-11th gengeration on 30-700 hectares. The donor DNA as well as the recombinant DNA traversed the pathway of the pollen tube to enter the embryonic sac through the nucellus and transformed the germ line cell(s). The pathway of the pollen tube is not the pollen tube itself. It is formed physiologically after pollination for passing the pollen tube from the microphyle into the embryonic sac.
A domestic symposium of plant molecular breeding was held in Dezhou, China on May 1988 and a Society of Plant Molecular Breeding Research was thus organized.

M 164 GENETIC TRANSFORMATION OF GRAIN LEGUMES
D.Mariotti,G.S.Fontana,L.Santini, Istituto di Radiobiochimica ed Ecofisiologia Vegetali CNR,via Salaria Km29.300, 00016 Monterotondo Scalo,(Roma) ITALY. Legumes are widely cultivated crops so far only partially utilized in plant biotechnology devoted to genetic improvement. We report here the successful transformation of Phaseolus,Pisum and Cicer by means of the Agrobacterium-derived vector pBI 121, carrying the reporter gene GUS. The transformation procedure (co-cultivation) used for these species is based on a rapid and efficient method of plant regeneration from organs which strongly reduces the in vitro culture phase. The transformed state of regenerated plantlets was assessed by both Kanamycin resistance and GUS activity tests.

Gene Systems

M 200 SOME DIFFERENCES BETWEEN DOUGLAS FIR AND ANGIOSPERMS IN LIGHT REGULATED GENE EXPRESSION, M. Carol Alosi, David B. Neale, and Claire S. Kinlaw, Institute of Forest Genetics, USDA Forest Service, Berkeley, CA 94704. Conifer seedlings are green when grown in the dark and do not etiolate as most angiosperms do. This suggests that the expression of some photosynthetic genes in conifers may not be as dependent on light as is generally true for angiosperms. We are investigating three aspects of the development of the photosynthetic apparatus in Douglas-fir (Pseudotsuga menziesii) to identify differences between conifers and angiosperms in light-regulated gene expression. (1) Dark-grown Douglas-fir seedlings have 1/7th the chlorophyll per unit fresh weight as light-grown seedlings, while dark-grown oats have less than 1/600th that of light-grown oats. The 350-720 nm absorbance spectrum of dark-produced Douglas-fir pigment extract is similar to that produced by both angiosperm and gymnosperm tissues grown in the light, but is dissimilar to dark-grown oat tissue extracts. (2) Chlorophyll a/b binding (CAB) protein is not detectable in dark-grown angiosperm tissue but it accounts for over 9% of the total protein in thylakoid fractions of dark-grown Douglas fir. (3) Northern blots show that while cab messenger RNA is undetectable in preparations of dark-grown angiosperm tissue, there is significant expression in dark-grown Douglas-fir tissue. These results demonstrate that there are fundamental physiological differences between angiosperms and gymnosperms in response to light.

M 201 EFFECTS OF LIGHT QUALITY, IRRADIANCE LEVEL, AND TISSUE AGE ON MAIZE LHCP2 GENE EXPRESSION, Phillip D. Beremand and Kenneth Eskins, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1815 N. University, Peoria, IL 61604.
LHCP2 family member-specific clones (1) have been used to probe the accumulation of individual LHCP2 messages in maize plants (OP Golden Bantam) grown for 14 days under red or blue light (14 h light/10 h dark cycles). Irradiance levels examined ranged from 1.6 to 25 μmol m^{-2} s^{-1}. Separate preparations of total mesophyll RNA from first (old) and second (young) leaves were subjected to Northern blot analysis. Under controlled light, different LHCP2 family members were expressed at different levels. All family members that were expressed showed irradiance-dependent non-linear variation in mRNA accumulation. Differences between red-grown and blue-grown plants were most apparent in older tissue and were most pronounced at 8 μmol m^{-2} s^{-1}. The levels of individual LHCP2 RNAs in red-grown old tissue ranged from 2.5- to 7.3-fold higher than those seen in blue-grown old tissue at this irradiance level. We have also observed irradiance-dependent light-quality and tissue-age effects on various chloroplast-encoded photosystem I and II messages, although the patterns of expression were different. The results confirm and extend previous observations on the effects of light quality, irradiance level, and tissue age on chloroplast pigments, pigment-proteins, and LHCP2 mRNA accumulation (2).
1. Sheen, J. and Bogorad, L. 1986, Proc. Natl. Acad. Sci. USA, 83:7811.
2. Eskins, K. Westhoff, P. and Beremand, P.D. Plant Physiol., submitted.

M 202 **COMPARATIVE MOLECULAR STUDIES OF CHALCONE ISOMERASES.** E. Richard Blyden[a], Richard A. Dixon[b], Christopher J. Lamb[c], Eli Vriglandt[d] Arjen van Tunen[d], and Jos Mol[d]. Department of Biochemistry,Royal Holloway and Bedford New College, University of London, Egham Hill, Egham, Surrey TW20 OEX, U.K.;[a] Plant Biology Division, The Noble Foundation , P.O. Box 2180, Ardmore, Oklahoma 73402;[b] Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, California 92138, USA;[c] Department of Genetics The Free University, De Boelelaan 1087, 1081 HV, Amsterdam[d]
Chalcone isomerase (CHI) catalyses the isomerization of chalcones to their corresponding flavanones, a key step in the biosynthesis of flavonoid pigments in most plants and in the synthesis of microbially-induced antifungal phytoalexins in legumes (1). In bean cell cultures exposed to elicitor polysaccharides, CHI activity is induced as a result of transient increases in its mRNA (2). In petunia and Antirrhinum majus, CHI activity and its mRNA are highly expressed during floral development (3). We have recently published comparative enzymological and immunochemical data on CHI from bean and petunia (4). The absence of cross-hybridization between CHI clones from petunia and bean is explained by an unusually low level of DNA sequence homology. At the polypeptide sequence level however, differences between bean and petunia are relatively conservative. We present a comparative analysis of the CHI cDNA sequences and the deduced polypeptide sequences and structures from bean, petunia and Antirrhinum majus.

(1) Robbins, M.P. and Dixon R.A. (1984) Eur. J. Biochem. 145, 195
(2) Mehdy, M. and Lamb, C.J. (1987). EMBO J. 6, 1527.
(3) van Tunen, A.J. et al. (1988) EMBO J. 7, 1257
(4) Dixon et al. (1988) Phytochemistry 27, 2801

M 203 ANALYSIS OF FLORAL-SPECIFIC GENES, Kim A. Budelier, Charles S. Gasser, Alan G. Smith, Dilip M. Shah, and Robert T. Fraley, Plant Molecular Biology, 700 Chesterfield Village Parkway, St. Louis, MO 63198.
We are investigating genes whose expression is induced during the development of the floral organs of tomato (*Lycopersicon esculentum*). We have used a differential screening procedure to isolate cDNA clones of RNAs that are preferentially present in either pistils or anthers. *In situ* RNA hybridization to sectioned flowers shows that the expression of the genes corresponding to these cDNAs is confined to specific subsets of cells within the floral organs. The cDNAs have been used to isolate genomic clones from a library of tomato DNA. The transcription start sites for the genes have been identified by primer extension, and we have isolated the putative promoter regions for several of the clones. Assays of transgenic plants containing a chimeric gene construct in which the promoter region of a floral-specific gene is fused to the *E.coli* B-glucuronidase coding sequence showed identical tissue-specific expression as revealed in the *in situ* hybridizations.

M 204 STRUCTURE AND EXPRESSION OF TUBULIN GENES IN ARABIDOPSIS THALIANA, Jeffrey L. Carpenter, Steven D. Kopczak, Nancy Haas, D. Peter Snustad, and Carolyn D. Silflow, Department of Genetics and Cell Biology, University of Minnesota, St. Paul MN 55108
The small genome of A. thaliana contains at least four alpha-tubulin genes and at least eight beta-tubulin genes. We have constructed 3' noncoding gene-specific hybridization probes for three alpha-tubulin and seven beta-tubulin genes. These probes have been used in northern blot analyses to investigate the spatial and temporal expression patterns of these genes. The alpha1-, beta1-, and beta5-tubulin genes exhibit organ-specific patterns of transcript accumulation. Transcripts of the other seven genes are prevalent in all organs of the plant. Work is in progress to identify cis-acting regulatory sequences that control flower specific expression of the alpha1-tubulin gene. The minimal sequence required for normal expression will be identified using promoter deletions fused to the beta-glucuronidase reporter gene. The promoter will be analyzed in more detail using linker scanning techniques to selectively alter specific regions, thereby identifying promoter sequences that control tissue specific expression.

M 205 FERREDOXIN IN *ARABIDOPSIS* IS REGULATED BY PHYTOCHROME. Timothy Caspar, David Somers, and Peter Quail, Dept. of Molecular Plant Biology, Univ. of Calif., Berkeley, CA 94720 and USDA Plant Gene Expression Center, Albany, CA 94710 Ferredoxin is a soluble Fe-S protein which mediates electron transfer in chloroplasts. The ferredoxin transcript has been shown to be regulated by phytochrome in pea (Dobres, et al. Plant Mol Biol 8,53-59). In order to study the phytochrome regulatory system in *Arabidopsis*, we have cloned an *Arabidopsis* ferredoxin gene using a ferredoxin cDNA from *Silene pratensis* as a probe. Nucleotide sequencing of the genomic clone identified a 444 bp open reading frame which codes for a 148 amino acid polypeptide. The carboxy-terminal 97 amino acids of this sequence are 75% identical to the deduced *Silene* ferredoxin sequence, and the amino terminal 51 amino acids correspond well to known transit peptide sequences. S1 nuclease protection and primer extension analyses of poly A^+ RNA indicate a putative transcription start site 49 bp upstream of the initiator ATG codon. Genomic Southern analysis identified a second, weakly hybridizing band, suggesting the presence of another ferredoxin-related sequence. Northern blots using RNA from etiolated and white-light irradiated seedlings showed a 6-8 fold light-induced increase in ferredoxin mRNA. A 5 minute red light treatment produced a rapid, 3-5 fold increase in ferredoxin mRNA. The red light response was partially reversed by a subsequent far red treatment, indicating that the response is phytochrome mediated.

M 206 PCR SELECTION OF GENES BY A HOMOPOLYMER-PRIMED CDNA PRIMER A.A. Christen and B. Montalbano, U.S.D.A.-S.R.R.C., New Orleans, LA 70179 The polymerase chain reaction (PCR) has recently been developed to amplify defined regions of DNA. The technique utilizes two primers synthesized to complement a given sequence of each strand of DNA. In the present study, the use of PCR without custom oligonucleotides was shown to be possible in applications where sequence information was not available or where a standard primer is adequate. Poly d(A)'s or d(T)'s were used as primers on DNA templates containing a string of A's or T's. Single or double stranded DNA's were generated. Further use of the PCR products as specific primers was made possible by manipulation of incubation conditions. This enabled the short cDNA generated to be used to select the corresponding genomic DNA by a subsequent PCR.

M 207 EXPRESSION OF THE GRP1 GENE OF PETUNIA, Carol M. Condit*, B. Gail McLean and Richard B. Meagher, Department of Plant Sciences, University of Nevada-Reno, Reno NV 89557* and Department of Genetics, University of Georgia, Athens GA 30602. We have previously isolated, sequenced and transcriptionally characterized the GRP1 gene (glycine-rich protein 1) of petunia. We had hypothesized that this gene codes for a cell wall structural protein based on the fact that 1) the predicted amino acid sequence of the protein is 67% glycine, 2) the carboxy-terminal 317 amino acids of the protein should be capable of forming a ß-pleated sheet and 3) the first 27 NH_2 terminal residues comprise an optimal signal sequence for transport out of the cytoplasm. Using an antibody raised against a synthetic peptide comprising amino acids 22 thru 37 of the mature protein, in conjunction with cell fractionation studies, we have shown that this gene codes for a cell wall protein. In addition, we find that this protein is made at, at least, a 30-fold great rate in dividing cells or those just beginning to elongate than in mature fully elongated cells. We also find that the synthesis of this protein is increased after wounding. This pattern of GRP1 gene product expression is identical to its' transcriptional expression.

M 208 IDENTIFICATION OF A BRASSICA NAPUS GENE WHICH SHOWS CORTEX-SPECIFIC EXPRESSION. R. A. Dietrich, J. J. Harada, and C. S. Baden. Botany Department, Univ. of California, Davis, CA 95616. A cDNA clone designated pAX92 was obtained by differentially screening a cDNA library made from mRNA of B. napus seedlings harvested 14 hours after imbibition. The expression of the corresponding gene was characterized at various stages of embryogenesis, germination, and post-germinative growth using mRNA dot blots and in situ hybridization techniques. The results of the in situ hybridization experiments showed that the AX92 gene is expressed at detectable levels only in the ground meristem and mature cortex cells of the seedling axis. In the root meristems of both seedlings and mature plants, the message is present in cortex cells within one or two divisions of the cortical cell initials, but is not detected in the vascular cylinder, the root cap or their initials. The mRNA is abundant in the dividing and elongating cells of the ground meristem, but is less abundant in mature cortex cells. The mRNA dot blots showed that this message accumulates early in embryogenesis at a time that coincides with the stage when the embryonic axis is elongating at its maximal rate. The results indicate that AX92 mRNA accumulates specifically in cortex cells, and suggest that the gene may be involved in a determination event in cortical cell differentiation.

M 209 A BUD SPECIFIC TRANSCRIPT IN PEA IS POSSIBLY ASSOCIATED WITH CELL DIVISION

Michael S. Dobres[1] and William F. Thompson[2]. [1]Department of Bioscience, Drexel University, Philadlephia, PA 19104. [2]Department of Botany, North Carolina State University, Raleigh, NC 27695, USA.

The pEA207 transcript was previously characterized as a 1 kb nuclear encoded transcript that was abundant in both light and dark grown pea seedlings (1) . We have characterized the developmental expression of the transcript corresponding to the cDNA pEA207 and have found that it is only detected in the actively growing terminal bud of the pea plant. Expanded leaves immediately below the bud contain no detectable pEA207 RNA. In addition we are unable to detect this transcript in RNA isolated from roots, root tips, developing seeds and pods. A likely hypothesis is that pEA207 RNA only accumulates in dividing or recently divided cells. The deduced amino acid sequence pEA207 displays 49% identity with the phytohemaglutinin lectin of kidney bean (*Phaseolus vulgaris*)(2) and 37% identity to that of the major pea seed lectin (3) . The possibility that pEA207 is a cDNA for a non-seed lectin is considered.

1. Thompson et al., (1983) Planta 158, 487-500.
2. Hoffman and Donaldson (1985) EMBO J 4, 883-889
3. Higgins et al., (1983) J. Biol. Chem. 258, 9544-9549.

M 210 CYTOKININ TREATMENT OF *N. plumbaginifolia* CELLS INDUCES CHANGES IN mRNA LEVELS. Janice A. Dominov and Stephen H. Howell, Dept. of Biology C016, University of California, San Diego, La Jolla, CA 92093 and Boyce Thompson Institute for Plant Research, Tower Rd. Cornell University, Ithaca, NY 14853-1801. The mechanism of cytokinin action on plant cell growth and development is not well characterized, but numerous observations sugest it is likely to be complex, involving factors such as other hormones, light, changes in calcium levels, etc. Using *N. plumbaginifolia* suspension cells capable of growth in the absence of exogenous cytokinin, a culture system has been developed designed to directly identify changes in gene expression following cytokinin treatment. Addition of the cytokinin N^6-benzyladenine (BA) to these cultures in constant white light results in the gradual greening of the cells, characteristic of chloroplast development in response to cytokinin. A cDNA library has been constructed from 24 hr BA treated cells and differentially screened for clones representing mRNAs more abundant in the presence of BA than in untreated controls. Using several of these clones as probes, we have observed BA-induced changes in mRNA abundance within 2 hr of cytokinin treatment. Sequence analysis has revealed that among the selected population of clones are cDNAs homologous to calmodulin, S-adenosylmethionine (SAM) synthetase, and phenylalanine ammonia-lyase. This supports the concept that cytokinin treatment may indeed be affecting a variety of complex pathways such as those regulating calcium or SAM levels (the latter of which may affect ethylene or polyamine concentration and function) or pathways associated with a general stress response.

M 211 PHOTORESPIRATION AND LIGHT ACT IN CONCERT TO REGULATE THE EXPRESSION OF THE NUCLEAR GENE FOR CHLOROPLAST GLUTAMINE SYNTHETASE. Janice. W. Edwards and Gloria. M. Coruzzi, Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021

Several isoforms of glutamine synthetase (GS) are present in pea that differ in their subcellular location and pattern of expression. The GS isoforms are encoded by different nuclear genes that comprise a small gene family. One GS isoform (GS2) is located within the chloroplast where it functions in part to assimilate the ammonia generated during photorespiration. We have used gene-specific probes to study the external and metabolic factors involved in GS2 expression. The GS2 mRNA is present at low levels in etiolated pea and accumulates to high levels upon exposure to light. The light-induced accumulation of GS2 mRNA is mediated in part by the chromophore phytochrome. However, maximal accumulation of GS2 mRNA in white light occurs under conditions that promote photorespiration. Photorespiring peas (grown in 0.02% CO_2) contain four-fold more GS2 mRNA than nonphotorespiring plants (grown in 2% CO_2). The GS2 promoter has a complex structure that may reflect intricate gene regulation. Fragments of the GS2 promoter have been tested in transgenic plants to define the cis-acting DNA regions required for tissue-specific and light-regulated expression of the GS_2 gene.

M 212 **MOLECULAR CLONING OF E_1/E_0-TYPE ATPases IN HIGHER PLANTS.** N.N. Ewing, L.E. Wimmers, and A.B. Bennett. Mann Laboratory, Dept. of Vegetable Crops, Univ. of California, Davis, CA. 95616.

Two distinct families of cDNA clones, pPM1 and pPM2, corresponding to the E_1/E_0 class of ATPases were isolated from a tomato root cDNA library by screening with an oligonucleotide probe corresponding to a highly conserved ATP-binding domain. pPM1 hybridized to a relatively abundant 4.2 kb mRNA present at similar levels in both root and leaf tissue. pPM2 hybridized to less abundant mRNAs of 3.0, 4.5, and 6.0 kb in root tissue, and to a single and even less abundant 4.5 kb mRNA in leaf tissue. Genomic Southern analysis indicated that pPM1 and pPM2 hybridized to different restriction fragments and are present at low copy number in the tomato genome.

Sequence analysis of pPM2, a partial-length cDNA of 1.8kb, indicated that the cDNA encodes a polypeptide that includes a 37 amino acid region corresponding to the ATP-binding domain. Within this domain pPM2 is 78% similar to the CA^{2+}-ATPase of rabbit sarcoplasmic reticulum, and 73, 62, and 57% similar to the Neurospora H^+-ATPase, mammalian Na^+/K^+-ATPase, and the E. coli Kdp-ATPase, respectively. A 128 amino acid sequence immediately downstream from the ATP-binding domain is 80% similar to the Ca^{2+}-ATPase. This region is not conserved between Ca^{2+}- and H^+-, Na^+/K^+-, or Kdp-ATPases, but is 96% conserved between the fast and slow twitch forms of the Ca^{2+}-ATPase. Based upon this sequence similarity, pPM2 may encode a Ca^{2+}-ATPase while higher mRNA levels suggests pPM1 may encode the plasma membrane H^+-ATPase.

M 213 **ISOLATING DEVELOPMENTAL GENES FROM ARABIDOPSIS THALIANA USING THE T-DNA OF AGROBACTERIUM AS AN INSERTION MUTAGEN.** Kenneth A. Feldmann. E.I. DuPont, Wilmington, DE 19898.

More than 800 transformed lines of A. thaliana, segregating for a kanamycin resistance marker, are being screened for mutations that segregate in a Mendelian fashion. These transformed lines were generated by the seed infection method as described by Feldmann and Marks (MGG, 208:1, 1987). This methodology precludes variants induced by the tissue culture process (somaclonal variants). Mutants thus far observed include those that are affected in size, flower or trichome morphology, embryo-, gametophyte-, or seedling-lethality, etc. The first three mutants studied, a dwarf, a stem trichomeless and a flower mutant, have been shown to cosegregate with the kanamycin resistance marker. However, these particular mutations occur at a low frequency, even in chemically mutagenized populations, and as such the probability was high from the outset that they would have been caused by a T-DNA insert. By comparison, mutations that cause lethality, either at the embryo, gametophyte or seedling stage of development, occur frequently both in traditionally mutagenized and T-DNA tagged populations due in part to the large number of genes which control these characters. As such the probability that these mutants are caused by a T-DNA insertion may be reduced. To test these classes a group of mutants that are affected in fertility were tested for cosegregation of the mutation with the Kan^R marker. This class of mutants includes those affected in gametophyte and flower development. In fact, in one of these mutants the timing of filament elongation is affected such that pollen is not shed on the stigmatic surface. In another line, the anthers fail to shed pollen. Both mutations are recessive. These mutants will be described in detail along with cosegregation data showing the linkage of the T-DNA with the mutation. In addition, progress on the characterization of the dwarf mutant and cloning of the affected gene will be presented.

M 214 A GC-BOX IN A PLANT VIRUS PROMOTER BINDS NUCLEAR FACTORS AND ACTIVATES TRANSCRIPTION IN MAIZE CELLS, Carmen Fenoll*, Michel Schneider, Diane M. Black and Stephen H. Howell,D. Biology, UCSD, La Jolla, CA. *Present address: Dep. Biologia, Univ. Autnoma de Madrid, 28049 Madrid, SPAIN.

The common intergenic region of the geminivirus Maize Streak Virus (MSV) contains a major rightward promoter able to support transient expression of a reporter gene in maize protoplasts (Fenoll et al., EMBO J., 7:1589, 1988). We had identified a 122 bp fragment in this promoter that behaves as an Upstream Activating Sequence (UAS) of its own and of an heterologous core promoter, and that specifically binds maize nuclear factor(s). We have created a series of deletional and insertional mutant UASs and have studied their activity as transcriptional activators and their ability to bind the maize nuclear factors. The results, confirmed by footprinting techniques, indicate that the active element within the UAS both for gene expression and for factor binding is a direct repeat of a GC-box with homology to the animal Sp1-binding sequence. We have started the characterization of the nuclear factor(s) that bind to the GC-boxes and that are putative plant transcriptional activators, in terms of saturation kinetics and specific salt requirements for the formation of DNA-protein complexes. We have also demonstrated that a synthetic, multimeric GC-box competes efficiently for the binding of nuclear factor(s) with the native promoter sequence, indicating that the purification of the putative transcriptional factor by affinity chromatography (and subsequent clonnig of the gen) should be possible.

M 215 FURTHER CHARACTERIZATION OF THE Pi STIMULON IN TOMATO. Alan H. Goldstein, Liansheng Zheng and Avihai Danon, Dept. of Plant Sciences, The University of Arizona Tucson, Arizona 85721

We have characterized several molecular and physiological events associated with phosphate starvation inducible (psi) metabolism in tomato. Pi starvation increased the secretion of at least 6 proteins in suspension cultured cells. Cells exhibited a two phased response to Pi starvation. Secretion of three proteins was enhanced after transfer to Pi-depleted media while growth continued at the unstressed rate. Three additional proteins showed increased secretion only after the cells stopped growing. Immunoblots using antibodies that reacted specifically with Golgi-processed oligosaccharides showed that enhancement in secretion during both phases was Golgi-mediated. Nongrowing cells had a respiration rate twice that of growing (unstressed cells). These nongrowing cells secreted about 4.4 times the protein per unit cell as the unstressed cells. We also present data to show psi enhancement of Pi uptake. Three days after transfer to Pi-depleted medium, -Pi cells continued to grow at the rate of +Pi cells. The -Pi cells were capable of taking up Pi at about 2.7 times the rate of the unstressed cells. We also present data to show the involvement of protein phosphorylation in the early psi response. Three day old -Pi cells showed a unique phosphorylated protein of about 94 kDA. Finally, we have identifed several psi cDNA clones via +/- screening. Northern blot analyses confirmed that Pi starvation induced unique mRNAs that hybridized with these clones. Our data demonstrate that the higher plant Pi stimulon involves an integrated response that includes several molecular components. Our data further suggest that de novo gene expression is involved in some aspects of this response.

M 216 THE 5SrRNA (5S DNA) GENES OF PINUS RADIATA, Susan W. Gorman, Chris A. Cullis and Robert Teasdale[1], Department of Biology, Case Western Reserve University, Cleveland, OH 44106,[1]Bond University, Gold Coast, Australia. The gymnosperm genomes have been poorly characterized in comparison to those of the angiosperms. The multicopy 5SrRNA (5S DNA) gene was selected to begin investigating the genome organization of the conifer Pinus radiata (Monterey pine). Genomic DNA from P. radiata was enriched for ribosomal DNA on a CsCl/actinomycin D gradient and used to clone into the EcoRI site of pUC 19. Concurrently, a P.radiata library was constructed in EMBL 4 using Sau3A partially digested genomic DNA. Clones containing the 5S sequence were identified using an heterologous probe from flax. Genomic partial restriction digests indicate that the 5S DNA is organized in tandem arrays. A plasmid clone has been sequenced completely and has little variation in the transcribed region compared to 9 angiosperms. Restriction mapping reveals several different classes of phage clones, some of which do not exhibit crosshomology with the plasmid clone. The chromosomal organization of 5S genes was determined using in situ hybridization. The 5S genes are not located at a small number of specific sites, but are widely dispersed over the chromosomal complement. Some RFLPs for 5S genes from individuals taken across the P. radiata gene pool were found. Polymorphisms appear to be as varied between individuals derived from the same provenance as between the seven pine provenances.

M 217 MOLECULAR CLONING AND SEQUENCE OF A cDNA ENCODING THE PLASMA MEMBRANE PROTON PUMP (H^+ ATPase) OF *Arabidopsis thaliana*, Jeff Harper, Terry K. Surowy and Michael R. Sussman, Department of Horticulture, University of Wisconsin, Madison, WI 53706

In plants, the transport of solutes across the plasma membrane is driven by a proton pump (H^+ ATPase) that produces an electric potential and pH gradient. We have isolated and sequenced a full-length cDNA clone (AHA1, *Arabidopsis* H^+ ATPase) that encodes this enzyme in *Arabidopsis thaliana*. The protein predicted from its nucleotide sequence encodes 959 amino acids and has a molecular weight of 104,207 Da. The plant enzyme shows structural features common to a family of cation-translocating ATPases found in the plasma membrane of prokaryotic and eukaryotic cells, with the greatest overall identity in amino acid sequence (36%) to the H^+ ATPase observed in the plasma membrane of fungi. The structure predicted from a hydropathy plot contains a least eight transmembrane segments, with most (73%) of the protein extending into the cytoplasm and only 5% of the residues exposed on the external surface. Unique features of the plant enzyme include diverged sequences at the amino and carboxy termini as well as greater hydrophilic character in three extracellular loops.

Southern blot analysis suggests that the *Arabidopsis* genome contains two H^+ ATPase genes. We have isolated a genomic clone encoding the second H^+ ATPase (AHA2) and the predicted proteins from AHA1 and AHA2 are being compared.

In order to examine the effects of over-expressing a H^+ ATPase gene in plant cells we have cloned AHA1 downstream of the CaMV promoter in the binary vector pGA643 and used it to transform tobacco cells.

M 218 MOLECULAR CLONING, CHARACTERIZATION AND WOUND-INDUCTION OF TOMATO 3-DEOXY-D-ARABINO-HEPTULOSONATE-7-PHOSPHATE SYNTHASE.
John M. Henstrand, William E. Dyer, Klaus M. Herrmann[1] and Avtar K. Handa, Departments of Biochemistry[1] and Horticulture, Purdue University, West Lafayette IN 47907.
Wounding induces the synthesis of a variety of products involved in the plant repair/defense response. Many of these metabolites are derived from the shikimate pathway. 3-Deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, tne first enzyme of this pathway, was induced in mechanically wounded tomato (Lycopersicon esculentum var. Rutgers) immature green pericarp tissue. Using a potato probe encoding DAHP synthase (Dyer and Herrmann, J. Biol. Chem., submitted), we isolated the tomato clone by screening a turning stage tomato pericarp cDNA library. The tomato clone was used to probe total RNA from wounded tissue. DAHP synthase mRNA accumulated dramatically within 12 hours, suggesting transcriptional regulation. Immunoblots developed with antibodies raised against potato DAHP synthase revealed a concomitant increase of a single protein in tomato. Induction of DAHP synthase was not inhibited by ethylene action inhibitors. Wound ethylene, therefore, does not appear to participate in DAHP synthase regulation.

M 219 IDENTIFICATION OF DEVELOPMENTALLY REGULATED POLLEN SPECIFIC mRNAs AND PROTEINS IN BARLEY. R.D. Hill, J. D. Astwood, A. Hamel, and A. Silvanovich. Department of Plant Science, University of Manitoba, Winnipeg, Canada. Available Kentucky Bluegrass (KBG) pollen allergen cDNA clones and monoclonal antibodies directed against KBG pollen allergen proteins have been exploited to study pollen development in the agronomically important cereal crop, barley. We have previously described the identification and primary sequences of KBG pollen allergen cDNA clones. Total and poly(A^+) RNA obtained from mature pollen of both KBG and barley reveal transcripts of 1.5 and 1.2 kb, which correspond to clones c7.2 and c14.4 respectively. Total and poly(A^+) RNA was prepared from Barley pollen at the uninucleate, binucleate, and trinucleate stage of development. In vitro translation of barley RNA species revealed them to be biologically active. Northern analysis reveals that the 1.2 kb transcript appears only when barley pollen reach the trinucleate stage. Water extractable proteins from mature barley pollen were separated by SDS-PAGE and immunodetected with an enzyme-linked assay using monoclonal antibodies directed against KBG pollen allergens. Proteins approximately 33 and 37 kD are revealed in mature barley pollen. However, proteins extracted from developing barley pollen contain the 37 Kd protein at all stages, whereas the 33 kD protein appears in trinucleate stage pollen only. Therefore, the 1.2 kb mRNA species and the 33 kD protein are developmentally regulated.

M 220 A COMPARATIVE STUDY OF Ac AND Tam3 TRANSPOSITION IN TRANSGENIC PLANTS

Michel Haring, Caius Rommens, Tarcies Kneppers and Jacques Hille. Free University, Dept. of Genetics, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

With the aim to develop a "transposon tagging" system in tomato the transposable elements Ac and Tam3 have been studied for their ability to transpose in transgenic plants. A phenotypic assay has been applied to determine the occurrence and frequency of transposon excision. The Ac and Tam3 cassettes were cloned between promoter and coding region of a reporter gene: HPT-II or GUS. For both Ac and Tam3 this resulted in restoration of hygromycin resistance in 20-40% of the transformants analyzed. When the GUS gene was used to detect transposition a lower excision frequency (10-20%) was found. Southern blot analysis established that excision events of Ac and Tam3, as phenotypically monitored, coincided with integration elsewhere in the plant genome. The ability for continued transposition was subsequently shown both at the somatic cell level and after meiosis. Experiments aimed at influencing the transposition frequency in transgenic plant cells will be described.

M 221 CHARACTERIZATION OF GENES CODING FOR FIBER SPECIFIC mRNAs IN COTTON (Gossypium hirsutum L.)

Maliyakal John, Laura Crow, Grant Johnson, and Michael Petersen, Agracetus, Middleton, WI 53562. Five fiber specific cDNA clones were isolated through differential screening of cotton cDNA library. They were characterized in terms of transcript sizes, protein products, gene numbers and developmental expression. Soybean, corn and tobacco plant DNAs do not contain homologous sequences to these cDNAs, while fiber producing and non-fiber producing wild cotton species possess homologous genes. Structural characterization of these genes are underway. In cotton fiber, the steady state concentration of RNAs of three genes follow a pattern similar to the known cellulose deposition profile, suggesting functions in cellulose synthesis and/or deposition.

M 222 RETROTRANSPOSABLE ELEMENTS IN ARABIDOPSIS THALIANA

Andrzej Konieczny, Daniel F. Voytas and Frederick M. Ausubel, Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02114

Tal is a transposable element family in Arabidopsis thaliana which was identified by characterizing restriction fragment length polymorphisms caused by DNA insertion among 16 A. thaliana geographical races. Sequence analysis of a Tal element from the Landsberg race, Tal-3, showed that it is closely related to retrotransposons and to the Drosophila melanogaster copia element in particular. We have completed the nucleotide sequence of five additional Tal insertions, which together encompass all of the Tal elements found in three A. thaliana races. Two of these elements are identical in size (5.2 kbp) and appear to encode all of the necessary functions required for retrotransposition; the remaining elements have suffered either internal or terminal deletions. In addition, we have identified a second retrotransposable element family, Ta2. A member of this element family, Ta2-1, encodes a putative protein with a high degree of amino acid similarity to the Tal protein, despite significant divergence between this element and Tal at the nucleotide level. We have designed gene fusions to assay for transcriptional activity of these elements, as well as their ability to carry out reverse transcription and integration into target DNAs. We are currently testing these constructs in transient gene expression assays with plant protoplasts.

M 223 DIFFERENTIAL GENE EXPRESSION IN LATICIFERS OF RUBBER TREE (HEVEA BRAZILIENSIS), Anil Kush & Nam-Hai Chua, Lab of Plant Molecular Biology, The Rockefeller University NY, NY 10021-6399. Hevea braziliensis is a perennial tropical plant that belongs to the family Euphobiaceae & is used commercially to produce natural rubber, cis 1,4-polyisoprene. The latter is synthesized in the cytosol of specialized laticifer vessels which are arranged as sheaths concentric with the bark in the inner cortex of the tree trunk. Upon tapping, the laticifers are severed and their cytoplasmic contents are discharged as latex. We are interested in the regulation of genes involved in rubber biosynthesis as well as biogenesis of laticifers. To this end we have developed a method for the isolation of translatable polyA RNA and construction of a cDNA library from latex. In vitro translation profile of latex mRNA was very different from that of leaf, suggesting differential gene expression in the laticifers. Northern blot analyses using characterized cDNA probes revealed that genes encoding rubber biosynthetic enzymes (HMG-CoA reductase, HMG-CoA synthase, rubber elongation factor) are highly expressed in laticifers but not in leaf cells, whereas the reverse is true for photosynthetic genes (rbcS and Cab). Surprisingly, we found that latex contains high levels of mRNA encoding proteins that are normally associated with plant defense (e.g. chitinase, polygalacturonidase, phenylalanine ammonia lyase, chalcone synthase, and hydroxyprolein-rich cell wall proteins). These defense genes may be preferentially induced by tapping and/or application of ethapone which is used to stimulate the latex flow. We have also isolated and characterized cDNA clones encoding a new cell wall protein that is highly expressed in laticifers. This protein may be a component specific to the cell wall of laticifer vessels. Characterization of other latex specific cDNA clones are in progress.

M 224 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSES OF PLANTS PRODUCED BY *IN VITRO* ANTHER CULTURE OF *SOLANUM CHACOENSE* (BITT.). Benoit S. Landry, Sylvain Rivard, Gilles Vincent, Normand Brisson and Mario Cappadocia, Agriculture Canada Research Center, P.O. Box 457, St-Jean-sur-Richelieu, Québec, Canada, J3B 6Z8. In this study, a novel approach was used to characterize the genetic architecture of plants produced by *in vitro* anther culture of two lines of self-incompatible *Solanum chacoense* Bitt.(2n=2x=24). We used cytological observations to determine the ploidy level of the regenerated plants and scanned genomic DNA of the anther donor plants to identify heterozygous sequences. Restriction fragment length polymorphism (RFLP) analyses permitted the visualization of DNA variations. Several heterozygous DNA markers were found within single anther donor plants. Completely homozygous lines could be easily identified. Somatically derived plants could be separated from diploid plants produced from 2n (unreduced) microspores. Our results demonstrate first division restitution (FDR) as the mechanism operating during the production of 2n microspores in one of our *S. chacoense* line. Potential applications of RFLP analyses for genetic mapping, identification of lethal alleles and quantitative trait loci (QTL) with haploid or homozygous diploid plants and determination of gene-centromere distance with diploid plants derived from 2n microspores will be discussed.

