# THE GENETIC DISSECTION OF PLANT CELL PROCESSES

*Organizer: Robert Goldberg*

January 10-17, 1991

## *Plenary Sessions*

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## The Genetic Dissection of Plant Cell Processes

*Keynote Address*

**A 001** GENETIC DISSECTION OF DEVELOPMENT IN FUNGI, William E. Timberlake, Department of Genetics, University of Georgia, Athens, GA 30606

*Aspergillus nidulans* is an Ascomycete that has become a model for studying the mechanisms controlling cell differentiation and development in fungi due to 1) an extensive background of genetic analysis, 2) a highly tractable transformation system, and 3) easily controllable developmental pathways that result in formation of complex, multicellular, reproductive structures. The molecular and genetic processes controlling elaboration of the asexual sporophore, the conidiophore, have been studied most intensively. Several hundred genes are expressed specifically during conidiophore development. Inactivation of many of these genes results in very subtle, although informative, alterations in conidiophore structure, indicating that their products contribute incrementally to morphogenesis. By contrast, mutations in a few genes lead to major developmental abnormalities. The functions of three such genes, designated *brlA, abaA,* and *wetA* have been examined in detail. These genes make up a *central* regulatory pathway that controls the major steps of conidiophore development. *brlA* is activated by poorly defined signals that include nutrient deprivation and exposure of cells to red light. *brlA* in turn activates early morphogenetic loci and *abaA*. *abaA* is a positive feedback regulator of *brlA*, enhances expression of early morphogenetic loci, and activates *wetA*. *wetA* interacts with *brlA* and *abaA* to stimulate expression of late morphogenetic loci. However, *wetA* alone induces expression of genes whose products make up the final wall layers of the dormant conidiospore. The *brlA* and *abaA* products are probably transcriptional inducers, but *wetA* may function through selective transcript stabilization. The patterns of autoregulation displayed by this pathway, in association with physiological restructuring within the cells undergoing differentiation, assure that sporulation will proceed independent of the environmental ques that initiated the process.

*Genetic Control of Animal Development*

**A 002** **INITIAL MECHANISMS OF SPATIAL GENE REGULATION IN THE SEA URCHIN EMBRYO**, E. H. Davidson, Division of Biology, California Institute of Technology, Pasadena, CA 91125

This presentation will concern the molecular basis of the process by which differential patterns of regional gene activity are instituted in the embryo. The cytoskeletal actin gene CyIIIa provides an excellent molecular marker for the zygotic program of gene expression characteristic of the embryonic aboral ectoderm. According to our recent lineage tracer studies the aboral ectoderm derives clonally from 11 specific cleavage stage founder cells. However, the egg is initially radially symmetric, and interblastomere interaction as well as an early cytoplasmic polarization are probably required for aboral ectoderm specification. Regulatory sequences of the CyIIIa gene have now been shown to accurately direct spatial and temporal expression of exogenous reporter genes, after injection of the appropriate fusion gene constructs into sea urchin eggs. By using this method of gene transfer the cis regulatory domain of the CyIIIa gene has been delimited. Coinjection of excess quantities of DNA fragments containing subregions of the regulatory domain results in in vivo competition, and thus it is possible to analyze the functional significance of individual regulatory elements. DNA-protein binding studies in vitro demonstrate at least 20 sites where highly specific interactions occur within this domain ($10^4$-$10^6$ fold preference for CyIIIa site vs. random DNA sequence). Some of the factors that bind to these sites are probably zygotic gene products, since their concentration increases as development proceeds and they cannot be detected in extracts of unfertilized eggs. Analysis of the origin, the cytological distribution and possibly the activation of certain of these CyIIIa regulatory factors in the egg and early embryo, considered in conjunction with the lineage and location of the aboral ectoderm precursors will provide a molecular interpretation of how the CyIIIa gene becomes differentially expressed as the aboral ectoderm is formed. An interesting result follows from introduction of the CyIIIa regulatory sequences into eggs of a different sea urchin species. Though the exogenous fusion construct is regulated temporally in a correct manner, spatial regulation is wholly deranged. The same pattern of ectopic expression is observed in in vivo competition experiments, when two specific cis-trans interactions in the CyIIIa regulatory domain are prevented. These are mediated by factors P3A2 and P7II. P3A2 is cloned, as are a number of other factors involved in early regulation of this and other lineage-specific genes in the S. purpuratus embryo. The P3A2 mRNA is a rare maternal species, but calculations show that its prevalence suffices to generate functional levels of P3A2 factor in blastomere nuclei during the process of specification. By immunocytology we also show that P3A2 is localized to the cell types where a CyIIIa transcription is repressed, in accord with its known role as a negative spatial regulator. An interpretation of sea urchin embryogenesis integrating classical and molecular evidence will be presented.

**A 003** SEX DETERMINATION AND DOSAGE COMPENSATION IN THE NEMATODE *C. elegans*, Barbara Meyer, Michael Nonet, Robert Klein, Leslie DeLong, John Plenefisch and Leilani Miller, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The choice of sexual fate is a developmental decision made by most organisms. Because of its ubiquity, the process of sex determination provides an opportunity to compare, among evolutionarily divergent organisms, the molecular basis for the variety of developmental strategies that have evolved to solve this major developmental decision. These strategies used to determine sexual fate range from those specified by a chromosomal mechanism, to those in which sex is determined solely in response to the environmental conditions during embryogenesis, such as temperature or population density.

**The basis for the male/hermaphrodite decision: coordinate control of sex determination and dosage compensation.** It has been known for some time that the primary sex-determining signal in *C. elegans* is the X/A ratio (the ratio of the number of X chromosomes to the number of sets of autosomes). Only recently has it become clear that this same signal serves as a trigger for another developmental process, X chromosome dosage compensation, which equalizes the expression of X-linked genes between the male (XO) and hermaphrodite (XX) sexes. How the primary sex-determining signal is assessed and subsequently translated into the choice of either the hermaphrodite or male mode of sexual differentiation remains a mystery. However, insight into understanding the basis of sex determination has been achieved with our observation that the sex determination and dosage compensation processes share common early steps prior to their divergence into separate pathways. In hermaphrodites, the sex determination and dosage compensation processes are coordinately controlled through at least two X-linked genes, *sdc-1* and *sdc-2*; mutations in either gene disrupt both processes, shifting them to the male mode, resulting in masculinization, overexpression of X-linked genes and XX-specific lethality. Genetic and molecular experiments indicate that the effects of *sdc* mutations on sex determination and dosage compensation are ultimately implemented by two independent pathways. The *sdc* genes appear to play a role in either assessing the X/A ratio or transmitting this signal to both the sex determination and dosage compensation pathways. We have also shown that the autosomal gene *sdc-3* is involved in controlling both processes in XX animals. This gene is different from the other *sdc* genes in that the sex determination and dosage compensation functions are separately mutable. Moreover, only XX animals carrying non-null alleles are masculinized, while XX animals carrying null alleles exhibit only the phenotypes associated with the disruption of dosage compensation (XX-specific lethality and dumpiness), but no masculinization. The X-linked gene *xol-1* controls the male modes of both sex determination and dosage compensation. Mutations in *xol-1* shift both processes to the hermaphrodite mode, resulting in the feminization and death of XO animals. Genetic interactions suggest that *xol-1* is the earliest acting gene in the known hierarchy that controls the male/hermaphrodite decision and is likely to be the gene nearest to the primary sex-determining signal. The wild-type *xol-1* activity appears to promote male development by ensuring that the *sdc* genes (or gene products) are inactive in XO animals. All four of these global regulatory genes have been cloned; the genetic and molecular nature of their regulation will be discussed.

## *Genetic Analysis of Cell Processes in Prokaryotes and Lower Eukaryotes*

**A 004** HETEROCYST DIFFERENTIATION IN THE CYANOBACTERIUM ANABAENA, Robert Haselkorn, William J. Buikema and Bianca Brahamsha, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

Cyanobacteria such as Anabaena grow in long filaments containing hundreds of identical vegetative cells. These cells carry out green plant photosynthesis, evolving oxygen in the light, as long as the growth medium contains a source of combined nitrogen. Deprived of such a source, cells specialized for the fixation of atmospheric nitrogen, called heterocysts, differentiate at regular intervals along the filaments. The heterocyst has an extra double-layered envelope of glycolipid and polysaccharide, wholly reorganized photosynthetic membrane systems, no $CO_2$ fixation capacity, new metabolic paths to produce ATP and low-potential reductant for nitrogen fixation, and the nitrogenase complex. We would like to elucidate the regulatory cascade that transmits the environmental cue of nitrogen starvation to the transcription apparatus for the differential gene expression required to make a functional heterocyst. To this end, we have isolated Anabaena mutants defective in aerobic nitrogen fixation and selected, among these, ones that have obviously defective heterocysts. For each of these, a cosmid containing the corresponding wild-type gene was isolated by complementation of the mutant with a library of wild-type DNA fragments. Repeated subcloning and conjugation were used to isolate gene-sized fragments still capable of complementation. In the case of one mutant blocked at a very early stage of differentiation, the smallest complementing fragment was sequenced and found to contain a single gene, hetR, whose transcript increases about five-fold early in normal development. When present in wild-type cells in extra copies, the hetR gene provokes the differentiation of extra, clustered heterocysts. The hetR gene encodes a 35 kD protein, whose predicted amino acid sequence does not contain any of the motifs associated with transcription factors. On the other hand, the gene encoding the major vegetative cell RNA polymerase sigma factor, sigA, has been cloned, sequenced and found to have five transcription start sites ranging from 328 to 867 bp prior to the translation start. One of these sites is not used in cells growing on combined nitrogen but is turned on abundantly under heterocyst-inducing conditions. The relationships among hetR, sigA and other elements of the regulatory circuits are being pursued.

**A 005** MATING TYPE IN NEUROSPORA. Robert L. Metzenberg, Jeff Grotelueschen, and Mary Anne Nelson, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706, and N. Louise Glass, Biotechnology Laboratory, University of British Columbia, Vancouver BC, CANADA V6T 1W5. Neurospora crassa is a bipolar heterothallic ascomycete with two mating types, each specified by a single gene, and designated A and a. A thallus of either mating type, growing on medium appropriate for mating, makes protoperithecia, the precursors of the fruiting bodies. Trichogynes associated with protoperithecia grow toward conidia or hyphae of the opposite mating type and fuse with them. Conjugate division occurs in the resulting ascogenous hyphae, followed by caryogamy, meiosis, and ascospore formation. During vegetative growth, fusion between thalli of opposite mating types to form a heterocaryon results in cell death along the line of fusion -- that is, "incompatibility".

The cloning (1), characterization (2) and sequencing of A and a has opened these phenomena to molecular analysis (3, 4). Surprisingly, the two forms are dissimilar in sequence over a distance of 5301 bp and 3235 bp, respectively. Hence they are not "alleles", and we refer to them instead as "idiomorphs" (5). In N. crassa, there is only one idiomorph per haploid genome so that switching of the sort seen in S. cerevisiae or S. pombe is not possible. Many but not all true homothallic Sordariaceae have homologs of both idiomorphs in each haploid gene complement. The exceptional homothallic species, exemplified by N. africana, have only the A idiomorph.

We have isolated DNA sequences transcribed under conditions appropriate to mating but not under conditions appropriate for vegetative growth and have carried out disruption of some of these sequences. Analysis of the sexually aberrant phenotypes of such disruptants is in progress.

(1) Vollmer, S. J. and Yanofsky, C. (1986) Proc. Nat. Acad. Sci. U.S.A. 83: 4869.
(2) Glass, N. L., Vollmer, S. J., Staben, C., Grotelueschen, J., Metzenberg, R. L., and Yanofsky, C. (1988) Science 241:570.
(3) Glass, N. L., Grotelueschen, J., and Metzenberg, R. L. (1990) Proc. Nat. Acad. Sci. U.S.A. 87:4912.
(4) Staben, C. and Yanofsky, C. (1990) Proc. Nat. Acad. Sci. U.S.A. 87:4917.
(5) Metzenberg, R. L. and Glass, N. L. (1990) BioEssays 12:53.

*Genetic Analysis of Plant/Microbe Interactions*

**A 006** DEVELOPMENT OF A MODEL PATHOGENESIS SYSTEM INVOLVING INFECTION OF *Arabidopsis thaliana* BY *Pseudomonas syringae*, Frederick M. Ausubel, Michael Mindrinos, Xinnian Dong, and Eric Schott, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

We have developed a model system that utilizes *Arabidopsis thaliana* to study the signal transduction pathways leading to the expression of plant defense genes in response to pathogenic pseudomonads. We have identified virulent and avirulent *Pseudomonas syringae* strains, infiltrated them into *A. thaliana* leaves, and characterized the accumulation of mRNA corresponding to two major defense related gene families, the phenylalanine ammonia lyase (PAL) and β-1,3,-glucanase genes. To monitor β-1,3-glucanase mRNA, we cloned three adjacent *A. thaliana* genes that encode β-1,3-glucanases (BG1, BG2, and BG3). Following inoculation of *A. thaliana* leaves with virulent strains, the bacteria multiply 5 logs *in planta* and mRNA corresponding to BG1, BG2, and BG3 accumulates 10 to 20 fold over the course of 48 hours. There is only a modest effect on the levels of PAL mRNA. In contrast, following inoculation with an avirulent strain, bacteria multiply one to two logs *in planta* and PAL mRNA transiently increases 10 to 30 fold within 6 hours. There is a modest effect on the levels of BG1, BG2, and BG3 mRNA. To address the question of whether the differences between the virulent and avirulent strains can be attributed to the presence of individual *avr* gene(s), we have cloned two putative *avr* genes from an avirulent strain and transferred them to a virulent strain. The virulent strain carrying either of the cloned *avr* genes mimicked the avirulent strain when infiltrated into the *A. thaliana* ecotype Columbia in that it elicited a visible hypersensitive response, grew more poorly *in planta* than the virulent strain, and strongly elicited PAL mRNA but not β-1,3-glucanase mRNA accumulation. On the other hand, the *A. thaliana* ecotype Bensheim appears to respond to the presence of only one of the two cloned *avr* genes, suggesting that *A. thaliana* is responding to *avr* genes through single or a limited number of "resistance" genes. These experiments demonstrate for the first time that an individual *avr* gene can affect the expression of host defense genes and lay the foundation for the isolation of *A. thaliana* mutations that affect the defense response. Our current goal is to devise simplified genetic screens in which we monitor the induction of the phenylalanine ammonia lyase (PAL) and β-1,3-glucanase promoters in response to cloned *avr* genes.

**A 007** SIGNALS AND RESPONSES IN THE *RHIZOBIUM*-ALFALFA SYMBIOSIS, S.R. Long, D. Ehrhardt, M. Atkinson, M. Barnett, J. Schwedock, H. Brierley, J. Swanson, J. Ogawa, B. Rushing and R. F. Fisher, Department of Biological Sciences Stanford University Stanford CA 94305-5020

*Rhizobium meliloti* causes nodulation on alfalfa, in a process requiring common *nod* genes *nodABC* and additional genes *nodH*, *nodFE*, *nodG* and *nodPQ*. We have studied the regulation of these genes by the *nodD* gene activator family, including the action of NodD proteins and the circuitry of control of *nodD* and other genes. We have found that NodD interacts with the upstream *nod* box[1] in a way that includes pronounced bending of the target DNA, and have defined contacts between protein and individual bases in the promoter. The expression of *nod* genes becomes independent of inducer due to the action of the NodD3 protein; we have found that the *nodD3* gene and a higher level regulator, *syrM*, constitute a self-amplifying cascade. We have also discovered that a *groEL* like gene on the chromosome of *R. meliloti* is required for full activity of NodD. We are also studying nod gene proteins and sequences to obtain clues as to their individual functions. In pursuit of functions for *nodP* and *nodQ*, we have recently found that *E. coli* has a *nodP* equivalent[2], which we cloned and mapped to a location between 58 and 59 minutes of the *E. coli* chromosome. The restriction map suggested that *nodP* and *nodQ* were equivalent to *cysD* and *cysN*, enzymes carrying out ATP sulfurylase activity, and we have confirmed this by *in vitro* assay. This is consistent with the model of Lerouge et al.[3], who propose that the *R. meliloti* signal molecule NodRm-1 is sulfurylated at the 6-C position of the reducing-end N-acetyl glucosamine residue. We have used purified signal molecules including NodRm-1 and related structures to study reactions of alfalfa root hairs, as monitored by microscopy and electrophysiology. We wish to follow cytoplasmic events in root hairs and roots during infection, such as changes in calcium and the cytoskeleton. We have cloned and sequenced genes for calmodulin[4] and tubulin, as a preliminary step towards such analysis.

[1]R.F. Fisher and S.R. Long (1989) J. Bacteriol. 171:5492-5502.
[2]J. Schwedock and S.R. Long (1989) Mol. Plant-Microbe Interactions 2:181-194.
[3]P. Lerouge, P. Roche, C. Faucher, F. Maillet, G. Truchet, J.C. Promée, and J. Dénarié (1990) Nature 344:781-784.
[4]M. Barnett and S.R. Long (1990) J. Bacteriol. 172:3695-3700.