M 225 **GENETIC TRANSACTIVATION OF *Ds* IN TRANSGENIC TOMATO, Michael W. Lassner and John I. Yoder, Department of Vegetable Crops, University of California, Davis, CA 95616. We have cloned maize transposable elements *Ac* and *Ds* into *Agrobacterium* vectors and transformed tomato. We have used wild type elements from maize and have constructed synthetic *Ds* and stable transposase (*Ts*) elements. The synthetic *Ds* elements contain the ends of *Ac* flanking beta-galactosidase genes; beta-galactosidase allows recloning of the elements by marker rescue. The *Ts* elements have one or both inverted repeats removed from the ends of *Ac* and transcription of the elements are controlled by several different promoters. The *Ts* and *Ds* elements were stable in transgenic tomato plants. We constructed F_1 hybrids between *Ds* and *Ac* or *Ts* transgenotes, and we monitored the activation of *Ds* elements by Southern hybridization. In F_1 plants containing both *Ds* and either *Ac* or *Ts*, *Ds* elements excised from their original locations and reinserted at new locations in the tomato genome.**

M 226 **CHARACTERIZATION OF RAPESEED MYROSINASE**, M Lenman, J Rödin, L-G Josefsson, and L Rask, Dep. of Cell Research Swedish Univ. of Agricultural Sciences, Uppsala, Sweden.
Many cruciferae contain glucosinolates, low molecular weight compounds consisting of a glucose residue derivatized in a thioglukoside bond to an amino acid. The glucosinolates seem to always be accompanied by a group of isoenzymes, thioglucoside glucohydrolase, also called myrosinase, which catalyzes the hydrolysis of the nontoxic glucosinolates to goitrogenic isothiocyanates, nitriles or thiocyanates. Partly purified rapeseed myrosinase was used as antigen to produce mouse monoclonal antibodies. Two sets of monoclonal antibodies were obtained: One of them reacts with at least three rapeseed components of approximately 70 kD, all of them with myrosinase activity. The other set of monoclonal antibodies reacts with two components of 52 and 55kD, both lacking myrosinase activity. Gelchromatography experiments resolved two myrosinase-containing fractions one with approximate m.w. 200.000, the other is size heterogeneous with m.w. ranging from 400.000 to 700.000. The high molecular fraction contains a complex consisting of two myrosinases and the 52 and 55kD proteins whereas the smaller fraction only contains 70kD chains.

M 227 **MANIPULATING BIOCHEMICAL DIFFERENTIATION IN THE CHILLI PEPPER FRUIT,**
Keith Lindsey and Jennifer F. Topping, Leicester Biocentre, University of Leicester, Leicester LE1 7RH, U.K.
The pungent principle of the chilli pepper fruit (*Capsicum frutescens*) is the acid amine capsaicin, being the product of the convergence of two metabolic pathways, the phenylpropanoid pathway from phenylalanine and a fatty acid pathway from valine. The accumulation of capsaicin in the fruit is regulated both spatially and temporally, being linked to the morphological development of the fruit. Biochemical aspects of chilli fruit development, and the synthesis of capsaicin in cell cultures, have been and are currently being characterized. A strategy for the isolation and manipulation of genes encoding enzymes of a complex metabolic pathway, and the relevance of this system to our understanding of tissue-specific biochemical differentiation, are discussed.
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M 228 CHARACTERISATION OF A RETROTRANSPOSON-LIKE ELEMENT IN WHEAT,Helene Lucas(1,2), Graham Moore(1), Richard B. Flavell(1).(1) IPSR, Maris Lane, Trumpington, Cambridge CB221Q, United Kingdom. (2) INRA, Station d'amelioration des plantes, domaine de Crouelle, Clermont Ferrand 63039, France
An insertion of about 8 kb called Wis-2 has been isolated from a HMW glutenin gene of the wheat variety Chinese Spring. Initial analyses indicated that it was not a Ac type transposon. Further analysis show that this inserted sequence shares structural features with retrotransposons. A 5bp duplication is found at its insertion site, it has 1.8 kb Long Terminal Repeats, small inverted repeats at both ends of the LTRs, and the internal domain has homology with a core sequence of reverse transcriptase genes.Related sequences are present in a high copy number in wheat as well as in Aegilops species, rye, barley and oat. Some individual plants of a variety of cultivated wheat have additional copies of specific variants of the retrotransposon-like element, suggesting its relatively recent activity.

M 229 **A NOVEL MAIZE DS TRANSPOSABLE ELEMENT CONTAINING A PLANT SELECTABLE MARKER TRANSPOSES IN ARABIDOPSIS THALIANA AND TOBACCO**, Robert V. Masterson[1], Douglas B. Furtek[2], Christoph Grevelding[1], and Jeff Schell[1]. [1]Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG; and [2]Institut für Genetik der Universität zu Köln, D-5000 Köln 41, FRG.

A mouse dihydrofolate reductase gene (DHFR) under the control of the cauliflower mosaic virus (CaMV) 35S promoter has been inserted within a maize nonautonomous transposable Ds element. This transposon (Ds-DHFR-1) can easily be monitored using methotrexate selection in plants which contain at least one element. The identification of mutants in which methotrexate resistance cosegregates with a mutant phenotype is facilitated by the use of an element containing a selectable marker. This chimeric element is able to transpose at a frequency similar to an unmodified element in transgenic tobacco protoplasts containing an autonomous Ac element. A phenotypic assay was used in which restoration of NPTII activity, hence conferring kanamycin resistance, resulted from the excision of Ds-DHFR from the leader sequence of an NPTII gene (Baker et al. 1987. EMBO 6:1547-1554).

M 230 PHYSICAL ANALYSIS OF ACTIN GENE CLUSTERS IN Petunia hybrida, Michael McLean and Richard B. Meagher, Dept. of Genetics, University of Georgia, Athens, GA 30602

A superfamily of actin genes has been characterized in several cultivars of Petunia hybrida. More than one hundred actin-related sequences have been observed in the genome of Petunia Mitchell (cv.). At least six subfamilies of actin genes have been characterized within this superfamily, and each subfamily contains multiple members. The segregation of restriction fragment length polymorphisms (RFLPs) among progeny from a series of genetic crosses between the Violet 23 cv. (V23) and Red 51 cv. (R51) demonstrated that the multiple members within each subfamily were genetically linked and that these six subfamilies resided on five of the seven Petunia chromosomes. Genetic recombinations have not been observed within each subfamily in more than ninety progeny, suggesting tight linkages for each cluster. The PAc4 subfamily and the PAc3/PAc7 subfamilies are the two largest clusters of actin genes. The PAc4 subfamily, which is on chromosome III, contains between four to eight members in V23 and R51 and might exist as a cluster on greater than 40 kilobase pairs (kbp) of DNA. The PAc3/PAc7 subfamilies, which comprise another cluster on chromosome IV, contain between five to eighteen members in V23 and R51 on perhaps more than 50 kbp. Because multiple probes and RFLPs exist for the members of these three subfamilies, the technique of pulsed field electrophoresis is being used to estimate the physical sizes of these two actin gene clusters.

M 232 CLONING AND SEQUENCING OF A CDNA CLONE ENCODING AN ETHYLENE-INDUCED PEROXIDASE FROM CUCUMBER COTYLEDONS, Peter H. Morgens, Ann M. Callahan, Linda J. Dunn, and Fred B. Abeles, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 24530

Ethylene-induced senescence of cucumber cotyledons is accompanied by a fifty-fold increase in a cationic, 33-kDa, pI 8.9 peroxidase (33-CPO) over a 2 day period. The function of peroxidases during senescence is not known, but peroxidase involvement in lignification and hence plant defence mechanisms has been proposed. A cyanogen bromide peptide fragment of 33-CPO was sequenced and found to be homologous with amino acid sequences in horseradish, turnip, and tobacco peroxidases. Using a 20-base mixed oligonucleotide synthetic probe based on the protein sequence, cDNA clones encoding a peroxidase were isolated from a cDNA library constructed from ethylene-induced cucumber cotyledons. mRNA homologous to these clones was induced by ethylene by 3 hours and plateaued by 15 hours. The induction is about 10 fold at the RNA level. DNA sequencing indicated that the two clones were derived from the same RNA species and that the cucumber amino acid sequence is homologous with a tobacco (55%), a horseradish (55%), a potato (41%), and a turnip (45%) peroxidase.

M 233 MOLECULAR STUDIES OF THE HIGH LYSINE GENES OPAQUE-2 AND OPAQUE-6 IN MAIZE, Natale Di Fonzo, Hans Hartings, Massimo Maddaloni, Jaume Palau, Francesco Salamini, Richard Thompson, Mario Motto,Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, 24100 Bergamo (Italy) and Max-Planck Institut für Züchtungsforschung, Köln, W. Germany.

We are interested to elucidate at the molecular level the mechanisms controlling the accumulation of zein, the major maize seed storage protein component. To date we have isolated clones for two genes giving rise to the high-lysine phenotype, Opaque-2 (O2) and the b-32 gene which is tightly linked to Opaque-6 (O6).

In order to study at the molecular level the b-32 gene, which appears to play a key role in mediating the action of the O2 locus, cDNA clones, containing sequences related to b-32 were isolated by screening a cDNA library. The nucleotide sequence of the putative full-length mRNA clone contains an open reading frame of 909 nucleotides which codes for a polypeptide of mol wt 32,430. From the structural analysis of the b32 protein it was possible to predict the existence of an acidic central domain separated by two compact domains, which may accomplish a regulatory role by interacting with specific chromatin structures located nearby the transcriptional machinery at the 5' flanking region of zein genes. The cloning and sequencing of three different b-32 genes, from two different inbred lines, indicated that the b-32 genes form a gene family showing polymorphism. Sequence heterogeneity affecting the whole central domain of the protein afford evidence for a complex role of the b-32 genes and b-32 products within a coordinated regulatory pathway. As far as the O2 locus is concerned, genetic analyses suggested that this locus codes for a positive, trans-acting, transcriptional activactor of the zein protein genes. Because the molecular cloning of the O2 locus and the analysis of its structure and expression, could contribute to an understanding of the nature of its regulatory effects, transposon tagging with the transposable element Ac (Activactor) was carried out and 14 mutable o2 alleles were obtained. One of these alleles (o2-m5) contains an autonomous Ac element. A DNA sequence flanking the Ac insertion was found to be O2 specific and provided a probe for the molecular analysis of the O2 locus. The structure of this gene was determined by sequence analysis of both genomic and cDNA clones. A putative protein, 499 amino acids in length was deduced from the sequence of the transcript. Translation of the O2 transcript in a reticulocyte system produces a polypeptide with an apparent mol wt of 58,000. The implication of the results will be discussed.

M 234 ISOLATION OF GENES FROM *Arabidopsis* BY COMPLEMENTATION OF MUTATIONS IN THE YEAST, *S. cerevisiae*. John T. Mulligan and Ronald W. Davis, Department of Biochemistry, Stanford University, Stanford, CA 94305

Mutations in a large number of basic metabolic, cell cycle and regulatory genes are available in *S. cerevisiae*, and the technology for genetics and molecular genetics is well developed. We are developing a system for isolating plant analogues of yeast genes by complementation of mutations in yeast. We have constructed an *Arabidopsis* cDNA library in a yeast centromeric vector. Expression of the cDNA is under the control of a tightly regulated promoter, pGAL1. We plan to test the library by complementing mutations in amino acid biosynthetic genes.

M 235 GENETIC ANALYSIS OF HEAT SHOCK PROTEIN SYNTHESIS IN WHEAT. Henry T. Nguyen, Department of Agronomy, Horticulture, and Entomology, Texas Tech University, Lubbock, Texas 79409. Improving heat tolerance in wheat using cellular and molecular technology will require a greater understanding of the genetic mechanisms of cellular and molecular responses of wheat to high temperature stress. Our research objectives are (1) to evaluate the genetic variability in the synthesis of heat shock proteins, and (2) to determine the regulation of genotype-specific heat shock gene expression and relationship to cellular thermal tolerance. Results will be presented to show substantial genetic variation, both qualitatively and quantitatively, in the synthesis of heat shock proteins (HSP) among several wheat genotypes. It appears that the higher level of synthesis of low molecular weight heat shock proteins (15-33 kDa) is correlated with cellular thermal tolerance, suggesting that genes coding for these proteins or regulating their synthesis may be involved in the genetic control of heat tolerance in wheat. Preliminary results on the in vitro translatable protein assays and Northern hybridizations using heterologous probes indicate that the higher level of HSPs synthesized in the heat tolerant genotype are correlated with the higher level of the corresponding HS mRNAs.

M 236 MUTATIONAL ANALYSES OF FLOWER DEVELOPMENT OF ARABIDOPSIS THALIANA, Kiyotaka Okada, Masako M. Komaki, Hideaki Shiraishi, Yoshiro Shimura, National Institute for Basic Biology, Okazaki, 444, Japan.

We have isolated a number of mutants of Arabidopsis thaliana that have defects in flower development and morphogenesis. The mutants have been classified in several groups on the basis of the stages of floral development and morphogenesis where presumptive genetic defects appear. Group 1 mutants have no flowers on the inflorescence axis, and are possibly defective in the initial step of reproductive growth. Group 2 mutants show altered number of floral organs, namely a mutant with no petals and a mutant having a huge pistil composed of 4 to 6 carpels. It is suggested that these mutants have genetic defects in stages forming primoridia of the corresponding floral organs. Group 3 mutants have abnormalities in mature structure of the floral organs, possibly due to defects in development of the organ primordia. Group 4 mutants in which we are mostly interested are homeotic mutants that have, for example, sepals in the position of petals, or carpels in the position of sepals. These mutants would have mutations in genes which correspond to developmental determination of floral organs. Most of the mutants isolated so far have been shown to have single, nuclear and recessive mutations. Several mutational loci have been mapped.

These mutants may prove useful for analysis of the genetic control of flower development and morphogenesis. Identification and isolation of the corresponding genes are currently in progress.

M 237 GENE EXPRESSION DURING PSEUDOMONAS SYRINGAE PV. TOMATO INFECTION, V. Pautot, B. Gaut, F. Holzer, and L. Walling, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

We are interested in identifying, isolating, and characterizing tomato genes which are induced during Pseudomonas syringae pv. tomato infection. A lambda gt10 cDNA library synthesized from infected tomato leaf mRNA was constructed and screened with a probe that was enriched for sequences that are expressed in a disease-resistant plant during infection. The cDNA clones isolated were classified using cDNA probes made from healthy, wounded, or infected disease-sensitive and disease-resistant plant leaf mRNA. Clones were classified into 9 distinct expression programs. Cross-hybridization studies are being done with each group of clones to determine the number of genes in each class. Selected clones of each class are being used in Northern blots studies to determine the abundance of their mRNA in healthy, wounded, infected disease-sensitive and disease-resistant tomato plant leaves. In addition, RNA blots will be used to establish the temporal and the spatial expression of these genes after infection by P. s. tomato.

We have defined the expression of a number of genes that encode known gene products (i.e., proteinase inhibitors I and II, phenylalanine ammonium lyase, chalcone, synthase) during P. s. tomato infection. Temporal variations in expression of these genes during P. s. tomato infection was observed.

M 238 RAPID SEQUENCE ANALYSIS OF MEMBERS OF DIVERSE ACTIN GENE FAMILIES USING DEGENERATE OLIGONUCLEOTIDES. Leslie Pearson, Wm Vance Baird and Richard B Meagher, Department of Genetics, University of Georgia, Athens GA 30602. The amino acid sequence of actins from all four eukaryotic kingdoms were compared and regions of conservation were determined. Oligonucleotides homologous to both the sense and nonsense strands of the corresponding DNA sequence encoding these conserved regions were synthesized. The oligomers are distributed throughout the entire actin coding sequence. The diverse superfamily of petunia actin genes were characterized by dot blot hybridization using these oligonucleotides as probes. A method is described in which degenerate oligonucleotides are used as primers in di-deoxy DNA sequencing reactions. Using the oligomers described here it should be possible to sequence actin genes isolated from most organisms.

M 239 EVIDENCE THAT TRANSPOSITION OF AN ARABIDOPSIS TRANSPOSON, Tat1, MAY OCCUR VIA AN EXTRACHROMOSOMAL INTERMEDIATE, Johan Peleman, Marc Van Montagu, and Dirk Inzé, Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium)

Analysis of eleven genomic clones containing the S-adenosylmethionine synthetase-1 gene (sam-1) [1] of Arabidopsis thaliana revealed the presence of a 431-bp insertion in the 3' end of sam-1 in one of these clones. The inserted sequence, called Tat1, has structural features of a transposon. It is flanked by a 5-bp duplication of the target site DNA and has 13-bp inverted repeats at its termini. A highly homologous element situated in a different genomic context was isolated from a genomic library. Genomic Southern analysis shows that there exist approximately 5 copies of Tat1 in the Arabidopsis genome. Different hybridization patterns are observed with DNAs derived from different varieties of Arabidopsis indicating that the element has moved since the divergence of these varieties . Moreover, analysis of individual plants from one Arabidopsis line revealed plants that were heterozygous for one Tat1 locus. These and additional data indicate that Tat1 is an active transposable element. Genomic Southern analysis of hundreds of individual tissue culture-derived plants revealed a few plants that contain extrachromosomal Tat1 DNA, presumably representing a transposition intermediate. Currently, experiments are in progress to study the excision of Tat1 from a reporter gene.

Reference

[1] Peleman, J., Boerjan, W., Engler, G., Seurinck, J., Botterman, J., Alliotte, T., Van Montagu, M., and Inzé, D. (1989) The Plant Cell 1, in press.

M 240 GENE RESCUE AND TRANSFER IN MAIZE VIA TRANSPOSON TAGGING, Peter A. Peterson, Agronomy Department, Iowa State University, Ames, IA 50011. With current needs for genes to transfer, prospects for genes to be isolated are more significant. Use of **transposon tagging** has already proven expedient for many of the genes that have been isolated. The transposon tagging method is not simply the use of any available transposon for use as a "tag." There are several features of transposons that aid in the tagging process. The **transposon neighborhood** of the originating transposon is the most likely site for a transposon to insert following transposition. The site of the **originating transposon** at a gene that can be monitored for the excision event in order to visualize and recover revertants and imperfect excisions (generally null) alleles is equally significant. Thirdly, use of an insert that excises at a high rate germinally (the **elixer transposon**) is the most important feature. Coupling the three features, neighborhood, gene site, and high exciser, would result in a most expeditious means of tagging a targeted site. This can be done by a crossing program that translocates chromosome arms containing the **"elixer transposon"** to the target site. This completes the process prior to assaying a population for the desired gene. Of course, one must not forget the efficient molecular probes (such as Ac or En) to complete the process because a high-copy number element would make the molecular tagging operation difficult. These features are illustrated.

M 241 STRUCTURE AND EXPRESSION OF TWO FAMILIES OF DEVELOPMENTALLY REGULATED GENES FROM ZEA MAYS. Vallés, M.P., Montoliu, L., Ruiz-Avila, L., Stiefel, V., Torrent, Ludevid, M.D., M., Rigau, J. and Puigdoménech, P. Departamento de Genética Molecular. CID-CSIC. Jorge Girona Salgado, 18. 08034 Barcelona. Spain.

Two groups of clones have been isolated from coleoptile and root cDNA libraries from maize hybridizing in parts of the young plantlet rich in meristematic tissues. One corresponds to a cell wall protein with a proline-rich highly repetitive structure, the other one to alpha-tubulin. The mRNAs corresponding to these cDNAs are abundant both in the meristematic zones of coleoptile and root but the cell wall mRNA is highly expressed in the coleoptile node and tne main alpha-tubulin mRNA is highly expressed in radicular tissues. The expresion of these genes in different types of calli in the presence and in the absence of auxin has been studied and they appear to be expressed mainly in highly dividing tissues. Therefore, a double control on the expression of these genes, organ-specific and cell-cycle dependent seem to act upon them. Both families of cDNAs correspond to small (less than 5) multigene family. In the case of alpha-tubulin genes specific probes for two of the genes show a differential expression in distinct parts of the root. Genomic clones have been identified for both families of genes and the corresponding structure will be presented.

M 242 DIURNAL VARIATION IN THE LEVEL OF MAIZE *CATALASE3* mRNA, *Margaret Redinbaugh and John Scandalios, Genetics Department, North Carolina State Univ., Raleigh, NC.*

In the green leaf of the maize seedling the three isozymic forms of catalase (CAT-1, CAT-2 and CAT-3) are all found, indicating that all three catalase genes (*Cat1*, *Cat2* and *Cat3*, respectively) are expressed. We observed that the steady-state level of the *Cat3* transcript varied with the time of day at which the plants were sampled. In order to further investigate this phenomenon, maize plants (*Zea mays* L. W64A) were grown for 6 days with a 12 h day at constant temperature and relative humidity. The leaves were sampled every 3 h for 3 days and used for the isolation of total RNA. The steady-state level of each of the catalase transcripts was then determined using S_1-nuclease protection analysis. The steady-state levels of the *Cat1* and *Cat2* mRNA's did not vary with any discernible pattern. However, the level of *Cat3* transcript clearly varied with a period of 24 h. The mRNA began to accumulate 4 to 7 h into the light period, with the highest levels found after 10 h of light. The mRNA was present 4 h into the dark period, but was not detectable after 7 h of darkness. To further investigate this phenomenon, plants were grown as described above, then transferred to continuous light or darkness for the sampling period. The fluctuation in the steady-state levels of the *Cat3* mRNA persisted in continuous darkness with the same pattern as described above. When the plants were transferred to continuous light, the variation in the level of *Cat3* mRNA continued, but with a slightly different periodicity. Thus, the diurnal variation in transcript level appears to be a circadian rhythm. We are currently investigating whether or not the changes in mRNA level are due to differential transcription of the *Cat3* gene.

M 243 ANAEROBIC TREATMENT ALTERS THE CELL-SPECIFIC EXPRESSION OF Adh-1, Sh, AND Sus GENES IN ROOTS OF MAIZE SEEDLINGS, Lisa J. Rowland, Yen-Ching Chen, and Prem S. Chourey, USDA/ARS, Department of Plant Pathology, University of Florida, Gainesville, FL 32611

We have examined the in situ expression pattern of Sh and Sus which encode sucrose synthase isozymes SS1 and SS2 respectively and Adh-1 which encodes alcohol dehydrogenase 1 (ADH1) in the lower region of the primary root of maize seedlings in response to anaerobiosis. In situ hybridization and/or immunolocalization experiments revealed a unique spatial pattern of expression for each of the three genes. Anaerobic induction of ADH1 RNA was localized to the epidermis and cortex. Induction of Sh was marked by highly elevated SS1 RNA levels in the vascular elements, pith, and epidermis. A significant but less drastic increase in SS protein was found in these same tissues as well as the root cap; the increased level of immunosignal was, however, restricted to cells within about 1 centimeter of the root apex. The specific response of the Sus gene to anaerobic stress was determined using a sh deletion mutant; Sus responded with a slight reduction in SS2 RNA and protein levels except in the root cap where SS2 protein, but not SS2 RNA, was induced. These data indicate that multiple regulatory controls including cell-specific post-transcriptional mechanisms modulate SS levels in anaerobically-stressed seedlings.

M 244 **HOST-SPECIFIC BYPASS OF THE POLYADENYLATION SIGNAL IN THE CAULIFLOWER MOSAIC VIRUS (CaMV),** **Helene Sanfacon, Jean-Marc Bonneville, Johannes Futterer and Thomas Hohn, Friedrich-Miescher Institut, P.O. Box 2543, CH-4002, Basel, Switzerland.**

CaMV is a DNA plant virus replicating through reverse transcription. Its genomic RNA (35S RNA) has a terminal redundancy including the polyadenylation signal. This signal is therefore recognize only at the 3' end of the molecule.

With a transient expression system, we could show that the bypass of the upstream polyadenylation signal occurs efficiently in CaMV host plants (Crucifers) but very inefficiently in the non-host plants tested (Solanaceas). We propose that the specificity of this bypass is one of the factors limiting the CaMV host range.

The CaMV polyadenylation signal was further analysed in host and non-host plants with deletion or point mutations. As in animal cells, the AATAAA consensus sequence is required for mRNA processing. This is true in all the plants tested. Several additional sequences upstream of this AATAAA are also necessary for mRNA processing, especially in non-host plants.

M 245 ANALYSIS OF 5S rRNA GENES IN FLAX (Linum usitatissimum); ORGANIZATION OF 5S rRNA GENE VARIANTS IN FLAX GENOTROPHS, Richard G. Schneeberger, Gary P. Creissen and C.A. Cullis, Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106, * John Innes Institute, Norwich, U.K.

Large changes in the copy number of 5S rRNA genes as well as several other repetitve sequences have been observed in flax genotrophs. The copy number is decreased by over 50% in the genotroph LH. The 5S rRNA genes in flax are organized as dispersed tandam arrays of several size and sequence classes. *in situ* hybridizations indicate that the 5S genes are dispersed on many chromosomes unlike most plants. Several sequence classes of 5S rRNA genes have been identified through characterization of 11 LH genomic clones. Sequence analysis indicates a high degree of variation between and within the different classes. One class which is present at approximately 50 copies per 2C identifies a set of genotroph specific RFLPs. The RFLP patterns for the genotrophs derived from several independent induction experiments are the same. The nature of the RFLPs and their use in studing environmentally induced variation is discussed.

M 246 MOLECULAR ANALYSIS OF DICOT AND MONOCOT snRNA POPULATIONS, Daniel B. Egeland, Brian Hanley and Mary A. Schuler, Departments of Plant Biology and Biochemistry, University of Illinois, Urbana IL 61801

Oligonucleotides directed against conserved small nuclear RNA (snRNA) sequences have been used to identify the individual U1, U2, U4, U5, and U6 snRNAs in dicot and monocot nuclei. The plant snRNA populations are significantly more heterogenous than the mammalian or *S. cerevisiae* snRNA populations. U6 snRNA exists as a single species of the same size in monocot and dicot nuclei. The abundance and molecular weights of the U1, U2, U4 and U5 snRNAs expressed in monocot and dicot nuclei are significantly different. Whereas dicot nuclei contain one predominant form of U2 snRNA and a small number of U4 snRNAs, monocot nuclei contain multiple forms of U2 snRNA ranging from 208 to 260 nucleotides and multiple forms of U4 snRNA from 159 to 176 nucleotides. Multiple forms of U1 and U5 snRNA exist in both plant groups. All size variants of U1, U2, U4 and U5 snRNA identified in monocot nuclei can be immuneprecipitated with anti-trimethylguanosine antibody. We conclude that the sizes and number of snRNA molecules involved in intron excision differ considerably in dicot and monocot nuclei. We have cloned several of the U1-U6 snRNA variants and have demonstrated that the U1 and U5 molecular weight variants have primary sequence differences that may be responsible for establishing different snRNP structures. In addition, we have purified plant snRNP particles in order to examine RNA:protein interactions involved in pre-mRNA splicing.

M 247 MOLECULAR GENETICS OF THE CAROTENOID-ABA PATHWAY IN TOMATO PLANTS, Norberto Iusem, Dolores Bartholomew, Glenn E. Bartley and Pablo A. Scolnik, E. I. du Pont de Nemours and Co., Central Research and Development Department, Plant Science Group, Experimental Station, Wilmington, Delaware 19880-0402. Carotenoids are essential to protect photosynthetic and non-photosynthetic organisms against photooxidative damage. In plants, they also appear to be precursors of the phytohormone ABA. To analyze the structure and function of phytoene dehydrogenase, a key enzyme in the biosynthetic pathway, we used the corresponding gene from the photosynthetic bacterium *Rhodobacter capsulatus*. An antibody prepared against the overexpressed protein *E. coli* recognizes an intrinsic membrane protein from *Rhodobacter capsulatus*. Genetic analysis of this locus suggests that this enzyme has both a catalytic and a structural function. We will discuss the possible implications of these observations on chloroplast differentiation in higher plants. To study the effect of the carotenoid-ABA pathway on plant nuclear gene expression, we used the tomato *sit* mutant, which is blocked in the synthesis of ABA. We determined that water stress has both positive and negative effects on the transcription of nuclear genes, and that this effect is mediated by ABA.

M 248 CHARACTERIZATION OF IN VIVO RNA STRUCTURE IN SOYBEAN LEAVES BY CHEMICAL MODIFICATION AND PRIMER EXTENSION, Julie F. Senecoff and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, Georgia 30602. The structure of RNA molecules is important in many biological processes. In order to study the native structure of various RNA molecules, we have developed a method to modify RNA directly within leaf tissues. Dimethyl sulfate is used as a reagent to probe single stranded adenine and cytosine residues within the RNA. This method has been used to partially characterize the secondary structure of soybean 18S ribosomal RNA. We have also begun to study the modification pattern of the messenger RNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. This gene is transcriptionally induced in response to light. Additional evidence suggests that this RNA is differentially stable in light and darkness. We are investigating the structure of this RNA under different growth conditions to ascertain whether structural changes correlate with altered stabilities.

M 249 POST-TRANSCRIPTIONAL REGULATION OF RUBISCO SMALL SUBUNIT GENE EXPRESSION IN RESPONSE TO LIGHT IN SOYBEAN SEEDLINGS, Brenda W. Shirley and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, GA 30602. Transcription of the Rubisco small subunit (SSU) genes varies over a 32-64 fold range in response to light in soybean seedlings. In contrast, the SSU steady state RNA levels vary only 8 fold, indicating that SSU RNA turns over more rapidly in the light than in darkness. This high turnover rate appears to be rapidly induced by light and to be maintained for some time in darkness. An increase in RNA turnover is not a general response to light in these plants as actin genes have equivalent transcription rates and mRNA levels under all conditions. The distribution of RNA in polysomal and nonpolysomal fractions under different light conditions has been examined. These experiments indicate that the large transcriptional increase observed for the SSU genes in the light is reflected in a large increase in SSU RNA associated with ribosomes. These observations suggest that the light regulated turnover of SSU RNA is associated with translation.

M 250 CHARACTERIZATION OF S-LOCUS GENES FROM PETUNIA HYBRIDA, Thomas L. Sims, K. Reed Clark, John Okuley, and Pamela Collins, Dept. of Molecular Genetics, Ohio State University, Columbus, OH 43210
As an initial step in investigating the molecular basis of gametophytic self-incompatibility, we have cloned putative S-locus genes from Petunia hybrida lines containing S_1, S_2, and S_3 alleles. One clone, termed PHSB, has been partially characterized by DNA and RNA hybridization, restriction mapping and DNA sequencing. The PHSB clone hybridizes specifically to a 950 nt mRNA from $S_{1,2}$ and $S_{1,1}$ styles, and hybridizes to $S_{3,3}$ style mRNA when low strigency conditions are employed. PHSB does not hybridize detectably to mRNAs from leaves, petals, or anthers. The mRna detected in these experiments is highly prevalent in mature styles, but is present at much lower amunts in styles from immature floral buds. The PHSB gene is single or low copy, and DNA sequence analysis indicates that the PHSB cDNA is homologous to the S_2 cDNA of Nicotina alata [Anderson et al, (1986) Nature 321 38-44]. Taken together, these data suggest that the cDNA clones we have isolated represent S-locus genes of Petunia hybrida. We are presently using these cDNAs as tools for the initial characterization of the structure and expression of Petunia S-locus genes.

M 251 REGULATION OF SOYBEAN GENE EXPRESSION BY JASMONIC ACID, Paul E. Staswick, Dept. of Agronomy, University of Nebraska, Lincoln, NE 68583 Soybeans accumulate a storage glycoprotein which accounts for about 10% of the soluble protein in leaves, stems and pods. This vegetative storage protein (VSP) is preferentially degraded during seed development, but accumulates to 50% of the leaf protein if seeds are continuously removed. VSP mRNA levels increase rapidly when export of leaf nutrients via the phloem is blocked. Nitrogen deficiency depresses VSP gene expression while it is greatly elevated in nitrogen enriched plants. These results are consistant with the VSP's function as a transient storage molecule when sufficient precursors are available. Treatment of soybean suspension cell cultures with sub-micromolar concentrations of jasmonic acid (JA) increase VSP mRNA levels by 2-fold within an hour and about 30 fold within 24 hr. JA also increases VSP mRNA and protein accumulation when sprayed on leaves or when taken up by the transpiration system of soybean explants. An ELISA assay for JA is being developed to determine whether endogenous JA levels in soybean correlate with the regulation of VSP gene expression.

M 252 CARROT SOMATIC EMBRYOGENESIS DEPENDS ON THE PRESENCE OF CORRECTLY GLYCOSYLATED EXTRACELLULAR PROTEINS, P. Sterk, H. Booij, J. Cordewener, F. van Engelen, S.C. de Vries, A. van Kammen - Department of Molecular Biology, Agricultural University Wageningen, Dreijenlaan 3, 6703HA Wageningen, the Netherlands.
Carrot somatic embryogenesis was shown to depend on the presence in conditioned medium of several correctly glycosylated extracellular proteins. This result was based on the ability of extracellular protein preparations to complement several non-embryogenic variant carrot cell lines and tunicamycin-blocked embryogenic cell lines (De Vries *et al.* 1988, Genes and Development 2, 462-476). Polyclonal antibodies were raised against λgt11 lacZ fusion proteins corresponding to embryo extracellular proteins. Two of the extracellular proteins were found to be present as $CaCl_2$-extractable cell wall proteins of 52/54 kDa, that may depend on proper glycosylation to retain their presence in the cell wall. The predicted amino acid sequence of the 52/54 kDa protein showed no homology with known plant cell wall proteins. Based on the finding that complementation of tunicamycin blocked embryogenesis was only possible during the first day after transfer of proembryogenic masses to hormone-free medium, the observed disruption of proembryogenic mass integrity after treatment with tunicamycin and the localization of the 52/54 kDa protein we propose a function for these proteins in the maintenance of proper cell-cell adhesion in proembryogenic masses. Apparently the aggregation of a limited number of meristematic cells characteristic for proembryogenic masses is of crucial importance to the subsequent highly organized development of somatic embryos from proembryogenic masses.

M 253 EVIDENCE FOR DEVELOPMENTAL CONTROL OF SSU RNA TURNOVER, Deborah Marin Thompson and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, Georgia 30602. The RNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (SSU) is regulated in response to light. Changes in the transcription rate and steady state levels of RNA of two genes encoding SSU were compared in mature (6 week old) petunia plants. In these plants, changes in transcription rates were accompanied by equivalent changes in steady state levels of the SSU RNAs. Hence, mature petunia plants regulate SSU RNA expression in response to light primarily by regulating transcription. In contrast, SSU expression is regulated by changes in both transcription rate and turnover rate in soybean seedlings. We propose that the differential turnover of the SSU RNA is related to the developmental change which occurs when etioplasts are induced by light to develop into chloroplasts. By comparing changes in transcription rates with changes in the steady state levels of the SSU RNA in light grown and dark-adapted mature soybean plants and in etiolated and light grown petunia seedlings, we will determine if the differential stability of SSU RNA is developmentally regulated.

M 254 REGULATION OF GENES ENCODING ASPARTATE AMINOTRANSFERASE, Frank J. Turano, Barbara J. Wilson and Benjamin F. Matthews, Plant Molecular Biology Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705

The molecular mechanisms that regulate and orchestrate the expression of genes encoding isoenzymes in various plant organelles are not fully understood. To gain a better understanding of these mechanisms, we have chosen to investigate the differential expression of aspartate aminotransferase (AAT) isoenzymes in plants. AAT plays a key role in nitrogen metabolism, hydrogen shuttles (malate-aspartate), and carbon shuttles. We have purified a carrot AAT isoenzyme to apparent homogeneity (> 150 fold) and are characterizing the isoenzyme physically and biochemically. The isoenzyme has a molecular weight of 105±5 kD. It appears to be comprised of two subunits of approximately 48±3 kD. Antibodies to this AAT isoenzyme will be used to screen expression cDNA libraries and for purification of AAT by immunoaffinity chromatography. Heterologous probes were used to screen a cDNA library. Five clones containing AAT genes have been identified and isolated. The size of the cDNA inserts ranges from 200 bp to 600 bp. This approach will enable us to 1) gain a better understanding of the function and regulation of the AAT isoenzymes in plants, 2) study and understand the complexity of the AAT gene family and 3) identify and characterize the gene(s) encoding the different isoenzymic forms of AAT.

M 255 EFFECTS OF T-DNA CYTOKININ AND AUXIN GENES ON HORMONE CONCENTRATIONS, GROWTH AND DIFFERENTIATION OF WILLOW (*SALIX* SP.) CALLUS.
Tiina Vahala, Peter Engström and Tage Eriksson, Department of Physiological Botany, University of Uppsala, Box 540, S-751 21 Uppsala, Sweden.

Shoot regeneration of willow is difficult to obtain in tissue culture. In attempts to induce differentiation by altering endogenous levels of plant growth regulators, we transformed willow with strains of *Agrobacterium tumefaciens* that carry the T-DNA cytokinin biosynthesis gene (gene 4) of strain C58 and with the wildtype strain C58. Stem segments of willow clones were cocultivated with the bacteria and stably transformed callus was obtained. In the transformants, the growth rate was increased and root formation, characteristic to non-transformed callus, was inhibited, but no shoot differentiation was observed. This is in contrast to tobacco, where expression of the T-DNA gene 4 induces shoot development. We are currently determining concentrations of plant growth regulators in the transformed callus of both species using a procedure that includes purification with an immunoaffinity column, separation with HPLC and an ELISA assay. Relationships between these results and the morphological and physiological changes observed will be discussed. Furthermore, effects of expression of a heat shock inducible cytokinin gene in transformed callus are compared to those of a constitutively expressed gene.

M 256 BEHAVIOR OF Ac IN DAUCUS COROTA AND ARABIDOPSIS THALIANA, Marie Anne Van Sluys and Jacques Tempe, Institut de Microbiologie, Batiment 409, Universite de Paris Sud, 91405 Orsay, France

The maize transposable element Ac has been shown to transpose in carrot and Arabidopsis thaliana hairy root transformants (Van Sluys et al., EMBO J. 6, 3881-3889, 1987). Further investigations on carrot hairy roots and regenerants demonstrated continued transposition of Ac. We have studied the excision process by restriction analysis of empty donor sites. In several instances excision was accompanied by methylation of sequences close to the original insertion site. When several copies of Ac had been present in the same cell, it was found that they all had excised and the same pattern of methylation was observed for all the empty donor sites. In other instances excision was accompanied by loss of the transposable element, even when several copies of Ac had been present in the same cell. These results are suggestive of concerted excision-transposition processes in cells containing several copies of Ac element. No further transposition was observed in the progeny of A. thaliana lines in which this process had been previously detected.

M 257 REGULATION OF CHALCONE FLAVANONE ISOMERASE (CHI) GENE-EXPRESSION IN *PETUNIA HYBRIDA*: THE USE OF ALTERNATIVE PROMOTERS IN COROLLA, ANTHERS AND POLLEN. A.J. van Tunen and J.N.M. Mol, Department of Genetics, Section Biosynthesis Secondary Metabolites, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Flavonoids are plant secondary metabolites which serve important functions in flower pigmentation, protection against environmental stress and induction of nodulation genes. One of the enzymes of flavonoid biosynthesis is Chalcone Flavanone Isomerase (CHI). CHI in *P. hybrida* is encoded by only two genes designated as CHI-A and B. Both genes are extensively characterized with respect to genomic organization, structure and expression pattern. The CHI-A gene does not contain intervening sequences and its expression is regulated by two distinct promoters: PA_I and PA_{II}. PA_I is active in corolla, tubes and in UV-irradiated seedlings whereas the PA_{II} promoter is activated late in anther development and specifically in pollengrains. The CHI-B gene contains three introns and is regulated by a single promoter P_B. The P_B promoter is only active in immature anthers (eg. tapetum cells). Comparison of P_B with other flavonoid gene promoters active in anther tissue (Chalcone Synthase and Dihydroflavonol-reductase) revealed a highly homologous region, called the antherbox which is presumably involved in regulation of anther-specific gene expression.

The specificity of P_{AII} and P_B suggests their use in influencing pollen development and fertility. We have therefore started to fuse these promoters to genes known to possess lethal effects in plant cells and analyze their effect in *planta*.

M 258 MOLECULAR GENETICS OF TOBACCO NITRATE REDUCTASE

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Nitrate reductase (NR) catalyses the second step of nitrate assimilation and is considered as a limiting step of nitrogen metabolism in higher plants. NR deficient mutants have been isolated on the basis of chlorate resistance. The genetic analysis of 200 mutants of Nicotiana plumbaginifolia lead to the determination of 7 complementation groups. One of them (NIA) corresponds to the structural gene encoding the apoprotein. The 6 other (CNX 1 to 6) are involved in the biosynthesis of the molybdenum cofactor of the enzyme (MoCo). We have cloned and sequenced NR cDNAs and the corresponding genes from tobacco, an amphitetraploid species. cDNAs have been used as probes to determine the level of NR mRNA under different physiological conditions including nitrogen starvation, continuous light or darkness, or to study the regulation of NR deficient mutants. Several levels of regulation have been deduced from these experiments. Nitrate and light promote the accumulation of the NR mRNA in leaf tissues. The mRNA level is also under the control of a circadian rythm. Mutants defective for nitrate reductase are deregulated with respect to the circadian rythm but not for nitrate induction. Genomic clones carrying the wild type allele of the tobacco NR gene have been transferred to tobacco and N. plumbaginifolia NR deficient lines. Complemented transformants, able to grow on nitrate, have been rescued and analysed. The introduced gene is expressed at a low level but is correctly regulated. Promoter sequences have been fused to the glucuronidase (GUS) coding sequence and transferred into tobacco. Studies including deletion of cis-acting domains and isolation of trans-acting factors are under progress.

M 259 ENZYMOLOGY OF THE UBIQUITIN CONJUGATION SYSTEM IN PLANTS

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Ubiquitin is a highly conserved eukaryotic protein involved in several important regulatory processes through its covalent attachment to a variety of target proteins. We have purified several of the enzymes involved in ubiquitin conjugation from wheat germ, including ubiquitin activating enzyme, 4 functionally different ubiquitin carrier proteins, and ubiquitin isopeptidase. Three isoforms of ubiquitin activating enzyme are present *in vivo* with molecular masses ~120 kDa. Antibodies against this enzyme also recognize ubiquitin activating enzyme from yeast and rabbits indicating that substantial conservation among these proteins exists. Of interest is a 23-kDa ubiquitin carrier protein that has amino acid sequence homology to the RAD6 gene product required for DNA repair in yeast. One stretch near the putative active site is 76% homologous when conservative substitutions are included. The RAD6 protein has been shown recently to be a ubiquitin carrier protein that specifically ubiquitinates histones. Like RAD6 protein, the 23-kDa species transfers ubiquitin specifically to wheat germ histones in the presence of ATP and ubiquitin activating enzyme. Homologous proteins to the 23-kDa species also have been detected immunologically in a variety of plant species. This ubiquitin carrier protein represents one of the first enzyme identified to be involved in DNA repair in plants. Efforts to characterize ubiquitin conjugating enzymes at the molecular level are in progress.

M 260 IDENTIFICATION OF AN ASSEMBLY DOMAIN IN THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE. Catherine C. Wasmann, Robert T. Ramage, Hans J. Bohnert and James A. Ostrem, Department of Biochemistry, University of Arizona, Tucson, AZ 85721, USA In higher plants the mature small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase contains a highly conserved sequence of 16 amino acids that is absent in the SSUs of cyanobacteria. To determine whether this region of the SSU of higher plants has a specific function, portions of the SSU genes (rbcS) of pea (Pisum sativum) and the cyanobacterium Anacystis nidulans were fused to create chimeric genes which either lacked or contained the coding sequence for the 16 conserved amino acids. Radioactively labeled precursor proteins synthesized from mRNAs transcribed from the chimeric genes were incubated with isolated pea chloroplasts to assay import and assembly into the holoenzyme. Fusion proteins lacking the 16-amino acid sequence were imported and processed but failed to assemble with endogenous large subunit (LSU). Addition of a region from a pea rbcS containing the 16 amino acids to the rbcS of Anacystis enabled the imported SSU fusion protein to assemble with pea LSU. This amino acid sequence is encoded by a separate exon in certain rbcS genes of some higher plants. We propose that the conserved 16-amino acid sequence constitutes a domain acquired to facilitate assembly of the eukaryotic holoenzyme.

M 261 ISOLATION OF PROBES FOR RFLP MAPPING OF THE SOYBEAN GENOME, Jane M. Weisemann, Dennis A. Schaff, and Benjamin F. Matthews, U.S. Dept. of Agriculture, Plant Molecular Biology Laboratory, Beltsville, MD 20705.
The identification of restriction fragment length polymorphisms has proved to be a valuable tool for developing genetic linkage maps. We are using this technique for mapping the soybean (Glycine max) genome. Towards this end we have isolated probes for RFLPs. Three sources have been explored for probes: random cDNA fragments from lambda libraries, random genomic DNA fragments from a PstI digest of total soybean DNA, and cloned DNA fragments from known soybean genes. The probes have been used to test five soybean cultivars for polymorphisms. All three sources of probe DNA have yielded polymorphic probes.

M 262 ISOLATION AND CHARACTERIZATION OF TWO cDNA CLONES FOR RICE LECTIN DERIVED FROM TWO DEVELOPMENTALLY REGULATED mRNA SPECIES, Thea A. Wilkins and Natasha V. Raikhel, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824. Two cDNA clones encoding rice lectin have been isolated and characterized to investigate the expression of rice lectin at the molecular and cellular levels. Immunoprecipitation of *in vitro* translation products and nucleotide sequence analysis indicate that both cDNA clones code for the identical polypeptide. The clones, which differ solely in the length of their 3'-untranslated region, correspond to two mRNA species that are differentially expressed during embryo development. Southern blot analysis established that two lectin mRNAs are derived from a single gene via alternative polyadenylation site selection. At the cellular level, lectin mRNA accumulates in specific cell-layers of developing embryos. Thus, this is the first report of the temporal regulation of multiple mRNA transcripts in plants.

M 263 CHARACTERIZATION OF TWO HIGHLY HOMOLOGOUS GENES FROM PEA WITH VERY DIFFERENT ORGAN-SPECIFIC EXPRESSION PATTERNS, Mary E. Williams and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021-6399.
Using a cDNA isolated from a Pisum sativum (var. Feltham First) stem library as a probe (de Vries, S.C., de Vos, W.M., Harmsen, M.C., and Wessels, J.G.H., 1985, Plant Molecular Biology 4:95-102), we have cloned and sequenced two pea genes with extensive sequence homology. One of these genes is expressed only in stems and the other only in pods. The stem gene has four 26 amino acid repeats in the coding region, while the pod gene has only two such repeats. An antibody which specifically reacts to both protein products has been raised against a synthetic peptide based on the repeat sequence. In an in vitro translation/immunoprecipitation system, a single protein each is recovered from pea stem and pod RNA; no translation products from any other tissues were recognized by this antibody. The stem protein is 28 kd and the pod 22 kd, a difference which corresponds to the size of two repeats. This antibody is being used to localize the gene product to specific cell types. We are attempting to define the regulatory sequences involved in the distinct organ specificities of these two genes by the use of chimeric constructs in transgenic plants. As the stem-specific regulatory sequences are not active in tobacco, we are currently using the legume alfalfa, a more closely related species to pea, for these promoter analyses.

M 264 FLORAL SPECIFIC CDNAS FROM TOMATO ENCODE PROTEINS HOMOLOGOUS TO PECTATE LYASE FROM THE PLANT PATHOGEN *ERWINIA*. Rod Wing, Judy Yamaguchi, SusanLarabell, Virginia Ursin and Sheila McCormick, Plant Gene Expression Center, USDA-ARS/UC-Berkeley, 800 Buchanan Street, Albany, CA 94710.

Pollen has been shown to contain many hydrolytic enzymes that may play an important part in pollen germination and pollen tube growth. We have identified two cDNAs expressed in tomato pollen whose proteins share similarity to pectate lyases sequenced from *Erwinia* (1). The similarity extends across 103 amino acids and includes the consensus "region I" and "region II" found in all the pectate lyases sequenced from *Erwinia* . Within these regions, the similarity is approximately 58% (identical amino acids) to "region I" and 53% (identical amino acids) to "region II". When evolutionarily conserved amino acids are considered these percentages increase significantly. We are now attempting to demonstrate that these genes indeed encode plant pectate lyases. Using RFLP analysis we have shown that these two cDNAs are linked by 5 recombination units. The protein coding regions are 54% homologous. However, the genomic structure and flanking DNA of these tomato genes are divergent. Both genes contain introns located at different positions. The promoters have been sequenced and show no significant sequence homology. 1) Tamaki, S, S. Gold, M. Robeson, S. Manulis, and T Keen. (1988). Structure and Organization of the *pel* Genes from *Erwinia chrysanthemi* EC16. J.Bacteriol. **170**, 3468.

M 265 **A DEVELOPMENTALLY-INDUCED PROTEINASE INHIBITOR I FROM LYCOPERSICON PERUVIANUM FRUIT.** Vincent P. M. Wingate, Roxanne M. Broadway*, and Clarence A. Ryan. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164. *Department of Emtomology, Cornell University, Ithaca, NY 14853.

Both the mature polypeptide and the corresponding cDNA of a proteinase Inhibitor I were isolated from *Lycopersicon peruvianum* (L.) Mill. (LA107) fruit. The proteinase Inhibitor I mRNA represents 0.06% of the polyA RNA, while the Inhibitor I proteins comprise about 50% of the total soluble proteins of the fruit.Gene copy number calculated from reconstruction experiments give an estimate of 2 to 4 genes per hapliod genome. The protein is a member of the serine proteinase Inhibitor I family and the first member of the family that has a trypsin inhibitor specificity. The open reading frame of the cDNA codes for a protein of 111 amino acids having a 41 amino acid prepropolypeptide. An Inhibitor I protein corresponding to the cDNA was purified to homogeneity and the N-terminal sequence of the first 22 amino acids of the mature polypeptide determined. The amino acid at the N-terminus of the mature protein was thus established and the protein appears to be identical to that coded by the cDNA. The *Lycopersicon peruvianum* fruit Inhibitor I amino acid sequence contains 85% identity with the wound-inducible tomato Inhibitor I and 86% identity with the potato tuber Inhibitor I. The gene coding for the fruit Inhibitor I is presently being isolated to further investigate its developmental regulation. (Supported in part by grants from the National Science Foundation and EniChem Americas, Inc.)

M 266 GERMINAL TRANSPOSITION RATES OF *Ac* IN TRANSGENIC TOMATOES, John I. Yoder, Francois Belzile, Michael Lassner, Ranjiv Khush, Department of Vegetable Crops, University of California, Davis, CA 95616
We have used Southern hybridizations to examine the behavior of the maize transposable element *Ac* in progeny of transgenic tomato plants. By using hybridization analysis, we were able to follow the segregation of both transposed Ac elements and empty donor sites. In over sixty percent (8/12) of the progeny families, a germinally transposed *Ac* was segregating at a new chromosomal location; some families segregated for more than one forward transposition. In seven of twelve families, at least one progeny contained a transposed Ac but no donor plasmid sequences, indicating meiotic assortment of the donor plasmid and transposed Ac. From these observations, we determined that at least 49 of 121 selfed progeny (40%) had inherited an Ac at a new chromosomal location. An empty donor site resulting from germinal excision of Ac in primary transformants segregated in 50% of the progeny. In one family, an empty donor site segregated but there was no evidence of Ac anywhere in the genome. Even though our criteria for germinal transposition were stringent, the germinal transposition rate in primary tomato transformants was about ten times higher than has been reported in maize.

M 267 A ONE BASEPAIR REPAIR OF A PHA PSEUDOGENE RESTORES THE ACCUMULATION LEVELS OF ITS TRANSCRIPT AND GENE PRODUCT TO NORMAL IN TRANSGENIC TOBACCO, Toni A. Voelker[1], Joaquin Moreno [2], Maarten J. Chrispeels, Dept. Biology, University of California, San Diego, La Jolla, CA 92093. present adress: [1] Calgene, 1920 Fifth Street, Davis, CA 95616 [2] Dept. Bioquimica i Biologia Molecular , Universitad de Valencia, 46100 Spain.
The seeds of the Pinto cultivar of the common bean, *Phaseolus vulgaris* , are deficient in the lectin phytohemagglutinin (PHA), which is normally composed of two different polypeptides (PHA-E and PHA-L). In Pinto seeds there is no PHA-E and only small amounts of PHA-L. The gene coding for the Pinto PHA-E, Pdlec1, is a pseudogene as a result of a single basepair deletion in codon11, causing a frameshift and premature termination of translation. This explains the complete absence of the PHA-E but not the several hundred fold reduction of the cytoplasmic Pdlec1 mRNA in developing cotyledons when compared to a normal PHA-E allele. To find the cause for the reduction of transcript accumulation, we swapped the promoter of Pdlec1 with the promoter of normal PHA allele. Analysis in the seeds of transgenic tobacco revealed that the Pdlec1 promoter is fully functional and contained in the 400 bp immediately 5' to the frame. In addition, we repaired the Pdlec1 coding frame *in vitro* and inserted the repaired and unrepaired versions into a PHA gene expression cassette. In transgenic tobacco, both constructs showed Pdlec1 transcript accumulation in the second half of seed maturation. The frame repair boosted the transcript levels by a factor of 40 and a PHA-E like polypeptide appeared. We propose that the premature translational stop caused by the frame shift causes the obvious instability of the Pdlec1 mRNA.

Gene Regulation

M 300 ANALYSIS OF A HEAT SHOCK INDUCIBLE EXPRESSION CASSETTE, W. Michael Ainley, Joscelyn W. Hill, Robert B. Simpson[1] and Joe L. Key, Botany Department, [1]ARCO Plant Cell Research Institute, Dublin, CA. 94568, University of Georgia, Athens, GA. 30602. We are testing the potential usefulness of the soybean 2019E heat shock gene promoter for expression of sequences of interest in heterologous systems. Analysis of the expression of a chimeric HS promoter-ß-glucuronidase (GUS) fusion gene in leaves of independently transformed transgenic tobacco plants shows high heat induced expression with generally a low ratio of control temperature/heat shock temperature level of expression of GUS, with most less than 0.5%. There was variation in this ratio between plants, ranging from 0.05% to 5%. To test the possible usefulness of the heat shock promoter during various stages of plant development, a HS promoter-isopentenyl transferase gene was constructed and transferred into tobacco plants. In addition, using the same construct, we have been testing whether the isopentenyl transferase gene can be used as a screenable marker for identification of mutants incapable of expressing heat shock genes. The expression of the *ipt* gene coding sequence would be expected to produce increased levels of cytokinin and hence a dramatic phenotype that can be easily distinguished from plants not expressing the gene. For these experiments, we have subjected either germinating seeds or 9 day old seedlings that were grown from F2 seeds of transgenic plants containing the HS promoter-*ipt* gene construct to a 2 hr daily 37°C heat shock. Using germinating seeds, this heat shock treatment does not prevent or markedly reduce the initial rate of seed germination, cotyledon unfolding and expansion or radical elongation. Further heat shock treatments, however, results in radial expansion of the hypocotyl, severe retardation in the development of the root and ultimately adventitious shoot formation at the hypocotyl base. Whereas all of the transgenic germinating seedlings responded uniformly to heat shock, if the heat shock treatment was not initiated until after the seeds had germinated and the primary leafs had emerged (9 day old seedlings), the seedlings showed large differences in their responses. In all experiments, seedlings from nontransformed plants subjected to heat shock developed normally although possibly at a slightly slower rate relative to untreated controls.

M 301 A SEED SPECIFIC NUCLEAR FACTOR BINDS TO AN ENHANCER DOMAIN OF A ß-CONGLYCININ GENE, Randy D. Allen, François Bernier, Philip A. Lessard and Roger N. Beachy, Department of Biology, Washington University, Saint Louis, MO 63130

Subunits of ß-conglycinin, a 7S seed storage protein of soybeans, are encoded by a gene family. The α'subunit gene is expressed at extremely high levels during middle and late stages of seed maturation. Sequences located approximately 200 bp upstream of the transcription start site of the α' gene are required for expression and this domain has been shown to function as a seed specific transcriptional enhancer in transgenic tobacco plants. We have identified a number of DNA binding factors in nuclear extracts from immature soybean seeds which interact with upstream sequences of the α' subunit gene. One of these factors (SEF 3) binds to sequences that correspond with enhancer activity. Nuclease protection and mutational analyses indicate that this factor interacts cooperatively with two sites at -183 to -169 and -153 to -134. The hexanucleotide AACCCA is conserved in both sites and may serve as the core recognition sequence for SEF 3. Experiments are under way to confirm the regulatory function of SEF 3 and to determine the possible functions of other sequence specific DNA binding factors.

M 302 SPATIAL AND TEMPORAL PATTERNS OF GENE EXPRESSION IN HIGHER PLANTS: PAL AND WIN 2 TRANSCRIPTIONAL PATTERNS, Michael W. Bevan, Anne Stanford and Diane Shufflebottom, Department of Molecular Genetics, AFRC Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB, U.K.
The development of histochemical methods for detecting transcription in higher plants (Jefferson et al., 1987) has allowed an important new level of analysis of gene activity to be undertaken. We are now in a position to relate the anatomy of higher plants with differential gene expression. Surprisingly, nearly all of the promoters studied using these techniques appear to have a more restricted pattern of gene expression than predicted. Firstly, we have used a histochemical analysis to determine that there are two important upstream regulatory elements for a Phaseolus phenylalanine-ammonia lyase gene promoter in transgenic tobacco that confer xylem specific expression and expression in pigmented regions of the plant. Secondly, an analysis of win 2 (wound-induced gene 2) glucuronidase gene fusions in transgenic potato showed a strong localised induction of transcription immediately surrounding the wound.

Jefferson, R.A. et al. (1987) EMBO J. 6: 3901-3907.

M 303 COMPARISON OF PATATIN INDUCED GUS EXPRESSION IN DIFFERENT CULTIVARS OF SOLANUM TUBEROSUM, Keith S. Blundy, Margaret A.C. Blundy, Fiona Wilson, Dawn Carter, Peter J. Mooney, William D. Park, Michael M. Burrell, Advanced Technologies (Cambridge) Ltd., Cambridge Science Park, Cambridge, England, CB4 4WA. Six cultivars of potato (Solanum tuberosum) have been transformed with Agrobacterium containing constructs to express ß-glucuronidase (GUS) under the control of a class I patatin promoter. Two cultivars have been transformed with constructs containing class I or class II patatin promoters. The effect of variety and effect of promoter on GUS expression will be presented.

M 304 A STUDY OF 5' COMPONENTS WHICH AFFECT PLANT GENE EXPRESSION,
Alan N. Brunelle, Frank Cannon, Vicky Buchanan-Wollaston, BioTechnica International Inc., 85 Bolton Street, Cambridge, MA 02140.

The duplication of the CaMV 35S promoter sequences has been shown to cause a significant increase in expression of nearby promoters (Kay et al., 1987, Science 236, 1299). Also, it has been shown that fusions of upstream enhancer-like sequences that occur close to transcriptional start sites can cause enhancer-mediated regulation of those heterologous promoters (Timko et al., 1985, Nature 318, 579 and Nagy et al., 1987, EMBO J. 6, 2537). We have explored the effects of various promoter/enhancer combinations and enhancer duplications on gene expression in transgenic plants. We have also investigated the effect that the sequence of the untranslated mRNA leader has on protein levels obtained from a particular promoter.

Statistical analysis of the results indicates that each component has a significant contribution to the pattern of gene expression.

M 305 *IN VIVO* AND *IN VITRO* TISSUE-SPECIFIC INTERACTIONS OF A β-PHASEOLIN ENHANCER-LIKE ELEMENT WITH EMBRYONIC AND VEGETATIVE NUCLEAR PROTEIN FACTORS, M. Bustos, J. Jordano, R. Klassy, M. Guiltinan, D. Begun, A. Kalkan and T.C. Hall, Department of Biology, Texas A&M University, College Station, TX 77843-3258. A chimeric gene containing 800 bp of a β-phaseolin promoter region linked to the coding sequence of bacterial β-glucuronidase (GUS) displayed the same temporal regulation and tissue-specificity in transgenic tobacco that the native phaseolin gene had in *Phaseolus vulgaris*, indicating that most of the control occurs at the level of transcription. An A/T rich 55-bp sequence located 626 bp upstream of the transcription start site acted as an enhancer when fused upstream of the CAAT-box of a CaMV-35S/GUS reporter gene in tobacco, but it yielded a novel pattern of tissue specificity, different from that of both precursor promoters. Reporter activity appeared much earlier in development and did not undergo the strong induction seen with the full promoter after 12 DAF. Significant levels of activity also occurred in vegetative tissues. This indicates that a negative regulatory element present in the intact promoter is involved in conferring tissue specificity. Furthermore, the maximum level of activity seen in developing embryos is 6-7 fold lower than that seen with the full promoter, suggesting that either the distance between the A/T rich enhancer and the TATA-box or a second tissue-specific enhancer are also involved. We will show that this sequence can interact *in vitro* with different protein complexes from *Phaseolus vulgaris* embryonic cotyledons or vegetative tissues. While footprinting analysis shows that the site of interaction is the same, gel retardation and south-western blots indicate that the protein constituents of each complex are different.

M 306 THE UBIQUITIN GENE FAMILY IN ARABIDOPSIS THALIANA.
Judy Callis, John Shanklin and Richard D. Vierstra, Horticulture Department, University of Wisconsin-Madison, Madison WI 53706.

Ubiquitin is a highly conserved 76 aa protein that becomes covalently ligated to the epsilon amino group of lysine residues in cellular proteins through its C-terminus. This ligation serves multiple functions; one is to target proteins for degradation. We have isolated 9 genes that encode ubiquitin from an Arabidopsis genomic library. The coding regions have been identified and the nucleic acid sequence determined. Two genes encoding polyubiquitin contain a different number of the 228 bp repeat unit, but terminate in the same two additional amino acids not found in the mature protein. Four genes encode single ubiquitin polypeptides with C-terminal extensions of either 52 or 81 amino acids. These extensions are highly basic, homologous to extension polypeptides from yeast and humans and contain potential nucleic acid binding domains. Three other genes encode ubiquitin proteins with amino acid substitutions. Preliminary data suggest that these genes are not expressed. The promoters for several of the ubiquitin genes were placed upstream of βglucuronidase and introduced into tobacco plants. The expression of these ubiquitin promoters in different tissues will be described.

M 307 CONTROL OF GUS EXPRESSION IN TRANSGENIC PLANTS USING ANTIGUS, Maura C. Cannon, Jerry G. Platz, Maureen C. O'Leary, Cathleen Cornell and Frank C. Cannon, BioTechnica International, 85 Bolton St., Cambridge, MA 02140.

Inhibition of gene expression by antisense RNA has been reported for bacteria, yeast, plants and animal cells. It has also been shown to be a naturally occurring mechanism for gene regulation in bacteria. Therefore the use of antisense RNA as a tool for the regulation of gene expression is an attractive possibility.

We have examined the regulation of gene expression in transgenic plants using the ß-glucuronidase gene (GUS) as a reporter gene expressed from the 35S promoter and various antisense constructs expressed from the Arabidopsis thaliana ca/b promoter. Experiments were designed to show ca/b mediated control of GUS expression and to determine the efficacy of antisense constructs of different sizes and parts of the GUS gene. Our results show that antisense constructs of less than 50 base pairs can provide a wide range of control over gene expression in plants.

M 308 CONSTRUCTION OF PHASEOLIN 5' DELETION MUTANTS FOR GENE EXPRESSION STUDIES, Caryl A. Chlan and Norimoto Murai, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana, 70803.

The expression of the major storage proteins of Phaseolus vulgaris is developmentally controlled and tissue specific. We wish to identify and characterize cis and trans elements that are involved in tissue specific, developmentally regulated genes, such as phaseolin. Studies of the phaseolin gene may enable us to identify components that central to gene regulation.

To study the effect of cis elements on the expression of phaseolin, we have constructed a series of binary transfer vectors that contain the phaseolin gene linked to nested deletions of the wild - type 5' sequences. These vectors contain an NPT II marker for selection of transformant plants, the phaseolin/5' deletion constructs, and the T DNA boarder fragments on a broad host range plasmid. These vectors were introduced into Agrobacterium strain pC2760 by triparental mating. We have transformed tobacco plants by the leaf disk co-cultivation procedure, and are in the process of regenerating and scoring plants. Effects of the 5' deletions at the transcriptional level will be determined by northern blot analyses, and translational effects will be studied by ELIZA and western blot analyses.

M 309 IDENTIFICATION AND CHARACTERIZATION OF TWO FUNCTIONALLY REDUNDANT *CIS*-ACTING SEQUENCES IN THE WHEAT STORAGE PROTEIN GENE *Lmwg-1d1*, Vincent Colot, Richard A. Jefferson, Michael W. Bevan, Richard B. Flavell *, Department of Molecular Genetics, Inst. of Plant Science Research, Trumpington, Cambridge, CB2 2JB and * John Innes Inst., Colney Lane, Norwich, NR4 7UH, U.K.

We and others have previously shown that transgenic tobacco can be used effectively as a surrogate system to study endosperm-specific expression of several cereal storage protein genes (1-3). We now report the identification of two fragments within the 5'flanking sequence of a wheat LMW glutenin gene (*Lmwg-1d1*, 4) that can independently activate transcription from the *Lmwg-1d1* promoter in a tissue (endosperm)-specific manner in tobacco. Although we have not directly tested wether the 5' distal fragment works in its native arrangement, quantitative analysis of our data suggests that this is the case and that the two fragments work additively rather than in synergy. Also, activity of both fragments is strongly dependent on them being in their native orientation with respect to transcription. On the other hand, experiments with a truncated CaMV35S promoter region (-58/+2) revealed that the 5' flanking sequence of *Lmwg-1d1* which covers both fragments acts as an endosperm-specific enhancer. Taken together, these results indicate that there are at least two levels of organisation in the regulatory region of gene *Lmwg-1d1*.

(1) Colot et al. (1987) EMBO. J. 6: 3559, (2) Marris et al. (1988) Pl. Mol. Bio. 10: 359, (3) Schernthaner et al. (1988) EMBO. J. 7: 1249, (4) Colot et al. (1989) Mol. Gen. Genet., in press

M 310 PLANT TRANSFORMATION AS A TEST OF THE RELATIONSHIP BETWEEN CYTOPLASMIC MALE STERILITY, RESPIRATORY PHENOTYPE, AND THE *PCF* GENE, M. B. Connett, P. L. Lehman, and M. R. Hanson, Genetics Department, Cornell University, Ithaca, NY 14853
The presence of a gene (*pcf*) composed of a fusion between the promoter and coding region of *atp9*, parts of both exons of *coxII*, and an unidentified reading frame (*urf8*) is correlated with cytoplasmic male sterility (CMS) in *Petunia*. In addition, isonuclear lines of *Petunia* carrying the CMS cytoplasm or a normal cytoplasm differ consistently with regard to partitioning of mitochondrial electron transport between the cyanide-sensitive (cytochrome oxidase) and alternative pathways. Nuclear transformation of *Petunia* plants with *pcf* in a construct targeting the product to the mitochondria with a transit sequence is being carried out and the phenotypic differences in respiration and male fertility will be used as a test of the hypothesis that the presence of the gene product in *Petunia* mitochondria causes these phenotypes.

M 311 FUNCTIONAL COMPLEXITY OF UV-LIGHT RESPONSIVE DOMAINS IN THE PARSLEY CHALCONE SYNTHASE PROMOTER. J.L. Dangl, P. Schulze-Lefert, M. Becker-Andre, K. Hahlbrock and W. Schulz; Biochemistry, Max-Planck Institute, Cologne, Fed. Rep. of Germany.
Chalcone synthase (CHS) is the key enzyme of flavonoid biosynthesis. Transcriptional activity of the CHS gene is induced by several environmental stimuli in different species, leading ultimately to the accumulation of diverse classes of flavonoids with various functions. In parsley (*Petroselinum crispum*), flavonoids accumulate in vacuoles of epidermal cells after exposure to UV-containing white light, and are thought to serve as UV protective compounds. This accumulation is preceeded by a massive increase in CHS transcription rate. We identified *cis* elements in the CHS promoter required for UV-light activation using a combination of *in vivo* footprinting and transient expression assays in parsley protoplasts which are responsive to UV-light. We identified four UV-light dependent *in vivo* footprints on the CHS promoter from -130 to -275bp. Their appearance strongly correlates to the onset of maximal CHS transcription. Analysis of CHS-GUS reporter gene fusions in protoplasts allowed us to define a minimal UV-light responsive element consisting of the TATA proximal -180bp. Two *in vivo* footprints reside in this fragment, and both are required for light activation as demonstrated by site directed mutagenesis. Interestingly, sequences from -180 to -615 bp constitute an independent light responsive system. This system contains the other two *in vivo* footprints, which alone are only very weakly light responsive, and an upstream enhancer. Two of the *in vivo* footprints, one in each "system", are homologous to a conserved *cis* element found in a plethora of SSU genes. Our results prove that induced changes in DNA-protein interaction are necessary for UV-light response of the CHS gene, and suggest strongly that diverse light activated biosynthetic pathways share regulatory elements.

M 312 2S ALBUMIN STORAGE PROTEIN GENE EXPRESSION IN *BRASSICA NAPUS* MICROSPORIC EMBRYOS AND TRANSGENIC PLANTS, Janice M. DeMoor*, Maria-Jesus Gines+, V.N. Iyer* and Brian L. Miki+, *Department of Biology, Carleton University, Ottawa, Canada, K1S5B6, +Agriculture Canada, Ottawa, Canada, K1A0C6
Gene expression during embryo development was monitored in *B. napus* embryos derived from isolated microspores, using a number of probes. The pattern of transcription of the genes for the two major storage proteins, napin and cruciferin appeared to parallel that for zygotic embryos. However, napin mRNA was unexpectedly detected at high levels after the four-day 32.5° C treatment used to induce embryogenesis in microspores. This response appeared to be specific to embryogenic cells.
A gene coding for the corresponding storage protein in *Arabidopsis thaliana*, (arabin), was isolated from a genomic library. The gene was sequenced and found to be equivalent to the AT2S1 gene recently reported by Krebbers, *et al.* (1988, Plant Physiol. 87:859-866). A comparison of the arabin sequence to gNa, a napin genomic clone from *B. napus* (Scofield *et al.*, 1987, J. Biol. Chem. 262:12202-12208) revealed nucleotide homologies of 68% in the region 250 bp upstream of the ATG and 81% in the signal peptide sequences. Transformation vectors have been constructed containing arabin signal peptide and/or promoter sequences fused to the GUS reporter coding region. GUS activity will be monitored in transgenic tobacco and *B. napus* plants, including microsporic embryos.

M 313 ANTISENSE RNA INHIBITION OF BETA GLUCURONIDASE IN TRANSGENIC TOBACCO PLANTS. Steven Fabijanski, Paladin Hybrids Inc. P.O. Box 219 , Brampton, Ontario, L6V 2L2. Laurian S. Robert and Pauline Donaldson, Dept. of Biochemistry, University of Ottawa, 40 Marie Curie, Ottawa, Ontario, K1N 6N5, CANADA.

A model system was devised to critically examine antisense RNA regulation in transgenic tobacco plants. The strong constitutive cauliflower mosaic virus 35S promoter was chosen to regulate the expression of both the sense and antisense copies of the versatile reporter gene Beta-glucuronidase (GUS). A single tobacco transformant that contained an intact copy of the GUS gene was re-transformed with an antisense GUS vector. Plants recovered that contained both sense and antisense GUS genes showed reduced GUS levels relative to those containing only the GUS gene. In some of the plants examined a nearly complete inhibition of GUS activity was seen. Southern blot analysis confirmed the presence of 1-5 copies of the antisense gene, Northern blot analysis demonstrated the presence of sense and antisense RNA, and Western blot analysis demonstrated reduced GUS enzyme levels in the plants containing the antisense gene. These results demonstrate that stable and effective antisense RNA inhibition of gene activity can be achieved with identical strength promoters driving the sense and antisense genes.

M 314 MOLECULAR ANALYSIS OF *FED I* GENE EXPRESSION. Denis Falconet Robert C. Elliott, Lynn F. Dickey, Brian Fristensky, George C. Allen and William F. Thompson, Departments of Botany and Genetics, North Carolina State University, Raleigh, NC 27695-7612.

Ferredoxin I is a soluble electron transport protein involved in the photosynthetic reduction of NADPH. In pea plants, it is encoded by a single copy gene, *Fed I*. Expression of this gene is under the control of phytochrome, and steady state levels of the *Fed I* mRNA increase substantially after plants have been exposed to light. We have isolated, mapped, and sequenced a *Fed I* genomic clone. Expression of an intact pea *Fed I* gene with normal flanking sequences is regulated by light in the leaves of transgenic tobacco plants. Using different constructs containing either a GUS reporter gene, the 35S promotor, or parts of the *Fed I* gene, we have shown that in transgenic plants, the sequences responsible for light regulation are contained within the transcribed region. Experiments using alpha-amanitin suggest that the regulation is at the level of message stability.

M 315 REGULATION OF THE ARABIDOPSIS THALIANA CHALCONE SYNTHASE GENE BY LIGHT Rhonda L. Feinbaum and Frederick M. Ausubel. Department of Molecular Biology, Mass. General Hospital, Boston, MA 02114. Chalcone synthase (CHS), the first enzyme unique to flavonoid biosynthesis, is highly regulated both during development and in response to environmental stimuli in many plant systems. We have previously reported the isolation and characterization of the CHS gene from Arabidopsis thaliana. We have shown that the Arabidopsis CHS gene is transcriptionally induced in response to increased light intensity. Our current work is focused on defining the regulation of the CHS gene by light. Using northern blot analysis we have examined the accumulation of CHS mRNA under various light conditions in order to determine which of the known photoreceptor systems play a role in the regulation of the CHS gene in Arabidopsis. Data will be presented describing the effect of red, blue, and u.v. light on endogenous CHS expression. In addition, we have generated transgenic Arabidopsis plants carrying a fusion of the CHS promoter (consisting of sequence 2.0 kb 5′ of the CHS transcriptional start site) to the B-glucuronidase marker gene. This fusion appears to be induced in response to high intensity light and we are currently testing the response of this construct to red, blue, and u.v. light.