**A 008** CROWN GALL TUMOR FORMATION BY AGROBACTERIUM, Eugene W. Nester, Milton P. Gordon, Departments of Microbiology and Biochemistry, University of Washington, Seattle, WA 98195.

*Agrobacterium* induces plant tumors by inserting a fragment (T-DNA) of its tumor inducing (Ti) plasmid into the plant chromosome. Some understanding of the functioning of genes required for this process is being elucidated. The *vir* genes are activated by two classes of plant signal molecules, plant phenolic compounds and a variety of plant sugars. These molecules interact with the VirA protein which then activates the VirG protein, which in turn transcriptionally activates all of the *vir* genes following binding to their promoter regions. The VirA and VirG proteins, members of a two component regulatory system undergo the phosphorylation and phosphate transfer demonstrated by other members of the group. The *virD* operon is concerned with early stages in the processing of T-DNA. The VirD1 protein has topoisomerase activity for the Ti plasmid. The *virD2* locus codes for a site specific endonuclease which nicks at the right and left borders of the T-DNA. The VirC1 protein interacts with the overdrive sequence and in conjunction with the VirD1 and VirD2 proteins promotes T-strand formation and the efficiency of transfer of the T-DNA. The *virE* operon codes for a single stranded DNA binding protein which associates with the T-DNA. The *virB* operon has now been sequenced and many of its protein products have been shown to be associated with the cytoplasmic membrane. Presumably these protein components provide the pore through the bacterial envelope for the exit of the T-DNA. The *virB11* gene product has ATP'ase activity and autophosphorylates. Its DNA sequence is similar to that of a gene in *B. subtilis* required for development of competence. The VirB10 protein is oligomeric and anchored in the inner membrane. Open reading frames 9, 10 and 11 all code for gene products which are essential for tumor formation.

The genes of the T-DNA code for enzymes of auxin and cytokinin synthesis and presumably one or more genes code for the alteration of cytokinin and perhaps auxin activity. Another has been reported to code for transport of opines out of the plant cell.

**A 009** AGROBACTERIUM, RHIZOBIUM AND PLANT MORPHOGENESIS, Jeff Schell*, Csaba Koncz, Angelo Spena, Thomas Schmülling, Michael John and Jürgen Schmidt, Max-Planck-Institute for Plant Breeding, D-5000 Köln 30, FRG. The $T_L$-DNA segments of A.tumefaciens strains harbour genes that code for enzymes catalizing the synthesis of plant hormones such as auxins and cytokinins. In addition gene 5 of these T-DNA's was recently found to be responsible for the synthesis, in transformed plants, of an auxin-analogue: Indol-lactic acid. Transgenic tobacco plants expressing gene 5 under the control of the 35S RNA promoter of CAMV, and producing Indol-lactic acid, appear to develop without readily observable alterations. Their seedlings do however tolerate levels of exogenously added auxins that are toxic to isogenic non-transgenic tobacco seedlings. Preliminary evidence will be provided indicating that ILA might function by competing for binding to some specific auxin receptors. The $T_L$-DNA of A.rhizogenes strains carry so-called rol genes, that function in a cellautonomous fashion. Evidence will be reviewed indicating that the product of the rol B and rol C genes modify the sensitivity of plant cells to the action of plant hormones. The nod A and nod B genes of Rhizobium sym plasmids were recently found to be involved in the synthesis of low molecular weight molecules that appear to represent a new class of plant growth regulators. Other genes carried by the Rhizobium sym plasmid such as nod C and hns appear to produce enzymes that modify these new plant growth factors. Transgenic tobacco plants expressing nod A and B genes have characteristic morphological abnormalities.

**A 010** GENETIC INTERACTIONS SPECIFYING BACTERIAL DISEASE RESISTANCE IN *ARABIDOPSIS*, TOMATO, AND PEPPER, S. Barker, A. Bent, F. Carland, D. Dahlbeck, R. Innes, B. Kunkel, M. Roy, J. Salmeron and B. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720.

We are currently working on three model systems to elucidate the molecular basis of plant-bacterial specificity and the expression of host resistance. Our initial effort is to clone and characterize both the avirulence gene from the bacterium and the corresponding resistance gene from the host in our three model systems. These systems have been carefully chosen because we are able to genetically manipulate both the host and the pathogen employing the tools of classical and molecular genetics.

To date, we have cloned and characterized three avirulence genes that correspond to three genetically defined resistance genes in the above hosts. Data will be presented on the genomic organization and expression of the avirulence genes, *avrPto*, *avrRpt2*, and *avrBs2*. We are also developing strategies to clone the corresponding plant genes employing the tools of transposon tagging, chromosome walking, and genomic subtraction. Finally, we are currently mutagenizing and screening M2 seed for mutations from resistance to susceptibility in the above three systems in order to assist our efforts in both genomic subtraction and chromosome walking.

# The Genetic Dissection of Plant Cell Processes

## *Genetic Dissection of Viral Pathogenicity*

**A 011** THE ROLE OF THE TMV MOVEMENT PROTEIN ON PLASMODESMATA FUNCTION, Roger N. Beachy, C. Michael Deom, Patricia Moore, Ron Gafney, Csilla Fenczik, Anne Berne, and William D. Lucas[1], Department of Biology, Washington University, St. Louis MO 63130, and [1] Department of Botany, University of California Davis, CA 95616

Tobacco mosaic virus (TMV) and other plant viruses move between leaf mesophyll cells by as yet poorly defined mechanism(s) that involve a virus encoded protein(s) loosely referred to as "movement proteins" (MP). MPs potentiate virus movement through a single or multiple mechanism(s), one of which is correlated with modification of the plasmodesmata function. Transgenic plants that express genes encoding the TMV - MP compliment MP(-) TMV mutants allowing local spread of infection of the mutants. MP( + ) plant lines that are systemic or local lesion hosts of TMV are modified to different degrees as evidenced by differences in the size exclusion limits of their plasmodesmata.

Immunogold localization studies showed that MP was associated with plasmodesmata in MP( + ) plant lines: however, MP accumulation in plasmodesmata was much greater in older than in younger leaves. Furthermore, accumulation of MP was correlated with altered size exclusion limits of plasmodesmata. Cell fractionation studies documented that the majority of MP was associated with a cell wall fraction, and with the plasma membrane, but little or none with ER or Golgi. Site specific mutagenesis of the MP is in progress to identify sequences of the MP that are important to subcellular localization, modification of plasmodesmal function, and local movement of TMV.

**A 012** ANALYSIS OF PATHOGENICITY DETERMINANTS OF BARLEY STRIPE MOSAIC VIRUS. Andrew O. Jackson[1], I. T. D. Petty[1], R. W. Jones[1], M. C. Edwards[2], and R. French[3]. [1]Department of Plant Pathology, University of California, Berkeley, CA; [2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND and [3]Department of Plant Pathology, University of Nebraska, Lincoln, NE.

Barley stripe mosaic virus (BSMV) has a genome comprised of three RNAs designated α, β and γ which encode seven polypeptides respectively designated αa, βa, βb, βc, βd, γa and γb. Use of in vitro transcripts derived from cDNA clones reveals that all three RNAs are required for systemic infection of plants but that protoplast infections require only RNAs α and γ. The first RNAγ gene (γa) is essential for RNA replication in protoplasts, but a second gene (γb) is dispensable. Thus, the αa and γa proteins, which contain helicase and RNA polymerase motifs respectively, comprise the BSMV-encoded replicase components. RNAβ is necessary for systemic movement in plants, although βa, the coat protein gene, can be deleted without detrimental effect on systemic movement in barley. Mutations within the γb protein vary in their ability to alter systemic movement in barley depending on the background sequences present in RNAγ. A deletion of the γb gene in the ND18 strain background results in a systemic infection characterized by an attenuated and erratic mosaic pattern. However, barley inoculated with an equivalent deletion in the Type strain fails to develop systemic infections. Cloned derivatives of these RNAs differ by having a direct repeat of 372 nucleotides near the 5' terminus of the Type strain RNAγ which alters the size of the encoded γa protein. When this repeat is deleted, systemic movement in the presence of the γb mutation is acquired. Thus, mutations in both the γa (replicase) and γb proteins can affect the systemic movement phenotype of BSMV in barley. The Type and ND18 strains also differ in their ability to infect Nicotiana benthamiana systemically, and in the timing and phenotype of lesions induced on Chenopodium amaranticolor. Reassortment of the genome components demonstrates that RNAγ determines both these phenotypes. Mutational analyses indicate that a small open reading frame (ORF) encoded in the 5' leader of the γa gene prevents systemic movement of the Type strain in N. benthamiana, possibly via a translational attenuation mechanism that results in reduced synthesis of the γa protein. The local lesion phenotype in Chenopodium is affected by at least three genetic elements within RNAγ. The timing of lesion formation is appreciably delayed by the presence of the repeat in the Type strain RNAγ, and a point mutation resulting in an amino acid change within the γb protein can alter the phenotype induced by the Type strain from chlorotic to necrotic lesions. However, mutations that obstruct expression of the short ORF in the 5' leader of the Type strain RNAγ are dominant to the repeat and to the point mutations, as they alone are sufficient to restore the lesion phenotype exhibited by the ND18 strain. Thus, symptom and pathogenicity determinants in different hosts are complex and require interactions of several BSMV gene products with the host.

**A 013** MOLECULAR GENETIC DETERMINANTS OF PATHOGENICITY OF SMALL RNA VIRUSES AND ASSOCIATED DEFECTIVE INTERFERING RNAS, T. Jack Morris, School of Biological Sciences, University of Nebraska, Lincoln, NE 68588-0118.

Molecular genetic studies on members of two groups of closely related small RNA plant viruses, turnip crinkle virus (TCV), a carmovirus and tomato bushy stunt virus (TBSV), a tombusvirus, have provided new insights into factors which affect viral pathogenicity and the molecular events important in virus-host interactions. Studies using infectious transcripts derived from complete cDNA clones of both viral genomes have demonstrated that many factors are involved in the development of disease symptoms. We have dissected the TCV genome and shown that only a single ORF (p28-88) is necessary for replication but that a second small gene (p8) and the coat protein (p38) are needed for systemic movement and symptom development. In addition, we have shown the coding sequence of the coat protein is also a symptom determinant. We have also identified "slow" mutants which exhibited a delay in systemic invasion of plants due entirely to structural alterations made in the viral genomic RNA in the region of the readthrough codon of the polymerase gene. Infectivity studies in plants and protoplasts has revealed a spectrum of phenotypes with altered replication competency. Finally, we have demonstrated that defective interfering RNAs arise spontaneously upon plant infection with these viruses. The marked symptom attenuation attributable to the TBSV DI RNAs results from competition during replication of the DI RNA and genomic RNA templates. Studies on the evolution of DI RNAs during continuous passage in plants suggests that certain sequences, other than those important in the replicative competitivity, may affect DI attenuation ability.

**A 014** INDUCTION OF TOBACCO MOSAIC VIRUS RESISTANCE IN TOBACCO BY TRANSFORMATION WITH A VIRAL NON-STRUCTURAL GENE SEQUENCE, Milton Zaitlin, Daniel B. Golemboski, George P. Lomonossoff, and John P. Carr, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Tobacco mosaic virus (TMV) RNA encodes 4 polypeptides. One of these, the 183kDa protein is a readthrough of the 126kDa protein; both are considered to be a part of the viral replicase complex. In addition, there is an open reading frame for a 54 kDa protein in the readthrough region of the 183 kDa sequence, although no such protein has been detected in virus infected tissues. To seek a function for the putative 54 kDa protein, we transformed *Nicotiana tabacum* cv. Xanthi (nn) plants with a cDNA clone containing TMV nucleotides 3472-4916, which encompass all of the 54 kDA protein codon except for 3 nucleotides at the 3' end. When we attempted to infect these transgenic plants, to our surprise we found that they were completely resistant to infection, utilizing either virions or viral RNA as inoculum. The copy number of the 54 kDa sequence in individual transformants ranged from 1 to 5, but the level of resistance was high in all of them. A sequence-specific 54 kDa RNA transcript of the expected size was found in the transformants, but no 54 kDA protein has been detected. The plants were resistant only to the virus strain from which the 54 kDa sequence was derived and to a closely-related mutant, but not to more distantly related TMV strains nor to 3 unrelated viruses. The details of this discovery have been published (Proc. Natl. Acad. Sci. USA 87:6311-6315). The mechanism of action of this novel type of virus resistance will be discussed.

This work was supported by the Cornell Biotechnology Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, the US Army Research Office and the National Science Foundation.

*Gene Control by Insects, Pathogens, and Environment*

**A 015** EXPRESSION AND TARGETING OF TOBACCO PROTEINS INDUCED BY VIRUS INFECTION, John F. Bol, Huub J.M. Linthorst, Miranda D. van de Rhee, Isabelle Dore, Bauke Zelle, Department of Chemistry, Gorlaeus Laboratories, Leiden University, 2333 CC Leiden, and Ben J.C. Cornelissen, Leo S. Melchers, Charles P. Woloshuk, MOGEN Int., 2333 CB Leiden, The Netherlands.

Infection of tobacco with tobacco mosaic virus (TMV) or other necrotizing pathogens results in the de novo synthesis of a number of host proteins, including five groups of "pathogenesis-related proteins" (PR proteins). These proteins are induced in the primary inoculated leaves as well as in the virus-free upper leaves. Concomitantly, the plants aquire a systemic resistance to further infection with viruses, fungi or bacteria, suggesting a role of PR proteins in defence mechanisms. We have characterized cDNA clones and genomic clones encoding PR proteins from groups 1, 2, 3 and 5. Groups 1 and 5 contain PR-1 and "thaumatin-like" proteins, respectively, of unknown function. Groups 2 and 3 contain β-1,3-glucanases and chitinases, respectively. Each group contains acidic isoforms accumulating in pocket-like structures in the intercellular space of the leaf, and basic isoforms accumulating in vacuolar inclusion bodies (for a review see ref. 1). We have analyzed regulatory sequences involved in the coordinate induction of PR genes by TMV infection, and we have studied the possible role of PR proteins by expressing them constitutively in transgenic plants.

Regulatory sequences in the PR-1 promoter have been analyzed by fusing upstream sequences to the GUS reporter gene. In addition, a number of constructs including either the core promoter or enhancer sequence of the CaMV 35S promoter were studied. The results indicate that TMV induction of the reporter gene in transgenic plants requires the concerted action of elements located between -689/-625 with either upstream elements (between -902/-689) or downstream elements (between -625/-287). Some of these elements are active in both orientations. Two other promoters have been characterized in less detail. An upstream sequence of about 1 kb of a gene encoding an acidic PR-5 protein was required for induction of the reporter gene. In a gene encoding a TMV-inducible glycine-rich protein regulatory sequences were mapped between -400 and -645 of the upstream sequence.

Genes encoding an acidic PR-1 protein, an acidic PR-5 protein, acidic and basic β-1,3-glucanases, acidic and basic chitinases and the glycine-rich protein were constitutively expressed either alone or in various combinations in transgenic tobacco plants. In most cases the expression level was similar to that in TMV-infected plants. All plants were phenotypically normal. So far, no antiviral function of these PR proteins was detectable. The signals involved in the extracellular and vacuolar targeting of PR proteins in transgenic plants are being studied by exchanging domains of acidic and basic isoforms.

1. Bol, J.F., Linthorst, H.J.M. and Cornelissen, B.J.C. Plant pathogenesis-related proteins induced by virus infection. Annu. Rev. Phytopathol. 28, 113-138 (1990).

**A 016** CHITINASE EXPRESSION IN TRANSGENIC PLANTS: A MOLECULAR APPROACH TO FUNGAL DISEASE RESISTANCE, Richard Broglie[1], Karen Broglie[1], Ilan Chet[2], Dominique Roby[3] and Mark Holliday[1], [1]Agricultural Products Department, E. I. DuPont de Nemours & Co., Wilmington, DE 19880, [2] Hebrew University, Faculty of Agriculture, Rehovot, ISRAEL, [3] Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganisms, CNRS/INRA, Toulouse, FRANCE

An important method by which plants protect themselves against potentially pathogenic organisms is the production of lytic enzymes that are capable of cell wall hydrolysis. Among these is the enzyme chitinase which catalyzes the hydrolysis of chitin, a β-1,4-linked homopolymer of N-acetyl-D-glucosamine and a ubiquitous component of many phytopathogenic fungi. In healthy, uninfected plants, chitinase levels are low or undetectable. However, treatment with ethylene, oligosaccharide eliciltors, or infection with fungal pathogens results in an increase in chitinase mRNA levels and an increase in enzyme activity. Analysis of chitinase gene expression in transgenic tobacco and electroporated bean protoplasts have identified two promoter regions responsible for optimal gene expression: a quantitative region required for maximal gene expression and an ethylene-responsive region required for induction by ethylene. Transgenic tobacco plants containing a chimeric gene composed of a bean chitinase promoter fused to the coding region of the reporter gene β-glucuronidase (GUS) have been used to study the induction of chitinase gene expression in response to fungal infection. Our results indicate that gene activation occurs locally in tissues immediately surrounding the site of fungal infection and coincides with the induction of endogenous tobacco defense genes. Additionally, the role of chitinase in plant protection is being evaluated by studying transgenic tobacco plants which express a bean chitinase gene modified so that the native inducible promoter is replaced by a high level, constitutive promoter. Transgenic tobacco seedlings constitutively expressing the bean chitinase gene are delayed in the development of disease symptoms and show an increase ability to survive in soil infested with the fungal pathogen *Rhizoctonia solani*.