M 316 REGULATORY ELEMENTS OF ZEIN GENES FROM MAIZE, Günter Feix, Uwe Maier, Michael Schwall and Tom Quayle, Institute of Biology III, University of Freiburg, 7800 Freiburg, W-Germany.
Towards an analysis of the zein genetic system of maize and its regulation, we have previously isolated and characterized representative genes of the zein multigen system and are now focussing our analysis on specific protein-DNA interactions occuring in the 5'flanking regions of zein genes. By a variety of techniques we have identified several tissue dependent and independent (as well as stage specific) binding-sites for nuclear proteins from various maize tissues. These extend as far as 2 KB upstream of the coding region. Some of the analyzed interactions are multicomponent and involve Zn-finger proteins. Several of the regions with protein binding sites show enhancing or silencing activities when tested in front of the 35s promoter with CAT or LUC as reporter genes in transient transformation assays. Likewise, zein promoter regions were tested in transient transformations and showed, depending on the size of the promoter fragment used, a tissue specific response. The future analysis of the zein system will profit from our recent success in obtaining stable maize transformants by a pollen transformation method.

M 317 **Influence of a Bacterial Repressor-Operator-Complex in Different Locations of a Plant Promoter**, Christiane Gatz, Institut für Genbiologische Forschung , Ihnestr 63, 1 Berlin 33, FRG.
The Tn10 encoded TET-repressor-operator-system can be used to regulate transcription from the 35S-Cauliflower (CaMV)-promoter in a transient expression system using electroporated tobacco protoplasts. Tet TET repressor, being expressed in plant cells under the controll of eucaryotic transcription signals, blocks transcription of a CaMV-promoter-chloramphenicolacetyltransferase constructs when the operators are located flanking the TATA-box. In the presence of sublethal concentrations of tetracycline - the inducer molecule - expression is restored to full activity. The influence of the TET-repressor-operator-complex in different locations of the promoter on promoter activity is presented. Results on the effect of the TET-repressor on gene expression in transgenic tobacco and Arabidopsis are shown.

M 318 IDENTIFICATION OF PROMOTER SEQUENCES FOR THE MAJOR RNA TRANSCRIPTS OF FIGWORT MOSAIC AND PEANUT CHLOROTIC STREAK VIRUSES (CAULIMOVIRUS GROUP). Siddarame Gowda, Fang C. Wu and Robert J. Shepherd, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.
DNA sequences containing the putative promoter elements of the major RNA transcripts of Figwort mosaic virus (FMV) and Peanut chlorotic streak virus (PCSV) were fused to marker chloromphenicol acetyl transferase gene. 3' and 5' deletions were introduced in order to define the boundaries of regulatory sequences. In FMV, 'TATA' box, 'CAAT' box sequence and 5' enhancer elements were observed to be present in the 3' end of Region VI, while in PCSV these sequences are located in the large intergenic region downstream from Region VI. In comparative tests in suspension cell protoplasts of Nicotiana edwardsonii, both promoters exhibited activity approximately equal to that of 35S promoter of CaMV.

M 319 ANALYSIS OF THE SUPPRESSOR PHENOTYPE OF THE MAIZE TRANSPOSON EN IN TRANSGENIC TOBACCO, S.R. Grant, A. Pereira, A. Gierl, H. Cuypers, G. Hombrecher and H. Saedler, Max-Planck-Institut fuer Zuechtungsforscung, D-5000 Koeln 30, West Germany.
The autonomous En transposon is unusual in that it encodes gene regulatory factors as well as transposase. The regulatory factors control expression of genes with any one of a number of related non-autonomous transposable elements (I elements) inserted therein. Through analysis of many different En or I elements and genes affected by the regulatory factors, a model has been proposed to explain the mechanism of regulation. This model predicts that tight binding of an En protein called tnpA to sequence motifs reiterated several times at the termini of the elements blocks progression of RNA polymerase. Truncation of transcripts at the site of element insertion in a gene "suppresses" expression of that gene resulting in the "Suppressor" phenotype. In order to test the predictions of the model, we have mimicked "suppression" in a transient expression assay in transgenic tobacco protoplasts. Mutant transposon sequences have been tested for their ability to reproduce "suppression" in order to identify the En proteins and *cis*-acting sequences essential for suppression. We have found that tnpA expressed from a cloned cDNA will suppress transient expression of the bacterial gene, GUS, from a plant promoter if tnpA binding sites are located between the plant promoter and the bacterial gene.

M 320 The characterization and expression of a disease resistance response gene (DRRG) of peas. Chin Chiang and L. A. Hadwiger. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164.

Disease resistance response genes of peas are expressed in temporal correlation with the expression of both "race-specific" and "non-host" resistance. A single pea genomic clone DRRG49 has been cloned and sequenced. In addition to conserved TATA and CAAT boxes in the 5′ flanking region, several putative regulatory elements have been found. This includes dyad symmetry, enhancer-like sequences, binding sequences and direct repeats. Interestingly, clusters of topoisomerase II consensus sites in both 5′ and 3′ segments are homologous with those in other eucaryotes and are the first to be reported in plants. The 5′ flanking region of DRRG49 has been fused to reporter genes and expression has been monitored in both protoplasts and transformed tobacco plants following treatment with a fungal elicitor or an incompatible pathogen.

M 321 THE *a* LOCUS SPATIALLY REGULATES THE EXPRESSION OF ONE MEMBER OF THE CHALCONE SYNTHASE (CHS) MULTIGENE FAMILY IN *Pisum sativum*, Carole L. Harker, Enrico S. Coen and T. H. Noel Ellis, John Innes Institute and AFRC Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.
The *a* locus has been shown to be essential for general flavonoid biosynthesis in the plant and for anthocyanin biosynthesis in flowers, stipules, stems, axil rings, pods and testas of *P. sativum*. By using a combination of genetic and immunological analyses we have shown that the *a* locus of *Pisum sativum* regulates CHS gene expression in *trans*. The *a* locus regulates CHS gene expression in pigmented regions of the plant but has no effect in the $CuCl_2$-induced CHS gene expression in root tissue. CHS detected in $CuCl_2$-induced root tissue and pigmented regions of the plant arises from expression of different members of the *P. sativum* CHS multigene family. Our results suggest that the response to different stimuli (e.g. heavy metals) may be mediated by genes regulating the expression of individual members of subclasses of the CHS multigene family.

M 322 DESIGNED RIBOZYMES TO STUDY AND MANIPULATE GENE EXPRESSION IN PLANTS.

Thomas Herget, Jeff Schell, and Peter H. Schreier.
Max-Planck-Institut fuer Zuechtungsforschung, 5000 Koeln 30, FRG

Genomic and satelite RNAs of some plant viruses are able to undergo autocatalytic self-processing. The catalytic activity of the endonucleolytic cleavage is due to a complex secondary structure of the RNA, the so called "hammer-head". The specificity of the reaction resides in the flanking sequences of the hammer-head. In our case,we designed a ribozyme by cloning an oligonucleotide behind the SP6 promoter. The hammer-head is flanked by 10 and 8 bases complementary to the transit-peptide of the rbcS mRNA of potato. The activity of this ribozyme is analyzed in vitro and in vivo. Essential for sequence specific digestion of the target RNA in vitro are neutral pH and magnesium-ions. The temperature optimum is 50°C. Transformation of plants with the designed ribozyme should answer the following questions: -does the ribozyme also work in vivo as an alternative to anti-sense RNA approaches? -is the sequence-specificity sufficient to knock out the expression of one member of a multigene-family? -how does the plant react upon inactivation of one specific rbcS gene?
In vitro and in vivo data will be presented and discussed.

M 323 SEQUENCE-SPECIFIC INTERACTIONS BETWEEN A CARROT ROOT NUCLEAR PROTEIN AND THE CARROT EXTENSIN GENE.

M.J.Holdsworth and G.G.Laties. Biology Dept., UCLA, 405 Hilgard Ave, Los Angeles, CA 90024, USA.

As one approach to investigating the mechanism for the activation of the expression of the carrot extensin gene following the wounding of carrot roots, we have studied the interaction of nuclear-located DNA binding proteins with the extensin gene. Analysis of results from experiments conducted in-vitro using the gel-retardation assay system has shown that a protein (designated EGBP-1) present in nuclear extracts from non-wounded carrot roots interacts with a single region of the extensin gene. This interaction is sequence-specific as judged by the failure of other plant gene sequences to compete with the extensin gene for EGBP-1 binding. EGBP-1 activity is organ specific, not being expressed in nuclear extracts obtained from carrot leaves or stems. The activity of EGBP-1 is diminished in nuclear extracts obtained from carrot roots following wounding. We are currently investigating EGBP-1 expression in nuclear extracts from carrot roots subjected to other stress regimes.

M 324 BINDING OF COMMON NUCLEAR FACTORS TO DISSIMILAR AT-RICH UPSTREAM SEQUENCES IN SUNFLOWER AND BEAN SEED STORAGE PROTEIN GENES. Juan Jordano, Concepcion Almoguera, Mauricio Bustos, Timothy Hall and Terry Thomas, Department of Biology, Texas A&M University, College Station, TX 77843.

Sunflower seed proteins include the 2S albumins and 11S globulins, or helianthinins. The accumulation of these proteins during seed development and maturation requires the regulated expression of the genes encoding these proteins. We report here the detection of nuclear proteins in 13 days post-flowering sunflower embryos that interact with sequences upstream of two different helianthinin gene promoters. These sequences have been delimited by *in situ* 1,10-phenanthroline-copper ion footprinting experiments. The sequence of the binding sites is very AT-rich and differs only by one nucleotide between the two helianthinin genes. Binding competition, southwestern blot and footprinting experiments suggest that the same sunflower embryo proteins also bind with comparable affinity to a putative upstream regulatory element in a *Phaseolus vulgaris* phaseolin seed protein gene. These phaseolin upstream sequences have been shown also to bind nuclear factors present in *Phaseolus vulgaris* embryo nuclear extracts (Guiltinan *et al.* submitted and Jordano *et al.* in preparation). Although the phaseolin binding site is also AT-rich, its nucleotide sequence is remarkably different to that of the helianthinin binding sites. This suggests that a family of evolutionarilly conserved proteins that bind to different AT-rich motifs may participate in controlling the coordinate expression of plant seed proteins. The possible functionality of the helianthinin upstream elements is being investigated in transgenic tobacco using various β-glucuronidase reporter gene constructs.

M 325 ONE MEMBER OF THE POTATO PROTEINASE INHIBITOR II GENE FAMILY IS RESPONSIBLE FOR BOTH WOUND-INDUCIBILITY AND CONSTITUTIVE TUBER EXPRESSION, Michael Keil, Jose Sanchez-Serrano, Jeff Schell and Lothar Willmitzer, Institut für Genbiologische Forschung GmbH Berlin, FRG. A chimaeric gene consisting of 1.3 kb of the 5'regulatory region of a potato proteinase inhibitor II gene, the bacterial ß-glucuronidase gene (GUS) and 260 bp of the proteinase inhibitor II untranslated downstream region was introduced into tobacco and potato using the Agrobacterium tumefaciens mediated transformation system. Assays on the enzymatic activity of the ß-glucuronidase and northern analysis of transgenic plants revealed constitutive expression of the chimaeric gene in potato tubers and systemic, wound-inducible expression in stem and leaves of tobacco and potato, thus following the expression of the whole potato proteinase inhibitor II gene family. These data demonstrate that within the proteinase inhibitor II gene family there is no division into wound- and tuber-specific genes, but that one single gene of this gene family can be regulated by both developmental and environmental factors. The transgenic plants will be further used for the histochemical analysis of the cellular localisation of the GUS-activity.

M 326 DIFFERENTIAL TARGETING OF PATATIN GENE EXPRESSION, Meike Köster, Mario Rocha-Sosa, Uwe Sonnewald and Lothar Willmitzer, Institut für Genbiologische Forschung Berlin GmbH, D-1000 Berlin 33, FRG - Patatin, which contributes up to 40% of the total soluble protein content in potato tubers (*Solanum tuberosum*), is encoded by a gene family comprising between 12 and 15 genes per haploid genome. Several patatin genomic clones have been isolated and sequenced indicating a highly conserved coding region and divergent 5' upstream sequences. Based on structural differences, the genes were arranged in two main classes. To study the biological meaning of their occurence within the patatin gene family, a class I as well as a class II gene were transcriptionally fused to the ß-glucuronidase gene to direct expression in transgenic plants of both potato and tobacco. Histochemical analysis reveals expression in different cell layers of tubers for both gene classes. However, only the class II gene shows activity in roots. Moreover, detailed analysis indicate that class II genes are primarily developmentally regulated. Thus, in contrast to the analysis of other gene families, for the patatin gene family certain specialized functions could be addressed to specific members.

M 327 ANALYSIS OF TRANS-ACTING NUCLEAR FACTORS WHICH INTERACT SPECIFICALLY WITH GA_3/ABA REGULATED PROMOTERS IN BARLEY. Michael B. Lanahan, T-H. David Ho. Department of Biology, Washington University, St. Louis, Mo. 63130.

Gibberellic acid (GA_3) transcriptionally activates genes encoding hydrolases in barley aleurone cells. Abscisic acid (ABA) suppresses this expression. We have assayed nuclear extracts from aleurone cells for factors which bind to sequences 5' of the 'TATAA' box of a number of different GA_3/ABA regulated promoters. Nuclear extracts were prepared and fractionated by salt gradient elution from a mono-Q column. Fractions were assayed for binding activity by mixing ^{32}P end-labeled DNA fragments, derived from α-amylase promoters, with the protein fraction in the presence of non-specific competitor DNAs. DNA-protein complexes were detected as radioactive bands with decreased electrophoretic mobilities in non-denaturing polyacrylamide gels (gel retardation/shift assays). The specificity of the DNA-protein interactions was determined on the basis of competitions with increasing amounts of unlabeled promoter DNAs. $Cu(phen)_2^+$, methylation interference, and exonuclease III footprinting experiments are underway to more clearly define binding sites.

M 328 DIFFERENTIAL EXPRESSION OF MONOCOTYLEDON AND DICOTYLEDON RuBisCO SMALL SUBUNIT GENES IN TOBACCO

Bernard LEROUX, Bernard PELISSIER, Michel LEBRUN, Alain SAILLAND, Stéphanie BRILLATZ and Georges FREYSSINET. Rhône-Poulenc Agrochimie, Biologie Moléculaire et Cellulaire Végétale, BP 9263, 69263 LYON Cédex 09, France.

Two genes encoding the RuBisCO small subunit have been isolated, one from sunflower and one from maize. These two genes have been shown to be the most highly expressed genes in the small multigene family from either sunflower or maize. The upstream regions of these genes have been fused to the nitrilase coding region. This enzyme confers resistance to the antidicotyledon herbicide Bromoxynil. The 3'region used for the chimeric gene was from the nos gene.Tobacco plants were transformed using the leaf disc technique with Agrobacterium. The regenerated plants show a striking difference in the resistance to bromoxynil depending on the origin of the promoter. Only the sunflower promoter leads to a high level of resistance. Transgenic tobaccos containing the maize promoter-bromoxynil fusion and the non-transgenic tobacco are very sensitive to this herbicide. In order to understand this difference in behavior we fused each promoter with the Cat reporter gene and tested their activities in transient electroporated leaf protoplasts and in Agrobacterium mediated transgenic plants. Results will be presented showing that part of the maize RuBisCO SSU promoter seems to be responsible for the absence of expression in transgenic tobacco.

M 329 EXPRESSION OF A DELETION SERIES OF THE SOYBEAN LECTIN GENE IN TOBACCO,

Jon T. Lindstrom[1], Roy W. Harding[2], and Lila O. Vodkin[1], [1] Department of Agronomy, University of Illinois, Urbana, Illinois, 61801, and [2] Department of Chemistry, Indiana University, Indiana, Pennsylvania 15705. A deletion series containing the developmentally regulated soybean lectin gene (*Le1*) was used to transform tobacco (*Nicotiana tabacum* cv. Xanthi) leaf disks with an *Agrobacterium tumefaciens* Ti plasmid and pMON200 intermediate vector. Lectin constructs used ranged in size from 5.5 kilobases (kb) to 1.1 kb. The largest construct contained 3.0 kb of the 5' flanking region and 1.0 kb of the 3' flanking region. The smallest construct contained no 5' flanking region and 200 base pairs of the 3' flanking region. Integration of foreign DNA in regenerated tobacco plants was shown using Southern blots and Elisa assays of lectin in single tobacco seed confirmed that the inserted gene was inherited as a single Mendelian event. Expression of the soybean lectin gene, determined by northern and western blotting, occurred in all but the smallest constructs. Maximal expression of lectin, however, required greater than 340 bp of 5'sequence indicating that far upstream factors are involved in quantitative control of lectin expression. Transcripts of *Le1* from the constructs that contained at least 190 bp of 5' sequence were developmentally controlled and limited to the mid-maturation phase of tobacco seed development. The lectin promoter offers a potential means to target high levels of gene expression to the developing soybean seed and may have applications in other plant systems for the recovery of foreign gene products from the seed.

M 330 IDENTIFICATION OF THREE CIS-ELEMENTS DIFFERENTIALLY REGULATING EXPRESSION OF THE UV-LIGHT INDUCIBLE ANTIRRHINUM MAJUS CHS GENE.

Susanne Lipphardt, Reinhold Brettschneider, Jeffrey Dangl, Klaus Fritze, David Wing and Jeff Schell. Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG

Parsley protoplasts retain differential responsiveness to UV-light as do intact cells. UV-inducible transient expression of a chimeric A.majus Chalcone Synthase gene (CHS-NPTII) in parsley protoplasts showed similar UV-induction kinetics as found for both the endogenous parsley CHS and the A.majus CHS gene in vivo. Transient expression of various CHS-NPTII promoter mutants led to the identification of a cis element (-39 to -197) being essential and sufficient for UV-induction but leading to low expression. The following cis-element (-198 to -357) increases gene activity exclusively after UV-light application. The third element (-564 to -661) enhanced gene expression generally in a completely UV-independent manner. Constructs carrying the CaMV 35S enhancer in front of the -39 to -197 element rise to higher overall expression and were alsoUV-inducible. Expression from a fusion of the general enhancer element with the -39 to -197 element compared to the activity of the 35S -39 to -197 construct revealed relatively low enhancing capacity of the general CHS-enhancer. Studies of stably transformed tobacco plants gave evidence for a developmentally regulated and flower specific spacial pattern of CHS-NPTII expression.

M 331 5' UPSTREAM SEQUENCES FROM THE wun1 GENE ARE RESPONSIBLE FOR GENE-ACTIVATION BY WOUNDING AND TISSUE SPECIFIC EXPRESSION IN TRANSGENIC PLANTS. Jürgen Logemann, Barbara Siebertz, Susanne Lipphardt, Lothar Willmitzer and Jeff Schell. Max-Planck-Institut fuer Züchtungsforschung, 5000 Köln 30, FRG

A 1,2 Kb fragment of the 5' upstream region of a potato wound-inducible gene (wun1) was fused to different markergenes (CAT; NPTII; GUS). Stable integration of a wun1-CAT chimeric gene into the tobacco genome led to a marked wound-inducible CAT-activity in leaves. Transient expression experiments in potato protoplasts showed that wun1 carries a strong promotor sequence similar in strenght to the 35S-promoter. The same intensity of expression was also observed using wun1-constructs in transient expression experiments with protoplasts derived from a monocot plant (rice). In transgenic wun1-GUS tobacco plants GUS-activity was localized specifically in the epidermis of leaves and in the stomium of anthers. Wun1 promotor deletion analysis indicates that a region between -111 and -571 is responsible for woundinduction whereas sequences at -572 to -1022 must contain general enhancer like functions and increase activity up to 400 fold. wun1-mRNA was shown to accumulate to high levels in potato leaves collapsing as a result of infection with the phytopathogen Phytophthora infestans. Although the function of wun1 is still unknown similarities to enzymes involved in suberinisation or in callose formation are likely.

M 332 **CHARACTERIZATION OF AN ABSCISIC ACID RESPONSE ELEMENT (AARE) AND ANALYSIS OF THE ELEMENT IN TRANSGENIC PLANTS,** **William R. Marcotte, Jr., S. A. Schmitz, S. H. Russell, C. C. Bayley and R. S. Quatrano, E. I. DuPont de Nemours and Co., Inc., Experimental Station, P.O. Box 80402, Wilmington, DE 19880-0402.**

The Em gene of wheat has been shown to direct the accumulation of Em protein in wheat embryos during late embroygenesis. This accumulation parallels increases in the levels of abscisic acid (ABA). A chimaeric construct containing a promoter region (642 bp) from the Em gene and the reporter GUS have been previously shown to be responsive to ABA in a transient assay using rice protoplasts. This construct has been introduced into tobacco plants and has been shown to direct the accumulation of GUS activity in transgenic seeds and not in leaves. Deletion analysis of this region has defined sequences which promote this responsiveness and these sequences have been shown to impart ABA responsiveness on the CaMV 35S promoter in protoplasts. These same constructs have been introduced into transgenic tobacco plants and the results of these studies will be presented. A consensus sequence for the response element and analyses of post-transcriptional controls will also be presented.

M 333 DETECTION OF AN ELICITOR DEPENDENT IN VIVO FOOTPRINT WITHIN THE PROMOTER OF THE PATHOGENESIS-RELATED PR1 GENE OF PARSLEY, Iris Meier, Imre E. Somssich and Klaus Hahlbrock, Max-Planck Institut für Züchtungsforschung, 5000 Köln, F.R.G.

We are investigating the hypersensitive nonhost response of parsley to the soybean pathogen Phytophthora megasperma f.sp. glycinea (Pmg). Upon treatment of parsley suspension cultures with a cell wall preparation of the fungus (Pmg-elicitor) there is a rapid activation of a large number of genes which have been identified by differential screening of a cDNA library. The most abundant cDNA encodes for pathogenesis-related protein 1 (PR1), a small acidic protein (17.000 D). Rapid and massive PR1 mRNA accumulation occurs not only in elicitor-treated cells but also around fungal infection sites of parsley leaves, as was shown by in situ hybridisation. Two expressed PR1 genes are present in parsley as deduced by sequence-comparison of the cDNAs. The promoter of one of them, PR1-1, was used to identify cis-acting elements by in vivo footprinting. This method enables us to gain information about DNA-protein contacts within the living cell at single nucleotide resolution. We identified G-residues within the first 250 bp upstream of the transcriptional start site that are protected specifically during activation of the gene. The promoter of the second PR1 gene shares no homology to the PR1-1 promoter according to cross-hybridisation. Sequence comparison of both promoters as well as of those of other elicitor-induced genes with respect to the identified sequences will be presented.

M 334 CONTROL OF PLANT GENE EXPRESSION USING WILD-TYPE AND ALTERED-SPECIFICITY BACTERIAL REPRESSOR MOLECULES. Andrew Merryweather, Raj. K. Beri, Gillian M. Smith, David Pioli, Diane Shufflebottom*, Mike Bevan*, William J. Brammar and Wolfgang Schuch†, I.C.I. Joint Laboratory, University of Leicester, University Road, Leicester, LE1 7RH, England. *IPSR, Trumpington, Cambridge, CB2 2LQ, England. †I.C.I. Seeds, Plant Biotechnology Section, Jealott's Hill, Bracknell, England.

One of the major mechanisms acting to control gene expression in a wide diversity of organisms involves the interaction of regulatory proteins with specific DNA sequences. Although such proteins are at present poorly characterised in plants, the protein-DNA interactions between bacterial repressor proteins and their specific operators are well understood at the molecular level.

This project investigates the efficacy with which prokaryotic repressor proteins can be used to control the expression of plant genes. The bacterial *lacI* gene, modified to contain an ATG initiation codon, has been fused to both the CaMV 35S and maize CAB promoter sequences and transgenic tobacco plants have been generated. Suitable plants have been selected for further analysis. We have also inserted the *lac* operator sequence at a number of sites in target promoters fused to the *gus* marker gene. Data will be presented on the effectiveness with which the interaction between bacterial repressor and operator sequences can be used to control plant gene expression.

A potentially more powerful corollary to this approach is the isolation of altered-specificity repressor mutants which recognise naturally occurring "pseudo-operator" sequences within the target gene. Such a strategy obviates the *in vitro* manipulation of the target gene. Details of the positive selection system developed to isolate altered-specificity mutants of bacterial repressor will be presented.

M 335 Structural and functional analyses of Arabidopsis thaliana chlorophyll a/b-binding protein (cab) promoters, Amitava Mitra, Hong K. Choi and Gynheung An, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

A. thaliana carries three functional copies of the cab gene which code for an identical mature protein. DNA sequence comparison of all three cab promoters indicated that cab2 and cab3 are more closely related compared to cab1. The highest degree of homology was found between the TATA box and -256 of cab3 promoter, suggesting that this region plays a major role in promoter function. To identify cis acting regulatory elements of the cab genes, the promoters were mutated by progressive deletions and the effects on the promoter activity were measured in either transgenic plants or cultured cells. It was found that the minimum sequence necessary for the light-dependent tissue-specific promoter activity of the cab3 is the 89 bp DNA fragment (between -74 and -164) at the region of the TATA and the CCAAT boxes. However, an additional 45 bp DNA fragment (between -164 and -209) at the upstream of the CCAAT box was necessary for the full promoter activity in the leaves. The regulatory element in the 45 bp region appears to be a positive regulator or enhancer which is specific to photosynthetic cells, since the region did not enhance the promoter activity in cultured cells. This region contains an octamer, TGCCACGT (cab2) or TGCCACAT (cab3), which is similar to the previously identified elements from Arabidopsis cab1 and Nicotiana plumbaginifolia cabE promoters.

M 336 CRYPTIC POLYADENYLATION SITES IN PLANT GENES, Brad Mogen and Arthur G. Hunt, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

We have identified cryptic polyadenylation sites in two plant transcription units, those from the pea *rbcS*-E9 gene and the cauliflower mosaic virus (CaMV) 19S/35S genome. In the *rbcS* gene, these sites lie within 350 bases of the array of sites that are utilized in the intact gene. In the CaMV transcription unit, two predominant sites are seen. One of these is approximately 230 bases upstream from the normal poly(A) addition site, and some 50 bases upstream from the 35S RNA transcription initiation site. The other is approximately 300 bases downstream from the normal poly(A) site. These cryptic sites are utilized when the normally-used sites are removed by mutagenesis. The occurrence of cryptic polyadenylation sites seems to be another manifestation of the multiplicity of poly(A) sites that is seen in most plant transcription units. We suggest that this multiplicity has functional significance and that efficient mRNA 3' end formation in plants requires interactions between adjacent polyadenylation signals.

M 337 **Functional Analysis of the PR-1a Promoter from Nicotiana tabaccum cv. xanthi: Identification of cis-acting sequences sufficient for induction by Salicylic Acid and Tobacco Mosaic Virus.** A. L. Montoya, L. Bethards, L. A. Buchanan, S. Dincher, G. Howe, H. Thompson-Talor, N. Desai and J. Ryals. CIBA-GEIGY Agricultural Biotechnology Unit, Research Triangle Park, N.C., 27709.

Various gene fusions between the 5′ sequences of the pathogenesis-related protein-1a (PR-1a) gene and the β-Glucuronidase (GUS) reporter gene were constructed in a binary vector for Agrobacterium mediated transformation of N. tabaccum cv. xanthi leaf discs. Shoots were propagated and subdivided to obtain multiple copies of each transgenic plant before rooting and transfer to the green house for induction by salicylic acid, or TMV treatment. The transgenic tissue was analyzed for GUS activity and mRNA expression of both the reporter GUS gene and the native PR1a genes. Cis-acting sequences sufficient for induction by both virus and chemical treatment were found in the 932 base pairs 5′ to the translation initiation site. The results of a deletion analysis of this sequence further defines the 5′ end of the cis-acting sequences required for induction by pathogen and chemical.

M 338 WATER-STRESS AND ABA RESPONSIVE RICE GENES, John Mundy, K. Y.-Shinozaki, K. Oeda, N-H. Chua, Department of Plant Molecular Biology, Rockefeller University, New York, N.Y. 10021. We have identified a rice gene (RAB 21) Responsive to ABA and water-stress (EMBO J. (1988) 7: 2279-2286). It encodes a glycine-rich, cytosolic protein of unknown function homologous with the cotton D 11 protein (PMB (1988) 11: 277-291). RAB 21 mRNA accumulates upon treatment with NaCl and/or ABA. The effects of NaCl and ABA are not cumulative, suggesting these inducers share a response pathway. Induction of RAB 21 mRNA accumulation by ABA is rapid (less than 15') and does not require protein synthesis, indicating preformed factors mediate this response. We have sequenced four RAB 21 genes (mapped to a locus < 30kB in length). Gene-specific probes show that all four genes are coordinately expressed. The four promoter regions share a single conserved decanucleotide sequence. Functional analysis of RAB 21 promoter-CAT constructs in transformed rice cells indicate that 1) negative regulatory sequences lie upstream of -440, and 2) ABA responsive element(s) (AREs) lie between -290 and -55 (CAAT box). Gel shift assays identify sequences in this region which specifically bind a protein factor(s) from nuclei of ABA-treated leaves. DNAse 1 foot-printing defines two homologous GC-rich binding sites, one of them overlapping the conserved sequence found in all four RAB genes. We are currently assaying these sequences in vivo to determine whether they are AREs.

M 339 PATATIN PROMOTER-DIRECTED TUBER-SPECIFIC GENE EXPRESSION IN POTATO, Jan-Peter Nap, Joke Onstenk, Wim G. Dirkse, Jeanine Louwerse*, Freek Heidekamp*, and Willem J. Stiekema, Molecular Plant Breeding, Research Institute Ital, P.O. Box 48, 6700 AA Wageningen, The Netherlands. *Present address: Centre for Phytotechnology RUL/TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.
Aiming at crop improvement, we search to obtain leaf- and tuber-specific expression of newly introduced genes in potato. To achieve tuber-specific expression, we have isolated members of the patatin gene family from a genomic library. Analysis showed these genes to belong to the so-called class II patatin genes, which are characterized by a relatively low level of tuber-specific expression. A 3.0 kb promoter fragment of one of these genes has been fused to the reporter gene β-glucuronidase and introduced in the Dutch commercial potato cultivar Bintje by tuber disc transformation using A. tumefaciens LBA 4404. None of the twelve obtained kanamycin resistant transgenic potato plants showed any detectable GUS activity in tubers. In contrast, Twell & Ooms (Plant Mol. Biol. 9,365,1987) reported a patatin promoter similar to ours to be active. This discrepancy will be discussed. Experiments to modulate the level of tuber-specific gene expression are in progress.

M 340 INTRODUCTION OF GLYCININ GENE INTO POTATO CULTIVARS USING AGROBACTERIUM TUMEFACIENS VECTOR, Ohtani T.[1], Momma T.[1], Okada K.[1], Fukazawa C.[2], and Kato T.[1], 1: Plant Laboratory, Kirin Brewery, Kitsuregawa-Machi, Shioya-Gun, Tochigi-Ken 329-14, Japan. 2: Genetic Engineering Laboratory, National Food Research Institute, Tsukuba, Ibaraki 305, Japan. Tuber discs were transformed with disarmed Agrobacterium tumefaciens vector, carrying glycinin cDNA (one of the subunits of soybean 11S storage protein) fused to the cauliflower mosaic virus 35S promoter. Tuber discs were co-cultivated with Agrobacterium for 48h. After co-cultivation, the tuber discs were plated onto selective regeneration medium containing 100 ug/ml kanamycin. Regeneration of plants commenced after a three or four week culture. Approximately 50% of the Agrobacterium-treated tuber discs regenerated shoots on selective medium. Shoots did not regenerate from control tuber discs. Plants were micropropagated and tested for their ability to root on a medium containing 100 ug/ml kanamycin. Rooted transformants were transferred to soil in a growth-chamber. These plants were analyzed by Southern blotting and Western blotting. The result showed introduction and expression of a glycinin subunit in potato cultivars.

M 341 Expression of glycinin gene in transgenic tobacco plant,
Okada K.[1] Ohtani T.[1] Momma T.[1] Fukazawa C.[1] and Kato T.[1]
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[2]National Food Resarch Institute Tsukoba Ibaraki 305 Japan

Transformation of tobacco protoplasts was achieved by introduction of chimeric genes using electroporation. Plasmid containing the chimeric gene consisting of cauliflower mosaic virus 35S promoter, the cDNA of glycinin A_2B_{1a} which is one of the subunits of soybean 11S storage protein and polyadenylation signal region of the nopalins synthase gene was used for transformation. Introduction of the plasmid DNA was performed with electroporation, which was generated by discharge of capacitor of 100 uF at 500 V/cm. Transformed colonies were obtained from cells electroporated with the plasmids at the frequency of 10^{-3} , whereas no colony was obtained with calf thymus DNA. Proteins were extracted from leaves and seeds of transgenic plants and analyzed by Western blot procedure. We could detect the glycinin proteins in leaves and seeds and we also could detect the glycinin polypeptides were not processed in leaf, although processed in seed.

Expression of glycinin cDNA and process of glycinin polypeptide in tansgenic plant will be discussed.

M 342 PROTEINS FROM NUCLEI OF WOUNDED TOMATO LEAVES WHICH BIND TO THE PROMOTER OF A WOUND INDUCIBLE PROTEINASE INHIBITOR II GENE, Curtis J. Palm, Gyn An and Clarence A. Ryan, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164.

The wound-inducible expression of a potato proteinase Inhibitor II gene is transcriptionally regulated. Deletion analysis of the promoter region of the gene indicates that sequences at approximately -100 and -600 are important for correct gene expression. Protein factor(s), which bind to DNA sequences from these regions of the gene have been found in nuclear extracts from tomato leaves. In gel retardation assays the binding of factor(s) to specific DNA fragments is greatly increased when using extracts made from wounded tomato leaves. The binding is sensitive to both the cation and anion components of the buffer. UV-crosslinking studies using bromodeoxyuridine labeled DNA indicate a single protein with MW ca. 29, 000 binds to the -600 region, where deletions show a major effect on expression. The protein is being isolated for further analysis of its role in regulating wound inducibility of the Inhibitor II gene. (Supported in part by grants from NSF and Enichem American, Inc.)

M 343 **HIGH-LEVEL, SUCROSE-INDUCIBLE EXPRESSION OF A CHIMERIC PATATIN-GUS GENE IN LEAF EXPLANTS OF TRANSGENIC TOBACCO PLANTS,** William D. Park, Herman C. Wenzler, Linda M. Fisher, and Greg A. Mignery, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128

The objective of this study was to compare the regulation of a patatin-GUS (β-glucuronidase) chimeric gene in related solanaceous species. We focused our effort on the genes for patatin, a family of lipid acyl hydrolases that accounts for 30-40% of the total protein in potato tubers. We found that a chimeric patatin-GUS gene containing 2.5 kb of 5'-flanking sequence from the patatin genomic clone PS20 can be induced to be expressed at high levels in explants from leaves of both tobacco and potato plants by 0.3 to 0.4 M Sucrose. Sucrose was the most effective of several simple sugars tested for GUS expression in the explants and the dose response for maximal GUS expression was similar for both tobacco and potato. These results show that the mechanisms that regulate somatic storage protein gene expression are also present in nontuberizing plants. This strongly suggests that tuberization evolved from a widely distributed mechanism in which gene expression is regulated by levels of available photosynthate.

M 344 GENETIC REGULATION OF TRANSIENT EXPRESSION OF MAIZE ANTHOCYANIN PATHWAY GENES INTRODUCED INTO INTACT MAIZE TISSUES BY MICROPROJECTILE BOMBARDMENT, Bradley A. Roth[1], Theodore M. Klein[1], John C. Sanford[2] and Michael E. Fromm[1], [1]USDA-ARS, Plant Gene Expression Center, Albany, CA 94710 and [2]Department of Horticultural Sciences, Cornell University, Geneva, NY 14456

The maize anthocyanin biosynthetic genes have been studied extensively at the genetic level and recently genomic clones corresponding to these loci have been isolated. *C* and *R* are genetically defined loci which regulate the expression of *A1*, *A2*, *Bz1*, *Bz2*, and *C2*, the structural genes in this pathway. The pathway produces purple anthocyanin pigments in the aleurone of the endosperm when *C* and *R* are present. When either *C* or *R* functions are missing due to mutations (*C, r; c, R or c, r* genotypes), the anthocyanin pigments are not accumulated.

In order to further study the regulation of the structural genes *A1* and *Bz1* by *C* and *R*, we have developed a system for introducing genes into intact aleurone tissues by microprojectile bombardment. Bombarded *A1* and *Bz1* structural genes complement mutant *a1* and *bz1* alleles, respectively, as evidenced by the production of purple anthocyanin pigment in *C, R* aleurones. To characterize the regulation of expression of the *A1* and *Bz1* promoters apart from the endogenous anthocyanin pathway, chimeric genes consisting of the *A1* promoter and firefly luciferase gene and a similar *Bz1* promoter/luciferase construct were bombarded into aleurones of various *C* and *R* allele content. Following bombardment, wildtype aleurones (*C, R*) express luciferase at high levels, while (*C, r*), (*c, R*) and (*c, r*) aleurones only express luciferase at low levels. Thus, the regulation of the transient expression of these chimeric genes is consistent with the regulated expression of the anthocyanin pathway *in planta*. This system has been used for a deletion analysis of the *Bz1* promoter to study the sequence elements necessary for its regulated expression.

M 345 **THE EXPRESSION OF THE NAPIN GENE UNDER THE CONTROL OF ITS OWN PROMOTER IN TRANSGENIC TOBACCO PLANTS.** Anderson J. Ryan, Christopher Royal, Sue Robinson, Robert Shields, Charles H. Shaw. Department of Biological Sciences, University of Durham. DH1 3LE. United Kingdom.

A 12.4kbp fragment of genomic DNA from *Brassica napus*, carrying a napin gene plus 11.7kbp of flanking sequences, was introduced into *Nicotiana tabacum* by agrobacterium mediated gene transfer. A sensitive ELISA assay detected napin gene product in 10/12 transformed plants at a level of 0.03-0.52% of total seed protein. Napin protein and mRNA were detected only in the developing tobacco embryo, and not in leaf, root, or stem tissue. Transformed tobacco plants expressing napin processed the precursor protein to 9kd and 4kd polypeptides, as found in the mature napin protein in *Brassica napus* embryos.

These results indicate that the *Brassica napus* DNA introduced into tobacco contains genetic information directing embryo-specific gene expression, and *Nicotiana tabacum* contains all the factors necessary for the processing of immature napin precursor to the mature protein.

M 346 SEQUENCES CONTROLLING THE TISSUE SPECIFIC PROMOTER ACTIVITY OF A LEGUMIN GENE FROM PEA, Anil H. Shirsat, Neville Wilford, Ronald R.D. Croy and Donald Boulter, Dept. of Biological Sciences, Plant Molecular Biology Group, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, U.K.
Maturing pea cotyledons accumulate large quantities of storage proteins at a specific time in seed development. To examine the sequences responsible for this regulated expression, a series of deletion mutants of the legA gene coding for the major seed storage protein legumin were made, and transferred to tobacco using the Bin 19 disarmed Agrobacterium vector system. 97 bp of promoter sequence including the CAAT and TATA boxes were insufficient for expression. Expression was first detected in a construct with 549 bp of upstream flanking sequence which contained the leg box element, a 28 bp conserved sequence found in the legumin type genes of several legume species. Constructs containing -833 and -1203 bp of promoter sequence significantly increased levels of expression. All expressing constructs preserved seed specificity and temporal regulation. The results indicate that promoter sequences between positions -97 and -549 bp are responsible for promoter activity, seed specificity, and temporal regulation of the pea legA gene. Sequences between positions -549 and -1203 bp appear to function as enhancer like elements, to increase expression.

M 347 EXPRESSION REGULATION OF THE ARABIDOPSIS THALIANA FERREDOXIN AND PLASTOCYANIN GENES. Sjef Smeekens, Oscar Vorst and Peter Weisbeek, Dept. of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.
In several species it has been shown that the expression of ferredoxin (FD) and plastocyanin (PC) genes is tissue-specific and light-controlled, most likely by a phytochrome-mediated mechanism. We are interested in the mechanism of this expression regulation and want to identify the cis- and trans acting regulatory factors involved in FD and PC gene expression. For this purpose genes encoding FD and PC were isolated and characterized from a Arabidopsis thaliana genomic library. In this species both proteins are encoded by single genes. The presumed 5'-regulatory regions of the FD and PC genes were fused to the reporter gene glucuronidase (GUS) and the fusions inserted into the A. tumefaciens plasmid pBIN19. Tobacco leaf discs were transformed and plants regenerated. These experiments show that 1.2 kb and 1.8 kb DNA fragments derived from FD and PC 5'-promoter regions, respectively, can induce expression of the reporter gene in transgenic tobacco plants.
For a more detailed analysis of cis-acting 5'-regulatory elements we constructed deletion mutants in the FD and PC promoter regions. These mutants were also tested in transgenic tobacco plants and the results of these experiments will be presented.

M 348 IDENTIFICATION AND CHARACTERISATION OF A TRANS-ACTING FACTOR INVOLVED IN THE REGULATION OF A CHALCONE SYNTHASE GENE IN TOBACCO.
Dorothee Staiger and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, 5000 Cologne 30, FRG

We have chosen the chalcone synthase promoter from Antirrhinum majus to study plant nuclear proteins involved in the regulation of light dependent gene expression. Using gel retardation assays we have identified a DNA-binding activity within the promoter region essential for UV light induction. A 15 bp sequence element responsible for specific binding of the trans-acting factor was delimited by oligonucleotide competition assays. The introduction of three G->T mutations completely inhibited the binding of the nuclear factor.

The DNA-binding protein was found in several different plant species including tobacco, snapdragon, petunia and arabidopsis. This suggests it may be involved in an universal mechanism of gene regulation. The factor was detected in extracts from noninduced as well as from UV light induced tobacco seedlings, indicating that de novo synthesis of the factor is not required for the activity. Purification of the factor from crude nuclear extracts of tobacco seedlings using a combination of conventional chromatography and sequence-specific affinity chromatography is in progress.

M 349 POLLEN-SPECIFIC EXPRESSION DIRECTED BY CHIMAERIC GENES IN TRANSGENIC TOMATO AND TOBACCO PLANTS. David Twell, Judy Yamaguchi, Rod Wing, Susan Larabell and Sheila McCormick, Plant Gene Expression Center, USDA-ARS/UC Berkley, 800 Buchanan Street, Albany, CA 94710.
We have isolated genomic clones from tomato corresponding to floral-specific mRNAs that are detectable in pollen, anthers and at lower levels in petals. The nucleotide sequences of three different genes (LAT52, LAT56, LAT59) were determined and their transcriptional start sites mapped. Transgenic tomato and tobacco plants containing 1.4kb 5'flanking DNA of LAT59 linked to the bacterial b-glucuronidase (GUS) and firefly luciferase (LUC) coding DNAs were generated. Quantitative analysis of GUS and LUC reporter enzyme activity in these plants showed anther-specific and developmental regulation that agreed well with Northern blot analysis of LAT59 mRNA levels in untransformed tomato plants. When assayed histochemically, GUS activity was detectable only in mature pollen with no staining in the anther wall. In contrast, plants containing the CaMV 35S promoter linked to GUS coding DNA showed GUS activity in the anther wall but not in pollen. These data demonstrate that 1.4kb of 5'flanking DNA of the LAT59 gene are sufficient to direct pollen-specific expression both in the homologous plant species (tomato) and in a heterologous one (tobacco). The differential regulation of the LAT59 and 35S constructs in pollen is of particular interest since the 35S promoter has previously been thought of as constituitive. The results of initial experiments designed to modify anther development in transgenic tomato plants by the use of the Agrobacterium tumefaciens iso-pentenyl transferase coding DNA linked to the LAT59 promoter will be presented.

M 350 REGULATION OF THE CELL TYPE-SPECIFIC EXPRESSION OF MAIZE *Adh1* AND *Sh1*: ELECTROPORATION-DIRECTED GENE TRANSFER INTO PROTOPLASTS OF SEVERAL MAIZE TISSUES, Julie M. Vogel, R. Kelly Dawe, and Michael Freeling, Department of Genetics, University of California, Berkeley, CA 94720.
Although expression of the maize genes, alcohol dehydrogenase-1 (*Adh1*) and *Shrunken-1* (*Sh1*), are similar in their ability to be induced to high levels in the anaerobic maize primary root (1), these genes exhibit distinct differences in their aerobic and anaerobic tissue-specific expression patterns in the maize plant. In an effort to identify and study cis-regulatory sequences in these genes that may contribute to these similarities and differences, we have begun a structure-function analysis of their promoter regions using electroporation-mediated gene transfer into maize protoplasts. Both nested deletion and in vitro-mutagenized derivatives of the *Adh1* and *Sh1* upstream sequence regions, each fused to the CAT reporter gene, are currently being constructed. In particular, in vitro mutagenesis is being used to mimic in vivo mutations that are known to lead to changes in organ-specific gene expression. Freshly-isolated protoplasts from primary root cortex, mesocotyl, and mesophyll are being used as electroporation recipients. Our preliminary results show that the intact *Adh1* promoter region can drive transient CAT expression in such primary tissue protoplasts. This study should provide a means for identifying regulatory sequences that are necessary for expression of each gene in various maize tissues.
(1) Springer et al. (1986) *Mol. Gen. Genet.* 205; 461.

M 351 ANALYSIS OF A CHIMERIC CLASS-I PATATIN-GUS GENE IN TRANSGENIC POTATO PLANTS: HIGH LEVEL EXPRESSION IN TUBERS AND SUCROSE-INDUCIBLE EXPRESSION IN LEAF AND STEM EXPLANTS, Herman C. Wenzler, Gregory A. Mignery, Linda M. Fisher and William D. Park, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128
Patatin is a family of lipid acyl hydrolases that accounts for 30-40% of the total soluble protein in potato tubers. We constructed a chimeric gene containing 2.5 kb of 5' flanking sequence from the class-I patatin genomic clone PS20 transcriptionally fused to β-glucuronidase (GUS) and introduced it into potato plants using a Ti-plasmid vector. The chimeric gene was expressed at high levels in tubers, but was not normally expressed in leaves, stems, roots or stolons before tuberization. However, expression of the class-I patatin-GUS construct was not "tuber specific" since leaf and stem explants cultured on medium containing 300 to 400 mM sucrose showed GUS activity equal to or greater than that of tubers. Induction of GUS activity in leaf and stem explants was accompanied by accumulation of patatin protein and large amounts of starch, but not by the morphological changes that normally are associated with tuberization. In contrast, the GUS reporter gene under control of the 35S promoter of cauliflower mosaic virus showed an essentially uniform pattern of expression in transgenic potato plants and was not inducible in transgenic leaf and stem explants by sucrose.

M 352 A TOBACCO ROOT-SPECIFIC GENE: CHARACTERIZATION AND REGULATION OF ITS EXPRESSION, Yuri T. Yamamoto, Gregoria N. Acedo and Mark A. Conkling, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614

We have isolated several tobacco cDNA clones which are highly expressed in roots but not in leaves or suspension culture cells. One of them, B7, shows a significant homology to both soybean nodulin26 and lens major intrinsic protein isolated from bovine(MIP26). In situ hybridization of the cDNA probe to tissue sections have revealed that the gene is highly expressed at meristematic regions of root tips. We are constructing a lacZ fusion to this gene product in order to raise antibodies to be used for immunocytochemical localization of the B7 product. The genomic clones corresponding to each cDNA have also been isolated in our laboratory. Sequence analysis of B7 genomic clones revealed that there are at least one intron, possibly two, in this gene. Various deletions of upstream region of the gene have been fused to bacterial beta-glucuronidase (GUS) gene and the constructs have been transformed into tobacco via Agrobacterium-mediated transformation. Tissues of regenerated plants will be tested for the GUS expression.

M 353 CELL TYPE EXPRESSION OF 35S CaMV-GUS REPORTER GENE IN TRANSGENIC SOYBEAN PLANTS, Ning-Sun Yang, Barry Cohen and Paul Christou, Plant Genetic Engineering, Agracetus, Middleton, WI 53562. Transformed soybean plants expressing a chimeric glucuronidase (GUS) gene under the control of the 35S CaMV promoter and a NOS polyadenylation signal were recovered using direct DNA transfer via electric discharge particle acceleration. Transgenic plants were selfed and F_1 progeny obtained. Expression of 35S GUS activity in F_1 plants was localized using thin tissue sections. Most tissue types expressed 35S GUS activity at various levels. Microscopic analysis at the cellular level revealed that three cell types exhibited the highest levels of enzyme activity. These were the phloem cells in root, stem and leaf, the parenchyma cells in the seed coat and the epidermal guard cells. GUS activity was detected at a lower level in palisade and spongy parenchyma cells of leaf or cotyledon and in colenchyma cells in the stem and petiole. Specific cell types, including cortex cells in the stem and root, non-guard epidermal cells and non-parenchyma seed coat cells (e.g. aleurone and endosperm cells) expressed little or no GUS activity. Our results suggest that, at least in the soybean system, the 35S CaMV promoter, is cell-type specific.

Signal Transduction; Receptors; Stress

M 400 TRYPTOPHAN ACTIVATED EXPRESSION OF A SEGMENT OF T-DNA OF Agrobacterium rhizogenes. Chaitali Bhaumik-Banerjee and Richard S. Berk, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI 48201.

A 3 Kb fragment of the T-DNA of a cucumopine strain of Agrobacterium rhizogenes (NCPPB 2659) was expressed in the E. coli maxicell system. Expression of the fragment showed two polypeptides of ca 28,500 and 24,500 daltons, only when the media was supplemented with L-tryptophan.

The T_L-DNA of agropine Ri plasmids share significant homology to T-DNA of this strain. So far, no 'auxin synthesising' genes have been located on the T_L-DNA. Instead, 'auxin sensitive' regions are present, raising the possibility that the T_L-DNA of pRi 2659 may likewise contain some 'auxin sensitive' segments. Tryptophan is a precursor of Indole-3-acetic acid in the auxin biosynthetic pathway; therefore it is likely that 'auxin sensitive' genes present in this 3 Kb T-DNA fragment are being trigerred by tryptophan into expressing these two polypeptides.

M 401 GENETIC ORGANIZATION OF AN OSMOTOLERANCE FUNCTION, Lesley Boyd[a], Alan McHughen[a] and Gopalan Selvaraj[b]. [a]Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, [b]Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Road, Saskatoon, Canada S7N 0W9.

Glycine betaine is synthesized and accumulated in response to water deficit or salt-stress in grasses and chenopods by oxidation of choline via betaine aldehyde[1,2]. A similar pathway exists in Escherichia coli which affords salt tolerance on this organism when an exogenous supply of choline is available[3]. We have cloned the bacterial genes that encode choline dehydrogenase (cdh; choline → betaine aldehyde) and betaine aldehyde dehydrogenase (bdh; betaine aldehyde → betaine). A physical and genetic map of these genes has been constructed. Our studies indicate that cdh gene product can also catalyze the conversion of betaine aldehyde to betaine in vivo.

M 402 SYNTHESIS OF OAT PHYTOCHROME IN TRANSGENIC TOMATOES. Margaret T. Boylan and Peter H. Quail, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

Monocot and dicot phytochromes differ in molecular size and immunological characteristics, although they share similar spectral properties and biological roles. In order to establish the degree of functional homology between phytochromes from evolutionarily diverse species, we have used the CaMV 35S promoter to express a full-length monocot (Avena sativa, oat) cDNA in a dicot plant (Lycopersicon esculentum, tomato). Immunoblot analysis shows that approximately 50% of the transgenic tomato plants synthesize oat phytochrome. The oat polypeptide is stable in re-etiolated transformants, often accumulating to higher levels than endogenous tomato phytochrome. Two pieces of evidence indicate that the heterologous oat polypeptide contains a covalently bound chromophore: first, leaves of light-grown transformants contain appreciably less oat phytochrome than leaves from re-etiolated plants; second, the oat polypeptide exhibits a differential pattern of proteolysis in tomato extracts dependent on irradiation with red vs. far-red light. We conclude that a monocot phytochrome can be synthesized and correctly processed to a photochemically active form in a dicot cell.

M 403 COMPARATIVE STUDIES ON THE FUNCTIONS OF Ri PLASMIDS OF AGROBACTERIUM RHIZOGENES, J. Brevet, D. Clerot, C. David, G. Hansen, M. Larribe, J. Tempe, CNRS-INRA Institut de Microbiologie, Batiment 409, Universite de Paris Sud, 91405 Orsay, France

Heteroduplex mapping of sequence homologies of Ri plasmids showed the presence of two conserved regions flanking a central region of reduced homology. Essential functions for root induction were mapped in the non-homologous region. Complementation studies between derivatives of different plasmids showed a high degree of functional homology even in the non-homologous region. Determinants involved in suppression of the geotropic response were mapped in one of the homologous regions.

M 404 INTERACTION OF A NUCLEAR FACTOR WITH THE UPSTREAM REGION OF AN rbcS GENE IN LEMNA GIBBA L. G-3; Jeffrey S. Buzby and Elaine M. Tobin, Department of Biology, University of California, Los Angeles, CA 90024 USA.

Lemna gibba has a twelve to fourteen member family of RuBisCo small-subunit genes (rbcS). The expression of this gene family and the steady-state level of transcripts from six of the genes have been previously shown to be under phytochrome control. The upstream region from one of the most strongly phytochrome-regulated genes, rbcS5B, was selected for use in identifying regulatory factors involved in this response. Since significant homologies between rbcS5B and its tandemly linked partner, rbcS5A, have been found in the region extending to -300, electrophoretic mobility shift assays were used to search for factors in nuclear protein extracts which interact with this region.

A nuclear factor has been identified which binds specifically at more than one site in this region. The factor does not seem to bind to the corresponding region of a small-subunit gene (rbcS13) which is not very highly expressed. Its interaction with that of rbcS5B can be competed by an upstream region from the CaMV-35S gene. The amount of binding activity appears to be significantly greater in light-grown plants than in those which have been dark-adapted for six days.

M 405 REGULATION OF THE AGROBACTERIUM RHIZOGENES ROOT INDUCING GENE rolB. Maura Cardarelli, Imerio Capone and Paolo Costantino, Dipartimento di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", P.le A.Moro 5, 00185 Rome, Italy.

Of the genes present on the TL-DNA of agropine-type A.rhizogenes rolB plays a crucial role in root differentiation.On carrot discs, rolB cloned in Bin19 is capable of directing morphogenesis only in the presence of a source of auxin and provided an extended (600 bp) 5′ non coding region is included in the construction (B600). A reduced version of the 5′ (300 bp, B300) does not allow this gene to control plant cell differentiation even in the presence of auxin. Infections with GUS under the control of B600, B300 and 35S promoters show the presence of an upstream regulatory element responsible for strong expression of rolB on the side of the discs where auxin accumulates, suggesting that auxin regulates the expression of this T-DNA gene. The regulation of rolB by auxin, the presence of protein factors mediating its activation and the localization of the activating sequence are currently being investigated.

M 406 ANAEROBIC TREATMENT ALTERS THE CELL-SPECIFIC EXPRESSION OF Adh-1, Sh, AND Sus GENES IN ROOTS OF MAIZE SEEDLINGS, Lisa J. Rowland, Yen-Ching Chen, and Prem S. Chourey, USDA/ARS, Department of Plant Pathology, University of Florida, Gainesville, FL 32611

We have examined the in situ expression pattern of Sh and Sus which encode sucrose synthase isozymes SS1 and SS2 respectively and Adh-1 which encodes alcohol dehydrogenase 1 (ADH1) in the lower region of the primary root of maize seedlings in response to anaerobiosis. In situ hybridization and/or immunolocalization experimets revealed a unique spatial pattern of expression for each of the three genes. Anaerobic induction of ADH1 RNA was localized to the epidermis and cortex. Induction of Sh was marked by highly elevated SS1 RNA levels in the vascular elements, pith, and epidermis. A significant but less drastic increase in SS protein was found in these same tissues as well as the root cap; the increased level of immunosigan1 was, however, restricted to cells within about 1 centimeter of the root apex. The specific response of the Sus gene to anaerobic stress was determined using a sh deletion mutant; Sus responded with a slight reduction in SS2 RNA and protein levels except in the root cap where SS2 protein, but not SS2 RNA, was induced. These data indicate that multiple regulatory controls including cell-specific post-transcriptional mechanisms modulate SS levels in anaerobically-stressed seedlings.

M 407 REGULATION OF PHYTOCHROME MRNA ABUNDANCE IN GREEN OAT LEAVES, James T. Colbert* and Cindy L. Edwards, Department of Biology, Colorado State University, Ft. Collins, CO 80523.

The ability of green Avena sativa seedlings to accumulate phytochrome mRNA during extended time periods (24-48 hours) in the dark has been previously shown (1). However, in these experiments no distinction was made between the green portions of the leaves that were present prior to the dark treatment and the etiolated basal portions of the leaves that developed during the dark treatment. We harvested these distinct portions separately from leaves of oat seedlings grown four days in continuous white light followed by three days in darkness. Probes derived from an oat phytochrome cDNA clone (pAP3.2) were used to measure the abundance of phytochrome mRNA. Both the green and etiolated portions accumulate phytochrome mRNA to a level of about 85% of the four-day-old etiolated oat level. Subsequent experiments with similar seedlings showed that both green and etiolated portions are capable of inducing a dramatic decline in phytochrome mRNA abundance in response to a saturating red light pulse. Despite the ability of green oat leaves to accumulate phytochrome mRNA and to down-regulate phytochrome mRNA abundance, we detect no diurnal variation in phytochrome mRNA abundance in green oat seedlings maintained on a 12 hour day/night cycle. This work was supported by the Colorado State University Agricultural Experiment Station and by USDA 88-37261-3681.

1. Colbert, J.T., H.P. Hershey and P.H. Quail (1985) Plant Molec. Biol. 5:91-101.

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M 408 MOLECULAR CLONING AND DEFENSE-RELATED EXPRESSION OF A TOMATO HMG CoA REDUCTASE GENE, Carole L. Cramer, Hee Sung Park, Cynthia J. Denbow, Zhenbiao Yang and George H. Lacy, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) mediates a major rate-limiting step in isoprenoid biosynthesis leading to the production of carotenoids, sterols, electron transport components, rubber, gibberellins, and abscisic acid in higher plants. In the Solanaceae, HMGR is also involved in disease resistance, directing the synthesis of sesquiterpenoid phytoalexins. We isolated HMGR gene sequences to analyze the regulation of phytoalexin accumulation in biotically stressed tomato and potato tissues. A full-length genomic clone encoding HMGR was isolated from a tomato genomic library based on cross-hybridization with a probe containing yeast HMG1* sequences. Partial sequence analysis reveals regions exceeding 65% nucleic acid and 70% derived amino acid identity with yeast and human HMGR. HMGR sequences hybridize to tomato and potato mRNA species of about 2.4 kb suggesting that the encoded plant HMGR (estimated at 65-70 kD) is substantially smaller than yeast or mammalian HMGR (97-100 kD). Northern hybridizations of HMGR mRNA from tomato cells and seedlings suggest that HMGR is induced by a very specific subset of disease-related elicitors compared to another defense-related gene, phenylalanine ammonia-lyase. In potato, HMGR mRNA levels are greatly elevated in tuber slices inoculated with the soft rot bacteria, Erwinia carotovora, but not in wounded tubers.

* Basson, et al. (1986) PNAS 83:5563.

M 409 ELICITOR-MEDIATED CHANGES IN DEFENSE GENE EXPRESSION IN CULTURED ALFALFA CELLS. Karen Dalkin, Brent Edington, Robert Edwards, Jesus Jorrin and Rick Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402, USA.

We are initiating a program aimed at cloning genes involved in resistance of alfalfa to pathogenic fungi, and here report experiments defining the molecular responses of cultured alfalfa cells to elicitor molecules. Of a range of potential elicitors, material from the cell walls of Colletotrichum lindemuthianum was the best inducer of the accumulation of the isoflavonoid phytoalexin medicarpin. Induction kinetics are presented for the following enzymes involved in the synthesis of phytoalexins and in other defense responses in alfalfa: phenylalanine ammonia-lyase, cinnamic acid 4-hydroxylase, 4-coumarate CoA ligase, chalcone synthase, chalcone isomerase, caffeic acid O-methyl transferase, isoflavone O-methyl transferase(s), chitinase, 1,3-β-D-glucanase and glutathione S-transferase. Both chalcone synthase and phenylalanine ammonia-lyase exhibit subunit polymorphism and are most likely encoded by multigene families. We outline some properties of these polymorphic forms, and describe elicitor-induced changes in chalcone synthase transcripts and other mRNA species.

M 410 TOWARDS A GENETIC SYSTEM TO IDENTIFY AND ENUMERATE LOCI REQUIRED FOR THE GENERATION OF A HYPERSENSITIVE RESPONSE IN *ARABIDOPSIS THALIANA*. J.L. Dangl, A. Horrchiss and K. Hahlbrock, Biochemistry., Max-Planck Inst., Cologne, FRG.

The hypersensitive reaction (HR) of plant cells to invasion by pathogens is governed by either non-host or "R gene" mediated factors. HR phenotype is often characterized by limited pathogen multiplication and localized necrosis of plant cells at the point of infection. Much information exists regarding induced biochemical events which accompany the HR. There is, however, a paucity of knowledge regarding the number of loci required for a successful HR. In some systems, many cDNAs corresponding to pathogen induced mRNAs have been isolated. It is, unfortunately, difficult to ascribe genetically causal roles to these gene products. We began development of an admittedly artificial patho-system in *Arabidopsis* aimed at identification of loci required for a successful HR against common bacterial phytopathogens. After low density inoculation of *Xanthomonas campestris* pathovars into leaves of 4 week old *Arabidopsis* plants, rapid bacterial growth accompanied by chlorosis and rotting of the inoculated leaf was observed over 7 days. Similar symptoms and growth curves were observed after inoculation with several *Pseudomonas syringae* pathovars. Two, however, grew poorly and caused local browning after 3-5 days. We used one to screen 700 M2 plants from seeds mutagenized with either X-ray or EMS. Our aim was to identify plants rendered susceptible to bacterial invasion. Two criteria were met by 4 putative mutants: These plants showed symptoms and bacterial growth kinetics observed after inoculation of wild type plants with more virulent *P. syringae* pathovars. Importantly, *P. syringae* isolated from these putative mutants and inoculated back into wild type plants were unable to grow significantly. We are analyzing segregation of this trait the next generation from these putative mutants.

M 411 CHARACTERIZATION OF A PEA NUCLEAR FACTOR BINDING TO LIGHT REGULATED RBCS AND CAB GENES. Neeraj Datta and Anthony R.Cashmore, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia PA - 19104.

We have identified a DNA binding protein from the nuclei of pea plumules which binds a specific sequence, the N box (consensus sequence AATATTTTTATT) in light regulated genes - rbcS ss3.6 from pea, rbcS 3A from tomato and cab E from tobacco. We call this factor the N box binding factor (NBF). This factor is regulated by phosphorylation and is active in the dephosphorylated form and inactive in the phosphorylated from. We are currently determining whether the activity of NBF is affected by exposure of etiolated pea seedlings to light.

M 412 THE FUSICOCCIN-RECEPTOR: PART OF A SIGNAL TRANSDUCTION CHAIN IN THE PLASMA MEMBRANE? Albertus H. de Boer[1] and Robert E. Cleland[2], Department of Plant Physiology, University of Groningen, POB 14, 9750 AA Haren, The Netherlands[1]; Department of Botany, University of Washington, Seattle WA 98195[2].

Fusicoccin (FC), a fungal phytotoxin, has a strong and rapid effect on plant cell metabolism. The membrane potential hyperpolarizes, H^+-extrusion and K^+-uptake increase and cell elongation is stimulated. It is likely that the first event in the FC action is binding of FC to a protein located in the plasma membrane (PM). Recently, this binding protein of FC (FCBP) has been identified and purified by means of a FC-affinity column after solubilization with octyl-glucoside (1). On SDS-PAGE there were 2 major bands with an approx. MW of 31.0 and 32.5 kD in a stoichiometry of 1:2. These results indicate that the FCBP is a heterotrimer of 96 kD.

The FCBP is found in all plant species tested, so it must be a highly conserved protein. In animal cells, potent toxins often interfere with key-proteins of a signal transduction chain; e.g. cholera and pertussis toxin interfere with the α-subunit of the G-protein. Binding of FC to the FCBP has some features in common with the binding of GTP/GDP to G-proteins: the importance of Mg^{++}, the sensitivity to mercurials, the stimulatory effect of ammonium sulfate and the binding kinetics. Interestingly, it was recently reported (Marmé, pers. comm.) that pertussis toxin ADP-ribosylates two polypeptides in the PM of plant cells with an apparent MW of 31 and 33 kD.

FC could exert its effects also by interference with a cation channel (H^+, K^+ ?), either directly or indirectly. We now investigate this possibility by reconstitution of the purified FCBP in giant liposomes and by studying its behaviour with the patch-clamp technique.

(1) De Boer, A.H., Watson, B.A., Cleland, R.E. Plant Physiology (in press).

M 413 CELL CYCLE REGULATION IN HIGHER PLANTS. Heidi S. Feiler and Thomas W. Jacobs, Department of Plant Biology, University of Illinois, Urbana, IL 61801. Plant morphogenesis is the spatial and temporal integral of simultaneous meristematic activities. Cell division regulation thus directly impinges upon the morphogenetic product. We have begun to investigate the molecular basis of mitotic cell cycle control in higher plants. We have obtained antibodies directed against a 34 kd phosphoprotein, p34, which regulates the mitotic cycle in the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Encoded by *S. pombe*'s *cdc2* gene, p34 is a protein kinase essential for passage of cells through the G1 and G2 stages of the cell cycle. We have found 34 kd cross-reacting material (CRM) on immunoblots of proteins extracted from all 6 plant species we have tested. As in *S. cerevisiae*, the CRM is associated with a particulate, extranuclear subcellular fraction and its abundance does not appear to be differentially regulated amongst developmentally divergent tissues. These findings suggest that a system homologous to the yeasts' multicomponent cell cycle regulatory network, of which p34 is only one of several identified elements, controls the plant cell cycle. Results will be discussed concerning our investigation into the biochemistry, cell biology and molecular genetics of p34 in plants.

M 414 PROGRESS TOWARD THE CLONING OF THE *ABI* LOCI IN *ARABIDOPSIS THALIANA* Jérôme Giraudat and Howard M. Goodman, Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114.
Abscisic acid (ABA) plays an important regulatory role; however, almost nothing is known about the molecular pathways involved in its action. In *Arabidopsis*, several "ABA-insensitive" mutant (*abi*) have been identified which produce normal levels of ABA, but are altered in ABA responsiveness. We are attempting to clone the genes corresponding to the *abi* loci by taking advantage of the combined physical and RFLP maps being developed in this laboratory. However, it is difficult to assign with confidence a precise position on the genetic map to an RFLP probe, as only two genetic markers per chromosome were used to align the genetic map with the RFLP map. Therefore, we have constructed a series of double mutant strains (Lansberg ecotype), each carrying an *abi* mutation flanked by a linked phenotypic marker. Each of these strains has been crossed to the Columbia ecotype, and F1 progeny were allowed to self. Recombinants between the *abi* locus and the flanking marker were selected in the F2 population for RFLP analysis. This allows us to enhance the resolution of the map (approx. 0.25 cM or 40kb respective to the *abi* locus) while minimizing the number of progeny to analyze. Using this approach it should be possible to localize the gene of interest to one or several clones. In which case, the gene can be identified by functional complementation or alternatively by physical means.

M 415 ANAEROBIC INDUCTION OF ALANINE AMINOTRANSFERASE IN BARLEY ROOT TISSUE, Allen G. Good and William L. Crosby, Molecular Genetics Section, Plant Biotechnology Institute, NRC Canada, 110 Gymnasium Road, Saskatoon, Saskatchewan, Canada S7N 0W9. Alanine aminotransferase (GPT) activity increases up to 4 fold during several days of anaerobic induction in barley roots, reaching a maximum activity of 13 IU gm^{-1} fw. This increase in activity paralleled the increase in ADH activity in the same root tissue. The isozyme profile of GPT in barley and maize root tissue comprised two bands of activity, the higher mobility band having much lower activity. Native PAGE indicated that the induction of GPT activity results from an increase in the level of activity of these two bands, no other activities were detected. When root tissue was induced under different levels of hypoxia (0%, 2%, 5% and 21% 0_2), changes in GPT activity were found to increase with lower levels of oxygen. Comparisons of GPT induction in barley, maize, rye and wheat indicate that this enzyme is induced in the root tissue of all of these cereals, however anaerobic root conditions do not result in the induction of GPT activity in leaf tissue. These results suggest that alanine aminotransferase shows a very similar pattern of induction to ADH in barley root tissue and may be important in anaerobic glycolysis.

M 416 GENETIC ANALYSIS OF ETHYLENE-MEDIATED SIGNAL TRANSDUCTION IN ARABIDOPSIS
Plinio A. Guzmán and Joseph R. Ecker, Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104.

The molecular mechanisms of plant hormone reception and signal transduction are poorly understood. The use of mutants to unravel such complex biological phenomena has proven to be a powerful tool in several systems. We have chosen the response of etiolated *Arabidopsis* seedlings to the gaseous hormone ethylene as a system for the molecular genetic analysis of hormone-regulated processes. The phenotype of etiolated seedlings exposed to ethylene is easily scored; the apical hook becomes exaggerated while elongation of the hypocotyl and root is inhibited. We have isolated ethylene-related mutants from independent lots of EMS and/or X-ray treated (M2) seeds. Five different classes of mutants were identified: 1) Ethylene insensitive (***ein***) mutants do not show ethylene-mediated inhibition of hypocotyl or root elongation and have no apical hook. Genetic analysis of 3 dominant and 11 recessive ***ein*** mutants indicates that the recessive mutations are either allelic or very closely linked. At least one of the dominant mutants is not allelic to this locus. 2) Hypocotyl insensitive (***hin***) mutants were selected as ethylene insensitive but, unlike ***ein*** mutants, display an apical hook. 3) Suppressor of ethylene insensitivity (***sin***) mutants were identified as second-site suppressors of the ***ein4*** mutation. ***sin1*** is a dominant suppressor that maps close to the original ***ein4*** mutation and suppresses all of the ***ein*** phenotypes. 4) Ethylene overproducer (***eto***) mutants mimic the response to exogenous ethylene in the absence of the gas. ***eto*** produces large amounts of ethylene under conditions were it is undetectable in the wild type plant. ***eto*** is a recessive mutation whose phenotype can be reversed by treatment with an inhibitor of ACC synthase, the key regulatory enzyme for ethylene production. 5) In an attempt to unequivocally target mutations in an ethylene receptor(s), mutants were selected which respond to competitive inhibitors of ethylene binding. Responsive to antagonist (***ran***) mutants display a typical ethylene response when treated with the ethylene antagonist 2,5-norbornadiene. We are currently RFLP mapping these ethylene-related mutations as well as constructing double mutant combinations.

M 417 CLONING OF COLD REGULATED GENES FROM ARABIDOPSIS THALIANA,
HAJELA, R. K., HORVATH, D. P., GILMOUR, S. J. AND THOMASHOW, M. F., Department of Crop and Soil Sciences, Michigan State University, EAST LANSING, MI 48824.

We previously demonstrated that Arabidopsis, like many other plants, becomes more tolerant to freezing temperatures when first exposed to low nonfreezing temperatures (Gilmour et al. 1987 Plant Physiol. 87:745). Further, in vitro translation of polyA$^+$ RNA isolated from control and cold acclimated plants indicated that the levels of certain mRNAs increase substantially in the cold. Here we confirm and extend these results. We have constructed a cDNA library in lambda Zap (0.5 to 1 X 10^5 independent inserts), screened it by differential hybridization (cDNA probes made to "cold" and "control" mRNA) and identified 4 clones that were cold regulated: pHH 28, pHH 29, pHH 67 and pHH 71. mRNA levels for all of these cDNA clones are very low or undetectable when plants are grown at 23°C, but begin to accumulate within 4 hours after shifting the temperature down to 5°C; they reach maximum levels within 24 hours. Steady state levels of these mRNAs remain high for up to 15 days (the longest time tested), but fall to low or undetectable levels within 4 to 8 hours when plants are returned to 23°C. The mRNAs corresponding to two of the cDNA clones, pHH 28 and pHH 29 are also induced by another environmental stress: high salinity (200 - 400 mM NaCl). Hybrid selected - in vitro translation experiments indicated that the mRNA represented in pHH 28 encodes a polypeptide of approximately 160,000 daltons with a pI of 4.5. Future experiments will focus on the roles that cold regulated genes have in plant acclimation to low temperature and the mechanism(s) responsible for their thermoregulation.