**A 017** THE ANAEROBIC INDUCTION OF GENES - CIS AND TRANS ACTIVITY COMPONENTS, E.S. Dennis, M.R. Olive, R. Dolferus, W.J. Peacock,
CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601 Australia

The cis acting sequences necessary for anaerobic induction of the maize alcohol dehydrogenase 1 gene (*Adh*1) have been localized to a 40 bp region (the anaerobic responsive element (ARE)). The ARE functions in both orientations relative to the TATA box. Promoter activity under anaerobic conditions is proportional to the number of complete ARE sequences in the *Adh*1 promoter. The ARE contains two subregions and dimers of sub-region II are as efficient as the wild type sequence in activating gene expression; sub region I dimers do not induce gene expression in response to anaerobic stress. Each subregion contains one copy of the anaerobic core sequence GGTTT present in the promoters of all anaerobically induced genes. The spacing between the two subregions can be increased by up to 64 bp with little effect but increasing the spacing by 136 bp or more abolishes expression under both anaerobic and aerobic conditions. This suggests the two subregions must be closely positioned for activity.

Nuclear extracts of maize suspension cultures contain a protein which binds specifically to the ARE. *In vitro* studies suggest anaerobically induced expression of the *Adh*1 promoter requires the binding of this protein to the ARE.

**A 018** Signalling Systems for Inducible Plant Defensive Genes

Clarence A. Ryan, Edward E. Farmer, Gregory Pearce and Barry McGurl, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Proteinase Inhibitor genes are among several plant defensive genes that are activated in cells nearby sites of pathogen or insect attacks. In a number of plant species, proteinase inhibitor genes are also activated systemically. Studies of the signal transduction mechanisms for both localized and systemic gene activation have revealed that several signalling components are involved. Oligouronide fragments of the plant cell walls, polypeptide fragments of intracellular proteins, and methyl jasmonate all activate proteinase inhibitor genes. The latter volatile chemical can also mediate interplant activation of proteinase inhibitor genes via the atmosphere. The possible roles of these molecules in localized, systemic and interplant signal transduction pathways will be presented. Supported in part by NSF Grants DCB 8703578 and DCB 8608954 and a Grant from EniMont Americas Inc.

# The Genetic Dissection of Plant Cell Processes

*Genetic Dissection of Plant Biochemistry (This session is sponsored by EniMont Americas, Inc.)*

**A 019** **GENETIC AND BIOCHEMICAL ANALYSIS OF α-1,4 GLUCAN SYNTHESIS IN PHOTOSYNTHETIC ORGANISMS.** **Jack Preiss, Brian Smith-White, Kathryn L. Ball, Genichi Kakefuda and Alberto Iglesias, Department of Biochemistry, Michigan State University, East Lansing, MI 48824**

**Regulation of starch synthesis in plants and of glycogen synthesis in cyanobacteria is observed at the ADPglucose synthetase step where 3PGA is an activator and $P_i$ is an inhibitor. A class of starch deficient mutants of pea seed, maize endosperm and of *Arabidopsis thaliana* having deficient ADPGlc synthetase activity strongly indicates that the ADPGlc pathway is the dominant if not sole pathway of starch synthesis. Control analysis using *Arabidopsis thaliana* ADPglc synthetase mutants show that the ADPglc synthetase is a major site for regulation of starch synthesis. The spinach leaf ADPGlc synthetase is composed of two different subunits that can be distinguished by their molecular masses (51 and 54 kd), their N-terminal sequences, their antigenic properties, their different amino acid compositions and different tryptic peptide maps as observed on HPLC. ADPGlc synthetases of maize endosperm, rice endosperm, potato tuber and *Arabidopsis* leaf, have also been shown to be composed of 2 different subunits. The amino acid sequence of the activator binding site of the spinach leaf 51 kd subunit has been determined. Isolation of cDNA clones of the spinach leaf and rice endosperm 51 kd subunit genes indicate that the 3PGA activator binding site is situated near the carboxyl end of the subunit. Amino acid sequences deduced from cDNA clones of at least 3 other plants show a very similar sequence at the carboxyl terminus of the peptide corresponding to the activator site seen in the spinach leaf and rice endosperm ADPGlc synthetase lower molecular mass subunit. Although a cDNA clone for the spinach leaf 54 kd subunit has not been isolated, amino acid sequence analyses of peptides generated from the 54 kd subunit by various proteases show about a 30% identity with the 51 kd subunit. In contrast to the higher plant ADPGlc synthetase, the enteric and cyanobacterial enzyme appears to be composed of only one subunit ranging in molecular mass from 49 to 53 kd. In neutralization tests, the cyanobacterial enzyme reacts with antibody prepared with the spinach leaf enzyme but not with *E. coli* anti-ADPGlc synthetase . Using 8-azido ATP as a photoaffinity analogue, it has been shown that the larger subunit of the spinach leaf enzyme participates in the binding of the substrate. Experiments are underway to determine the amino acid sequence of the substrate site, to obtain complete cDNA clones of both subunits and to determine the functional role played by both subunits.**

**A 020** GENETIC DISSECTION OF MEMBRANE AND STORAGE LIPID COMPOSITION IN *ARABIDOPSIS*. Chris Somerville, Vincent Arondel, John Browse, Sue Gibson, Erwin Grill, Ursula Hecht, Sue Hugly, Tom Johnson, Ellen Kearns, Bertrand Lemieux, Dieter Reiter, Carrie Schneider, John Shanklin, Sharon Thoma, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

The composition of lipids has been implicated as a determinative factor in many aspects of plant biology including the regulation of thermal or cold acclimation of plants, the regulation of organelle morphology, the regulation of the amount of cellular membranes and the economic value of many crop species. However, the mechanisms which regulate lipid metabolism are poorly understood because of a variety of technical problems related to the hydrophobicity and chemical diversity of lipids and the fact that most of the proteins involved are thought to be membrane bound. Therefore, we are using genetic methods to investigate the functional significance of lipid polymorphism, to evaluate the mechanisms which regulate lipid accumulation and to permit the isolation of otherwise intractable genes.

During the past few years we have isolated mutations affecting many of the enzymes which regulate membrane and storage lipid fatty acid composition. Biochemical analyses of these mutations have provided useful new information concerning the biosynthesis of lipids. Many of the mutations have been mapped relative to the RFLP map(s) and we are currently attempting to isolate several of the marked genes by chromosome walking using yeast artificial chromosomes. Although we have not, as yet, completed the isolation of any gene by this approach, our experience suggests that it is a widely applicable and feasible general approach to gene isolation. In addition, where possible, several of the genes involved in regulating lipid composition have been cloned by methods which involve purifying the protein as the first step. The preliminary evidence available to date indicates that these genes are regulated independently of enzymes involved in other aspects of cellular metabolism.

Although there has been substantial interest in the role of lipid composition in chilling stress, all of the evidence has been indirect. Analysis of the mutants has recently provided striking evidence for the role of lipid unsaturation in both chilling sensitivity and thermal tolerance. Mutants defective in the endoplasmic reticulum enzyme which converts $18{:}1^{\Delta 9}$ to $18{:}2^{\Delta 9,12}$ are severely injured by short exposure to non-freezing chilling temperatures. Mutants with a defect in the chloroplast isozyme exhibit impaired biogenesis of chloroplasts at chilling temperatures. Conversely, mutants deficient in lipid unsaturation have increased thermal tolerance to a degree which mimics thermal acclimation responses in many species which normally grow in habitats with seasonally variable mean temperatures.

**A 021** THE PLASMA MEMBRANE PROTON PUMP ($H^+$-ATPASE) OF ARABIDOPSIS THALIANA: NUMBER OF GENE ISOFORMS AND REGULATION OF ACTIVITY, Michael R. Sussman, Jeffrey F. Harper, Natalie D. DeWitt and G. Eric Schaller, Department of Horticulture and Cell and Molecular Biology Program, University of Wisconsin, Madison, WI 53706

Solute transport and signal transduction at the plasma membrane of higher plants is dominated by the protonmotive force generated by a proton pump ($H^+$-ATPase). In actively transporting tissue, such as root hairs, this enzyme consumes a large fraction of the metabolic energy. The purified enzyme contains a polypeptide of Mr=100,000 with sequence homology to other P-type ion-translocating ATPases. Using two short sequences conserved in all eukaryotic P-type ATPases, we have developed a PCR procedure which allowed us to isolate clones representing nine different $H^+$-ATPase genes from A. thaliana, all containing 80-90% amino acid sequence homology to the proton pump (see Harper, Surowy and Sussman, P.N.A.S. 86:1234-1238 (1989) and Sussman and Harper, Plant Cell 1:953-960 (1989)). Four other ATPase genes obtained by this procedure show only 60-70% homology and may represent pumps for cations other than protons. We have generated A. thaliana ATPase promoter fusions with the reporter gene, glucuronidase (GUS). 5' upstream sequences from two of the nine A. thaliana $H^+$-ATPase gene isoforms (AHA-2 and AHA-3) have been analyzed and each directs GUS to a different cell-type in transgenic plants. AHA-2 shows activity only in roots whereas AHA-3 produces GUS activity in companion cells of phloem tissue throughout the plant. These cells are thought to contain a plasma membrane $H^+$/sucrose transporter that uses the protonmotive force generated by the $H^+$-ATPase to load the leaf vein with sucrose synthesized in mesophyll cells. Our observations with the putative ATPase promoters suggest that specialized transport cells within the plant may express unique gene isoforms of the $H^+$-ATPase.

In collaboration with Dr. Alice Harmon (Univ. of Florida) we have also partially purified a calcium-dependent plasma membrane protein kinase that phosphorylates the $H^+$-ATPase. Starting with sequence derived from tryptic peptides, we have cloned and sequenced cDNA encoding this enzyme in soybean and A. thaliana. This enzyme contains a unique regulatory domain unlike that found in other calcium-dependent protein kinases.

Supported by DOE Grant No. FG02-88ER13938 and USDA Grant No. 87-CRCR-1-2357.

## *Genetic Dissection of Plant Cell Architecture and Membrane Function*

**A 022** ANALYSIS OF CELL WALL HYDROLASES IN TRANSGENIC TOMATO FRUIT, Alan B. Bennett[1], Coralie C. Lashbrook[1], Kathy W. Osteryoung[1], Tom S. Moore[1], Jamie Stott[2] and Robert L. Fischer[2]. [1]Department of Vegetable Crops, University of California, Davis, CA 95616 and [2]Department of Plant Biology, University of California, Berkeley, CA 94720.

Degradation of cell wall polymers plays an important role in fruit ripening. However, the complexity of the cell wall precludes precise predictions as to which polymer contributes significantly to the rheological properties of the wall and to the structural rigidity of the fruit. One means to dissect the processes that modify cell wall structure in ripening fruit is to clone cell wall hydrolase genes and modify their expression in transgenic plants.

Pectin and hemicellulose undergo extensive structural modification during fruit ripening and have been implicated as potential determinants of fruit softening. The enzymic basis for pectin degradation in tomato fruit is well established and recent research has focused on the implementation of molecular genetic strategies to alter pectin degradation by modifying expression of the gene encoding the degradative enzyme, polygalacturonase. In addition to defining the function of polygalacturonase, these experiments have provided a basis for elucidating the role of individual polygalacturonase isozymes in pectin degradation with one isozyme, PGI, proposed to play the major role in pectin degradation *in vivo*. Progress in elucidating the role of the non-catalytic subunit of the PGI isozyme will be presented.

Unlike pectin degradation, the enzymic basis of hemicellulose degradation in ripening fruit is not well-defined. A class of enzymes that are candidates for this role are the endo-$\beta$-1,4-glucanases, commonly referred to as cellulases. It is unfortunate that these enzymes have been referred to as cellulases since it is well-documented that they do not degrade crystalline cellulose as it occurs *in situ*. Although the endogenous substrate of endo-$\beta$-1,4-glucanases is not established, it has been shown that pea stem and avocado fruit endo-$\beta$-1,4-glucanases can degrade xyloglucan, suggesting that this may be the substrate *in situ*. Plant endo-$\beta$-1,4-glucanases have been cloned from avocado and bean and these sequences were used to isolate the corresponding cDNAs from tomato. The results of cDNA isolation indicate that multiple mRNAs encoding endo-$\beta$-1,4-glucanase in tomato. The expression of multiple related endo-$\beta$-1,4-glucanase genes in tomato is provocative. It is interesting to speculate that each gene product may have a unique substrate specificity, working in concert, to bring about the major alterations in cell wall structure that are associated with tomato fruit ripening. Progress in assessing the function of endo-$\beta$-1,4-glucanase genes in ripening transgenic tomato fruit will be presented.

**A 023** IDENTIFICATION AND CHARACTERIZATION OF GENES AND GENE PRODUCTS INVOLVED IN β-GLUCAN SYNTHESIS, Deborah Delmer, Yehudit Amor, Mazal Solomon, Esther Shedletzky, Miri Shmuel, Rafael Mayer, and Moshe Benziman, Departments of Botany and Biological Chemistry, The Hebrew University, Jerusalem 91904, Israel

Based upon current knowledge of cell wall structure in plants, hundreds of genes must be required to code for enzymes involved in wall biosynthesis. Genes which code for structural proteins, wall-bound hydrolases and peroxidases, and some involved in the biosynthesis of lignin precursors have been characterized, but no gene coding for any glycosyltransferase involved in cell wall polysaccharide synthesis has been identified. This is primarily due to the lack of characterization of these enzymes; even in cases where activity has been identified, detergent-solubilized forms of these membrane-bound enzymes have resisted conventional purification. Recently, we have used affinity labeling techniques to identify specific polypeptides involved in β-glucan synthesis in cotton fibers. Two membrane polypeptides which bind the substrate UDP-glc have been identified: 1) a 52 kD polypeptide which interacts specifically with UDP-glc in the presence of $Ca^{2+}$, and co-purifies with the $Ca^{2+}$-dependant (1→3)-β-glucan (callose) synthase; and 2) an 84 kD polypeptide which has properties consistent with being a component of a cellulose synthase. Photo-labeling of the 84 kD polypeptide with [$^{32}$P]UDP-glc is markedly enhanced by the presence of c-di-GMP, a specific activator of bacterial cellulose synthases. A polypeptide of the same mol wt (presumably the same polypeptide) can be photo-labeled with [$^{32}$P]c-di-GMP, and shows cross-reaction during Western blotting using an antibody directed against a fusion protein expressed by a clone of E. coli containing the cellulose synthase gene of A. xylinum. We have also identified a soluble polypeptide which interacts specifically with an analog of the cellulose synthesis inhibitor DCB. Current progress in isolation and characterization of these polypeptides will be discussed. Other approaches to gene identification include a search for mutants impaired in glucan synthesis and/or resistant to DCB. Selection of suspension-cultured, DCB-resistant tomato cells led us to the surprising selection of a cell line which can grow in the virtual absence of a cellulose-xyloglucan network. Such a finding implies that plant cells have remarkable flexibility for tolerating induced changes in wall structure, and that selection of specific mutations in wall biosynthesis is certainly feasible and a desirable future approach for studying wall biogenesis.

**A 024** ABSCISIC ACID ACTIVATION OF $Ca^{2+}$ PERMEABLE ION CHANNELS INITIATES SIGNAL TRANSDUCTION IN GUARD CELLS, Julian I. Schroeder, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Numerous signal transduction processes in higher plant cells have been suggested to be initiated by stimulus-induced opening of $Ca^{2+}$ permeable ion channels in the plasma membrane. However, direct evidence for activation of $Ca^{2+}$ channels by physiological signals in plants has not yet been obtained. In this regard, indirect evidence suggests that initiation of stomatal closure by abscisic acid requires $Ca^{2+}$ flux into the cytosol of guard cells. The plant growth regulator abscisic acid closes stomatal pores, thereby prohibiting loss of water by plants under conditions of drought.

To directly elucidate initial events in abscisic acid-induced signal transduction in guard cells, an experimental approach was developed which allows simultaneous measurement of changes in the cytosolic $Ca^{2+}$ concentration and patch clamp recordings of ion channels in *Vicia faba* guard cells. Resting levels of the cytosolic $Ca^{2+}$ concentration in the range of 0.2 μM were determined. Extracellular exposure of guard cells to abscisic acid (0.5 to 5 μM) caused transient repetitive increases in the cytosolic $Ca^{2+}$ concentration. Patch clamp recordings showed that each transient rise in the cytosolic $Ca^{2+}$ concentration was accompanied by a concomitant activation of an inward (into the guard cell) flowing ion current.

Abscisic acid-induced increases in the cytosolic $Ca^{2+}$ concentration and inward flowing ion currents could be terminated by depolarizing the membrane potential, showing that influx of $Ca^{2+}$ from the extracellular space via $Ca^{2+}$-permeable ion channels contributed to ABA-mediated $Ca^{2+}$ transients. Interestingly, detailed patch clamp analysis revealed that abscisic acid-activated ion channels are not highly $Ca^{2+}$ selective, thereby allowing the permeation of $Ca^{2+}$ as well as $K^+$ ions. Abscisic acid-mediated $Ca^{2+}$ influx into the cytosol and the resulting rise in the cytosolic $Ca^{2+}$ level of guard cells leads to regulation of $K^+$ channels and anion channels as previously demonstrated (1). Modulation of these ion channels by cytosolic $Ca^{2+}$ can provide the molecular basis for the $Ca^{2+}$-dependence of ABA-dependent closure of stomata. Activation of ion channels in guard cells by abscisic acid provides direct evidence for opening of $Ca^{2+}$-permeable channels by a physiological stimulus in a higher plant cell.

1. Schroeder and Hagiwara, *Nature*, 338, 427-430, 1989.

**A 025** TISSUE SPECIFIC EXPRESSION OF CELL WALL PROTEINS IN DEVELOPING SOYBEAN TISSUES.

Zheng-Hua Ye and Joseph E. Varner, Department of Biology, Washington University, St. Louis, MO 63130

We have examined the accumulation of cell wall hydroxyproline-rich glycoproteins (HRGPs) and glycine-rich proteins (GRPs) in developing soybean (Glycine Max cv. William) tissues by tissue print westerns and by immunocytochemistry. We have also examined in soybean the accumulation of HRGP mRNAs and GRP mRNAs by tissue print northerns and by in situ hybridization. In young soybean stems, HRGPs are expressed most heavily in cambium cells, a few layers of cortex cells surrounding primary phloem and in some parenchyma cells around primary xylem, whereas GRPs are highly expressed in primary xylem and also in primary phloem. In older soybean stems, HRGP genes are expressed exclusively in cambium cells, and GRP genes are most heavily expressed in newly differentiated secondary xylem cells. Similar expression patterns of HRGPs and of GRPs were found in soybean petioles, seedcoats and young hypocotyls, and also in bean (Phaseolus Vulgaris cv. Tendercrop) petioles and stems. HRGPs and GRPs become insolubilized in soybean stem cell walls. Three major HRGP mRNAs and two major GRP mRNAS accumulate in soybean stems. Soluble HRGPs are abundant in young hypocotyl apical regions and young root apical regions, whereas in hypocotyl and root mature regions soluble HRGPs are found only in a few layers of cortex cells surrounding vascular bundles. GRPs are specifically localized in primary xylem cell walls of young root. Our results show that the gene expression of HRGPs and GRPs is developmentally regulated in a tissue-specific manner. In soybean tissues, HRGPs are most heavily expressed in meristematic cells and in some of those cells which are under stress, whereas GRPs are expressed in all cells which are or are going to be lignified.

## *Genetic and Molecular Analysis of the Chloroplast*

**A 026** TRANSCRIPTION OF CHLAMYDOMONAS CHLOROPLAST GENES -- A VIEW FROM INSIDE,

Lawrence Bogorad, Alan D. Blowers, James DeCamp, George S. Ellmore, Uwe Klein and Maria Luisa Salvador, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Foreign DNA bordered by Chlamydomonas reinhardtii chloroplast DNA sequences can become integrated into the C. reinhardtii chloroplast chromosome by homologous recombination after the cloned DNA has been introduced into the cells by microprojectile bombardment. Transcription of an endogenous gene at its normal position has been compared to that of a chimeric gene composed of a GUS coding sequence reporter under the transcriptional control of a corresponding normal or modified C. reinhardtii chloroplast promoter. Promoters of atpA, atpB and rbcL genes have been studied in vivo. The chimeric gene has been inserted between a complete C. reinhardtii atpB and sequences normally adjacent and downstream of this gene. A C. reinhardtii strain incapable of carrying out photosynthesis because of a deletion in atpB is used as a recipient and selection is for acquisition of photosynthetic capacity. About half of the cells transformed to photosynthetic competence contain complete adjacent chimeric gene constructs. A single major GUS-hybridizing transcript accumulates in each chloroplast transformant. We have determined that: (a) Transcription of a chimeric gene begins at the same site as in the corresponding endogenous chloroplast gene. (b) The rates of transcription in vivo of the atpA promoter:GUS and atpB promoter:GUS genes relative to one another and to other genes are the same as those of the endogenous atpA and atpB genes, respectively. Thus, the functions of these promoters are not affected by fusion to a foreign coding sequence and relocation to another place on the chloroplast chromosome. (c) In contrast to the atpA and atpB promoters, the rbcL promoter directs transcription of the rbcL promoter:GUS gene introduced adjacent to the endogenous atpB gene at only one percent of the rate of the endogenous rbcL under standard cell culture conditions in these experiments. (d) We have also determined that transcription from a sequence of only 22 bp upstream of the 5' end of the atpB promoter:GUS transcript in the atpB promoter element is sufficient to confer wild type levels of promoter activity; indeed, even smaller portions of the atpB promoter region are adequate for transcription. (e) The effects of 3' sequences on transcription termination and transcripts ability have also been analyzed.

**A 027** THE ROLE OF CHAPERONINS IN THE ASSEMBLY OF MULTISUBUNIT PHOTOSYNTHETIC PROTEINS, Anthony A. Gatenby, John T. Christeller, Gail K. Donaldson, Pierre Goloubinoff, George H. Lorimer, Thomas H. Lubben and Paul V. Viitanen, Central Research and Development Department, E.I. DuPont de Nemours & Co., Wilmington, DE 19880-0402. Chaperonins are a conserved class of proteins found in bacteria, chloroplasts and mitochondria. In E. coli the GroEL and GroES chaperonins together facilitate the assembly of various forms of ribulose bisphosphate carboxylase/oxygenase (Rubisco). Thus, the assembly of dimeric, octameric and hexadecameric Rubisco molecules requires the chaperonins in vivo (1). In addition, recent data shows that the in vitro reconstitution of active dimeric Rubisco from denatured polypeptides depends on the presence of purified chaperonins (2). Chaperonins from bacteria or eukaryotic organelles can support Rubisco reconstitution, and this has formed the basis of an assay to identify the GroES homologue in organelles (3). In addition to Rubisco assembly, chaperonins may have a general role in chloroplasts in the folding of numerous proteins. Using isolated chloroplasts it has been observed that many proteins that are imported into the plastid will form a stable complex with the endogenous chaperonin (4). These binary complexes may be formed between chaperonins and incompletely folded imported proteins, and may represent an important intermediate step in the folding pathway of plastid proteins.

(1) Nature (1989), 337, 44-47. (2) Nature (1989), 342, 884-889. (3) Proc. Natl. Acad. Sci. U.S.A. "in press". (4) Plant Cell (1989) 1, 1223-1230.

**A 028** CHLOROPLAST RNA-BINDING PROTEINS AND THEIR ROLE IN mRNA PROCESSING AND STABILITY, Wilhelm Gruissem, Gadi Schuster, Petra Klaff and Susan Abrahamson, Department of Plant Biology, University of California, Berkeley, CA 94720

Transcriptional and post-transcriptional regulatory mechanisms are important control steps in the expression of genes for photosynthetic proteins. It appears, however, that transcription of several plastid genes is not stringently controlled during plant development. For example, plastid mRNAs for photosynthetic proteins accumulate to different levels in many plants and in specific plant organs, and in spinach and tomato plastids the differential accumulation does not always correlate with the transcriptional activity of the genes. From these results we have concluded that in addition to the transcriptional regulation, the temporal modulation of mRNA stabilities is also an important component in the control of plastid gene expression. We have investigated changes in plastid mRNA stability in vivo. The results for the psbA and rbcL mRNAs indicate that the stability of the psbA mRNA increases relative to the rbcL mRNA during leaf development. Furthermore, we have found that ribosome-protection does not increase the stability of both mRNAs, and they most likely decay by different mechanisms.

Most plastid mRNAs contain inverted repeat sequences in their 3' untranslated region. Based on their lack of efficient transcription termination, we have proposed that 3' IR sequences may function in transcript stabilization and differential mRNA accumulation. We are investigating the interaction of mRNA 3' ends with specific proteins, which may in part determine the relative stability of upstream mRNA coding region sequences. Analysis of protein-binding has revealed common and gene-specific proteins. Certain proteins may recognize specific sequences, since single base mutations can eliminate their binding. The cloning of the nuclear gene for a 28 Kd plastid 3' IR-binding protein has revealed two conserved RNA-binding domains. The expression of the gene of the 28 Kd protein correlates with the accumulation of plastid RNA during development and in different organs. The protein may be required for the correct processing of plastid mRNA 3' ends since depletion of this protein from a chloroplast extract interferes with the processing of 3' ends from different mRNAs. The function of the 28 Kd protein may be similar to the function of the polyA-binding protein for cytoplasmic mRNAs.

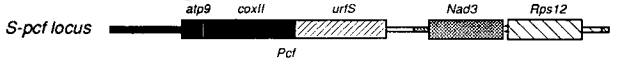
**A 029** PROTEIN TRANSPORT INTO CHLOROPLASTS, Kenneth Keegstra, Kathleen Archer, Cynthia Bauerle, Hsou-min Li, Jerry Marshall, Laura Olsen and Sharyn Perry, Department of Botany, University of Wisconsin, Madison, WI 53706

Most chloroplastic proteins are synthesized in the cytoplasm before being transported into chloroplasts. This transport process requires not only translocation across the envelope membranes, but also targeting to the proper compartment within chloroplasts. The general features of transport across the envelope membranes have been described in recent years and will be briefly review. Current work is focusing two unsolved problems. The first is identifying and characterizing the chloroplastic components that mediate precursor transport. The second is describing the mechanisms responsible for targeting proteins within chloroplasts. Recent progress in solving these problems will be presented.

## *Genetic and Molecular Analysis of the Mitochondria*

**A 030** CYTOPLASMIC MALE STERILITY IN PETUNIA, Maureen R. Hanson, Marie Connett, Otto Folkerts[1], Helen T. Nivison, and Kim Pruitt, Section of Genetics and Development, Cornell University, Ithaca, NY 14853 ([1]DowElanco Company, Agricultural Biotechnology Division, Midland, MI 48673)

Cytoplasmic male sterility (CMS) is a widespread phenomenon in the plant kingdom which results in disruption of pollen development in affected plants. In *Petunia*, a complex mitochondrial locus termed S-*pcf* has been genetically correlated with CMS. A single dominant nuclear allele *Rf* can restore fertility to genotypes containing the CMS cytoplasm. The CMS-associated locus contains three genes: *nad3*, *rps12*, and *pcf*, a unique gene derived from fusion of portions of *atp9*, *coxII*, and an unidentified reading frame termed *urfS*. Complete physical maps of mitochondrial DNAs of a CMS and a fertile *Petunia* line reveal a massive rearrangement near the S-*pcf* locus. Full-length transcripts encompassing all three genes have been detected in every tissue examined, while additional shorter transcripts are found in anther tissue. The amount of full-length transcript is somewhat reduced in *Petunia* lines carrying the *Rf* locus. A 25 kD protein product of the *pcf* gene is more abundant in anthers than in leaves. The amount of this protein is greatly reduced in lines carrying the *Rf* locus. Isonuclear CMS and fertile lines differ in the partitioning of electron transport through the cytochrome oxidase and alternative respiratory pathways. CMS lines exhibit lower alternative oxidase activity in cell cultures and young anthers than do fertile lines. The nuclear *Rf* gene affects the expression of the CMS-associated locus, restores fertility, and confers an increase in alternative oxidase activity.



**A 031** THE ROLE OF T-urf13 IN DISEASE SUSCEPTIBILITY IN cms-T MAIZE, Charles S. Levings III, Kenneth L. Korth, Mark E. Williams, Gerty C. Ward, Cyril I. Kaspi* and James N. Siedow*, Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695-7614 and *Department of Botany, Duke University, Durham, NC 27706

The T-urf13 mitochondrial gene of cms-T maize encodes a 13 kD polypeptide (URF13) that is localized in the inner mitochondrial membrane. URF13 is implicated with two traits, cytoplasmic male sterility and disease susceptibility. cms-T maize is uniquely susceptible to Southern corn leaf blight, a disease caused by the fungal pathogen Bipolaris maydis race T. B. maydis race T produces a pathotoxin, BmT-toxin, that adversely affects mitochondria from cms-T maize by causing mitochondrial swelling and ion leakage, inhibition of respiration and uncoupling of oxidative phosphorylation. Analogous responses to the pathotoxin also take place in E. coli cells expressing the transformed T-urf13 gene. Our studies indicate that the BmT-toxin binds to URF13, resulting in the permeabilization of the plasma membrane in E. coli and the inner mitochondrial membrane in maize. The URF13-toxin interaction that causes channel formation in mitochondrial membranes accounts for the capacity of B. maydis race T to rapidly colonize cms-T maize plants.

To learn how the URF13-toxin interaction is able to form membrane channels, we have characterized the topological structure of URF13 in the membrane. We have determined the structural orientation of URF13 in the membrane and shown that it exists as a dimeric or higher order structure. Using random and site-directed mutagenesis techniques, we have prepared URF13 mutations and analyzed their effects on toxicity, toxin binding and protein stability. These studies indicate that certain amino acid changes in URF13 result in toxin-insensitive mutations. Two types of toxin-insensitive mutations have been found: those with altered toxin-binding capacities and those with altered protein stabilities. These results provide insight into the URF13-toxin interaction and channel formation.

**A 032** **MITOCHONDRIAL MUTATIONS ASSOCIATED WITH ABNORMAL GROWTH PHENOTYPES.** Kathleen Newton, Marjorie Hunt and Mary Schramke. Division of Biological Sciences, University of Missouri, Columbia MO 65211.

Several maternally-inherited mutations characterized by reduced growth and yield have been associated with specific mitochondrial DNA rearrangements in maize. We have been studying one set of such mutants, known as nonchromosomal stripe (NCS). The mutations are usually lethal during kernel development and highly deleterious at other stages. Thus, plants carrying the mutant mitochondrial genomes are heteroplasmic and sectoring of defective and normal tissues results from the somatic sorting-out of mutant and normal mitochondria during development.

Each mutation is correlated with a deletion in an essential mitochondrial gene. We have characterized two different mutations in the cytochrome oxidase subunit 2 gene (NCS5 and NCS6), one in the NADH-dehydrogenase subunit 4 gene (NCS2) and two in the co-transcribed, overlapping S3/L16 ribosomal protein genes (NCS3 and NCS4). The progenitor mitochondrial genotype of NCS2 and NCS3 is the cytoplasmic male sterile, cms-T, while the progenitors for NCS4, NCS5 and NCS6 are fertile derivatives of cms-S. NCS-like plants have also been observed in plants carrying non-male-sterile N cytoplasms. NCS mutants arise most often in plants carrying a WF9-derived nuclear genotype, so nuclear genes appear to be involved in the generation and/or recovery of mitochondrial mutants affecting plant growth. Each NCS deletion appears to be generated by a similar series of events, beginning with recombination between very short repeats. The sizes of the repeated regions at the recombination junctions vary from 6 bp to 31 bp. Only one of the two products expected from a reciprocal recombination event is recovered in each of the mutant mitochondrial genomes. However, each deletion appears to be relatively short, not extending into nearby genes.

**A 033** mRNA EDITING AND tRNA IMPORT IN PLANT MITOCHONDRIA, Jacques-Henry Weil, Géraldine Bonnard, Jean-Michel Grienenberger, José Gualberto, Lorenzo Lamattina, André Dietrich, Pierre Guillemaut and Laurence Maréchal-Drouard, Institut de Biologie Moléculaire des Plantes, Université Louis Pasteur, 12 rue du Général Zimmer, 67084 Strasbourg, France

Sequencing cDNA corresponding to several wheat mitochondrial transcripts has revealed that a number of C residues are post-transcriptionally converted into U residues, causing a change in the encoded amino-acid, which results in a higher homology between the sequence of the protein and that of the corresponding protein in non-plant organisms (1).

In the case of wheat nad 4 transcripts (corresponding to NADH dehydrogenase subunit IV) a C to U conversion occurs at the junction between exon 2 and exon 3 (2).

Homologies can be found between the sequences flanking the editing sites in several wheat mitochondrial transcripts, suggesting that these sequences may play a role in the recognition of the editing sites (3).

Fractionation of tRNAs extracted from highly purified potato mitochondria yielded 31 tRNAs which have been identified by aminoacylation, sequencing or hybridization to specific oligonucleotides. This set could be sufficient to read all 61 sense codons and consists of 20 tRNAs encoded by mitochondrial DNA (5 of them are "chloroplast-like" and transcribed from chloroplast DNA sequences inserted into mitochondrial DNA) and 11 tRNAs encoded by nuclear DNA which must be imported into mitochondria (4).