M 418 STRUCTURE AND EXPRESSION OF ALCOHOL DEHYDROGENASE GENES FROM PINE, D.E. Harry, K.S Mordecai, Dept. of Forestry, University of Illinois, Urbana, IL 61801; C.S. Kinlaw, D.D. Sleeter, H. Burr, USDA Forest Service, P.O. Box 245, Berkeley, CA 94701; C. Loopstra and R.R. Sederoff, Dept. of Forestry, North Carolina State University, Raleigh, NC, 27695. The genes encoding alcohol dehydrogenase (ADH) are among the genes best characterized from angiosperms. We are studying ADH in two widely planted pine species (Pinus taeda and P. radiata) as a model for gene structure and expression in conifers, a commercially important and evolutionarily distinct group of woody plants. Isozyme data suggest that in pines, ADH is usually encoded by 2-4 genetically distinct loci. After pine seedlings are exposed to anaerobic conditions, the activity of one ADH isozyme is increased relative to another isozyme. This increased activity is paralleled by an increase in RNA abundance, as seen on northern blots. Our goal is to isolate and manipulate DNA sequences that correspond to the inducible ADH gene from pine. To this end, several pine ADH cDNA clones were identified from a lambda gt10 library using a maize cDNA clone (pZML793) as a heterologous probe. These cDNA clones share about 70% DNA sequence homology with the coding sequences from other plant ADH genes. Using these pine cDNAs as probes for Southern blots of genomic DNA, we observed more fragments than expected based on results from angiosperms. An examination of these sequences will be useful to understand the nature of the pine ADH gene family. We have now screened genomic libraries (EMBL3) from both pine species, and have identified many independent clones that share homology with our ADH cDNAs. These are being characterized, and results are discussed in terms of the structure of the pine ADH gene family.

M 419 A TWO ELEMENT TRANSPOSON TAGGING SYSTEM FOR *ARABIDOPSIS THALIANA*, Roger W. Innes, Barbara Baker, and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720 We have introduced derivatives of the Maize transposable elements *Ac* and *Ds* into *Arabidopsis thaliana* with the goal of developing an efficient gene tagging system. Our two element system is comprised of a stable *Ac* element that encodes and active transposase, but is incapable of transposition (Ac^*), and a *trans*-activatable *Ds* element. One of the initial questions we wish to address is whether *Ds* will preferentially transpose to nearby (genetically linked) sites in the *A. thaliana* genome, as has been demonstrated in Maize. If *Ds* transposes preferentially to linked sites, it should be feasible to "direct" a *Ds* element towards a gene of interest by providing a "launching pad" that is genetically linked to the target gene. To provide launching pads throughout the *A. thaliana* genome, we are currently obtaining numerous independent insertions of our *Ds* construct using *Agrobacterium tumefaciens* mediated transformation. We now have over 20 regenerated plants and are in the process of mapping insertions using RFLP analysis. The *Ds* elements will be activated by crossing *Ds* containing plants to Ac^* containing plants. As an initial test of the efficacy of gene tagging in *A. thaliana*, we are attempting to tag the alcohol dehydrogenase (*adh*) gene. Seeds in which *adh* has been inactivated can be selected by treating with allyl alcohol during germination. Comparison of the frequency of *Ds* transposition into *adh* from launching pads linked and unlinked to *adh* will provide data on the value of targeting. Should targeting prove efficient in *A. thaliana*, the collection of launching pads developed in this study should be valuable for tagging any gene of interest. Our primary goal is to tag a disease resistance gene.

M 420 THE INDUCTION OF MANGANESE SUPEROXIDE DISMUTASE IN RESPONSE TO STRESS IN NICOTIANA PLUMBAGINIFOLIA, Dirk Inzé, Chris Bowler and Marc Van Montagu, Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium)
Superoxide dismutases (SOD) are metalloproteins that catalyze the dismutation of superoxide radicals to oxygen and hydrogen peroxide. They are considered essential for an organism's defense against toxic reduced oxygen species which are generated in many biological oxidations. We have cloned a cDNA from Nicotiana plumbaginifolia encoding a manganese superoxide dismutase (MnSOD), an enzyme which is synthesized in the cytoplasm and subsequently translocated to the mitochondrial matrix. Analysis at the mRNA and protein level revealed that it is weakly expressed in whole plants but present in large amounts in cell suspension cultures. We were able to induce these high levels in leaves by incubating them with metabolisable sugars. This induction accompanies an enhanced cytochrome oxidase activity, which is likely to reflect an increase in mitochondrial activity as caused by the exogenous sugars. To determine whether MnSOD expression forms a part of the plant response to pathogens, we analyzed SOD protein and mRNA profiles in leaves after treatment with ethylene, salicylic acid, and Pseudomonas syringae. All these treatments enhance plant respiration and all increased the abundance of MnSOD transcript. These results highlight the importance of the protective role of MnSOD during conditions of increased mitochondrial activity. Currently, we are investigating MnSOD expresssion at the cellular level by the analyzing chimeric β-glucuronidase genes under the control of the MnSOD promoter.

M 421 *TMS2* ACTS AS A SUICIDE GENE IN TRANSFORMED *ARABIDOPSIS THALIANA* SEEDLINGS, George Karlin-Neumann & Elaine Tobin, Biology Dept., Univ. of California, Los Angeles, CA 90024. We are attempting to isolate mutants which will enable us to identify components of the signal transduction pathway through which phytochrome regulates the transcription of specific nuclear genes. We have devised a positive (suicide) selection for such mutants which involves fusing a presumed phytochrome-regulated promoter (PC) to the *tms2* gene of *Agrobacterium* (encoding an indole acetamide hydrolase) and transforming this PC:tms2 gene fusion into *Arabidopsis*. Mutagenized progeny of such a transformant which lack a functional transduction pathway should survive on the suicide substrate, 1-naphthaleneacetamide (NAM), when grown under red light (R), whereas seedlings with a functional pathway should convert NAM to the toxic product, naphthaleneacetic acid (NAA), and die (or be severely growth-inhibited). Initial studies of etiolated T2 seedlings (derived from a transformant containing a PC:tms2 gene fusion) grown on various NAM concentrations show: (i) the presence of the *tms2* gene increases seedling sensitivity to NAM by over 40-fold such that transformed seedlings germinate but fail to grow on a [NAM] which does not affect the growth of wild-type seedlings and (ii) Tms2 activity is inducible by R in T2 seedlings and thus presumably under the control of phytochrome.

M 422 GENETIC AND BIOCHEMICAL ANALYSES OF AUXIN RESISTANT MUTANTS OF ARABIDOPSIS, Cynthia Lincoln, James Britton, Jocelyn Turner and Mark Estelle, Biology Dept., Indiana University, Bloomington, IN 47405. We have identified a series of allelic recessive mutations in Arabidopsis thaliana by screening for mutants which are resistant to the synthetic auxin 2,4-D. Genetic analysis has shown that these auxin resistant mutants fall into one complementation group which we have named Axr1. The morphological phenotype of the axr1 mutants includes alterations in leaf shape, internode length and flower development. In addition, the mutants display a reduction in apical dominance and a loss of normal root geotropic response. Among the 15 axr1 alleles identified so far, several exhibit a more severe abnormal phenotype including a significant reduction in overall size of the plants and self-sterility. As a first step towards isolation of the Axr1 gene, we have determined its genetic location by using restriction fragment length polymorphisms (RFLPs) between two strains of Arabidopsis. We will use the most closely linked RFLP to initiate a chromosomal walk to the Axr1 gene. Along with the genetic analysis of the Axr1 locus, we have begun a biochemical characterization of the axr1 mutants. Endogenous IAA levels and the auxin induction of IAA-aspartate synthesis are being measured in both wild-type and mutant Arabidopsis plants.

M 423 SINGLE GENES FROM *AGROGACTERIUM RHIZOGENES* INCREASE THE SENSITIVITY OF TOBACCO PROTOPLASTS TO AUXIN. Christophe Maurel, Angelo Spena*, Hélène Barbier-Brygoo, Jacques Tempé+ and Jean Guern. C.N.R.S.-I.N.R.A., Physiologie Cellulaire Végétale, bât. 15, 91198 Gif-sur-Yvette Cedex, France - *Max Planck Institut für Züchtungsforschung, 5000 Köln, F.R.G. - +Institut de Microbiologie, Faculté des Sciences, 91405 Orsay Cedex, France.

The sensitivity to auxin of roots transformed by *Agrobacterium rhizogenes* was shown to be 100-1000 times higher than that of untransformed ones (1). Among different cellular effects induced by auxin, its action on the transmembrane electrical potential difference (Em) of isolated protoplasts allowed an accurate evaluation of the sensitivity to auxin. Tobacco plants regenerated from Ri-transformed roots display a typical "hairy-root" syndrome, and single genes from the Ri-T-DNA, corresponding to rol A, B and C loci, were recently shown to influence plant development (2). The sensitivity to auxin of tobacco plants transformed either by the whole Ri-T-DNA or by single rol genes was estimated by electrophysiological measurements on mesophyll protoplasts.
Here we show that, as already described for protoplasts from Ri-transformed roots, mesophyll protoplasts from transformed tobacco plants exhibit an increased sensitivity to auxin. Furthermore, rol loci A, B and C, together or as single genes, are able to induce various increases in the sensitivity to auxin, with a good correlation to their rhizogenic effects.

1- Shen W.H., Petit A., Guern J. and J. Tempé (1988) Proc Natl Acad Sci USA, 85, 3417
2- Schmülling, T., Schell J. and Spena A. (1988) EMBO J., 7, 2621

M 424 ELICITOR-INDUCIBLE GENES HOMOLOGOUS TO A MAMMALIAN CYTOCHROME P-450 GENE, Mona C. Mehdy, Jinsong Sheng, Department of Botany, University of Texas at Austin, Austin, TX 78713, Renato D'Ovidio, Dept. of Plant Biology, University of Rome, Rome, Italy and Chris Lamb, Salk Institute, La Jolla, CA 92037
The biosynthesis of isoflavonoid phytoalexins by leguminous plants requires the action of several cytochrome P-450 enzymes. To identify the genes encoding these enzymes, we have employed a rabbit P-450 cDNA clone as a hybridization probe. A cDNA library made from poly (A)+ RNA isolated from elicitor-treated Phaseolus vulgaris cell cultures was screened and yielded 8 positive clones. Two clones showed hybridization to RNAs of different sizes which accumulate in elicitor-treated cell cultures, in wounded hypocotyls, and during the infection of bean seedlings with Colletotrichum lindemuthianum. The kinetics of hybridizable mRNA accumulation parallel those previously observed for mRNAs encoding other enzymes in the bio-synthetic pathway: phenylalanine ammonia-lyase, chalcone synthase, and chalcone isomerase mRNAs. Sequence analysis of one clone revealed a region of approximately 60% nucleotide sequence homology with the rabbit P-450 gene. The complete sequence of these 2 clones and their genomic organization deduced from genomic blotting experiments will be presented.

M 425 GENETIC AND PHYSIOLOGICAL BASIS FOR THE TRANSFORMED PHENOTYPE CONFERRED BY THE RI TL-DNA OF *AGROBACTERIUM RHIZOGENES*, Anthony J. Michael[1], Daniel Burtin[2], Gilles Thomas[1], Li Yan Sun[1], Michel Paynot[2], Marie-Odile Monneuse[1], Josette Martin[2] and David Tepfer[1], [1]I.N.R.A., 78026, Versailles Cedex, France, [2]I.N.R.A., 21034, Dijon Cedex, France, *Agrobacterium rhizogenes* induces root formation in dicots through transfer of Ri T-DNA to a receptive cell. Three genes necessary for root induction are grouped on a 4.3 kb fragment in the TL-DNA. Analysis of their individual effects in a number of laboratories has produced variable results. We have re-examined the effects of several of these genes under the influence of their own promoter or under the 35S promotor of CaMV. In addition, we have established a correlation between the phenotypic alterations associated with the presence and expression of these genes and a repression of the accumulation of polyamines and their conjugates.

M 426 PATHOGENESIS-RELATED (PR) PROTEINS AS STRESS MARKERS IN MONO- AND DICOTYLEDONEOUS PLANTS. AN IMMUNOLOGICAL COMPARISON, Egon Mösinger[1] and Willi Fischer[2], Pflanzenphysiologisches Institut der Universität Bern, CH-3013 Bern, Switzerland, present address: 1) Agro Research, SANDOZ AG, CH-4002 BASEL, 2) F&E Pflanzenschutz, CIBA GEIGY AG, CH-4002 BASEL. The accumulation of acid-soluble proteins in the apoplast of stressed plants is a well described phenomenon. Some of these proteins were recently described as hydrolytic enzymes. Using antibodies against P14 of tomato and against glucanases and chitinases of tobacco we analized "western blots" of acidic protein extracts from about 10 crop plants. Extracts from noninfected controls were compared to extracts from plants that were infected by economically important pathogens. In nearly all plants tested acid-soluble proteins were identified showing immunological cross-reaction to P14 of tomato and the glucanases and chitinases of tobacco. In some cases the appearance of PR-proteins is correlated to the induction of acquired resistance.

M 427 STUDIES ON THE SELF-REGULATION OF HEAT SHOCK GENE TRANSCRIPTION IN SOYBEAN, Ron T. Nagao, Julie Y.-R. Lee, Janice A. Kimpel, C.-Y. Lin and Joe L. Key, Department of Botany, University of Georgia, Athens, GA 30602. Short-term heat shock (HS) regimes, cycles of heat followed by control temperature incubations and continuous heat treatments were used to study the induction and shut-off (self-regulation) of HS gene transcription. During continuous HS steady state levels of HS mRNA reach maximum levels at about 2 to 3 hr and were much reduced by 6 hr. Self-regulation was further characterized by nuclear run-on experiments which indicated that transcription at 40°C continues through 2 hr but was shut off by 4 hr. Treatment of soybean seedlings with the amino acid analogues, azetidine-2-carboxylic acid and to a lesser extent canavanine, induced HS gene transcription. The steady state accumulation of HS mRNA at 2 hr was somewhat less than the corresponding HS treatment but self-regulation was impaired because accumulation of HS mRNA was much higher at 6 hr than the corresponding heat treatment. Analogue treatment in combination with HS seemed to have an additive effect on mRNA accumulation; self-regulation was not apparent. Treatment with cycloheximide did not affect induction of HS mRNA following analogue or heat treatment. Not all families of HS genes were induced by analogue treatment, but analogue treatment during HS interfered with the self-regulation of both those that were induced by the analogues and those mRNAs that were specific to the HS treatment.

M 428 MOLECULAR AND PHYSIOLOGICAL ANALYSIS OF A MASKED STRAIN OF TOBACCO MOSAIC VIRUS (PV42) R.S. Nelson[1,2], R.A.J. Hodgson[1], C.A. Holt[1], C. Conesa[1], H.B. Pakrasi[1], R.N. Beachy[1], [1]Department of Biology, Washington University, St. Louis, MO 63130, [2]Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK 73402.
Tobacco mosaic virus (TMV) strain PV42 (ATCC designation) produces very mild disease symptoms in tobacco plants (Phytopathol. 24: 845-873, 1934). We are sequencing the viral genome as well as quantifying viral replication and movement to determine which component(s) of the viral genome promote these attenuated disease symptoms. Greater than 60% of the genome has been sequenced including parts of the 5' untranslated region, replicase gene, and 30kD movement gene, and all of the coat protein (CP) gene and 3' untranslated region. Compared with the common strain (U_1) sequence, seven nucleotide changes have been so far observed (see table). There are no base changes in the CP coding sequence. Studies of viral replication show that infectious PV42 and U_1 accumulate to the same concentration in inoculated leaves and old systemically infected leaves. In top (young) systemic leaves PV42 viral RNA and CP accumulate less rapidly than viral RNA and CP in U_1 infections. This may be the result of slower viral movement into these leaves, and/or reduced rates of PV42 replication, compared with U_1. We are currently investigating the cause of the slower accumulation of PV42 in leaf tissue.

Base Position	A.A.	Region Involved
209 (G→A)	silent	replicase
3272 (G→A)	silent	replicase
3231 (T→C)	silent	replicase
3622 (C→A)	Ser→Tyr	replicase
5585 (A→G)	Asn→Ser	30kD
6208 (U→G)	-	3' non-coding
6326 (U→G)	-	3' non-coding

M 429 MOLECULAR ANALYSIS OF NEMATODE-INDUCED GIANT CELLS IN POTATO ROOTS, Andreas Niebel, Dirk Inzé, Herman Van Montagu* and Marc Van Montagu, Laboratorium voor Genetica, Rijksuniversiteit Gent, and * Plant Genetic Systems N.V., B-9000 Gent (Belgium)
As in many interactions between sedendary root nematodes and plants, the second-stage larvae of Globodera induce the formation of giant cells after their penetration into potato roots. These giant cells result from cell wall breakdown and cell enlargement. The parasite then feeds upon these nurse cells until the end of its life cycle. Our aim is to isolate and study the plant genes involved in this complex host/parasite interaction in which the nematode appears to have tight control over the plant metabolism. By comparative two-dimensional gel electrophoresis of proteins from infected and uninfected roots, some proteins could be identified which are specifically present in giant cells. Subsequent microsequencing and screening of a cDNA library derived from giant cells with corresponding oligonucleotides should allow us to identify the genes involved. Alternatively, we intend to isolate giant-cell-specific genes by differential screening of the same cDNA library.

M 430 THE STRESS-INDUCED SHIFT TO CRASSULACEAN ACID METABOLISM IN MESEMBRYANTHEMUM CRYSTALLINUM, James Ostrem, John Cushman, Jutta Rickers, Gabriele Meyers, Dan Vernon, Jay DeRocher, Chris Michalowski, and Hans Bohnert, Biochemistry Dept., University of Arizona, Tucson, AZ 85721.
Water stress triggers a shift from C_3 photosynthesis to Crassulacean acid metabolism (CAM) in M. crystallinum. This change involves a rapid and coordinate increase in the activity of a number of enzymes involved in glycolysis, gluconeogenesis, and nighttime CO_2 fixation. We have shown that transcript levels for phosphoenolpyruvate carboxylase (PEPCase), pyruvate orthophosphate dikinase, NAD-glyceraldehyde 3-P dehydrogenase (NAD-GAPDH), and two unidentified polypeptides increase within 48 hours following irrigation with 0.5 M NaCl. The increase in the abundance of PEPCase transcripts in stressed leaf tissue may be due in part to enhanced mRNA stability. The steady-state level of PEPCase transcripts declines rapidly ($t^1/_2$ 2.5 h) when salt-stressed plants are watered thoroughly with a NaCl-free nutrient solution. Analysis of the transcription of PEPCase and other stress-regulated genes during CAM induction is in progress. We have isolated cDNA and genomic clones for a constituitively expressed gene encoding PEPCase, and for stress-induced genes encoding both PEPCase and NAD-GAPDH. Comparisions of the nucleotide sequences preceeding the transcription start sites for these genes should reveal whether conserved elements are present upstream of CAM genes in M. crystallinum.

M 431 MOLECULAR GENETIC ANALYSIS OF PHYTOCHELATIN BIOSYNTHESIS

David W. Ow, Lisa Kreppel, Gretchen Scheel, Gary McDonald and Michael Dumars. Plant Gene Expression Center, USDA/ARS/UC Berkeley, 800 Buchanan St., Albany, CA 94710.

Plants respond to **heavy metal stress** by inducing the synthesis of heavy metal binding peptides known as phytochelatins. Unlike the metallothioneins of animal cells, they are not products of primary translation. They have a unique structure: (gamma-glu-cys)$_n$-gly, with n = 2 to 11. Surprisingly, phytochelatins are also found in the fission yeast, Schizosaccharomyces pombe. Using S. pombe as a model system for studying the synthesis of these peptides that are ubiquitous in the plant kingdom, we have identified mutants with altered production of Cd-phytochelatin complexes. Some of these mutants lack the higher molecular weight form of Cd-phytochelatins that contains sulfide ions. Sulfide containing Cd-phytochelatin complexes are more stable and are believed to confer greater heavy metal tolerance. Another class of mutants is deficient in the synthesis of both high and low molecular weight Cd-peptide complexes. By functional complementation in S. pombe mutants, we have cloned DNA derived from the S. pombe genome that can complement hypersensitive phytochelatin-deficient mutants. Biochemical and genetic characterization of these mutants and their complementating DNA clones will be presented.

M 432 RECESSIVE MUTATION AT THE ETR-2 LOCUS OF Arabidopsis thaliana CONFERS RESISTANCE TO SOME EFFECTS OF ETHYLENE EXPOSURE, F. Bryan Pickett, Matthew L. Smith and Mark A. Estelle, Department of Biology, Indiana University, Bloomington, IN 47401 A recessive mutation conferring partial release from ethylene inhibition of root growth has been recovered by screening M2 mutagenized seeds of Arabidopsis thaliana with the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid(ACC). ACC is converted to ethylene by the plants' ethylene forming enzyme. Therefore, the etr-2 mutant was identified on the basis of its ability to elongate roots when exposed to an ACC concentration that causes high levels of endogenous ethylene production. The etr-2 mutation allows the root and hypocotyl of ethylene treated plants to elongate more than similarly treated wild type controls. However, some aspects of the plants' response to ethylene treatment (i.e. induction of chlorophyll loss) are not relieved by the etr-2 mutation. etr-2 is not linked to the previously isolated ethylene resistant mutation etr-1 and must represent a new gene involved in the plant response to ethylene. Further, the observation that lines carrying the etr-2 mutation exhibit some normal responses to ethylene treatment suggests that the normal activity of etr-2 is involved in events downstream of ethylene reception.

M 433 SAM:6A-HYDROXYMAACKIAIN 3-*O*-METHYLTRANSFERASE: PURIFICATION AND CHARACTERIZATION OF THE ENZYME AND ANTISERA, Carol L. Preisig, David E. Matthews, and Hans D. VanEtten, Cornell University, Ithaca, NY 14853.

The isoflavonoid phytoalexin pisatin is synthesized by *Pisum sativum* in response to microbial infection and certain other forms of stress. An enzyme which synthesizes pisatin by methylating the 3-hydroxyl of (+)6a-hydroxymaackiain (HMK) was extracted from $CuCl_2$-stressed pea seedlings. The enzyme has been enriched 250-fold by ammonium sulfate precipitation, DEAE chromatography and chromatofocusing, with a 16% yield and specific activity of 5.8 μkat g^{-1} protein. Further purification by HPLC utilized hydrophobic interaction chromatography followed by gel filtration.

A silver nitrate-staining band of 43 kd by SDS-PAGE was most intense in chromatographic fractions containing peak enzyme activity throughout purification; this protein was the only one in a partially purified preparation which was photoaffinity labelled with the substrate [^{3}H]*S*-adenosyl-L-methionine. The 43 kd band was cut from SDS gels and used to raise antisera. The antisera specifically recognized a 43 kd protein that was induced by $CuCl_2$ or wounding, and specifically inhibited HMK methyltransferase activity.

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

M 434 PROTEIN INDUCTION IN SALT-RESISTANT SUGARCANE CELLS, S. Ramagopal, USDA-ARS, Hawaiian Sugar Planters' Association, Aiea, HI 96701

Sugarcane is a moderately salt-sensitive species and salinity conditions are known to affect apparent sucrose concentration, juice quality, and yield in this crop. As in most other crops, the mechanism of salt-resistance in sugarcane is unknown. In an effort to understand the cellular basis of salt-resistance, several somaclonal variants tolerant to salt stress were developed from two sugarcane cell lines, H50-7209 NR (a nonregenerating line) and H50-7209 R (a regenerating line). The former cell line led to variants tolerant to a higher salt concentration than the latter. Protein synthesis was investigated by in vivo labeling of cells with ^{35}S-methionine, by in vitro translation of isolated mRNAs in a rabbit reticulocyte system and two dimensional gel electrophoresis. Preliminary findings indicated that the salt-tolerant variants originating from the two sugarcane cell lines exhibit differences in the types of proteins altered by salt stress. Although a 26 kDa protein has been shown to be induced by salt stress in other plant species, a similar protein could not be detected in sugarcane.

M 435 MOLECULAR CHARACTERIZATION OF RACE-SPECIFIC DEFENSE RESPONSES IN THE RICE -*XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* INTERACTION. P.J. Reimers and J.E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Race-specific resistance (incompatibility) in bacterial leaf blight of rice caused by *Xanthomonas campestris* pv. *oryzae* is correlated with reduced bacterial numbers and shorter lesion lengths in inoculated leaves. A hypersensitive response has not been described in this interaction. We adapted a technique for infiltrating leaves with bacterial suspensions, which allows observation of a rapid race-specific differential tissue reaction between the compatible (PXO61^{s}, race 1, streptomycin resistant) and incompatible (PXO86^{r}, race 2, rifampicin resistant) combinations on rice cultivar CAS 209. By 18-24 hours post-inoculation (PI), a light chocolate-brown shading was visible at the infiltration site of the incompatible combination, while faint water-soaked flecks were visible with the compatible combination. By 48 hrs. PI, the incompatible combination site was a camouflage-brown color, having a bleached tan-green center. The compatible combination site, conversely, was uniformly water-soaked, exhibiting a translucent yellowish-green color. The tissue reaction in the incompatible combination was similar to a classic hypersensitive response. Inoculation with sterile distilled water or UV-killed bacteria elicited no response.

To investigate the molecular events evoking this tissue response, a cDNA library was constructed from mRNA of CAS 209 leaves 6 hrs. after inoculation with PXO86^{r}. cDNA clones were screened by differential hybridization with total mRNA-directed cDNA probes prepared from control plants (PXO61^{s}- or water-infiltrated) and PXO86^{r}-inoculated plants. Several clones which hybridize more strongly with the incompatible-reaction probe than with the controls were identified. These clones appeared to be different from previously reported defense-response clones induced by fungal pathogens in monocots and dicots.

M 436 STRUCTURAL STUDIES OF POTATO CHITINASE GENES, Daniel Laflamme and Robert Roxby, Biochemistry Department, University of Maine, Orono, ME 04469 USA. A series of potato cDNA clones has been isolated using a bean cDNA clone [Broglie *et al., PNAS USA 83*, 6820-6884 (1986)] as a probe. The complete nucleotide sequence of one of these, CCH4, has been determined. It is 1092 nucleotides long, contains a single reading frame encoding a basic protein of 315 amino acids and bears a high degree of homology to published sequences of bean and tobacco chitinase genes. Two classes of cDNA clones can be distinguished on the basis of restriction sites near the middle of the reading frame. One class, which includes CCH4, has a *SmaI* site and the other class a *DraIII* site in this region. All clones contain an *EcoRI* site near the 3' end of the reading frame and an *AvaI* site near the 5' end. Partial sequencing of another clone of the *SmaI* class, CCH19, shows it to come from a different gene than does CCH4. One region of marked difference between CCH4 and CCH19 encompasses a sequence of 24 bases which comparisons with bean and tobacco clones would suggest encode the amino terminus of the mature peptide. Bean and tobacco clones are similar to each other in this region and different from either of the potato chitinase clones.

M 437 REGULATION OF A THIOL PROTEASE GENE INDUCIBLE BY LOW TEMPERATURE IN TOMATO, Mark A. Schaffer and Robert L. Fischer, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720.
Many plants of tropical or subtropical origin suffer severe damage when exposed to low, non-freezing temperature. Studying the induction of gene expression in response to cold treatment in tomato fruit, we have identified several cDNA clones representing mRNAs that respond rapidly to low temperature. DNA sequence analysis indicates that one of these mRNAs encodes a polypeptide homologous to plant and animal thiol proteases of the papain family. By analogy to heat shock's induction of the lon protease gene in prokaryotes, a cold-inducible protease might function by degrading polypeptides denatured by exposure to low temperature. Recent results in our laboratory indicate that heat shock itself can induce thiol protease gene expression in tomato fruit. We report nuclear run-on transcription analysis of the cold-inducible mRNAs, as well as immunological analysis of thiol protease polypeptide accumulation.

M 438 BARLEY YELLOW DWARF VIRUS EXPRESSION IN WHEAT PROTOPLASTS AND CONSTRUCTION OF SYNTHETIC GENES TO INTERFERE WITH VIRAL REPLICATION, Mark J. Young, and Wayne L. Gerlach, CSIRO Division of Plant Industry, Canberra, ACT 2601 Australia
Hydroxyproline-rich glycoproteins (HRGPs) are a family of structural proteins found in plant cell walls. HRGPs are known to accumulate in response to wounding and pathogen infection as an apparent defense mechanism. We report here on the isolation and characterization of several different cDNA and genomic clones from tomato encoding members of the HRGP gene family. The cDNA clones characterized to date fall into four distinct classes (I-IV). Class I clones encode an HRGP with $SP_4SPSP_4Y_3K$ repeats, class II clones encode an HRGP with SP_4SPSP_4(TYS) repeats, class III clones apparently encode a glycine-rich protein (GRP), and class IV clones encode both $SP_4SPSP_4Y_3K$ repeats and a GRP sequence. Two genomic HRGP clones have been isolated which correspond to the class I and class II cDNAs. In addition, two other tomato HRGP genomic clones have been identified which do not correspond to any of the above cDNA classes. These cDNA and genomic clones have been used as probes in RNA blot hybridizations of unwounded and wounded tomato stem tissue and were shown to hybridize to specific mRNAs which accumulate in response to wounding. This wound-induced accumulation of mRNA was especially marked in blots probed with the GRP cDNA sequence.

M 439 A CULTIVAR-SPECIFIC ELICITOR OF THE HYPERSENSITIVE RESPONSE IN SOYBEAN HAS BEEN IDENTIFIED AND MAY BE THE SIGNAL MOLECULE THAT INTERACTS WITH THE PLANT DISEASE RESISTANCE GENE PRODUCT TO TRIGGER HOST DEFENSE, Mark Stayton*, Stanley Tamaki**, Donald Kobayashi§ and Noel Keen§; *Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; **Cleargene, Inc., University of California - Richmond Field Station, Richmond, CA 94804-4698; §Department of Plant Pathology, University of California at Riverside, Riverside, CA 92521-0122. We have recently identified a novel, low molecular weight compound which functions as a cultivar-specific elicitor (SE) of the hypersensitive response in soybeans. The SE is secreted by a bacterial plant pathogen and may be the signal molecule that interacts directly with the plant disease resistance gene product to initiate the host defense response. Its biosynthesis is associated with expression of a bacterial avirulence gene, avrD, which was originally isolated from Pseudomonas syringae pv. tomato (Kobayashi et al., 1989, Proc. Natl. Acad. Sci. USA, in press). Expression of avrD in P. syringae. pv glycinea race 4 leads to the production of the SE and to the induction of the hypersensitve response on a unique subset of soybean cultivars. Escherichia coli strains, which overexpress the avrD gene product, produce the SE and elicit the HR on the same set of soybean cultivars. In addition, cell-free culture supernatants from E. coli (avrD) also induce the hypersensitive response in a cultivar-specific manner. Utilizing induction of the hypersensitive reaction as our assay, we have begun the purification of the specific elicitor. Determining its chemical structure will provide new insight into the function of avirulence gene products and their role in triggering plant defenses.

M 440 FUSICOCCIN BINDING IN MEMBRANES FROM ARABIDOPSIS THALIANA, **Richard G. Stout, Dept. of Biology, Montana State University, Bozeman, Montana 59717**

I have examined the activity and specific binding of the phytotoxin fusicoccin using Arabidopsis thaliana L. Col-0. Fusicoccin (10 micromolar) stimulates both proton extrusion and enlargement in isolated Arabidopsis leaf disks. Radiolabelled fusicoccin specifically binds to membranes (13,000 to 100,000 g subcellular fraction) from cultured cells of Arabidopsis. The specific binding of this phytotoxin to putative receptor sites in Arabidopsis membranes is both pH-sensitive (pH optimum = 5.5 to 6.0) and heat-labile (10 minutes at 70°C). The apparent dissociation constant for the specific binding at 20°C is approximately 1.3 X 10^{-8}M. The results of this study are in general agreement with previous reports of fusicoccin binding and activity in other plant species.

M 441 DEVELOPMENTAL REGULATION OF GENE EXPRESSION DURING HAUSTORIAL FORMATION IN THE PARASITIC ANGIOSPERM, STRIGA ASIATICA, Michael P. Timko, Christa S. Florea, Susan J. Wolf, and James L. Riopel, Department of biology, University of Virginia, Charlottesville, VA, 22901. Striga asiatica (witchweed) is a major parasite of agronomically important grasses. This parasitic angiosperm attaches to the host root vascular system through a specialized structure termed a haustorium. The development of this unique structure is initiated in response to specific chemical signals from the host. One such inducer of haustorial development has been isolated from Sorghum and identified as 2,6-dimethoxybenzoquinone (2,6-DMBQ). In order to understand how 2,6-DMBQ functions in haustorial induction we have been analyzing the transcriptional/ translational changes which occur during development. Protein synthesis inhibitors have been used to define critical aspects of 2,6-DMBQ induction. Changes in the transcriptional/ translational activities during the 24 hr development time of the mature organ are being analyzed by two-dimensional gel electrophoresis. Cloned cDNAs have been prepared from poly A^+ RNA isolated from uninduced radicles and developing haustoria (24 hr post-2,6-DMBQ induction). Differential screening of these cDNA libraries is now being used to identify and characterize developmentally expressed and haustorium-specific messages.

M 442 ISOLATION OF GROWTH REGULATING GENES FROM RADIATION-INDUCED TUMORS OF *Arabidopsis thaliana*, Christopher D. Town, Sharon M. Persinger, and Bruce A. Campell, Biology Department, Case Western Reserve University, Cleveland OH 44106.

In order to study of the molecular genetics of plant growth control, we have isolated tumors on the small cruciferous plant *Arabidopsis thaliana*. Tumors appeared on the hypocotyl or meristem of 30-60 day old plants which had been exposed to 20 - 100 krad of ^{60}Co gamma radiation at the seed or seedling stage. These tumors were found to be capable of hormone-autonomous growth when excised and placed in culture. The tumors show a number of different phenotypes, varying in color and texture of tissue, and degree of differentiation. Doubling time of the tumors on hormone-free medium varies from 4 to 20 days. The growth response of the tumors to exogenous hormones (auxin, cytokinin and giberellin) has been studied. Some are totally hormone-independent, while others are stimulated or inhibited by exogenous hormone, suggesting possible differences in hormone content or sensitivity. None of the tumors studied shows the marked sensitivity to exogenous hormone observed for *Agrobacterium*-induced tumors of tobacco. We hypothesize that the tumors are due to radiation-induced activation of "oncogenes", as can occur in animal cells. Such genes might be either directly involved in hormonal regulation or comprise other elements of the growth control network. Northern blots from some of the tumors have been screened at low stringency with a battery of some 20 animal oncogene probes, but no positives were found. The results of current strategies to detect genes specifically amplified or expressed in the tumors will be described.

M 443 CELL TYPE SPECIFIC EXPRESSION OF ROL C GENE OF Ri PLASMID,
H.Uchimiya, Y.Oono, S.Sugaya, K.Kanaya, K.Hayakawa,
Inst. of Biol. Sci. Univ. of Tsukuba, Ibaraki 305, Japan
A DNA fragment possessing ORF 12 (rol C) of Ri (A4) plasmid was inserted into tobacco through the binary vector system harbored by Agrobacterium tumefaciens. The transformed plants showed dwarfness in plant height and corolla length. Dwarfness in plants was related to shortening of internode length, and stably inherited to progenies.

Chimeric gene containing promoter of rol C and beta-glucronidase gene was constructed and introduced into tobacco plants. Histochemical anaylsis indicated that rol C promoter was expressed in phloem, trichome and pollen cells. Association of cell specific expression of rol C gene in relation to morphogenic alteration in plants will be presented.

M 444 ISOLATION AND CHARACTERIZATION OF IAA-RESISTANT MUTANTS OF ARABIDOPSIS THALIANA, Allison K. Wilson, Jocelyn Turner and Mark A. Estelle, Department of Biology, Indiana University, Bloomington, IN 47405. In order to identify genes involved in auxin response in Arabidopsis thaliana, a screen was done to identify mutants resistant to exogenously applied IAA. In a screen of 250,000 M2 seeds, twenty mutants were isolated that segregated IAA resistance in a Mendelian fashion and these are being characterized phenotypically and genetically. One of the mutants, designated axr2, has a distinctive morphological phenotype that co-segregates with the auxin resistance phenotype. The axr2 mutation is dominant and axr2 plants have shortened internodes, epinastic leaves and an altered response to gravity. Experiments are being done to compare the level of auxin resistance of axr2 plants to that of wild-type plants. We are also in the process of measuring endogenous IAA levels in wild-type and axr2 plants. The axr2 mutation has been mapped by using both visible markers and restriction fragment length polymorphisms (RFLPs) and it should now be possible to clone the axr-2 gene by initiating a chromosomal walk from the most closely linked RFLP.