One tRNA, transcribed from a mitochondrial gene which has a methionine anticodon (CAU), becomes an isoleucine-specific tRNA following a post-transcriptional modification of C into L*, a derivative of Lysidine (5).

1) J.M. Gualberto et al. Nature, 341, 660-662, 1989
2) L. Lamattina et al. FEBS Lett. 258, 79-83, 1989
3) J.M. Gualberto et al. Nucleic Acids Res. 18, 3771-3776, 1990
4) L. Maréchal-Drouard et al. Nucleic Acids Res. 18, 3688-3696, 1990
5) F. Weber et al. Nucleic Acids Res., in press

## *Mechanisms Controlling the Expression of Plant Genes*
## *(This session is sponsored by EniMont Americas, Inc.)*

**A 034** TRANSCRIPTIONAL REGULATION OF PAL AND PATATIN GENES.

Michael Bevan, Claire Nall, Michael Holdsworth, Jain-Sheng Du and Diane Shufflebottom, Molecular Genetics Dept, IPSR Cambridge Laboratory, Colney Lane, Norwich NR4 7UJ England.

Patatin is a major tuber protein encoded by two classes of genes. Class 1 genes are transcriptionally regulated by sucrose, and provide insight into the regulation of genes by metabolite concentrations. *Cis*-acting sequences conferring sucrose responsiveness have been identified and studies of the interaction of nuclear proteins with these domains is in progress.

PAL catalyses a key step in the biosynthesis of many phenolic compounds in plants, and the genes encoding PAL are transcriptionally regulated in response to numerous developmental and environmental signals. Different members of the gene family are expressed in different tissues and in response to different environmental signals. In one promoter two distinct regulatory sequences have been identified. A 100bp sequence confers xylem- and wound- inducibility on a heterologous promoter. Binding studies of proteins to this domain will be presented and the activity of PAL promoter sequences in yeast will be discussed.

**A 035** INDUCTION OF EARLY NODULIN GENE EXPRESSION BY RHIZOBIUM NOD FACTORS Ton Bisseling, Henk Franssen, Beatrix Horvath, Igor Kardailsky, Miklos Lados, Tetsuo Meshi, Marja Moerman, Julie Panagiota, Irma Vijn, Wei-Cai Yang.
Department of Molecular Biology, Agricultural University, Wageningen 6703 HA, The Netherlands.

The development of nitrogen fixing nodules on the roots of leguminous plants is induced by soil bacteria of the genes *(Brady) Rhizobium*. The formation of this plant organ involves the activation of genes in both plant and bacterium. The plant genes specifically expressed during the formation of the root nodule are named nodulin genes. We have isolated cDNA clones representing a set of nodulin genes that are expressed during early stages of *Rhizobium* -pea interaction. The location of the corresponding transcripts in specific cells and tissues of infected roots and pea root nodules was determined by *in situ* hybridization (1,2,3,). These studies revealed that we have a set of clones representing early nodulin genes that mark specific tissues of the nodule and moreover the corresponding gens are expressed at subsequent steps of development

The *Rhizobium* signal molecule that induces different development steps in nodule formation has recently been characterized (4). This is a substituted glucosamine tetrasaccharide and it is formed upon induction of the *Rhizobium nod*-genes. In this meeting we will present our studies on the mechanism by which the Nod factors induce early nodulin gene expression.

1. Van de Wiel *et al.* (1990) EMBO J. 9, 1-8
2. Scheres et al. (1990) Cell 60, 281-294
3. Scheres et al. (1990) The Plant Cell 2, 687-700
4. Lerouge et al. (1990) Nature 344, 781-784

**A 036** CHANGING BINDING AFFINITY OF A *CIS* ELEMENT FOR A TRANSCRIPTION FACTOR ALTERS TISSUE-SPECIFIC EXPRESSION IN TRANSGENIC PLANTS, Xiao-feng Qin and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University 1230 York Avenue, New York, NY 10021-6399

We have shown that the -90 deletion of the CaMV 35S promoter is preferentially expressed in roots with little or not activity in leaves. Transcription activity of the -90 35S promoter is determined by the 21-bp as-1 element which contains two related motifs, TGACGTAA and TGACGCAC. Both sequences serve as binding sites for the nuclear factor ASF-1. We have obtained a cDNA clone encoding a bZIP transcription factor called TGA1a. Based on their binding specificities, TGA1a is a good candidate for, or a component of, ASF-1. We suggested the lack of expression of the -90 35S in the leaf cells is due to the inability of as-1 to compete for ASF-1. To test this hypothesis, we created several mutants of as-1. One mutant (#A) contains two tandem copies of TGACGTAA and another (#B) contains two copies of the perfect palindrome, TGACGTCA. Gel mobility shift assays showed that mutants A and B possess 3 to 20 times higher binding affinity for ASF-1 and the recombinant TGA1a as compared to the wild-type (WT). Expression patterns of the mutants were analyzed in the context of the -90 35S promoter using GUS as the reporter gene. In contrast to plants containing the WT -90 35S promoter, transgenic plants containing mutant A or B show detectable GUS activity in mature leaves. Histochemical localization revealed that the activity is strong in vascular tissues, but weak in other cell types. In seeds, mutants A and B confer strong GUS activity in the radicle of the embryo. However, the expression domain appears to extend more towards the cotyledon end when compared to the wild-type expression pattern. These results provide evidence that, in certain cases, tissue specific expression can be controlled through an interplay between transcription factor concentration and the binding affinity of the *cis* element for the factor. Supported by a grant from Monsanto Company.

**A 037 DOMINANT AND RECESSIVE GENES REGULATING ZEIN DEPOSITION IN MAIZE ENDOSPERM** F. Salamini, S. Lohmer, R.D. Thompson, A. Marocco, C. Lorenzoni, M. Maddaloni, H. Hartings, N. Di Fonzo, M. Motto, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

The synthesis and deposition of maize zein proteins is affected by several mutantions. The three dominant mutations Fl2, De30 and Mc control the level of a protein body bound protein (b70) which is separable in two isoforms by 2D gel-electrophoresis. Both isoforms are 10-fold overexpressed in Fl2 and 5-fold in De30 and Mc. It is shown that b70 can bind ATP, cross react with antibodies directed against a conserved domain of the chaperon-like HSP70 from Drosophila and has a primary structure similar to that of several HSP-like proteins. The effect of the three mutations are thought to be mediated by an alteration of the zein transport or assembly system to which the HSP-70s participate. The locus Opaque-2 controls the transcription of the structural genes encoding the 22 kD zeins and the albumin-like protein b-32 (possibly the product of the O6 gene). The O2 locus was isolated and studied. The O2 protein contains a leucine zipper domain typical of transcription activators. The promoter of the b-32 gene can be activated in a protoplast bioassay by the O2-gene product. The information for the activation resides in a 440 bp DNA fragment containing five O2 binding sites ($GATGA^{PP}TG^{P}$). Two of these sites are embedded in the "endosperm box", a motif involved in endosperm-specific expression, which is also represented in 22 kD zein promoters. DNA-binding by O2 protein involves oligomer formation.

**A 038** MOLECULAR APPROACHES TO SINK-SOURCE RELATIONS

L. Willmitzer, Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1000 Berlin 33

Growth and development of a plant are dependent upon the energy gained by fixing carbon dioxide into carbohydrates during photosynthesis. Primary sites for photosynthesis are leaf and to a much lesser extent stem tissues, whereas other organs such as roots, seeds or tubers do not contribute to carbon assimilation but rather totally depend for their fixed carbon dioxide on photosynthetically active organs. Thus there is a net flow of energy from photosynthetically active tissues, representing the sources (defined as net exporters of fixed carbon) to photosynthetically inactive parts of the plant, representing the sinks (defined as net importers of fixed carbon). Plant development is accompanied by continuous changes with respect to an organ representing a sink or a source. Essentially all plant organs act as a sink at some stage. During plant development, however, the relative sink strength (defined as the ability of a sink organ to import assimilates) changes. Several models have been described in the literature dealing with the central problem of sink-source transitions as well as with the question of what factors determine sink strength and source capacity. In order to approach this problem on a molecular level we have created transgenic plants which display an altered expression of proteins supposedly involved in sink-source relations. Results obtained by ectopic expression of various genes supposedly involved in carbohydrate synthesis and/or partitioning will be described.

**A 039** GENETIC DESTRUCTION OF TAPETAL CELLS RESULTS IN THE PRODUCTION OF MALE STERILE PLANTS, Celestina Mariani, Marc De Beuckeleer, Véronique Gosselé, Anna Koltunow*, Willy DeGreef, Marc De Block, Jan Leemans, Robert B. Goldberg*, Plant Genetic Systems N.V., J. Plateaustraat 22, 9000 Gent, Belgium and *UCLA, Dept. of Biology, Los Angeles, California 90024

Male sterility in flowering plants is an important tool in plant breeding and an interesting object of studies to investigate the mechanism of gamete formation. Genic male-sterile (g-ms) and cytoplasmic male-sterile (c-ms) lines often exhibit an abnormal tapetum, the tissue that surrounds the developing microspores. Several tobacco tapetum-specific genes have been characterized. Although the function of these genes has not yet been identified, the analysis of their sequence may elucidate some of the features of the tapetum. We have used the 5' regulatory region of a tapetum-specific gene to target the expression of a ribonuclease specifically into these cells. The expression of this chimeric gene causes ablation of the tapetal cells and leads to male sterility. In breeding schemes of some crops it is necessary to restore full fertility of the offspring. To this aim we have engineered a Restorer line that is capable of restoring male sterility to full fertility. We present here that when the line carrying the engineered male sterility trait is crossed with this Restorer line, male sterility is restored to fertility and fertile plants exhibit a normal tapetum.

## *Molecular and Genetic Analysis of Embryogenesis*

**A 040** GENE REGULATION DURING EMBRYOGENY AND GERMINATION, John J. Harada[1], Robert A. Dietrich[1], Lucio Comai[1], James Z. Zhang[1], Kelly L. Matsudaira[1], and Sharon E. Radke[2], [1]Department of Botany, University of California, Davis and [2]Calgene Inc., Davis, CA 95616.

There are substantial differences in the developmental processes that characterize embryogeny and postgerminative growth in higher plants. A striking example of such a difference is the metabolism of storage reserve macromolecules. Storage proteins and lipids are synthesized and accumulate during a specific period of embryogeny, but they are hydrolyzed during postgerminative growth to provide nutrients for the growing seedling. It is also likely that, aside from general processes involved in cellular metabolism, similar events occur in both embryos and seedlings. For example, tissue differentiation in embryonic axes and in the apical meristems of seedlings may involve comparable processes. To investigate the basis for these similarities and differences, we are studying the regulation of gene sets that are expressed at specific stages of embryogeny and postgerminative growth in Brassica napus L. These sets include genes encoding the storage proteins cruciferin and napin, a late embryogenesis-abundant protein, the glyoxylate cycle enzymes isocitrate lyase and malate synthase, and an mRNA that accumulates during the early stages of cortical cell differentiation in roots. Some of these gene sets are expressed exclusively in either embryos or seedlings. By contrast, other sets are expressed in both embryos and seedlings, suggesting that the gene products are involved in similar processes at both stages of development. We will describe our studies of the regulation of these genes and discuss the implications of our findings to the control of plant development. This research is supported by a grant from the National Science Foundation.

**A 041** GENETIC FACTORS AFFECTING THE SYNTHESIS AND SPATIAL DISTRIBUTION OF STORAGE PROTEINS IN MAIZE ENDOSPERM, Brian A. Larkins, Mauricio A. Lopes, K.B. Geetha, and Craig R. Lending, Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721
Maize endosperm develops following the fusion of a sperm nucleus and two polar nuclei in the megagametopyte, or egg sac. After a period of free nuclear division lasting 10 days, cell walls form around each nucleus, and synthesis of food reserves, starch and storage protein, commences. These reserves accumulate primarily in the large central cells of the endosperm. Cells toward the periphery remain small and meristematic, and divisions by these cells increase the size and volume of the endosperm.

We have used immunocytochemical techniques to investigate the deposition of storage proteins, zeins, in developing endosperm. These studies have shown that in normal genotypes the sulfur-rich zeins appear first in the youngest endosperm cells. These proteins form accretions that are cross-linked by disulfide bonds in the rough endoplasmic reticulum. As the cells mature, the sulfur-poor zein proteins accumulate within these accretions and expand them into sperical protein bodies. Mutations such as opaque-2 affect the expression of certain zein genes and consequently alter the size and composition of the protein bodies. Genes called "opaque-2 modifiers" increase the expression of genes encoding the most abundant sulfur-rich storage protein, gamma-zein. Protein bodies in the "modified" mutants are similar in size to those in opaque-2 mutants, but contain 2- to 4-times more gamma-zein. As a consequence of the increased synthesis of gamma-zein, the mature seeds have a normal phenotype. Besides increasing the quantity of gamma-zein synthesis, the modifiers also appear to affect the spatial expression of gamma zein genes within the endosperm.

**A 042** DEVELOPMENTAL AND MOLECULAR GENETICS OF EMBRYOGENESIS IN ARABIDOPSIS THALIANA, David Meinke, David Patton, Deena Errampalli, Joe Shellhammer, Linda Franzmann, Linda Castle, and Leigh Mickelson, Department of Botany, Oklahoma State University, Stillwater, OK 74078.
Over 90 mutants of Arabidopsis thaliana defective in embryo development have been isolated and characterized in our laboratory. Many of these mutants are embryonic lethals with lesions in essential housekeeping functions required for the completion of embryogenesis. Others are embryonic defectives that resemble pattern mutants identified in other organisms. We believe that both types of mutants, not simply those with the most unusual phenotypes, must be analyzed to provide a clear picture of the relationship between gene function and embryo development in plants. Further analysis of an embryonic lethal defective in a known housekeeping function, a biotin auxotroph isolated several years ago in our laboratory, suggests that the defect may be in conversion of the biotin precursor 7-keto-8-aminopelargonic acid to 7,8-diaminopelargonic acid. We have used an RFLP mapping strategy that involves pooled $F_2$ plants to map this gene to within 0.5 cM of the λbAt558 RFLP marker on chromosome 5 in preparation for gene isolation through chromosome walking. Sixteen genes essential for embryo development have also been mapped relative to visible markers by analyzing progeny of selfed $F_1$ plants. Embryonic lethals are now the most common type of visible marker on the standard linkage map of Arabidopsis. Considerable effort will nevertheless be required to saturate for embryonic lethals in Arabidopsis and assign these mutants to complementation groups. The most efficient approach may be to first assign new mutants to linkage groups and then perform allelism tests with other mutants in the same region. We have also explored the importance of T-DNA insertional mutagenesis and analyzed 18 embryonic mutants isolated following seed transformation. Six of these appear to be tagged with T-DNA and three others may be tagged but require further analysis. The remaining mutants are clearly not tagged with a functional T-DNA insert and appear instead to contain mutations induced during the transformation process. Four of the putatively tagged mutants are embryonic lethals arrested early in development, one is an albino seedling lethal included because mutant seeds can be distinguished by their pale color prior to desiccation, and the other accumulates anthocyanin in the cotyledons and resembles the fusca mutants described previously by Andreas Müller. Marker rescue is currently being used to isolate the plant DNA flanking these T-DNA inserts. Further studies are planned to determine the structure and function of these genes and examine the molecular basis of abnormal development.

**A 043 HEAT SHOCK GENE REGULATION DURING CARROT SOMATIC EMBRYOGENESIS,**

J. Lynn Zimmerman, Nestor Apuya and Cynthia O'Carroll, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, 21228

We have analyzed the expression and regulation of heat shock genes during the development of embryos in culture, and have found both an interesting pattern of regulation and a very dramatic arrest of development when embryos are heat shocked. Specifically, globular embryos exhibit **no** transcriptional induction of HS mRNA, yet synthesize the whole spectrum of heat shock **proteins**, at rates equivalent to those of callus cells (which contain 12X more HS RNA) in spite of this low level of mRNA. Perhaps the most important implication of these and other findings from our laboratory is that they may suggest that the high level of HS mRNA normally synthesized during heat shock serves some function other than driving the synthesis of the hsps.

Superimposed on this unusual heat shock gene regulation is the observation that globular embryos are uniquely sensitive to heat shock. The timing of sensitivity is very specific and quite narrow; the embryos are not killed, but are arrested irreversibly, and the arrest can be avoided by pre-shocking the embryos on the day before the sensitive day. Thus, this may represent a "phenocopy" and may suggest that some essential developmental determinant required for the globular-to-heart transition is being produced during the "sensitive window". We are currently analyzing the genes being expressed during this time.

## *Genetic Analysis of Hormone Function*

**A 044** ISOLATION OF A GENE ASSOCIATED WITH THE etr LOCUS IN *ARABIDOPSIS* THAT CODES FOR A PUTATIVE TRANSMEMBRANE PROTEIN KINASE A.B. Bleecker*, C. Chang and E.M. Meyerowitz. *Department of Botany, University of Wisconsin, Madison, Wi 53706 and Division of Biology, California Institute of Technology, Pasadena Ca 91125.

The plant hormone ethylene mediates a number of developmental processes and responses to environmental stress in higher plants. Our research efforts over the last three years have been focused on developing an understanding of the molecular basis for ethylene action in plants. To this end, we have isolated mutants in Arabidopsis thaliana with altered responses to ethylene. One such mutant, designated etr, shows no measurable responses to ethylene and reduced ethylene binding in leaf tissue indicating that the mutation may directly affect the ethylene receptor (1). We have genetically mapped the etr mutation and by chromosome walking have isolated an 18kb fragment of genomic DNA which contains the mutant gene. Sequence analysis of cDNAs which map to the 18kb fragment has produced a candidate for the ETR gene which codes for a putative transmembrane protein kinase. Sequence analysis indicates a domain composed of 9 copies of a 23 amino acid leucine-rich repeat unit and a domain containing a serine/threonine type protein kinase. These two domains are separated by a single 24 amino acid hydrophobic domain. A model is presented which describes the possible mechanism of action of the protein kinase with respect to ethylene-mediated responses in plants.

1. Bleecker, A.B., Estelle, M.E., Somerville, C., Kende, H. (1988) Science 241, 1086-1089.

**A 045** REGULATION OF GENE EXPRESSION BY ETHYLENE DURING TOMATO FRUIT RIPENING, Jill Deikman, Julie Montgomery, Rodrigo Lois, Linda Margossian, Robert Fischer, Department of Plant Biology, University of California, CA 94720

An increase in ethylene hormone concentration has been shown to control the onset of ripening in many fruits, including tomato. The regulation of gene expression in ripening tomato fruit has been studied by cloning mRNAs that accumulate both during fruit ripening and in unripe fruit exposed to exogenous ethylene. Genes that encode two of the mRNAs (E4 and E8) have been studied in detail and have been shown to be regulated at the transcriptional level. To elucidate how gene expression is controlled by ethylene during fruit ripening, we have analyzed the effect of E4 and E8 promoter mutations on reporter gene expression in transformed tomato plants. Results from these experiments indicate that DNA sequences that control rapid ethylene-inducible gene expression in unripe fruit are distinct from the DNA sequences that control gene expression during fruit ripening. The nuclear proteins that react with DNA sequences that control gene expression during fruit ripening and in response to ethylene are currently being analyzed.

**A 046** HORMONAL CONTROL OF DIFFERENTIATION IN TRANSGENIC PLANTS, Harry J. Klee and Charles P. Romano, Monsanto Company, 700 Chesterfield Village Pkwy., Chesterfield, MO 63198.

Classical approaches to hormone analysis have utilized techniques such as exogenous application to define the roles of these molecules in development. The availability of transgenic technology and genes that control the levels of auxins and cytokinins provide more precise ways to elucidate the mechanisms of control. We have used bacterial genes that synthesize either auxin (iaaM) or cytokinin (ipt) and another that inactivates auxin (iaaL) to alter the pools of these hormones. In the case of auxins, expression of iaaM results in a 10-fold increase in IAA while expression of iaaL leads to as much as a 20-fold decrease in IAA. These alterations affect a number of developmental processes including apical dominance, vascular differentiation, root elongation and leaf growth. Our results indicate that in some instances, auxin and cytokinin have antagonistic effects. Thus, similar morphological effects can be achieved either by increasing the cytokinin content or decreasing the auxin content of a plant. The implications of these results will be discussed.

**A 047** A REGULATORY HIERARCHY IN SEED DEVELOPMENT: INTERACTION OF *Viviparous-1* AND ABSCISIC ACID IN REGULATION OF THE *C1* GENE IN MAIZE. Donald R. McCarty, Tsukaho Hattori, Vimla Vasil and Indra K. Vasil. Vegetable Crops Dept. University of Florida, Gainesville, FL 32611.

The *Viviparous-1* gene product is required for ABA regulated gene expression and anthocyanin synthesis in the developing maize seed. Mutant seed fail to express the *C1* gene which encodes a putative transcription factor for the anthocyanin pathway. Regulation of the *C1* promoter by the *Vp1* product and ABA was examined in a maize suspension culture protoplast transient expression assay using a *C1* promoter/βglucuronidase (GUS) reporter plasmid. ABA treatments resulted in a 10-15 fold induction of GUS expression. ABA was active over a 0.1 to 100 μM concentration range. Co-expression of a *Vp1* cDNA fused to the CaMV 35S promoter activated the *C1* promoter 5-7 fold independent of ABA. In combination, ABA and *Vp1* transactivation did not increase activity above the maximum level achieved with hormone alone. Deletion analysis and site directed mutagenesis were used to map ABA and *Vp1* responsive elements in the *C1* promoter. Disruption of a conserved 11 base sequence in the *C1* promoter abolished both ABA and *Vp1* activation. Deletion of 5 bases just upstream of the 11 base sequence abolished ABA regulation, but not *Vp1* trans-activation. The 5 base deletion reconstructs the promoter sequence of *c1-p*, an allele of *C1* that is expressed during germination, but not during seed maturation. These data also indicate that the ABA and *Vp1* responses are separable suggesting that the *Vp1* protein is not an integral part of the ABA signal transduction pathway. The *Vp1* protein has structural features consistent with a transcription activator function. We propose that *Vp1* functions to potentiate the hormone response in seed tissues by interacting with ABA regulated transcription factors at the level of the promoter.

**A 048** CIS- ELEMENTS AND TRANS- ACTING FACTORS INVOLVED IN THE ABSCISIC ACID REGULATION OF EM GENE EXPRESSION, Ralph S. Quatrano, Mark J. Guiltinan, William R. Marcotte, Jr. and Richard M. Bostock*, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280 and Department of Plant Pathology, University of California, Davis, CA 95616*.

Over a 3-5 day culture period in the presence of 50 uM ABA, immature wheat embryos increase in fresh and dry weight and undergo an accelerated but normal morphogenesis and accumulation of mature embryo proteins. In the absence of ABA, this maturation program is not engaged and the embryo germinates into a normal seedling, expressing a different set of genes characteristic of the germination program. Some proteins that accumulate in the presence of ABA include an abundant, hydrophilic protein found in the mature embryo (Em) and a 7S globulin storage protein (triticin). Characterization of these genes, and the pattern of accumulation of their respective proteins and mRNA's, reveal that regulation by ABA occurs at the level of transcription and mRNA stability (1). Expression of Em is not confined to the embryo (2). Increasing the levels of ABA or sensitivity to ABA in seedling tissue or in suspension cultures results in increased levels of Em mRNA. Fusions of the intact and deleted 5' promoter and leader as well as the 3' regions of the Em gene (3) with the glucuronidase (GUS) reporter gene have been tested for ABA-dependent expression in transient assays using rice protoplasts (4) and in transgenic tobacco plants. Results from both assays indicate that a 646bp region (-554 to +92) from the 5' untranslated region of the Em gene contains all the information needed for ABA regulation and embryo-specific expression (5). Further analysis of the cis elements in this 650bp fragment indicates three functional regions involved in the ABA response; a quantitative enhancer region (rich in A/T sequences), an ABA response element (ABRE) and an untranslated leader which appears to increase the stability/translatability of the mRNA. A detailed analysis of the sequences in the ABRE involved in ABA-modulated regulation of chimeric genes in a transient assay will be presented. Nuclear extracts from embryonic and suspension cultured cells contain protein factors which specifically bind to the ABRE, as evidenced by gel-shift and footprinting experiments. A 76bp oligonucleotide sequence from the active ABRE region was used as a probe to screen a lambda gt11 expression library. A cDNA clone was isolated whose product is a DNA binding protein (EmBP-1) that interacts specifically with an 8bp sequence (CACGTGGC) in the ABRE(6). A 2bp mutation in this sequence prevented binding of EmBP-1 and reduced the ability of the ABRE to confer ABA responsiveness on a viral promoter in a transient assay. The 8bp EmBP-1 target sequence was found to be conserved in several other ABA-responsive promoters and in promoters from plants that respond to signals other than ABA, e.g. light. The deduced amino acid sequence of EmBP-1 contains conserved basic and leucine zipper domains found in transcription factors in other plants, yeast and in mammals. Progress on the analysis of the role of EmBp-1 in the ABA response pathway of higher plants will be discussed. Supported by the U.S.D.A. Competitive Grants Program (89-37262-4456).

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*Use of Transposons to Isolate Plant Genes*

**A 049** MAIZE TRANSPOSABLE ELEMENTS AND GENE TAGGING. Nina V. Fedoroff, Patrick Masson, Mary Strem and Patricia Gary, Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210.

Transposable elements belonging to the *Activator-Dissociation* (*Ac-Ds*), *Suppressor-mutator* (*Spm*) and *Mutator* (*Mu*) families have all been used to clone genes with insertion mutations from the maize genome. The *Ac* and *Spm* elements have been shown to transpose in plants other than maize, including tobacco, tomato, potato, carrot and *Arabidopsis*, and therefore are likely to useful for mutagenesis and gene isolation. To date, most efforts to develop useful transposon tagging systems have concentrated on elements belonging to the *Ac-Ds* family and we will describe recent progress in our laboratory toward the development of *Ac-Ds* transposons for tagging genes in *Arabidopsis*. The *Spm* element is markedly more complex than the *Ac* element, encoding at least 4 alternatively spliced mRNAs. These have been designated *tnpA*, *tnpB*, *tnpC*, and *tnpD* and the protein-coding sequences of the transcripts overlap extensively. To facilitate development of *Spm* for transposon tagging, we have undertaken to identify the element-encoded gene products required for *Spm* transposition in tobacco, using cDNAs containing a single ORF to complement mutant *Spm* elements and each other. The results of these studies indicate that *tnpB* and *tnpC* are not required for transposition, while *tnpA* and *tnpD* are both directly involved.

**A 050** T-DNA INSERTION MUTAGENESIS IN *ARABIDOPSIS*: MUTATIONAL SPECTRUM OBTAINED FROM SCREENING 8,000 TRANSFORMED LINES, Kenneth A. Feldmann. CR&DD, The Du Pont Company, Wilmington, DE 19880-0402.

We have now generated more than 8,000 transformed lines using the seed infection method for transformation (1). The large number of transformants has been obtained due to our continued persistence. The frequency with which we can generate transformants has been, and continues to be, highly variable. We have grown >750,000 infected plants (T1) to maturity and have collected >225 million seeds (T2) from these selfed plants. We estimate that we have plated >80 million of these (T2) seeds on kanamycin-containing media to generate the transformants that we have to date. What we have ascertained about the mutants is that i) 90-95% segregate in a Mendelian manner for 1-4 linked or unlinked inserts (with an average of 1.4 inserts/transformant), ii) the vast majority of the inserts are concatamers of T-DNAs in direct and inverted repeats, and iii) numerous lines segregate for visibly altered phenotypes. We also know the mutations that are in many of these lines are due to a disruption of a gene by the T-DNA (2,3,4). In addition, there are lines segregating for visibly altered phenotypes that fail to segregate with the kanamycin resistance marker (Feldmann et al., Meyerowitz et al., Meinke et al., unpublished data). From testing the first 3-4 dozen mutants we feel confident that the majority of the altered lines are due to a disruption by a T-DNA insert. With this prospect we have decided to do large scale screening. Over the past several months the first 6,000 lines have been screened on plates containing agar-solidified medium. These plates have been placed in a vertical position to allow rapid screening of root, root hair, embryonic, pigment, dwarf and other miscellaneous mutant phenotypes. This work was done in collaboration with Phil Benfey (Rockefeller Univ.), Nam-Hai Chua (Rockefeller Univ.), Scott Poethig (Univ. of Pennsylvania) and Pablo Scolnik (Du Pont). We observed several mutants in each category listed above. This screen and the mutants observed will be described in more detail. Over the next several months we have tentatively planned to screen an additional 7,000 lines under greenhouse conditions. If we continue to observe phenotypes similar to those in the first 1,300 lines we should find ~200 embryo-defective mutants, ~200 reduced fertility mutants, 70 pigment mutants, hundreds of seedling-lethals and size variants, and 70-140 other dramatic developmental mutants including, 10-15 dwarfs and 20-30 floral mutants. The results of this screen will be summarized and the people participating will be listed.

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**A 051** THE USE OF TRANSPOSONS TO STUDY GENE FUNCTION AND CONTROL IN *ANTIRRHINUM MAJUS*, Cathie Martin, Clare Lister, David Jackson, Rachael Burton, Rosemary Carpenter, Justin Goodrich, Enrico Coen and Steve Mackay, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

Transposable elements are invaluable tools to assist in the isolation and study of plant genes. Many unstable mutations that affect anthocyanin biosynthesis have been identified, and because we are particularly interested in studying the mechanism and pattern control of flower colouration in *Antirrhinum majus*, we are using transposable elements to assist this study. Transposons can be used to isolate new genes by transposon tagging. This may involve extensive mutagenesis programmes to develop new unstable alleles, but we have also found that out-crossing between mutants isolated in Germany and our own lines often results in freshly observed instability. This provides a rapid method to isolate unstable alleles. Once a new unstable locus has been identified a transposon probe is required to isolate the locus. One colour gene (*incolorata*) has proved to be particularly useful as a transposon trap as it is semi-dominant and new unstable alleles can be observed in the $M_1$. Using this gene, six independent insertions of different transposons into *incolorata* have been observed and isolated at the molecular level. Transposon insertions at a locus can also be used for further mutagenesis, involving imprecise excision or transposon-induced chromosomal rearrangements. These mutations provide large allelic series of essentially isogenic material. We have used mutations at *nivea* and *pallida* (two genes involved in anthocyanin biosynthesis) to analyse promoter function *in vivo* with particular reference to regions controlling the pattern of gene expression within the flower. Transposon rearrangements can also place genes under novel genetic control providing another approach to isolating genes involved in determining spatial patterns.

**A 052** MOLECULAR CONSEQUENCES OF WIDE HYBRID CROSSES BETWEEN ORYZA SPECIES., Susan Wessler, Thomas Bureau, K.K. Jena*, Gurdev Khush* and Sylvestre Marillonnet, Department of Botany, University of Georgia, Athens, GA 30602, *International Rice Research Institute, Manila, Philippines

The mutant alleles of the maize *waxy* (*wx*) gene have provided the raw material for the isolation of several transposable elements (including *Ac* and *Spm*) and retrotransposons. Characterization of *wx* mutations in rice (*Oryza sativa*) was undertaken in the hopes of isolating the first endogenous rice transposable elements. Unfortunately, none of the mutations analyzed to date result from the insertion of DNA. In a last ditch effort to trap rice transposable elements at *wx*, we focused on a class of mutations with properties normally ascribed to transposable element alleles. These properties include (i) a high frequency of germinal instability and (ii) isolation following a wide hybrid cross (between the domesticated *Oryza sativa* and a wild relative *Oryza officinalis*).

Cloning and characterization of these unstable *wx* mutations in addition to the progenitor alleles and several stable revertants failed to detect any significant DNA insertion within or flanking the transcription unit. Rather, we have detected two consistent differences among these alleles: (i) the patterns of DNA methylation in their 5' flanking regions and (ii) the sequence of a 160 bp segment within a 260 bp *wx* intron. We have detected sequence variation at 10 positions scattered throughout the 160 bp region. Interestingly, this hypermutable intron sequence is repeated at least 20 times in the *O. sativa* genome and is not present in the genome of *O. officinalis*. I will report on the genetic propoerties of the unstable *wx* alleles and of other unstable mutations that arise following similar crosses. Possible connections between the hypermutable sequence, DNA methylation and the *wx* phenotype will be discussed. Finally, experiments designed to test the far-fetched notion that this sequence might prove useful as an "intron tag" in the isolation of other rice genes will be presented.

## *Genetic Analysis of Light Control*

**A 053** MOLECULAR GENETIC STUDIES OF LIGHT-REGULATED GENE EXPRESSION, Ulrike Schindler, Robert G.K.Donald, Peter Bringmann and Anthony R. Cashmore, Plant Science Institute, Department of Biology, University of Pennsylvania, PA 19104

Promoters of photoregulated genes encoding ribulose-1,5-bisphosphate carboxylase small subunit (RbcS) are characterized by conserved sequences in the -300 to -200 region. We have referred to these conserved sequences as L-, I-, and G-boxes and we have characterized a factor (GBF) that binds to the conserved G-box sequence [3]. An *Arabidopsis thaliana rbcS-1A* promoter fragment containing these conserved L-, I-, and G-boxes confers the ability to mediate light-regulated leaf expression upon fusion to a truncated ADH promoter. Mutagenesis of the I-box or the G-box sequences, in the context of the full-length *rbcS-1A* promoter, show that these sequences are essential for expression [2]. These results were derived by studying GUS expression, driven by the rbcS-1A promoter, in transgenic tobacco plants.

Similar studies on the *Nicotiana plumbaginifolia cab-E* promoter have characterized both positive (PRE) negative (NRE) and light-regulatory (LRE) elements [1]. The LRE contains a G-box-like sequence and an adjacent sequence (box 6), both of which are required for high levels of leaf expression. Mutants of this promoter that include deletion of box 6 result in root expression.