M 445 PHOTOSYNTHESIS GENE INDUCTION IN SALT TOLERANCE OF CULTURES OF ALFALFA, Ilga Winicov and Jane D. Button, Departments of Biochemistry and Microbiology, University of Nevada Reno, Reno, NV 98557.
We have selected alfalfa (Medicago sativa) cell lines that are tolerant to 1% NaCl in the growth medium. This phenotype is maintained in absence of selective pressure indicating a stable change in gene expression leading to tolerance. Plants regenerated from the tolerant callus in turn yield callus cultures that are salt tolerant without further selection, indicating the isolation of stable variant cell lines. Chloroplast gene expression shows selective changes in the salt tolerant cells. Chloroplast rRNA transcripts reflect the 50% increase in chloroplast genome copy number for the tolerant cells, as compared to the sensitive lines. In contrast, photosynthesis related chloroplast genes: psbA, psbD, atpB, psaB and rbcL mRNAs appear induced in the tolerant cells as measured by Northern blot hybridization. Nuclear genes: cab-4,cab-1 and rbcS also appear induced. These data indicate a basal level of general activation for genes involved in photosynthesis and suggest an alteration in a photosynthesis transacting regulatory element in the tolerant cells. Although the basal level of activation in expression can be detected in tolerant cells grown in absence of NaCl, the level of mRNA accumulation is further regulated by light and shows a large induction by growth in presence of NaCl. The salt tolerant phenotype is inhibited by inhibitors of PSII and cell growth in the dark, indicating that the gene activation is highly correlated with the salt tolerance phenotype.

Engineering for Desired Traits; Resistance

M 500 THE USE OF SOLANUM LYCOPERSICOIDES ALIEN ADDITION LINES AS AN AID TO MAPPING TOMATO MOLECULAR MARKERS, Kevin B. Alpert, Roger T. Chetelat, and Joseph W. DeVerna, Campbell's Institute for Research and Technology, Route 1 Box 1314, Davis, CA 95616.
A complete set of alien addition lines, consisting of 24 tomato chromosomes and 1 extra chromosome from S. lycopersicoides, are being used to localize molecular markers to individual chromosomes of tomato. As a prerequisite to this approach, the intactness of each S. lycopersicoides chromosome was evaluated using isozymes and genomic clone markers from the tomato molecular map. In addition, molecular markers were evaluated for polymorphisms relative to tomato. Once the molecular markers were localized to a particular homologue, an F2 population (L. esculentum x L. pennellii) was used to establish linkage to previously mapped tomato molecular markers (Tanksley, Miller, Paterson and Bernatzky, Stadler Genetics Symposium, 1987). This two step mapping technique permits direct chromosomal assignment of molecular markers followed by intrachromosomal linkage to previously assigned markers. Several cDNA, genomic and isozyme markers have been mapped in this manner. The use of the alien addition lines represents an efficient method of mapping molecular markers when linkage information is limited due to the lack of F2 linkage data or probes which saturate the molecular map. Also, these alien addition lines represent ideal material with which to assign dominant genes governing horticultural traits to their respective chromosomes.

M 501 DIFFERENTIAL EXPRESSION OF PROLAMIN AND GLOBULIN GENES DURING OAT EMBRYOGENESIS, Marc Giband, Bernard Potier, Joyce Byrne and Illimar Altosaar, Biochemistry Department, University of Ottawa, Ottawa Ontario K1N 6N5 Canada.
Oat genomic clones for both globulins and prolamins have been characterized and used to study the regulation of protein synthesis during cereal grain development. We have shown that the abundant prolamin-coding 12S mRNA in wheat, barley and rye is also present, but not well translated, in oat. Rather, the less-abundant globulin-coding 18S mRNA is preferentially translated causing globulins to constitute >70% of the mature grain protein in oats, whereas wheat etc. contain <5% globulins. We have sequenced a full-length prolamin cDNA clone specific for oat 12S mRNA. When compared to an oat globulin cDNA sequence, there is a striking high degree of homology in the 3'-untranslated region. Globulin and prolamin probes were used to follow the respective RNA levels during the grain filling period. The available sequences for oat storage proteins were used to generate predicted secondary structures for the corresponding mRNA molecules to test whether steric hindrance of ribosome attachment may explain the differences in their translational efficiencies during grain development.

M 502 MUTANT PLANT ACETOHYDROXYACID SYNTHASE GENE SHOWING SELECTIVE HERBICIDE RESISTANCE. Susan Armour, Joseph Di Maio, Ray Shillito, Christian Harms, George Jen. ABRU, Ciba-Geigy, P.O.Box 12257, Research Triangle Park, NC 27709.

Acetohydroxyacid synthase (AHAS), alternatively known as acetolactate synthase (ALS), is involved in the biosynthesis of branched-chain amino acids. It is the target of sulfonylurea, imidazolinone, and triazolopyrimidine sulfonamide herbicides. Mutagenized plant suspension culture cells were selected on Ciba-Geigy sulfonylurea CGA136,872. Resistant lines were screened for cross resistance to other AHAS-targeting herbicides, including other classes of sulfonylureas. An AHAS containing a mutation previously unknown in plants has been identified.

M 503 DETOXIFICATION OF THE HERBICIDE DALAPON BY TRANSFORMED PLANTS

Vicky Buchanan-Wollaston, Bert Gold, Andrea Naser and Frank Cannon, BioTechnica International Inc., 85 Bolton Street, Cambridge, MA 02140.

The engineering of plants resistant to herbicides has been achieved in several ways. Alteration of the protein that is sensitive to the action of the herbicide results in a protein that might have a reduced efficiency that in some cases could affect plant yield. Inactivation of the herbicide molecule by conjugation is possible but accumulation of the inactivated herbicide could be detrimental. A method by which the herbicide could be completely degraded and even utilised by the plant would seem to be the mechanism of choice for conferring herbicide resistance. To test this we have identified a degradation system for the herbicide Dalapon.

A gene coding for a dehalogenase active against MCPA (monochloropropionic acid) and 2,2 DCPA (dichloropropionic acid) the active ingredient of the herbicide Dalapon, was isolated from a strain of *Pseudomonas putida*. The gene was shown to have dehalogenase activity in *E. coli* by showing both growth on MCPA as a sole carbon source and chloride release from MCPA. Sequence of the gene was determined and the open reading frame identified was cloned downstream from the CaMV 35s promoter and transformed into *N. plumbaginifolia*. Transformed shoots could be selected on media containing 2,2 DCPA at a concentration at which untransformed tissue was killed. These shoots were rooted and grown up to obtain seed. Seedlings that showed resistance to 2,2DCPA were transferred to soil to test in spraying experiments with the herbicide. It was found that the presence of the degradation gene conferred 10 - 20 fold increased resistance to 2,2DCPA over the control plants.

M 504 BOTH IN SPACE AND TIME, A 3' DNA FROM A TOMATO PROTEASE INHIBITOR GENE IMPRESSES ITS OWN CONTROL PATTERN UPON THE 35S PROMOTER: IDEAL EXPRESSION OF THE BACILLUS THURINGIENSIS TOXIN GENE FOR INSECT PROTECTION

Wayne M. Barnes, Dept. Biological Chemistry, Washington Univ. School of Medicine, St. Louis, MO 63110.

The tomato protease inhibitor I gene (Tom PI I) is known to be systemically induced in the leaves of a tomato plant if a lower leaf is wounded. The CaMV 35S promoter is constitutive, but with a distinct anatomical pattern: in a leaf, it is much more highly expressed in the veins. I have discovered that when some 700 base pairs from the 3' half of the Tom PI I gene are inserted after the stop codon of a marker gene transcribed from the 35S promoter, the gene expression is repressed in the veins, and it is systemically induced in the rest of the leaf if the plant is wounded.

The marker gene used was a fusion between firefly luciferase cDNA and NPTII (kanamycin resistance). Transgenic tobacco shoots can be selected readily on kanamycin-containing medium, indicating either that the gene is expressed under shoot regeneration conditions, or that its uninduced level is sufficient for selection. After the transgenic plants have been potted in soil, the wound-inducible nature and the anatomical pattern of the expression are readily demonstrated by the observation of luciferase activity in explanted leaves imbibed with luciferin. Leaves slightly wounded with a forceps glow more brightly than unwounded controls, and the next (unwounded) leaf up on the wounded plant glows more brightly still. The pattern of expression is the opposite of that previously observed for the 35S promoter: the veins are darker, rather than much brighter, with the expression, at a high level, being confined to the interveinal regions of the leaf.

Insect larvae tend to eat the large veins last. Both systemic wound induction, and the pattern of induction favoring the interveinal areas of the leaf, present a superior pattern of gene expression for a Bt toxin gene. Such a gene, identical to the above except for HD73 Bacillus thuringiensis toxin codons in place of luciferase codons, is indeed, in transgenic tobacco, highly effective against Manduca sexta and Heliothis virescens larvae.

M 505 THE MAIZE *Glb1* GENE AS A MODEL SYSTEM FOR GENETIC ENGINEERING IN PLANTS, Faith C. Belanger and Alan L. Kriz, Department of Agronomy, University of Illinois, Urbana, IL 61821

Maize embryos contain large amounts of saline-soluble, water-insoluble proteins called globulins. The major globulin component, GLB1, is believed to serve as a storage protein. Genetic analysis has indicated that GLB1 is encoded by a single gene which exhibits allelic polymorphisms: several protein size alleles, as well as a null, have been described (MGG 174:233, 1979; Pl. Physiol. 82:1069, 1986). Kernels homozygous for the null allele exhibit no differences in pattern of development or germination when compared to kernels possessing functional *Glb1* alleles. The ability of the embryo to tolerate variability in *Glb1* expression makes this gene a model system for genetic engineering of seed proteins. Modification of *Glb1* may provide an alternative to modification of zein genes in the effort to improve the nutritional quality of maize grain protein. We have therefore isolated cDNA and genomic clones for *Glb1* and will report details of protein and gene structures. From RNA blots it appears that *Glb1* transcripts are present in the endosperm as well as the embryo but not in other plant tissues. The *Glb1* null allele produces a low level of transcript which is a different size than those encoded by normal alleles. [Supported by grants from SOHIO and the USDA (No. 88-37262-3427)]

M 506 ISOLATION OF A SUGAR BEET GENE ENCODING CHITINASE
Lars Berglund[1,2] and K.A. Marcker[1], Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark[1], the Danish Sugar Factories, Biochemical Lab., DK-1001 Copenhagen K, Denmark[2].
The hypersensitive response of plants to infection with viruses, fungi or bacteria induces a resistance to further infection by these pathogens by de novo synthesis of several classes of proteins. One class of induced proteins are the so called Pathogenesis-Related (PR) proteins. It has been reported that the PR proteins in tobacco include chitinases. In sugar beet PR proteins has also been observed after infection with pathogens. We have isolated a chitinase cDNA clone from a lambda gt-11 sugar beet cDNA library employing antibodies against wheat chitinase. A gene coding for sugar beet chitinase has been isolated with the cDNA as a probe. Details of the genomic clone will be presented.

M 507 CLONING THE GENE FOR AN ISOFORM OF STARCH BRANCHING ENZYME AND ITS ASSOCIATION WITH R LOCUS OF PEAS, Madan K. Bhattacharyya, Cathie Martin and Alison Smith, John Innes Institute, AFRC Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.
The first 'gene' described by Mendel in his study of heritable characteristics of peas was that conferring the round (R) compared to wrinkled (r) phenotype of seeds. This locus has a profound effect on seed storage product composition. Isolines differing in the R locus of peas also differ in one of the isoforms of starch branching enzyme (SBE) active early in pea seed development (Smith, 1988). An antiserum raised against this isoform was used to screen a cDNA library in λgt11. Six cDNA clones were obtained that were homologus to each other and have similar restriction maps. Northern blot analysis revealed a 3.2 kb transcript in round embryos. In the wrinkled embryos an abberent transcript about 1 kb larger than that in the line with round seeds was found. Analysis of genomic DNA from round and wrinkled seeds also indicated that in wrinkled peas there is an 1 kb insertion of new DNA towards the 3' end of the SBE gene. Linkage analysis of this polymorphism with round and wrinkled phenotypes of seeds showed 100% co-segregation of the gene with R locus establising the r phenotype to be due to the failure to produce active enzyme of this SBE isoform. Analysis of the mutation and its role in the expression of the wrinkled phenotype will be discussed. Possibilities for using SBE to modify starch quality in transgenic plants will also be considered.

M 508 CREATION OF FUSION PROTEINS FOR THE PRODUCTION OF ANTIBODIES TO PUTATIVE GENE PRODUCTS OF TOBACCO VEIN MOTTLING VIRUS. John D. Brantley and Arthur G. Hunt. Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

Tobacco vein mottling virus (TVMV) is a 10kb, (+)-sense, single-stranded RNA potyvirus. This virus encodes a single polyprotein which is proteolytically processed to yield at least five known polypeptides. In addition to these, two additional, as yet uncharacterized polypeptides are predicted to result from processing of the polyprotein. These have been termed the 34kDa and 42 kDa gene products.

A fusion protein of the 34kDa polypeptide with Protein A was created by inserting most of the 34k sequence into the polylinker region of the plasmid pRIT2T (Pharmacia). The reading frame was maintained at both ends. The resulting fusion product contains 34kDa sequences flanked at their amino and carboxyl termini by Protein A sequences. When expressed in *E. coli* strain N4830-1 (Pharmacia), the intact fusion protein and a characteristic breakdown product could be detected. These products were purified by column chromatography on I_gG-Sepharose (Pharmacia). Approximately 1mg of fusion protein suitable for antibody production could be obtained from a 1 liter culture. A 42 kDa-Protein A gene fusion was constructed in an analogous fashion. This gene, when expressed in N4830-1, yields a single, fusion polypeptide that, like the 34 kDa -Protein A fusion protein, breaks down during chromatographic purification. However, the intact fusion protein as well as breakdown products can be recovered.

The 34 kDa and 42 kDa fusions will be used to generate antisera against these putative gene products so that their occurrence and roles in virus infections can be established.

M 509 USE OF THE ALS GENE OF ARABIDOPSIS THALIANA FOR SULFONYLUREA AND IMIDAZOLINONE HERBICIDE RESISTANCE IN TRANSGENIC NICOTIANA TABACUM AND BRASSICA NAPUS.
Pierre J. Charest¶*, Jiro Hattori§, Janice DeMoor¶, V.N. Iyer¶ and B.L. Miki§.
¶-Dept. Biology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6 §-Plant Research Centre, Agriculture Canada, CEF, Ottawa, Ontario, Canada, K1A 0C6.
The wild type gene and mutant gene csr1-1 coding for the enzyme acetolactate synthase from Arabidopsis thaliana, with their own promoter sequences and with 35S promoter sequences from CAMV, were transferred to N. tabacum and B. napus by cocultivation with Agrobacterium tumefaciens. The mutant gene coding for ALS resistant to chlorsulfuron was evaluated as a selectable marker for transformation and compared with the NPT II gene. Resistance to chlorsulfuron and three short-residual sulfonylureas was obtained in B. napus and N. tabacum with the mutant gene regulated by 35S or ALS promoters. The 35S promoter gave much higher levels of gene expression. Tolerance to sulfonylureas and imidazolinones was obtained with either wild type or mutant ALS genes when there was overexpression of the gene.
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M 510 SITE-DIRECTED MUTATIONS IN THE psbD GENE OF PHOTOSYSTEM II IN THE CYANOBACTERIUM *SYNECHOCYSTIS 6803*.
Dexter Chisholm and Bruce Diner, Microbiology Group, CR&D Department, Dupont Experimental Station, PO Box 80173, Wilmington, DE 19880-0173
The psbD gene codes for the D2 protein of photosystem II. Two copies of this gene are present in Synechocystis 6803. One copy is known as $psbD_1$ and it overlaps psbC, the other copy is known as $psbD_2$. A strain was constructed in which $psbD_2$ was replaced with a spectinomycin resistance gene and $psbD_1$ replaced with a chloramphenicol resistance gene. Mutant versions of $psbD_1$, generated using site-directed mutagenesis, were tagged with a kanamycin resistance gene and then restored to the genome displacing the chloramphenicol resistance gene. Altered amino acids include Lys 264 which may play a role in bicarbonate binding, and possible ligands to iron. Some of the mutants have been characterized biophysically.

M 511 RISK ASSESSMENT OF GENE CAPTURE AND TRANSFER BY VIRAL SATELLITE-TRANSFORMED PLANTS.
David Coates*, Liu Yuan Yi, David Bertiolli, Mary-Lou Edwards, J. Ian Cooper. NERC Institute of Virology, Mansfield Rd., Oxford OX1 3SR, and *Genetics Laboratory, Dept. Biochemistry, S. Parks Rd, Oxford OX1 3QU, UK. One option for biological control of plant viral diseases is the use of disease mitigating "satellite RNAs", stable RNA molecules that multiply in cells only with the assistance of an appropriate "helper" virus. These satellites are found asociated with a range of plant RNA viruses, exemplified by the RNA-3 of arabis mosaic virus [AMV], a nepovirus with a bipartite genome. When RNA-1 and -2 are mixed with RNA-3 and manually inoculated, they lessen the disease symptoms in both *Nicotiana* and *Chenopodium* species. Transformation of susceptible plants with a transcription unit encoding the RNA-3 would confer a degree of resistance to all strains of AMV, whether or not they included the satellite. The satellite is 1122 bases long, and includes an open reading frame of 1068 bases. This is large enough to code for one of the two major proteins synthesized by RNA-3 *in vitro*, of 41 kD. A second product, of 36 kD, may be the result of a shortened transcript in the same frame. We have inserted a complete DNA copy of RNA-3 into the cloning site pf pRok1, a Bin-derived binary vector of *A.tumefaciens*, and transferred this into *Nicotiana tabacum*. These plants are being challenged with a range of AMV isolates and other viruses to assess the effect of the endogeneous production of RNA-3 on disease symptoms, as well as on the plant morphology and development.. In addition, the possibility of transfer of RNA-3 by transcapsidation and pollen transfer is being investigated to assess the risks inherent in this approach to disease resistance. The results will be presented at the meeting.

M 512 CHLORSULFURON-RESISTANT POTATOES VIA AGROBACTERIUM-MEDIATED TRANSFORMATION, A.J. Conner, M.K. Williams, D.J. Abernethy and R.A. Genet, Crop Research Division, DSIR, Private Bag, Christchurch, New Zealand

Potato (Solanum tuberosum) has been transformed using Agrobacterium tumefaciens strain LBA4404 harbouring the binary vector pKIWI110. This vector consists of the left and right T-DNA borders between which are a selectable kanamycin resistance gene (NOS-NPTII-NOS), a B-glucuronidase reporter gene (35S-GUS-OCS) and a gene encoding a chlorsulfuron insensitive form of the enzyme acetohydroxyacid synthase located on a 5.8 kb Xba 1 fragment cloned from chlorsulfuron-resistant Arabidopsis thaliana (Haughn et al. 1988. Mol. Gen. Genet, 211: 266). Transformed potato cells were selected and regenerated into plants using kanamycin resistance as a selectable marker. Six independent transformants of the cultivar 'Iwa' have been screened for B-glucuronidase activity and chlorsulfuron resistance. Five have B-glucuronidase activity as determined via fluorogenic assays and histochemical staining. These five transformants also have an increase in chlorsulfuron resistance, as judged from the rooting of excised shoots in chlorsulfuron supplemented medium. Control 'Iwa' shoots had greatly inhibited root growth at 1 ug/L chlorsulfuron, with no root initiation at 10 ug/L. In contrast, the five transformants showed good root development at 100 ug/L with root initiation (but very poor growth) occurring at 1000 ug/L. Small plants were established in soil and sprayed with the equivalent of the recommended field application of the herbicide Glean (20 g/ha). Control plants failed to continue growth and rapidly deteriorated from about 10 days post spraying. The five transformed lines with chlorsulfuron resistance continued to grow at the same rate as the unsprayed controls.

M 513 IS THE PR1 FAMILY OF "PATHOGENESIS-RELATED" PROTEINS IN TOBACCO A COMPONENT OF VIRAL DISEASE RESISTANCE? John Cutt[1], Mark Harpster[2], John Carr[1], David Dixon[1], Pamela Dunsmuir[2], Daniel Klessig[1] 1. Waksman Institute at Rutgers University, PO Box 759 Piscataway, New Jersey 08855 and 2. Advanced Genetic Sciences, 6701 San Pablo Ave. Oakland, California 94608.

We are investigating the function of the "pathogenesis-related" proteins of *Nicotiana tabacum*. The correlation of temporal and spacial induction of PR1 protein synthesis with viral resisistance in certain tobacco cultivars has implicated them as a component of viral disease resistance. To address whether they are involved in resistance to tobacco mosaic virus (TMV) transgenic tobacco (*N.t. cvs. Xanthi and Xanthi nc*) plants have been constructed which contain one or more of the PR1 genes under control of the CaMV 35S promotor. Several of the transgenic isolates constitutively express the PR1b gene from the 35S promotor. The PR1b protein synthesized by these plants is secreted by the cells into the extracellular space, as are the endogenous PR1 proteins after induction, and accumulates to high levels in the leaves. No adverse effect of constitutive PR1b synthesis on growth was noted. Transgenic *Xanthi* (susceptible cultivar) plants exhibit no change in the onset or severity of systemic symptoms after TMV infection. The number of viral lesions produced, their time of appearance, and their general morphology on transgenic *Xanthi nc* (resistant cultivar) plants are similar to the control plants after TMV infection. These data imply the PR1 proteins of tobacco either are not sufficient for TMV resistance or may not function as antiviral compounds.

M 514 IDENTIFICATION OF DOMAINS IN CMV SATELLITE RNA FOR SYMPTOM PRODUCTION, Martine J. Devic, Martine M.Jaegle and David C. Baulcombe,Department of Molecular Genetics, IPSR Cambridge, Maris lane, Trumpington , Cambridge CB2 2 JB, U.K. CMV satellite RNA is a small RNA molecules (around 335 bases) which modifies the symptom production of the virus but which needs the virus for its replication. It does not share significant sequence homology with the helper virus. cDNA of three different satellite isolates (I17N, Y and R) have been constructed in a transcription vector containing the PM1 promoter. The in vitro transcripts of these satellite cDNA were highly infectious in presence of the helper virus and produced the same symptoms as the natural satellite RNAs. In order to investigate the functional domains of the molecule, mutants of I17N and Y satellite have been produced using restriction sites. The results suggested that 1) this small molecule contains functional sequences in the termini as well as in the middle 2) the product of the three proposed ORFs are not likely to be involved in symptom production. Recombinant molecules involving Y and R satellite RNA have been constructed in order to determine which part of the molecule is responsible for the symptom production in tobacco and tomato. The determinant for symptom production on tobacco (yellow chlorosis) lies between nucleotides 81 and 219 but is not confined to the extra loop present in the sequence of Y. On the other hand, the domain involved in symptoms on tomato (lethal necrosis) resides on the 3' end between nucleotides 301 and 379.

M 515 A SHOTGUN STRATEGY FOR EXPRESSION OF FRAGMENTS OF A VIRAL GENOME IN TRANSGENIC PLANTS: PERSPECTIVES FOR STUDYING VIRAL FUNCTIONS RESPONSIBLE FOR PATHOGENESIS AND FOR OBTAINING NOVEL TOLERANCE GENES. Mylène Durand-Tardif, Christophe Robaglia, Sylvie Dinant* and Francine Casse-Delbart, Laboratoire de Biologie Cellulaire, Laboratoire de Pathologie Végétale*, INRA, Centre de Versailles, 78026-Versailles Cedex, FRANCE.
We have used random primed cDNA synthesis to construct a bank of the 10kb RNA genome of the Potato Virus Y (N strain). This bank was used to obtain the complete nucleotide sequence of the virus (Robaglia et al., J. Gen. Virol., in press). We are currently using the same method to construct a cDNA bank of the PVY and of the Lettuce Mosaic Virus genomic RNA in an expression vector for plants. We will insert random c-DNA fragments in between the promoter of the Cauliflower Mosaic Virus 35S RNA and the coding region of the kanamycine resistance gene, flanked by the terminator of the nopaline synthase gene. These banks of viruses genomes will be introduced in tobacco cells by electroporation. Transgenic plantlets will be selected for their resistance to kanamycine encoded by the viral-kanamycine resistance fusion protein. We will look for tobacco plants with disease symptoms in order to identify which part(s) of the genome are responsible for the pathogenicity. We will also investigate plants which show tolerance to the pathogens, in order to develop novel types of tolerance genes.

M 516 A SYNTHETIC GENE CONFERS RESISTANCE AGAINST THE BROAD SPECTRUM HERBICIDE L-PHOSPHINOTHRICIN IN PLANTS, Peter Eckes, Bert Uijtewaal, Gunter Donn, Hoechst AG, Pflanzenschutzforschung Biochemie, 6230 Frankfurt 80, W.-Germany,
L-Phosphinothricin (L-PPT), the herbicidal moiety of bialaphos, a compound produced by some Streptomyces strains, is the active ingredient of the herbicide BASTA. It can be inactivated by a specific N-acetyltransferase, which is also expressed in these Streptomyces strains. Based on the amino acid sequence of one such acetyltransferase, which acts during biosynthesis of bialaphos in S. viridochromogenes, a gene has been synthesized with a codon usage, optimized for expression in plants. This synthetic gene was fused between the 35S-promotor and -terminator of CaMV and transferred to plants such as N. tabacum, L. esculentum or M. sativa. Data on the expression of this gene in transgenic plants, the inactivation of the herbicide and the resulting resistance of these plants against L-PPT will be presented.

M 517 EXPRESSION OF FUNCTIONAL PEA LECTIN IN TRANSGENIC POTATO PLANTS, Glyn. A. Edwards, Andrew Hepher*, Stephen P. Clerk* and Donald Boulter, Department of Biological Science, Durham University, U.K.,* Shell Research Ltd., Sittingbourne, U.K. Lectins are a widely distributed group of carbohydrate-binding proteins. Their presence at relatively high concentrations in legume seeds has been associated with a possible role as a pest resistance mechanism. A clone encoding the preproprotein of the pea (Pisum sativum) lectin has been expressed in transgenic potato plants using either a CaMV 35S promoter or a tobacco Rubisco small-subunit promoter. Presence of the lectin to levels greater than 1% of total soluble protein was demonstrated by radioimmunoassay. The preprolectin was processed, generating α and β subunits that assembled to give isolectin forms observed in pea seeds. The fidelity of subunit assembly was verified by demonstrating that the haemagglutination activity of the pea lectin synthesised in transgenic potato leaves was comparable to purified lectin from pea cotyledons. Experiments are in progress to determine the intracellular location of the processed lectin in transgenic plants. Additionally, such plants are currently being assessed for enhanced pest resistance.

M 518 CLONING AND CHARACTERIZATION OF DNA COMPLEMENTARY TO THE SOYBEAN MOSAIC VIRUS GENOME, Alan L. Eggenberger, David M. Stark, and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130.

Soybean mosaic virus (SMV) is a member of the potyvirus group of plant viruses. It has a positive-sense RNA genome, approximately 10 kb in length. The long open reading frame encodes a large polyprotein which is post-translationally cleaved by one or more viral proteases to yield mature viral proteins. We have undertaken to clone the genome of SMV in order to better understand its organization and function. A cDNA from the 3' end of the genome that codes for the SMV coat protein has been sequenced and expressed in plants. Another cDNA coding for the putative viral replicase has been partially characterized. Work is in progress to clone and characterize the remainder of the SMV genome.

M 519 CLONING, SEQUENCING AND MUTAGENESIS OF GENES ENCODING PHOTOSYSTEM II REACTION CENTER POLYPEPTIDES IN CYANOBACTERIA, Jeffrey C. Gingrich [1], Kenneth Sauer [1], and Donald A. Bryant [2]. 1) Chemical Biodynamics Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720. 2) Department of Cell and Molecular Biology, The Pennsylvania State University, University Park, PA 16802.

Genes encoding the photosystem II (PSII) reaction center polypeptides D2 and CP43 from the transformable photoheterotrophic cyanobacteria *Synechococcus* sp PCC 7002 were cloned and sequenced. Two highly homologous genes (psbD1 and psbD2) encode D2 proteins of identical sequence. The CP43 protein is encoded by a single gene (psbC) which overlaps the 3' end of psbD1. The cyanobacterial psbD and psbC genes encode proteins ~90% homologous to the complementary higher plant proteins. We propose that the start codon for cyanobacterial and chloroplast encoded psbC genes is not an ATG but a GTG twelve codons downstream from the previously proposed start codon. The PSII genes were cloned with the intent to explore structure-function relationships within the PSII reaction center. To do this, a strain of cyanobacteria with a single psbD allele was constructed by replacement of the psbD2 gene with a gene containing an antibiotic resistance gene cartridge internal to the psbD2 gene coding sequences. Site-directed mutations are being created in the remaining psbD1 gene. Protein residues which we are targeting are those which have been postulated to be responsible for binding of the manganese cluster involved in photosynthetic oxygen evolution.

M 320 EXPRESSION OF A CHIMERIC POLYGALACTURONASE GENE IN TRANSGENIC <u>rin</u> (RIPENING INHIBITOR) TOMATO FRUIT RESULTS IN POLYURONIDE DEGRADATION BUT NOT FRUIT SOFTENING, James J. Giovannoni*, Dean Dellapenna+, Alan B. Bennett+, and Robert L. Fischer*, *Division of Molecular Plant Biology, University of California, Berkeley, CA 94720; +Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616

Tomato fruit ripening is accompanied by extensive degradation of pectic cell wall components. This is thought to be due to the action of a single enzyme, polygalacturonase, whose activity is controlled, at least in part, at the level of gene expression. At the onset of tomato fruit ripening, polygalacturonase enzyme activity, mRNA levels, and relative rate of gene transcription all dramatically increase. In order to elucidate the role of polygalacturonase during tomato fruit ripening, we utilized a pleitropic genetic mutation, <u>rin</u>, that blocks many aspects of ripening including the activation of polygalacturonase gene transcription. The polygalacturonase structural gene was ligated to a promoter that is inducible in mature <u>rin</u> fruit, inserted into the <u>rin</u> genome, and plants were regenerated. This allowed expression of the polygalacturonase gene in transgenic <u>rin</u> fruit at a time corresponding to ripening in wild-type fruit. Expression of this gene resulted in the accumulation of active polygalacturonase enzyme and the degradation of cell wall polyuronides in transgenic <u>rin</u> fruit. However no significant effect on fruit softening, ethylene evolution, or color development was detected. These results indicate that polygalacturonase is the primary determinant of cell wall polyuronide degradation, but suggest that this degradation is not sufficient for the induction of softening, elevated rates of ethylene biosynthesis, or lycopene accumulation in <u>rin</u> fruit.

M 521 TRANSGENIC PLANTS THROUGH CHROMOSOME INJECTION, R.J. Griesbach, USDA,ARS,BARC-w Beltsville, MD 20705 A new method was developed which allows the transfer of multigenic traits between species. Chromosomes from a drought tolerant species, Petunia alpicola, were isolated and microinjected into protoplasts of the cultivated petunia, P. hybrida cv. Pink Magic. The injected cells were screened for the expression of foreign proteins on polyacrylamide gels. Approximately 5% of the injected protoplasts showed stable differences in protein profiles. These transformed cells were regenerated into whole plants. The resulting transgenic plants expressed several flavonoid markers from the chromosome donor species. These flavonoids were sexually transmitted to offspring in a codominant Mendelian manner. In addition, these transgenic plants also expressed an increase in drought tolerance. It appears that chromosome-mediated transformation will allow the transfer of polygenic characteristics unlike Agrobacterium-meditaed gene transfer.

M 522 AGROBACTERIUM-MEDIATED INOCULATION OF VIROID cDNA REVEALS THE BIOLOGICAL EFFECT OF APPARENTLY LETHAL MUTATIONS, Rosemarie W. Hammond, and Robert A. Owens, Microbiology and Plant Pathology Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705 Potato spindle tuber viroid (PSTV) mutants which contain alterations in the terminal loops have previously been reported. PSTV-P contains mutations at positions 2, 4, and 6 in the left terminal loop, whereas PSTV-R contains mutations at positions 177 and 178 in the right terminal loop. Both of these mutants were noninfectious when either cDNA or SP6-generated transcripts were used as inoculum. Agrobacterium-mediated inoculation has shown that PSTV-R can replicate, though at a reduced level, and PSTV-P still appears to be incapable of replication. PSTV-R progeny and its replicative intermediates are restricted to the gall and root tissues and do not appear to travel upward through the stem to the newly developing leaves. The significance of these results and the use of Agrobacterium-mediated transformation for studying biological functions of viroids are discussed.

M 523 IDENTIFICATION OF POLYPEPTIDE MARKERS OF BARLEY YELLOW DWARF VIRUS-RESISTANCE AND SUSCEPTIBILITY IN NON-INFECTED BARLEYS, Peter Holloway and Robyn Heath, Biotechnology Unit, State Chemistry Laboratory, 5 Macarthur Street, East Melbourne, 3002, Australia. Barley Yellow Dwarf Virus (BYDV) is an economically important pathogen of over 100 cereal crops and pasture grasses throughout the world including wheat, rice, barley, triticale, oats, cereal rye and maize. We have undertaken a program to investigate the molecular basis of resistance to BYDV which is conferred upon barley by the Yd2 gene. When polypeptides from cvs. Shannon (BYDV-resistant) and Proctor (the BYDV-susceptible recurrent parent of Shannon) were compared by high-resolution two-dimensional electrophoresis, the polypeptide patterns obtained were almost indistinguishable. However, upon close examination cv. Shannon appeared to contain one polypeptide which could not be seen in cv. Proctor. Similarly, cv. Proctor contained another different polypeptide which was not present in cv. Shannon. An F1 hybrid between cvs Proctor and Shannon contained both polypeptides. These two polypeptides appeared to have the same molecular weight (approx. 40,000) but slightly different isoelectric points (7.2 for cv. Proctor and 7.0 for cv. Shannon). Peptide mapping and immunological studies strongly suggested that these two polypeptides are closely related to one another. When coleoptiles from a wide range of resistant and susceptible barley lines were examined for these two polypeptides, the polypeptide found in cv. Proctor was exclusively present in other BYDV-susceptible plants. The second polypeptide found in cv. Shannon was exclusively present in other resistant plants. Our data suggests that we have identified the gene products of 2 alleles of a locus that is closely linked to the locus which controls the Yd2 resistance gene on chromosome 3 of barley.

M 524 INSECT RESISTANT TRANSGENIC TOMATO PLANTS, Guy Honée, Theo van der Salm and Bert Visser, Research Institute Ital, P.O. Box 48, 6700 AA Wageningen, The Netherlands.
Bacillus thuringiensis produces parasporal crystals that are toxic against larvae of either lepidopteran, dipteran or coleopteran insect species. The crystals consist predominantly of Mr 130,000 to 160,000 proteins (protoxins) which can be proteolytically processed to yield smaller toxic fragments. A class B toxin gene was isolated from the lepidopteran specific strain *B. thuringiensis aizawai* 7.21.
Four constructs derived from this gene (BtII) and from the *Agrobacterium tumefaciens* binary vector pBI121 were made for plant transformation: ptox-1, containing the intact 3.5 kb gene; ptox-2, containing a 3'-end truncated 1.9 kb gene; ptox-3 and ptox-4 containing the 3'-end truncated gene fused to the NPTII and β-glucuronidase gene respectively. The constructs were used for transformation of MSK93 (*Lycopersicon esculentum* x *L. peruvianum*) and plants were analyzed for gene expression and toxicity.
Genes were also cloned in the *E. coli* expression vector PINIII^{113}A2 to study toxicity and enzyme activity of the gene products.
Constructs coding for two crystal proteins showing different insecticidal spectra are in preparation.

M 525 ENGINEERING NEMATODE RESISTANCE IN SUGARBEET, Frans A. Krens, Elma M.J. Salentijn, Harry Paul, Wouter Lange and Henk J. Huizing, Foundation for Agricultural Plant Breeding, SVP, P.O.Box 117,6700 AC Wageningen, The Netherlands.
A monosomic addition plant of Beta vulgaris L., containing an extra chromosome of B.patellaris L. with a gene for resistance to the beet cyst nematode (Heterodera schachtii Schm.)[1], gave rise to a diploid descendant with an additional chromosome fragment. This fragment was found to bear the gene for resistance. The estimated size of the fragment is 9-14 Mb while B.vulgaris chromosomes are as an average 134 Mb. Investigations are in progress in our laboratory to separate the fragment from the sugarbeet chromosomes by pulsed field gel electrophoresis (PFGE) or contour-clamped homogeneous electric fields (CHEF)[2]. After isolation the fragment will be subcloned in appropriate Agrobacterium rhizogenes vectors to allow application of the resistance assay which was developed in our laboratory[3]. In this in vitro assay hairy roots which have been induced on Beta plant material are aseptically inoculated with larvae. No cysts will occur when the plant material is resistant. The identification of the DNA sequences involved in nematode resistance will open interesting prospects for further research: a) transfer of the gene to commercial sugarbeet genotypes or other hosts like rapeseed; b) identification of homologous genes in other plant material; c) study of the mechanism involved in nematode resistance. This work presents one of the few projects on engineering pathogen resistance using a plant-derived gene.
1) Speckmann, G.J. et al. 1985. Z. Pflanzenzuechtg. 95,74.
2) Orbach, M.J. et al. 1988. Mol. Cell. Biol. 8,1469.
3) Paul, H. et al. 1987. Plant Cell Reports 6,379.