We have studied protein factors that interact with the rather complex regulatory elements of the *cab-E* promoter. Five different factors have been characterized and shown to bind to 25 different sites within the promoter [5]. DNA binding proteins encoded by cloned DNA sequences and similar in properties to GBF [3] and GT-1 [4] have been characterized.

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**A 054** GENETIC ANALYSIS OF LIGHT-REGULATED SEEDLING DEVELOPMENT IN *ARABIDOPSIS*, Joanne Chory, Lothar Altschmied, Terry Delaney, Punita Nagpal, Alan Pepper, and Ronald Susek, Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186.

The photoconversion of photoreceptors by light induces the diverse morphogenic responses which result in greening. Several regulatory photoreceptors are involved in the perception of light signals, but little is known of the transduction pathways that mediate light-regulated development. We are taking a combined genetic and molecular biological approach to identify potential components of the light signal transduction pathways. We have identified a class of *Arabidopsis thaliana* mutants that show many characteristics of light-grown plants even when grown in complete darkness. The mutants define at least 3 complementation groups, designated *det*1, *det*2, and *det*3 (*de-et*iolated). Loss-of-function mutations in any of these 3 genes result in dark-grown seedlings that constitutively display many characteristics of light-grown plants, including the development of leaves and chloroplasts, and the accumulation of anthocyanins and mRNAs for several light-regulated nuclear and chloroplast genes. Mutations in the *DET*1 gene are particularly interesting because they also affect cell-type-specific expression of light-regulated genes and chloroplast development. A second class of mutants have a long *hy*pocotyl (*hy*) when grown in the light. Sixty-five mutant alleles have been analyzed by M. Koornneef and by our lab. These efforts have identified 7 complementation groups; 3 of these (*hy*1, *hy*2, and *hy*6) show deficiencies in photoreversible phytochrome activity, and are presumably signal perception mutants. Biochemical analysis of *hy*1, *hy*2, and *hy*6 mutants indicates that these genes may encode enzymes in the chromophore biosynthetic pathway. We have constructed double mutants between the phytochrome-deficient *hy* mutants and the *det* mutants. The phenotype of the *hy*1-*det*1, *hy*2-*det*1, or *hy*6-*det*1 double mutant is *det*1, indicating that *det*1 is epistatic to *hy*1, *hy*2 and *hy*6. Likewise, *det*2 is epistatic to *hy*1, *hy*2, and *hy*6. These results are consistent with a model where formation of the active form of phytochrome results in a decrease in activity of DET1 or DET2, which in turn leads to the de-etiolation response. Clearly, it will be important to know the mechanisms by which *DET*1 and *DET*2 act. We are attempting to clone these loci by chromosome walking and complementation of the mutant phenotypes with the wild-type copy of the gene.

Since the phenotypes of regulatory mutations are often difficult to predict, a parallel molecular genetic approach using promoter fusions is also being pursued. The strategy involves fusing an *A. thaliana* light-regulated promoter (*CAB*3) to both selectable (hygromycin phosphotransferase) and screenable (GUS) marker genes, transferring these chimeric constructions into *A. thaliana*, and mutagenizing the transgenic line to identify mutations where the *CAB* promoter is aberrantly expressed. We are currently analyzing mutants where the *CAB* promoter is expressed inappropriately with regard to tissue-specific expression, light-dark expression, and in response to chloroplast signals. Characterization of these potentially interesting regulatory mutants will be presented.

**A 055** CELL-SPECIFIC GENE EXPRESSION IN TRANSGENIC $C_4$ PLANTS, William C. Taylor, Chris J. Chastain, Julie Chitty, Elizabeth Smith, John D. Stubbs and John G. Mason, CSIRO Division of Plant Industry, Canberra 2601, Australia

Enzymes of the $C_4$ photosynthetic cycle are compartmentalised in one or the other of two specialised leaf cell types. Accumulation of mRNAs encoding $C_4$ enzymes is cell-specific and strongly induced by light. Differentiated mesophyll and bundle sheath cells have long been recognized as one of the best experimental systems in which to study cell-specific gene regulation. However, the lack of a gene transfer system for any $C_4$ plant has severely inhibited progress to date. We have developed a reproducible, stable transformation system in a $C_4$ dicot, Flaveria bidentis. Initial experiments involved infection of seedling explants with Agrobacterium containing nos-nptII and 35S-GUS constructs. Following Kanamycin selection, calli were shown to be transformed by the presence of GUS activity and the formation of roots and shoots under Kanamycin selection. In leaves of regenerated plants, the 35S-GUS construct was expressed primarily in the vascular system and trichomes, and exhibited preferential expression in bundle sheath cells over mesophyll. Promoter activities have also been measured by transient expression in F. bidentis leaf protoplasts, the majority of which are from mesophyll cells. The highest level of GUS activity came from a mas-GUS construct, followed by a 35S(+4ocs enhancers)-GUS construct, with very low activity from the same 35S-GUS construct used for stable transformation. Current work is focused on two mesophyll-specific genes, PD (pyruvate Pi dikinase and Cab (chlorophyll a/b apoprotein). Progress in defining the mesophyll-specific regulation of both genes will be discussed. Another attractive feature of the genus Flaveria is that some species are $C_4$, others $C_3$, and some $C_3$-$C_4$ intermediates. We are currently developing transformation techniques for Flaveria pringlei, a $C_3$.

*Genetic Dissection of Vegetative Development*

**A 056 DEVELOPMENTAL AGE DICTATES CELL FATES IN THE MAIZE LEAF.** Michael Freeling and lab, Plant Biology Department, University of California, Berkeley, CA 94720

We study about two dozen mutants that affect the pattern and fates of cells in the leaf. These mutants fall into various categories. Mutants at the two liguleless genes remove the ligule and auricle and blur the boundary between sheath and blade. We used sectors of lg1 leaf to interrupt a cell-cell signal, a signal that is propagated from midvein to margin. New lg1 mutants have been isolated from transposon-laden lines, and-- as with all of the leaf mutants that are fully penetrant-- we either have a clone or a transposon- or homeobox-tagging strategy. Some mutants act in leaf primordia and elsewhere. Exemplary are the mutants that affect the midvein of the leaf: twi1-0, Abp1-0 and Lxm1, the former two being new Mu-induced mutants. These mutants alter midvein number or vein-dominance, disturb phylotaxis, and can slow developmental maturation of the midvein with the consequence that all events that depend on timely differentiation of a single midvein are altered. Another systemic mutant is Hsf1-0, originally defined as specifying large transformations of blade to sheath. We have shown that this dominant mutant slows the transitions from juvenile to adult phases all over the plant, and is strongly affected by the overall flowering-time of the genetic background. This mutant's phenotype helped us define "developmental time." Most of the mutants we study are dominant alleles that act to retard the maturation of cells at particular developmental times or places. The best studied is Knotted-1, defined by dominant mutants that affect maturation of cells around the lateral veins. Mutants of Rs1, Lg3, and Lg4 are similar to Kn1 in their genetic characteristics and mode of action. All seem to slow maturation, so cells behave "young for their age," and several behave predictably when crossed into lines that have very fast or slow generation times, or when combined with "upstream" developmental mutants. Our data on the acquisition of cell fate in plants demand that developmental age in a chronology be taken as seriously as position in a field. Our working hypothesis is that provascular tissue induces subsequent developmental events and that a cell's developmental age-- as well as its position-- encodes competence to make sense of these induction signals. References: See lab's contributions to Developmental Biology and Development and citations to previous work therein, in press for late 1990.

**A 057** TRICHOME FORMATION AS A MODEL FOR PLANT CELL DIFFERENTIATION, M. David Marks, Jeff Esch, and David Oppenheimer, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588.
The development of trichomes on Arabidopsis provides an excellent model for cell differentiation because: 1) trichome formation is relatively simple compared to that of other cell types; 2) trichome formation occurs on the plant surface where it can be easily observed; and 3) several mutations that result in altered trichome development define genes that are uniquely required for trichome formation. Our short-term goal is to isolate and characterize genes that are uniquely required for trichome formation. Our hope is that these genes will have counterparts that are uniquely for other types of cell differentiation. Finally, we hope to manipulate cell differentiation by altering the expression of genes controlling cell differentiation.

Most of our progress to date has been in the isolation and characterization of the GL1 gene. GL1 is required for the initiation of trichome formation. We have isolated this gene through the use of T-DNA insertional mutagenesis (1,2). DNA sequence analysis reveals that the GL1 product is a member of the myb class of DNA binding proteins. We have placed a CaMV 35S promoter in front of the putative coding region to determine the effect of constitutive GL1 expression on trichome development. We have also placed the GUS reporter gene behind the putative GL1 promoter in order to study the timing GL1 expression during trichome development. We are also characterizing three mutant alleles of GL1 that result in three distinct phenotypes. Results on our preliminary characterization of the myb gene family in Arabidopsis and on our search for other myb genes required for plant cell differentiation will be presented.

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**A 058** PATTERNS OF DEVELOPMENT IN C4 LEAVES. Timothy Nelson and Jane A. Langdale, Yale Biology Department, P.O. Box 6666, New Haven, CT 06511
The operation of the C4 cycle relies on the presence of one set of photosynthetic enzymes exclusively in bundle sheath (BS) cells (e.g., RuBPCase, NADP-ME) and a complementary set in mesophyll (M) cells (e.g., PEPCase, PPdK, NADP-MDH). We propose that the distinct patterns of differentiation shown by mature BS and M cells in C4 leaves represent an extreme state of a regulatory system capable of gene expression patterns permitting C3-type, C4-type, or combined modes of carbon fixation. In situ hybridization studies suggest that C4 genes are controlled in a radial fashion around developing vascular centers, such that BS genes are activated at one radius and M genes at a greater radius. This spatial pattern is evident before BS and M cells are morphologically distinct and is correlated with methylation near or within individual C4 genes. We suggest that methylation patterns mark the gene activity patterns established early in BS and M differentiation.

**A 059** GENETIC CONTROL OF POLAR CELL GROWTH IN ARABIDOPSIS,
John Schiefelbein, Susan Ford, Pablo Azcurrain, and Paul Rowse, Department of Biology, University of Michigan, Ann Arbor, MI 48109.

The ultimate shape that a plant cell attains is largely determined by the precise control of cell division and cell expansion. In some cells, like pollen tubes and root hairs, expansion is limited to a single growing point, which leads to the formation of a tubular-shaped cell. This highly-organized type of cell expansion, known as polar cell growth, provides a useful model system with which to investigate and define the mechanisms that control cell expansion in plants. With this objective in mind, we have chosen to study the process of root hair development in Arabidopsis thaliana. In addition to their rapid localized expansion, root hairs are advantageous because they can be examined when the plant is only 3-4 days old, they are produced continuously in a short zone near the root tip, and they play an important role in mineral ion acquisition and in plant-microbe interactions. A genetic dissection of root hair development was initiated by visually screening roots from 12,000 $M_2$ seedlings on Petri dishes and identifying more than 40 root hair mutants (1). These mutants display a variety of phenotypes, ranging from the absence of root hairs to the production of wavy or branched hairs. At least 9 different complementation groups have been defined by these mutations, and these RHD (root hair development) loci have been mapped and incorporated into a developmental genetic pathway. Current research from our group will be presented and includes work in three main areas. (A) Electron microscopy and cell biology techniques are being used to analyze the ultrastructure and cytoskeleton of the mutant root hairs and to compare them to wild-type hairs. (B) RFLP mapping and chromosome walking experiments are being performed to isolate DNA clones of specific RHD genes. (C) Additional mutant screens are being carried out in order to identify loci that are involved in the expansion of other cells that display polar growth (such as pollen tubes).

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*Molecular and Genetic Analysis of Plant Reproduction*

**A 060** OLIGOSACCHARINS CAN REGULATE PLANT DEFENSE, GROWTH, AND ORGANOGENESIS, P. Albersheim, A.G. Darvill, C. Augur, C. Bergmann, K. Brady, J.J. Cheong, S.H. Doares, S. Eberhard, T.A. Gruber, M.G. Hahn, V. Marfà, D. Mohnen, and S. Kauffman, Complex Carbohydrate Research Center and the Dept. of Biochemistry, The University of Georgia, Athens, GA 30602; F. Cervone and G. De Lorenzo, Dipartimento di Biologia Vegetale, Universita di Roma "La Sapienza", Rome, ITALY

Two seemingly unrelated lines of research--one, elucidating the walls of growing plant cells, and the other, studying how plants defend themselves against disease--have provided evidence for the functioning in plants of carbohydrate regulatory molecules. The combined results of these projects established that oligosaccharins, structurally defined fragments of plant and microbial cell wall polysaccharides, can function in plants as chemical messages with specific regulatory properties. Oligosaccharins can trigger plant defense responses against pathogens and other types of stress and are also able to regulate growth and organogenesis of plant tissues. This lecture will describe the biological assays, purification, structural characterization, and initial studies of the mode of action of several oligosaccharins. Acknowledgments: This work is supported by U.S. Department of Energy grant DE-FG09-85ER13425, and by DE-FG09-87ER13810 as part of the DOE/NSF/USDA Plant Science Centers Program.

**A 061** MOLECULAR ASPECTS OF SELF-INCOMPATIBILITY IN THE SOLANACEAE, Bruce A. McClure, Volker Haring, Marilyn A. Anderson, Antony Bacic and Adrienne E. Clarke, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

Self-incompatibility (SI) is the inherited ability of a flower to reject its own pollen. The effectiveness of self-incompatibility in maintaining outbreeding is believed to be one of the most important factors in ensuring the evolutionary success of flowering plants. In many, but not all cases, SI is controlled by a single gene, the *S*-gene, with multiple alleles. There are two major types of SI, gametophytic and sporophytic. The gametophytic system is the more widely distributed. In this type of SI, pollination with self-pollen or pollen bearing an *S*-allele identical to either of the *S*-alleles carried in the diploid tissues of the pistil does not lead to seed set. The pollen germinates and the tube grows into the style transmitting tissue. At some point within the female tissue, the growth of the incompatible pollen tubes is arrested. Gametophytic SI has been studied in most detail in members of the Solanaceae, such as *Nicotiana alata*. Style extracts of *N.alata* typically contain a major protein, each variant of which co-segregates with a particular self-incompatibility allele. The structure of the major glycosyl substituents of the glycoproteins has been established and the structure of the protein backbone deduced from cDNA cloning and peptide sequencing. The cDNAs encoding three of the predicted amino acid sequences show 56% amino acid identity between the three sequences. The *S*-glycoproteins are ribonucleases. Within the homologous regions of the three *N.alata S*-alleles, there are regions with precise homology to the fungal ribonucleases $T_2$ and Rh. These regions of homology between the *S*-glyproteins and the fungal ribonucleases include the two histidine residues which have been implicated in RNase $T_2$ catalysis. The isolated glycoproteins were recently shown to have ribonuclease activity [McClure *et al.* (1989) Nature **342**, 955-957]. This observation leads to the idea that uptake of this cytotoxic agent by the gametophyte may be involved in self-incompatibility.

**A 062** MALE GAMETOPHYTE EXPRESSED GENES, Joseph P. Mascarenhas, Department of Biological Sciences and Center for Molecular Genetics, State University of New York at Albany, Albany, N.Y. 12222

The messenger RNAs present in the mature pollen grains of maize are the products of a large number of different genes (1). Most of these genes are expressed in both the male gametophyte and in the sporophyte. A relatively small number of the total sequences transcribed in pollen appear to be pollen-specific, being expressed in pollen but not in other tissues of the plant (2). A pollen-specific genomic clone, Zm13 has been isolated and characterized (3). This clone includes extensive 5' and 3' sequences flanking the coding region. To identify the location of promoter sequences responsible for pollen-specific expression a set of 5' promoter deletions was fused to a ß-glucuronidase (GUS) reporter gene and introduced by transformation into tobacco plants (4). These experiments have shown that a fragment of DNA consisting of 314 base pairs 5' to the start of transcription is sufficient for the correct pollen-specific and temporal expression of the GUS gene (4). To map the promoter in finer detail we have used high-velocity microprojectiles to deliver DNA directly into pollen. Tradescantia pollen has been used for this purpose because of its experimental advantages. A construct containing 260 bp of DNA 5' to the start of transcription of the Zm13 gene coupled to a GUS gene and a nopaline synthase (NOS) terminator is able to induce GUS activity in pollen in such transient transformation assays. The 260 bp fragment is as active as a promoter fragment consisting of 1000 bp of 5' DNA. When just 100 bp of 5' promoter DNA is used GUS activity is seen in the transformed pollen grains although the activity is reduced compared to the 260 bp construct. Controls using the CaMV35S promoter or constructs with no promoter 5' to the GUS gene are unable to activate GUS activity in pollen. In addition the CaMV35S-GUS-NOS construct when introduced into Tradescantia leaves induces GUS activity in the leaf cells, whereas, the constructs containing fragments of 5' flanking DNA from the Zm13 gene are unable to activate GUS activity in leaf cells. The regulatory signals responsible for pollen-specific expression of the Zm13 gene thus reside within 100 bp from the start of transcription of the gene. Some quantitative elements might, however, be present further upstream than 100 bp.