M 526 MANIPULATION OF THE COAT PROTEIN GENE OF POTATO LEAFROLL VIRUS FOR TRANSFORMATION OF POTATO, Amar Kumar, Brian Reavy and Michael A. Mayo, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom.
The genome RNA of potato leafroll virus (PLRV) has been cloned and sequenced. From this sequence we have identified an open reading frame (ORF) that could code for PLRV coat protein. We have confirmed that this ORF can code for the coat protein by subcloning in a mammalian transient expression vector and introducing it into HeLa cells. The cells made a protein of the expected molecular weight and which reacted with an anti-PLRV monoclonal antibody in Western blots. The coat protein ORF was also subcloned into a binary plant expression vector and introduced into potato by Agrobacterium-mediated transformation using tuber discs and leaf discs. Kanamycin-resistant shoots have been regenerated from the discs and whole plants have been obtained. The transgenic plants are being analysed for the presence and expression of PLRV coat protein cDNA sequence.

M 527 INHERITANCE OF A FUNCTIONAL MOUSE METALLOTHIONEIN GENE IN TOBACCO, Indu B. Maiti, George J. Wagner, Ricky Yeargan and Arthur G. Hunt, Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091
Morphologically normal plants were obtained from progeny (Ro, R1 and R2) originating from tobacco leaf tissue transformed with an engineered *Agrobacterium tumefaciens* containing a chimeric gene for kanamycin resistance and the mouse metallothionein cDNA gene directed by the constitutive promoter 35S from CaMV. Integration and expression of the metallothionein gene in R1 progeny was demonstrated by Southern, Northern blot analysis and metallothionein assay. Kanamycin resistance analysis of R1 and R2 progeny showed the inheritance of the foreign gene to be as a dominant Mendelian trait. Seedlings obtained from self-fertilized transgenic tobacco are more tolerant to cadmium stress than nontransformed controls. Cadmium accumulation in leaves of transgenic seedlings exposed to a low, field-like Cd concentration (0.02μM) was about 20% lower than that in nontransformed controls. These results suggest the possibility of developing transgenic plants with increased tolerance to heavy metal stress, and food crops having lower Cd content.

M 528 TOWARD HERBICIDE RESISTANT PLANTS: CLONING OF THE GENES FOR GLYPHOSATE DEGRADATION FROM A SOIL ORGANISM, AND THEIR EXPRESSION IN E. coli, Paul A. McLean, Charng-Ming Liu, Cathleen Cornell and Frank Cannon, BioTechnica International Inc., Agricultural Biotechnology Group, 85 Bolton St., Cambridge, MA 02140.
The object of this study is to transfer glyphosate-degrading ability to crop plants to confer glyphosate resistance. A soil organism has been isolated which degrades the herbicide glyphosate by selection for growth with glyphosate as sole P source. Metabolism of glyphosate was monitored using 1-[^{14}C]- and 3-[^{14}C]- glyphosate, and sarcosine was identified as a transient breakdown product. This implies that the degradative enzyme was a CP lyase which cleaves the carbon-phosphorous bond of the molecule. Tn5-induced mutants were isolated which were glyphosate-negative, and three complementation groups were identified: Regulatory, uptake and CP lyase. Complementing cosmids were isolated from a gene library, subcloned and the relevant areas sequenced. The CP lyase genes comprise a 3.9 kb operon under phoB-control (phosphate starvation induced) containing 5 open reading frames with predicted polypeptides of mol. wt. from 17K - 41K. These polypeptides were expressed from the tac promoter in an in vitro transcription-translation system. The poster will present data on expression and characterization of these genes in E. coli.

M 529 DIRECTED ALTERATION OF THE PHOTOSYSTEM II D1 PROTEIN: EVIDENCE DEMONSTRATING *TYR* $_{161}$ IS THE PRIMARY DONOR TO P680$^{+\cdot}$ James G. Metz, Peter J. Nixon, Matthias Roegner, and Bruce A. Diner, Microbiology Group, CR&D Dept. E.I. DuPont Experimental Station, P.O. Box 80173, Wilmington, DE 19880-173. In the photosystem II complex electrons are sequentially extracted from water at a site containing Mn atoms and transferred through an intermediate carrier (Z) to the photooxidized reaction center chlorophyll (P680$^+$). Recently Debus et al. (1988, PNAS 85, 427-430), have suggested that Z is a tyrosine residue located at position 161 of the D1 protein. We have therefore engineered a strain of the cyanobacterium *Synechocystis* 6803 to produce a D1 protein in which Tyr-161 has been replaced by phenylalanine. Wild type *Synechocystis* 6803 contains three nonidentical copies of the *psb* A gene which encode the polypeptide D1. In the mutant strain, two copies were deleted by replacement with antibiotic resistance genes (kanamycin and chloramphenicol), and site directed mutations constructed in a cloned portion of the remaining gene (*psb* A$_{III}$) carrying a spectinomycin resistance gene downstream. Transformants were selected for spectinomycin resistance and then screened for a photosynthesis minus phenotype. The mutant genotype was verified by complementation tests and by PCR amplification and sequencing of genomic DNA. Cells of the mutant cannot evolve oxygen and are unable to stabilize, with high efficiency, the charge separated state in the presence of hydroxylamine and DCMU. Optical spectroscopic analysis of reaction centers, isolated from the mutant, shows that photooxidation of Z is no longer observed and is replaced with formation of a chlorophyll cation radical. In the wild type, charge recombination between Z^+ and Q_A^- occurs with a $t_{1/2}$ of 70-80ms. In the mutant, charge recombination between Chl^+ and Q_A^- occurs with a $t_{1/2}$ of 1ms. These observations provide strong evidence in favor of identifying Z with D1-tyrosine-161.

M 530 A SELF-CLEAVING SATELLITE RNA ASSOCIATED WITH BARLEY YELLOW DWARF VIRUS. W. A. Miller,* T. Hercus,+ P. M. Waterhouse, and W. L. Gerlach. *Dept. of Plant Pathology, Iowa State University, Ames, IA 50011, +Dept. of Biochemistry, Adelaide University, Adelaide, South Australia 5001, and CSIRO Division of Plant Industry, Canberra, Australia 2601.

An RNA molecule was found in purified virions of the RPV serotype of barley yellow dwarf virus (BYDV) which has many properties of a satellite RNA, including: (i) the RNA is 322 nucleotides long, (ii) circular and multimeric forms of the RNA were detected, (iii) multimers were capable of self-cleavage to monomers in an autocatalytic fashion, and (iv) structures flanking the self-cleavage sites of both strands were similar to the "hammerhead" structures in other self-cleaving RNAs (Forster and Symons, Cell 49, 211-220, 1987). However, the (+) strand cleavage site varied significantly from the consensus hammerhead structure. It lacks some conserved sequences, and probably folds into a different secondary structure. Such a satellite RNA may be exploitable as an antiviral gene, if it can be shown to reduce virus symptoms. In addition, the novel cleavage structure may provide information to reduce constraints on the design of satellite RNA-derived ribozymes (Haseloff and Gerlach, Nature 334, 585-591, 1988).

M 531 TRANSGENIC BRASSICA NAPUS AND TOBACCO PLANTS HARBORING HUMAN METALLOTHIONEIN GENE ARE RESISTANT TO TOXIC LEVELS OF HEAVY METALS, Santosh Misra, Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C. V8W 2Y2. A chimeric gene containing a cloned human metallothionein-II (MT-II) processed gene was introduced into Brassica napus and tobacco cells on a disarmed Ti plasmid of Agrobacterium tumefaciens. Transformants expressed MT protein as a nuclear trait, and in a constitutive manner. Seeds from self-fertilized transgenic plants were germinated on media containing toxic levels of cadmium and scored for tolerance/susceptibility to this heavy metal. The growth of root and shoot of transformed seedlings was unaffected by up to 100 μM $CdCl_2$, whereas, control seedlings showed severe inhibition of root and shoot growth and chlorosis of leaves. The results of these experiments indicate that agriculturally important plants such as B. napus can be genetically engineered for heavy metal tolerance/sequestration and eventually for partitioning of heavy metals in non-consumed plant tissues. (Supported by NSERC).

M 532 CONTROLLING THE EXPRESSION OF THE TOMATO POLYGALACTURONASE GENE: PROMOTION BY REGULATORY *cis* ACTING REGIONS AND INHIBITION BY ANTISENSE RNA P.C. Morris, C.J.S. Smith, C.W. Watson, J.E. Knapp, K. Davies, S. Picton, D. Grierson; Department of Physiology and Environmental Science, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK, and C.R. Bird, J. Ray, W. Schuch; ICI Seeds, Plant Biotechnology Section, PO Box 11, The Heath, Runcorn, Cheshire, WA7 4QE, UK

Ripening in the tomato fruit is initiated by autocatalytic ethylene production. This is thought to drive the expression of numerous ripening specific genes. Notable amongst these is the gene for the cell wall degrading enzyme polygalacturonase. Expression of this gene can be influenced by specific mutations, by environmental factors such as heat shock, and by inhibiting ethylene perception. We have isolated and characterised a genomic clone for this gene. We show that the DNA sequences 5' to the structural gene direct tissue and temporal specific expression of the CAT reporter gene in transgenic tomatoes in an identical manner to the expression of PG during ripening. We also demonstrate the successful down regulation of PG gene expression in ripening fruit by means of constitutive PG-antisense RNA expression in transgenic tomatoes.

M 533 RESTRICTION FRAGMENT LENGTH POLYMORPHISM MAPPING IN APOMICTIC Pennisetum HYBRIDS, Peggy Ozias-Akins, James W. McNay, Michel Dujardin and Wayne W. Hanna, Department of Horticulture and USDA/ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA 31793
Apomixis in Pennisetum squamulatum results in asexual reproduction through seed. Transfer of this trait into cultivated pearl millet (P. glaucum) would have tremendous impact on the maintenance of superior hybrids. Seed from F1 hybrids could be used directly to propagate genetically identical plants in large numbers. Conventional breeding has resulted in the production of one individual from the third backcross generation that is apomictic and presumably contains only one chromosome from P. squamulatum. RFLP mapping combined with in situ hybridization to chromosomes is being used to characterize apomictic backcross individuals. We hope to find molecular markers that remain tightly linked to apomixis in a segregating population. These markers would provide starting points for a chromosome walk.

M 534 EXPRESSION OF SPINACH ACYL CARRIER PROTEIN IN TRANSGENIC TOBACCO PLANTS, Martha A. Post-Beittenmiller, Katherine M. Schmid, and John B. Ohlrogge, Department of Botany and Plant Pathology, Michigan State University, E. Lansing MI 48824-1312
Acyl carrier protein (ACP) is a chloroplast-localized, integral cofactor of fatty acid synthesis. ACP serves as carrier for the growing acyl chain during the multiple enzymatic steps of fatty acid assembly, desaturation and acyl transfer. In order to examine the consequences of overexpression of ACP in vivo, we have constructed a plasmid containing a fusion between the tobacco small subunit promoter and transit peptide and sequences encoding mature spinach ACP-I. The recombinant gene is under light regulation and is expected to produce the precursor ACP whose transit peptide would be cleaved as it is transported into the chloroplast. Western blot analyses of leaf homogenates from ACP-transgenic plants and vector-control plants demonstrate that the spinach ACP accumulates to levels in excess of the endogenous tobacco ACPs and that the transit peptide is cleaved. ACP-I has a phosphopantetheine prosthetic group added to ser-38, converting the apo form to the holo form. Normally apoACP is not detected in vivo. However, in the plants transformed with the spinach ACP, approximately 50% of the spinach ACP is in the apo form.

M 535 IDENTIFICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) MARKERS LINKED TO DOWNY MILDEW RESISTANCE GENES IN LETTUCE. Ilan Paran, Richard V. Kesseli and Richard W. Michelmore. Department of Vegetable Crops, University of California Davis, CA 95616.
Lettuce downy mildew is one of the best genetically characterized gene-for-gene interactions between a fungal pathogen and its host. Thirteen resistance (*Dm*) genes have been identified in lettuce (Lactuca sativa) that are matched by avirulence genes in Bremia lactucae. The *Dm* genes are clustered into four linkage groups. A detailed genetic map using RFLP markers is being constructed with an intra-specific cross of lettuce, in which six *Dm* genes are segragating. Clones detecting RFLPs linked to *Dm* genes are being identified using near-isogenic lines. Six to eight cDNA clones are bulked together to probe Southern blots of three pairs of near-isogenic lines. Most bulks are monomorphic and therfore discarded. Polymorphic bulks are further dissected into individual clones. This procedure has enabled us to screen a large number of clones in a relatively short time. The linked RFLPs will be used to correlate physical and genetic distance in the genomic region containing *Dm* genes and will subsequently act as starting points for chromosomal walking to clone *Dm* genes.

M 536 PROTEINS WITH THE POTENTIAL TO CONFER ALUMINUM RESISTANCE ON PLANTS, Joanna J. Putterill[1,2] and Richard C. Gardner[1], [1]Department of Molecular and Cellular Biology, Center for Gene Technology, University of Auckland, Private Bag Auckland, New Zealand and [2]Ruakura Agricultural Center, MAFTech North, Private Bag, Hamilton, New Zealand. Aluminum toxicity is an extremely important problem in agriculture, affecting crop yields on up to 40% of the worlds arable soils. Metal chelation is the predominant defence mechanism in both plant and animals for metals such as Cd and Zn. We have decided to attempt to develop a protein which can chelate free aluminum as a strategy to protect plants against the toxic effects of the metal. Work will be described in which the ability of various proteins to bind aluminum is assessed. We have used equilibrium dialysis, and two additional assays which measure the ability of test compounds to compete with calmodulin for aluminum binding. Aluminum binding to calmodulin can be measured by monitoring ANS fluorescence or by measuring its stimulation of phosphodiesterase acitivity. The order of the test compounds in their ability to bind aluminum in the three assays was: poly-l-aspartic acid/poly-l-glutamic acid/transferrin > citric acid/calmodulin >> metallothioneins/enkephalins. Currently, we are attempting to express some of these proteins in plants in order to assess their aluminum-binding capacity in plants and to ascertain whether they have a protective effect on the plants under conditions of high aluminum.

M 537 THE ABILITY OF CMV-C COAT PROTEIN TO PROTECT AGAINST OTHER STRAINS OF CMV IN TRANSGENIC PLANTS

Hector Quemada, Jerry L. Slightom, and Dennis Gonsalves*
Molecular Biology Research, The Upjohn Company, Kalamazoo, MI 49007 and *Dept. of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456

Transgenic tobacco plants expressing the coat protein of CMV-C have been tested for resistance to CMV-C and CMV-WL, two strains which differ by approximately 30% at the nucleotide sequence level (20% at the amino acid level). Based on the development of symptoms and ELISA assays of virus level, these plants appear to have a higher level of protection against the C strain than against the WL strain. The ability of the WL strain to overcome protection differs between lines of transgenic plants. Possible reasons for this difference are being investigated. Strains other than C and WL are also being used to challenge plants expressing CMV-C coat protein.

M 538 INTRODUCTION OF CAPSID PROTEIN FROM A PLANT VIRUS INTO TOBACCO PROTOPLASTS CAN CONFER TRANSIENT PROTECTION AGAINST THAT VIRUS. EFFECT OF AGGREGATION STATE OF TMV CAPSID PROTEIN. James C. Register III and Roger N. Beachy. Department of Biology, Washington University, St. Louis, MO 63130.

Protection against tobacco mosaic virus (TMV) infection is present in protoplasts isolated from transgenic plants which express TMV capsid protein (CP) (Register III, J.C. and Beachy, R.N. [1988] Virology, 166, 524-532). To help address the mechanism of this protection, a transient protection assay has been developed. It is shown here that introduction of either purified viral CP or virus that has been inactivated by ultraviolet irradiation (UV) into protoplasts which do not express viral CP induces a transient protection in these protoplasts to challenge virus introduced concommitantly or shortly thereafter. The transient protection: i) shows virus specificity, ii) can be overcome either by decreasing the level of "protecting" CP (or UV-inactivated virus) or by increasing the amount of challenge virus, iii) can be overcome by TMV treated briefly at pH 8.0 (a treatment which also renders TMV capable of overcoming transgenic cross-protection), and iv) can be mediated by UV-inactivated potato virus X, a virus unrelated to TMV.

Monomers of TMV CP tend to self-associate into a variety of higher ordered structures and the effect of the different aggregation states on transient protection was assessed. Capsid protein preparations comprsed largely of helical, virus-like, aggregates provided better protection than preparations comprised primarily of smaller structures such as disks and A-protein. The possible significance of these findings is discussed.

M 539 RESISTANCE TO ISOXABEN EXPRESSED IN TISSUE CULTURE BY ixr MUTANTS OF ARABIDOPSIS

Jean L. Roberts, Phillip D. Pike, Dale R. Heim and Ignacio M. Larrinua, Lilly Research Laboratories, A Division of Eli Lilly and Co., Greenfield, Indiana 46140.

Isoxaben (EL-107, Flexidor®, Gallery™) is a novel, highly active, preemergence broadleaf herbicide used primarily on small grains, tree crops and woody species. Mutants highly resistant to isoxaben in vivo have been isolated in Arabidopsis thaliana. We have subsequently studied the herbicide response of callus cultures of these mutants. I50 values for inhibition of callus growth by the herbicide have been determined for homozygous and heterozygous mutant lines. The results show that the herbicide resistance gene is semidominant, is expressed in tissue culture, and confers a high degree of resistance to isoxaben in vitro. Further studies will examine effects of the ixr (isoxaben resistant) mutation on physiological and cellular responses to the herbicide.

M 540 CLONING AND CHARACTERIZATION OF A GEMINI VIRUS GENOME FROM THAILAND

Dean E. Rochester, Wichai Kositratana[1], Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO. 63130 1.Department of Plant Pathology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140 Thailand. This report describes the initial cloning and characterization of a gemini virus isolated in Thailand from tomato plants exhibiting symptoms similar to those produced by Tomato Yellow Leaf Curl virus. After extraction of the DNA from virions, a primer was used to generate a second strand. The genome was then cloned using a unique EcoRI site. Two recombinants containing approximately 2.8 kb inserts were subsequently isolated. Sequence analysis has shown the clones to be different and presumably represent the "A" and "B" components of the viral genome. Experiments are underway to transfer the coat protein gene into tomato plants to determine if genetically engineered cross protection is successful with a gemini virus.

M 541 *THE Pto- avrPto INTERACTON: CLONING OF GENES INVOLVED IN DISEASE RESISTANCE OF TOMATO.*

Pamela C Ronald, Brian Kearney, Doug Dahlbeck and Brian Staskawicz. Department of Plant Pathology, UC Berkeley, Berkeley, CA 94720. *Resistance of tomato to the bacterial pathogen Psuedomonas syringae pv. tomato (Pst) race 0 is controlled by a single gene Pto expressing incomplete dominance. The gene Pto, originally identified in Lycopersicon pimpinellifolium, has been introduced into L. esculentum and near isogenic lines are available. There exists virulent races of Pst that can overcome the resistance conferred by Pto suggesting that a gene for gene interaction may be functioning. Our goal is to clone the bacterial avirulence gene and the tomato resistance gene involved in the incompatible interaction. We have initially concentrated our efforts in cloning the avirulence gene, avrPto, by introducing a cosmid library from the avirulent Pst race into the virulent strain Ps pv maculicola (Psm). A single 25 Kb cosmid clone was isolated that, when conjugated into Psm, attenuated symptoms in the Pto line of tomato. The cosmid was subcloned to a 2.4 Kb fragment that reduced growth of Psm transconjugants in the resistant plant but grew as well as the wild type Psm in the susceptible plant. The maize transposable element Ac has been introduced into the resistant cultivar of tomato, Pto/Pto. A strategy for mapping Ac insertions and tagging the resistant locus Pto with Ac will be presented.*

M 542 INFECTION OF PROTOPLASTS OF BLACK MEXICAN SWEET (BMS) MAIZE WITH MAIZE CHLOROTIC MOTTLE VIRUS (MCMV). Kay Scheets[1], Robert C. Nutter[1] and Steve Lommel[2], [1]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078, [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695. Developoment of resistance to MCMV may be aided through studying the replication and gene expression of MCMV at the cellular and molecular level. A PEG procedure was used for the synchronous infection of BMS protoplasts with MCMV. The order of infectivity was MCMV virions, viral RNA, and in vitro synthesized RNA. The in vitro synthesized RNA was made from a near full length cDNA clone of genomic MCMV RNA inserted into Bluescript KS+. RNA transcribed from the T7 promoter included 75 bp of non-MCMV sequence at the 5' end and replicated at low levels. A cDNA clone with the non-MCMV sequence removed is under construction. Viral infection can be detected with fluorescein labeled anti-MCMV IgG, and by hybridization to total RNA on Northern blots. The 4.44 kb genomic and 1.4 kb subgenomic RNA can be detected as early as 4 hrs post-infection, and RNA accumulated for at least 72 hrs. Escherichia coli expression plasmids are under construction to produce antigen for nonstructural MCMV proteins. Antibodies to these proteins and coat protein will be used in Western blots to study viral replication and gene expression. We are also investigating how callus cultures of maize lines showing field resistance to MCMV support viral replication.

M 543 ELECTROPHORETIC ANALYSIS OF INTERSPECIES HYBRIDS OF Triticum aestivum L. X T.timopheevi ZHUK. USING MALATE DEHYDROGENASE AS A GENETIC MARKER, Surendra Singh, Department of Plant Breeding, Haryana Agricultural University, Hisar-125004, India.
The malate dehydrogenase spectrum using SDS-PAGE of four varieties (WH 147, C306, Chinese spring and Kalyansona, of bread wheat Triticum aestrivum (AABBDD; 2n=42), and of two tetraploid species T. dicoccum (AABB; 2n=28) and T. timopheevi (AAGG; 2n=28), and 42-chromosomes hybrids F_7B_1 obtained from T. aestivum x T. timopheevi cross, revealed the presence of interspecies polymorphism with respect to this enzyme. It was shown that the spectrum in variety C306 differes from that of other varieties in regard to the ratio of intensities of the bands of the three isoforms of malate dehydrogenase and that it is analogous to the spectrum of the tetraploid with an AABB genome. Among the F_7B_1 hybrids plants combination of the Kalyansona X T. timopheevi cross yielded forms with a malate dehydrogenase spectrum which differed from that of the original variety. Genetical, Biochemical and Cytological studies revealed that they contained a substitution of the 1A-Chromosome of T.aestivum and 1A-of T.timopheevi. The analysed data confirmed the porsible use of malate dehdydrogenase as a genetic marker in interspecific hybrids of Triticum.

M 544 EXPRESSION OF ANTISENSE RNA IN A GEMINIVIRUS, M.E.C. Siong, R.J. Hayes and K.W. Buck, Department of Pure & Applied Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, England.
Tomato golden mosaic virus (TGMV) is a member of the geminivirus group of plant viruses which are characterised by their unique double-icosahedral (geminate) particles and genomes of circular single-stranded DNA. It has two components, A and B, which replicate via a double-stranded (ds) form. The ds forms have been cloned, sequenced and shown to be infectious via mechanical inoculation after both excision from the vector, and with excision when present as dimers. In vitro mutagenesis of the open reading frames of both cloned components has shown that only the coat protein gene (AR1) is not required for DNA replication or systemic infection. Using agroinfection we have shown that large deletions in the AR1 gene are tolerated but elicit very mild symptoms compared to the wild type infection. A vector TAneo has been constructed in which AR1 has been replaced by the neomycin phosphotransferase gene of Tn5. Transgenic plants were then produced containing a partial dimer of the vector. Analysis of DNA from these plants shows that TAneo is formed resulting in the replication of the vector and amplification of the neo gene. The vector is presently being used in studies of plant gene function by increasing the efficacy of suppression of gene expression by amplification of antisense RNA.

M 545 A UNIQUE RFLP MAPPING SYSTEM IN PENNISETUM, Rex L. Smith, M. K. U. Chowdhury, and S. C. Schank, Department of Agronomy, University of Florida, Gainesville, FL 32611.
Pennisetum purpureum (napiergrass), a high yielding tropical and subtropical herbaceous biomass species, has been targeted for genetic improvement of its methane producing qualities by both plant breeding and molecular methods. Molecular study and genetic improvement in that species is hampered by the absence of a genetic database, genetic stocks, and inbred lines; in addition, partial sterility causes problems in controlling pollination and in producing hybrids. To avoid the complexities imposed by the lack of genetic information and inbred lines we have devised a unique scheme for mapping RFLPs that involves using interspecific progenies of napiergrass (A'A'BB) crossed to diploid P. glaucum (pearl millet) (AA). Those crosses are easily made on cytoplasmic male sterile pearl millet and result in hybrids where the pearl millet and napiergrass genomes are haploid. By using inbred pearl millet parentals, the pearl millet input into the progeny genotypes is predictable and consistent, so segregation of heterozygous napiergrass loci are observed in simple 1:1 expected ratios. Our RFLP analyses are being conducted on two napiergrass X pearl millet interspecific progenies using two napiergrass parentals selected for their high RFLP diversity which is indicated by about half of our probes showing polymorphism between those two napiergrasses. Segregating (heterozygous) loci are linkage mapped directly from those interspecific segregation data. Nonsegregating (homozygous) loci that differ between the two napiergrass parentals will be mapped using F_2 data of their hybrids. Specific results will be discussed.

M 546 EXAMINATION OF ENGINEERED COAT PROTEIN PROTECTION WITH VIRUSES OF THE POTYVIRUS GROUP David M. Stark and Roger N. Beachy, Biology Dept., Washington University, St. Louis, Mo. 63130. The phenomenon of coat protein protection has now been demonstrated with several different plant virus groups. We wish to extend this phenomenon to the potyvirus group. The sequences of several different potyviral coat proteins have been reported to date, giving some insights into the structure of these proteins. Sequence comparisons show strong amino acid homology in the carboxy two-thirds of the coat proteins, with the amino termini being less conserved but similar in that all are hydrophilic. Because of the conservation of amino acid sequence between potyviral coat proteins, we wished to test if the expression of one coat protein could protect a plant against infection by different members of the potyvirus group, i.e. heterologous protection. We also wished to test if truncated versions coding only the conserved portions of the coat protein could afford protection. To this end the coat protein genes of soybean mosaic virus (SMV) and tobacco etch virus (TEV), including truncated versions of the TEV coat protein, have been cloned and expressed in transgenic plants. Tests are currently under way to test the stability of the native and mutant proteins as well as the efficacy of chimeric gene products in protecting transgenic plants against potyvirus infection.

M 547 TRANSGENIC PLANTS EXPRESSING CMV SATELLITE RNA GENES: SEQUENCES REQUIRED FOR SATELLITE RNA REPLICATION OR INDUCTION OF THE NECROTIC RESPONSE IN TOMATO
M Tepfer, D Tousch, Laboratoire de Biologie cellulaire, INRA-Centre de Versailles, 78026 Versailles Cedex, France.
M Jacquemond, Station de Pathologie Végétale, INRA-Domaine St. Maurice, BP 94, 84180 Montfavet, France.

We have previously shown in transgenic tobacco plants that transcripts based on monomeric forms of CMV satellite RNA are recognized by CMV replicase to produce large quantities of biologically active satellite RNA. We have more recently transferred other genes to tobacco, based on either the necrogenic satellite RNA, N, or the non-necrogenic satellite RNA, R. The corresponding cDNAs were inserted in both orientations, giving rise to genes whose transcripts bear either (+) or (-) sense satellite RNA sequences. On infection with satellite-RNA-free CMV, plants bearing either N(+) or R(+) genes produced, as expected, large quantities of satellite RNA, while ones bearing N(-) or R(-) genes did not. In order to study the role of sequences flanking the satellite RNA in precursor transcripts, which may be essential for recognition by the CMV replicase, we are currently deleting part or all of the flanking homopolymeric regions.

The two satellite RNAs studied, N and R, are the most closely related members of the necrogenic and non-necrogenic groups, differing by only two substitutions and one deletion (Jacquemond and Lauquin, 1988, BBRC 151: 388-395). Experiments aimed at determining which of these differences are required for determining necrogenic potential will be described.

M 548 MOLECULAR CLONING AND ANALYSIS OF THE ANDEAN POTATO MOTTLE VIRUS, **Benedikt Timmerman, Paulo Ferreira, Ana Carolina Vicente, Adriana Silva, Tânia Jacinto, Mauro da Costa, Fernanda Silva, Nice Shindo e Paulo Brioso.**Department of Genetics, Federal University of Rio de Janeiro, CP 68011, CEP 21944 , Rio de Janeiro, RJ, Brasil.

The Andean Potato Mottle Virus (APMV) is a single-stranded RNA virus causing yearly increasing losses in potato crops in Argentina, Brasil and Peru. Virus particles sediment as 3 components: empty protein shells (53 S) and 2 nucleoprotein particles (95 S and 118 S), with identical protein content but different RNA species. Denatured PAGE analysis indicates the RNA's are ±3,5 Kb and ±5,6 Kb in lenght. The isometric particles (27-30 nmØ) consist of polipeptides with mol.wt. of 22,100 and 41,800. Complementary DNA to the viral genome was synthesized, ligated to EcoRI adapters and cloned into a pGEM-Z vector, Northern blot hybridization using characterized c-DNA probes indicate 70% of both RNA's have been cloned. Further analysis of molecular data will be presented.

M 549 EFFECT OF HYDROLYTIC ENZYMES ON THE GROWTH AND CELL WALL OF DIDYMELLA BRYONIAE,

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Gummy stem blight caused by the ascomycete fungus Didymella bryoniae is an important disease of greenhouse grown cucumbers. Chemical control is difficult and yet no satisfactory levels of resistance have been introduced in commercial cultivars. Resistance might be obtained by introduction of genes coding for enzymes which are able to attack the cell wall of the fungus. Analysis of the cell wall of D.bryoniae revealed that the structure in general is in agreement with the proposed model of the hyphal wall of Ascomycetes, but seems to be exceptional with regard to the highly crystalline form of chitin in the native wall. Resistance to several enzyme preparations may be due to the presence of this highly crystalline chitin. Several endo-chitinases were isolated from tomato and cucumber plants and their effect on mycelial growth and crystalline chitin will be presented.

M 550 STRATEGIES FOR INTERFERING IN VIVO WITH THE REPLICATION OF TURNIP YELLOW MOSAIC VIRUS

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F Cellier and M Tepfer, Laboratoire de Biologie Cellulaire, INRA-Centre de Versailles, 78026 Versailles Cedex, France.

Turnip yellow mosaic virus (TYMV) is the type member of the tymovirus group; its genome is composed of a single molecule of (+) sense RNA, with a tRNA-like structure at the 3' end. The tRNA-like region is recognized by the TYMV replicase in initiating the synthesis of (-) strand RNA from the (+) sense template.

We have introduced into *Brassica napus* plants several genes that might interfere with replication of TYMV. Transcripts of certain genes bear regions which are complementary to the the 3' region of the virus, such antisense ricRNA (replication interfering complementary RNA) could mask the site recognized by the replicase in initiating (-) strand synthesis.

A second strategy concerns the use of (+) sense RNA as a competitor for the replicase. Morch *et al.* (Nucl. Acids Res. (1987)15: 4123-4129) have shown that RNAs bearing the tRNA-like region are competitive inhibitors of the replication of TYMV genomic RNA *in vitro*. We have introduced genes into *B. napus* allowing us to test this "sense" strategy *in vivo* as well. Results of infecting such transgenic plants with TYMV will be described.

M 551 THE Tm-1 GENE ACTION: TENTATIVE INTERACTION BETWEEN 130K/180K PROTEINS OF TMV AND HOST FACTORS, Yuichiro Watanabe, Rika Yamafuji, Tetsuo Meshi and Yoshimi Okada, Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, JAPAN. Tm-1 gene donates tomatoes resistance against various wild isolates of tobacco mosaic virus (TMV) and expresses the resistance even in protoplasts. A resistance-breaking strain Ltal was spontaneously isolated from tomato strain L. Analysis of Ltal strain revealed that two concomitant amino acid substitutions mapped in 130K/180K proteins are likely to be responsible for overcoming the resistance and that the mutation does not suppress normal Ltal replication in tomatoes without Tm-1 gene background. Recent accumulating data concerning the function of these two large non-structural proteins and incomplete dominance character of Tm-1 gene, prompted us to imagine that a factor existing in Tm-1 gene tomato somehow interact with initially translated 130K/180K proteins of L strain and then positively interfere or suppress normal TMV multiplication. We are trying to investigate the very early stage of infection by L strain into Tm-1 tomates compared with the case with Ltal strain. For instance, we have constructed two types of Ltal derivatives having coat proteins of which internal portion was intervened by the critical partial 130K/180K protein coding region of L or Ltal. We have been analyzing the fates of expected fusion coat proteins in Tm-1 tomato protoplasts.

M 552 BARLEY YELLOW DWARF VIRUS EXPRESSION IN WHEAT PROTOPLASTS AND CONSTRUCTION OF SYNTHETIC GENES TO INTERFERE WITH VIRAL REPLICATION, Mark J. Young, and Wayne L. Gerlach, CSIRO Division of Plant Industry, Canberra, ACT 2601 Australia

Barley yellow dwarf virus (BYDV) possesses a single stranded RNA genome of about 6000 nucleotides. For many impotant ceral crops there is no known genetic resistance to this economically important virus. We are attempting to develope resistance to BYDV by constructing synthetic genes which interfere with viral replication. As a first step towards this goal, a wheat protoplast system has been developed for assaying the replication of BYDV. The protoplast system demonstrates for the first time that the purified virion RNA is infectious and provides a rapid assay for viral replication. We are currently using the protoplast asssay to test synthetic gene constructions designed to interfere with BYDV replication. Three types of gene constructions are being tested. These involve genes which direct the synthesis of (1) viral coat protein, (2) mutant viral replicase, and (3) catalytic antisense RNA ("ribozymes"). Transient expression of these genes and analysis of their effect on BYDV replication should provide a rational basis for engineering resistance into ceral crops.

Replacement for wrong abstract inadvertently published in Volume 13A as CA 407

CA 407 AUTOMATED PROTEIN ^{1}H, ^{13}C, AND ^{15}N NMR FIRST AND SECOND ORDER PEAK ASSIGNMENTS, Eldon L. Ulrich*, Zsolt Zolnai, William M. Westler, Michael D. Reily**, and John L. Markley, Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

Multinuclear protein NMR data from $^{13}C\{^{13}C\}$ double quantum correlation, $^{1}H\{^{13}C\}$ single bond correlation, $^{1}H\{^{15}N\}$ single bond correlation, $^{13}C\{^{15}N\}$ single bond correlation, $^{1}H\{^{13}C\}$ multiple bond correlation, $^{1}H\{^{15}N\}$ multiple bond correlation, $^{1}H\{^{1}H\}$ COSY, $^{1}H\{^{1}H\}$ NOESY, $^{1}H\{^{1}H\}$ RELAY, and $^{1}H\{^{1}H\}$ HOHAHA experiments have been collected for the protein cytochrome c_{553} from Anabaena 7120 in the reduced state. These data are used to demonstrate multiple methods for determining both first and second order protein resonance assignments. Redundant assignment pathways provide more reliable assignments but require the analysis of a wide range of experimental data. Computer software has been developed to aid the user in creating lists of peak coordinates for resonances in two-dimensional NMR spectra. Data files containing the resonance coordinates, standard amino acid ^{1}H and ^{13}C chemical shift data, and the amino acid sequence of the protein are used as input for additional software programs. These programs attempt to group together resonances that are related to individual amino acid spin systems, to identify the type of amino acid, and to assign the resonances to specific amino acid residues in the protein. [Present address: *The Upjohn Company, Control Division, Kalamazoo, MI 49001; **Park Davis, Research Division, Ann Arbor, MI 48105. Supported by National Institutes of Health Grant RR02301 from the Biomedical Research Technology Program, Division of Research Resources and Grant 88-37262-3406 from the U. S. Department of Agriculture.]