References: (1) Willing, RP et al, Theoret. Appl. Genet. 75, 751, 1988. (2) Stinson, JR et al, Plant Physiol. 83, 442, 1987. (3) Hamilton, DA et al, Sex. Plant Reprod. 2, 208, 1989. (4) Guerrero, FD et al, Mol. Gen. Genet. (in press)

**A 063** FUNCTIONAL ANALYSIS OF POLLEN-SPECIFIC GENES, Sheila McCormick, Plant Gene Expression Center, USDA/ARS–UC-Berkeley, 800 Buchanan St., Albany, CA 94710

Our overall objective is to understand the regulatory circuits that control differential gene expression in pollen. Towards this end, we have characterized three genes (LAT52, LAT56 and LAT59) that are predominantly expressed in pollen. LAT promoter-GUS constructs confer essentially pollen-specific GUS expression in transgenic tomato, tobacco and *Arabidopsis* (LAT59 shows weak expression in roots and immature seeds; LAT52 shows weak expression in endosperm of immature seeds). A series of 5' promoter deletions and base substitution mutations were assayed in transgenic plants and/or with a transient assay, in order to define both quantitative and tissue-specific regions of these promoters. The LAT56 and LAT52 promoters share a common sequence element, as do the LAT56 and LAT59 promoters. An expression library prepared from pollen mRNA is currently being screened with these promoter elements, in order to identify cDNA clones corresponding to pollen-expressed DNA binding factors. Mutagenesis of transgenic LAT59-GUS and LAT52-GUS *Arabidopsis* is being used to identify trans-acting factors that regulate the expression of these LAT genes.

*Genetic Dissection of Flowering*

**A 064** HOMEOTIC GENES CONTROLLING FLOWER DEVELOPMENT IN *ANTIRRHINUM*, Enrico Coen, Rosemary Carpenter, Sandra Doyle, Ruth Magrath, José Romero, Robert Elliott, Justin Goodrich and Desmond Bradley, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

In order to isolate and study genes controlling floral development, we have carried out a large-scale transposon- mutagenesis experiment in *Antirrhinum majus*. Ten independent floral homeotic mutations were obtained and these can be divided into three classes depending on whether they affect (1) identity of organs within the same whorl, (2) identity and sometimes also the number of whorls and (3) the fate of the axillary meristem that normally gives rise to the flower. The classes of floral phenotypes suggest a combinatorial model for the genetic control of primordium fate.

Using transposons as probes, we have isolated and analysed a class (3) gene, *floricaula* (*flo*). Plants carrying the *flo* mutation cannot make the transition from inflorescence to floral meristems and have indeterminate shoots in place of flowers. *In situ* hybridization shows that the *flo* gene is expressed from a very early stage in wild-type inflorescences in a specific temporal and spatial sequence. The earliest expression seen is in bract primordia and is followed by expression in sepal, petal and carpel primordia but no expression is seen in stamen primordia. Expression in each organ is transient and is not observed in later stages of floral development. This pattern of expression has implications for how *flo* affects phyllotaxis, organ identity and determinacy. In particular, we propose that *flo* interacts in a sequential manner with other homeotic genes (class 2) that affect the identity of whorls in the flower.

Reference

Carpenter, R. and Coen, E.S. (1990) Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. Genes & Development, in press.

**A 065 MOLECULAR GENETICS OF THE TASSEL-SEED GENES OF MAIZE,**
Stephen L. Dellaporta, Alison DeLong, Joy Chien, Alejandro Calderon-Urrea, Kevin Madden, Jychian Chen, and Roger Krueger, Department of Biology, Yale University, New Haven, CT 06511.

The condition of separating male and female reproductive organs on different inflorescences is termed monoecy. Monoecy increases the frequency of outcrossing and decreases the changes of self fertilization. In maize, the unisexual female and male inflorescences, ears and tassels, are formed by a selectively promoting or suppressing the development male or female organs in the floral primordium. The genetic control of monoecy in maize is complex and not well understood. The *tassel-seed* genes have been implicated in monoecy by determining the sexual identity of the male tassel. Mutant *ts/ts* plants produce functional female flowers in an otherwise unisexual male inflorescence. As a step toward understanding the genetic and molecular basis of monoecy, we have cloned mutant and wild-type *tassel-seed:2* genes. Unstable alleles of *Ts2* have used to determine the developmental time and spatial program of *Ts2* action. Imprecise excision of the transposon *Ac* from the *Ts2* gene has been used to create hypomorphic alleles of *Ts2* that condition incomplete sexual transformations. We will present molecular and genetic evidence that *Ts2* acts in subepidermal cells after branch primordia of the floral meristem are determined but before and during the development of spikelet and floral primordia.

**A 066** REGULATION OF FLOWER DEVELOPMENT IN ARABIDOPSIS THALIANA. K. Diane Jofuku[1], Bart den Boer[2], Marc Van Montagu[2], and Jack K. Okamuro[1]. [1]Department of Biology, University of California, Santa Cruz, CA 95064, and [2]Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000, Gent, Belgium. To isolate genes involved in the genetic control of flower development and morphogenesis, we have been developing strategies for generating molecularly-defined floral mutants in *Arabidopsis thaliana* by insertional mutagenesis using T-DNA from *Agrobacterium tumefaciens* and the transposable element systems *Ac/Ds* from maize and *Tam3* from snapdragon. Using T-DNA containing *Tam3* as an insertional mutagen, we have identified a T-DNA/*Tam3* induced homeotic floral mutation designated *flower-1* (*fl-1*). *fl-1* results in the loss of petals and the transformation of sepals to carpel-like structures. Complementation analysis indicates that *fl-1* is allelic to the genetically well-characterized *apetala2-1* (*ap2-1*) lesion (1-5). Genetic segregation and DNA gel blot studies correlate a single T-DNA insert with the *fl-1* mutation. Using T-DNA as a molecular probe, we have cloned the *flower-1* gene locus and its wildtype counterpart. We will present the *Apetala2* gene sequence and will discuss its implications on the function of the *Ap2* protein.

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**A 067** A GENETIC AND MOLECULAR MODEL FOR FLOWER DEVELOPMENT IN *ARABIDOPSIS*, Elliot M. Meyerowitz, John Bowman, Laura Brockman, Gary Drews, Thomas Jack, Leslie Sieburth and Detlef Weigel, Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA.

*Arabidopsis thaliana* flowers begin their development as small masses of undifferentiated cells. These masses mature to flowers with a series of specific organ types, each with its own characteristic cell types, and with the organs in specified numbers and positions. How the cells within the flower primordium learn their positions, and as a consequence differentiate into appropriate types, is still unknown. It does seem that there are at least two distinct stages by which this occurs: cells first form correct numbers of organ primordia, in appropriate positions, and then the cells in these primordia have their fates (or the fates of their descendents) specified. We have developed a model for one part of this process, the part in which cells in undifferentiated organ primordia chose a particular organ fate. The model is derived from a series of genetic experiments, in which homeotic mutants have been studied. Each of these mutants has organ primordia that have chosen an inappropriate fate for their positions. By analyzing many alleles of four different loci, and by studying plants homozygous for mutant alleles at two or three of these loci, we have devised a preliminary model. As a test of the model we have started cloning the homeotic genes. So far we have a detailed analysis of only one of them (*AGAMOUS*, Yanofsky et al., 1990, Nature 346, 35), which appears to be a transcription factor with specific functions in a limited subset of the cells of the developing flower. The *AGAMOUS* gene is spatially regulated by another of the homeotic genes, so that the eventual form of the flower depends not only on the individual action of the four homeotic genes, but also on an interaction between two of the four homeotic genes so far studied. *AGAMOUS* is also a member of a large gene family, which includes many *Arabidopsis* genes that are specifically expressed in developing flowers, as well as a homeotic gene in another plant species, and transcriptional regulators of yeast and human genes.

**A 068** FLOWER DEVELOPMENT IN ANTIRRHINUM MAJUS (SNAPDRAGON)
HEINZ SAEDLER, PETER HUIJSER, HANS SOMMER, ZSUZSANNA SCHWARZ-SOMMER
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Flower development is still a mysterious process, which, however is now available for molecular analysis, due to the recent cloning of homeotic genes. Several, at least half a dozen, homeotic loci have been described in A. majus. The cloning and the properties of the mutants at two of these loci, deficiens and globosa is presented. Moreover, the cloning of a locus involved in flower induction (squomosa) will be reported. Taking all information together a rather complex network of interacting gene products assuring flower development emerges.

*Late Abstracts*

THE MOLECULAR BIOLOGY OF PHOTOSYSTEM II IN CHLAMYDOMONAS

Erickson, J. M., Domian, A., Goga, A., and Tahtakran, S. Department of Biology, University of California, Los Angeles, CA. 90024

The eukaryotic green unicellular alga, *Chlamydomonas reinhardtii*, provides an excellent system for investigating the molecular processes which regulate the expression of nuclear and chloroplast genes encoding polypeptides required for photosynthesis, and for dissecting the function of these polypeptides in the photosynthetic process. Mutants unable to photosynthesize can be maintained on medium containing acetate, and the specific blocks affecting regulatory processes at the transcriptional, post-transcriptional, translational and post-translational levels, can be studied. The combination of classical and molecular genetics has allowed for the study of Photosystem II (PSII) assembly (for review see refs. 1, 2) as well as a detailed analysis of the structure-function relationships in the D1 reaction center polypeptide of PSII (3). With the advent of transformation systems allowing for the integration and stable maintenance of exogenous DNA into the chloroplast genome (4) and the nuclear genome of *Chlamydomonas (5)*, it is now possible to directly investigate molecular processes involved in PSII gene expression and the functional role of PSII gene products. To this end, we have used a chloroplast transformation system in which the photosynthetic mutant *Chlamydomonas* strain, FuD7, deleted for both copies of the chloroplast psbA gene encoding D1, is transformed with exogenous, mutant copies of psbA, and the resultant transformants analyzed. *Chlamydomonas* has one single, large chloroplast per cell, and each chloroplast contains 50 - 80 copies of the circular chloroplast genome on which two copies of psbA are located. Our system ensures that no endogenous D1 will interfere with the functional analysis on the role of mutant D1 polypeptides. In addition to the studies on structure-function relationships in D1, we are using the transformation system to investigate the process of chloroplast RNA splicing, and the role psbA Group I intron sequences play in the splicing of psbA mRNA.

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TRYING TARGETED TRANSPOSON TAGGING IN TRANSGENIC TOMATO

Jonathan DG Jones[1], G Bishop[1], M Dickinson[1], J English[1], K Harrison[1], D Jones[1], S Scofield[1], I Taylor[2], C Thomas[1] [1] Sainsbury Laboratory, Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, UK & [2] School of Agriculture, Sutton Bonington, LE12 5RD, UK. The maize transposon *Ac* has been shown to be active in tobacco (1) and tomato (2). Genetic studies in tobacco have shown that increasing copy number of *Ac* results in increased excision frequency, unlike the situation in maize (3), and also that, as in maize, *Ac* usually transposes to linked sites (4).

It is debatable whether tagging genes in a heterologous system is best effected with an autonomous element or with a two element system. We have argued (5) that for targeted tagging in tomato, a one element system will be more efficient, whilst for non-targeted tagging, a two element system is preferable. We are currently generating multiple tomato transformants carrying *Ac* in various excision marker genes, using inverse PCR to amplify the adjacent plant DNA, and mapping this plant DNA on the tomato RFLP map. To date we have mapped 10 T-DNAs, and identified T-DNAs which place *Ac* adjacent to several target loci on chromosomes 1, 2 and 7. To measure tagging efficiency, we are trying to tag both ABA and anthocyanin biosynthetic genes linked to some our *Ac*-carrying T-DNAs.

In tomato, the SPT:*Ac* variegation is difficult to detect because tomato cotyledons usually bleach poorly on streptomycin. Cotyledons are spectinomycin sensitive, but our chosen spectinomycin resistance gene (6) appears to be non-cell autonomous, in that genotypically variegated individuals act as phenotypically completely resistant. An approach to overcoming this problem will be presented. Overall, our data indicate that *Ac* is more active in tomato than in tobacco.

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APPROACHES TO UNDERSTANDING PHYTOCHROME REGULATION OF TRANSCRIPTION, Elaine M. Tobin, Judy A. Brusslan, Jeffrey S. Buzby, George A. Karlin-Neumann, D.M. Kehoe, P.A. Okubara, Stephen A. Rolfe, Lin Sun, and Sharlene C. Weatherwax, Department of Biology, University of California, Los Angeles, CA 90024

In order to understand the way in which phytochrome action can affect transcription of specific genes, we have been studying genes that can be phytochrome regulated in *Lemna gibba*, an aquatic monocot. Additionally, we have examined *cab* gene expression in *Arabidopsis thaliana* and have devised selection schemes to isolate mutants in the phytochrome transduction pathway leading to transcriptional changes.

In *Lemna gibba*, both the *rbcS* and *cab* gene families are regulated by phytochrome action. We have also isolated three genes, designated NPR 1, 2 and 3, that are negativley regulated by phytochrome action. We have developed a transient assay system in intact *Lemna* fronds, utilizing the Biolistics/DuPont particle acceleration system, to analyze the promoters of these genes *in vivo*. Using a promoter from a particular rbcS gene, SSU5B, we showed that the expression of a reporter gene could be regulated by phytochrome in this system, and we have carried out a deletion analysis of this promoter. Similar experiments are being done with promoters from the *cab* and NPR genes. Concurrently, nuclear factors that interact with these promoter regions and may be involved in the phytochrome regulation are being characterized. A light-regulated factor (LRF-1) has been found to interact with a specific sequence upstream of the SSU5B promoter (Buzby et al., 1990), and deletion of this region resulted in loss of phytochrome regulation in the transient assay described above. A similar activity can be demonstrated to be present in nuclear extracts of *Arabidopsis thaliana*.

Two different strategies are being used to isolate *Arabidopsis thaliana* mutants that have altered transcriptional responses to phytochrome action. In the first of these, EMS mutagenized M-2 seeds have been screened for failure to germinate in response to red light. The second approach is a directed one utilizing a "suicide" selection scheme in which we can screen for mutant survivors of treatment with a toxic substrate. Plant lines selected under both strategies are being tested for phytochrome regulation of the endogenous *cab* genes, and those showing an altered transcriptional response of these genes will be characterized further.

Reference: Buzby, J.S., Yamada, T. and Tobin, E.M. (1990) Plant Cell **2**: 805-814.

*Genetic Control of Plant/Microbe Interactions*

**A 100** SOLUBLE NOD GENE-DEPENDENT FACTORS AND EARLY PLANT CELL RESPONSES IN THE *RHIZOBIUM MELILOTI-ALFALFA* SYMBIOSIS. E. Morrey Atkinson, David W. Ehrhardt, Kym F. Faull and Sharon R. Long, Department of Biological Sciences and Department of Psychiatry, Stanford University, Stanford, CA, 94305, USA.

Cell-free extracts of *R. meliloti* cause distortion of alfalfa root hair growth (Had activity). This activity is abolished by mutations in several bacterial genes required for nodulation (nod genes). We are fractionating *R. meliloti* cell exudates by HPLC. The purified fractions are then analyzed by FAB Mass Spectrometry and assayed for biological activities. These analyses indicate the presence of the previously described molecule, NodRm-1, as well as at least one different molecule in the active exudates.

We are investigating the sufficiency of small soluble molecules as nodulation signals. A related issue is the mechanism of action of bacterial signals: are they transduced to plant secondary messengers? We are using a localized inoculation technique to test whether the bacterial molecules that deform hairs also cause the cell divisions that characterize early nodulation. Some signal transduction processes include ionic fluxes: to ask whether these accompany nodulation, we are measuring the membrane potential of individual root hair cells in the infectible zone of the root with intracellular microelectrodes. We have observed a membrane depolarization within 2 minutes of the application of exudates from $Nod^+$ bacteria.

**A 101** REGULATION OF CALMODULIN AND ß-TUBULIN mRNA EXPRESSION DURING NODULE INITIATION AND DEVELOPMENT IN ALFALFA, Melanie J. Barnett, Barbara Krummel and Sharon R. Long, Department of Biological Sciences, Stanford University, Stanford, CA 94305. Nodule growth is a result of the symbiosis of leguminous plants and specific bacteria. Bacterial infection of plant roots acts as a trigger of dedifferentiation of root cortical cells and provides a model system for the study of plant gene expression. Little is known about the regulation of plant genes during the very early stages of nodule development which include cell elongation and division. We are currently examining the expression of two ubiquitous genes, calmodulin and ß-tubulin, in this complex process.

**Calmodulin** (cam) regulates the activity of a diverse group of proteins and is essential in basic cellular processes such as signal transduction, ion transport and cytoskeletal function. We previously reported the nucleotide sequence of a cam cDNA from alfalfa (*Medicago sativa*). This *cal1* mRNA is expressed in both shoot and root tissue. We are using *in situ* hybridization to obtain a detailed picture of cam mRNA expression during nodule development. Additional cam cDNAs, some of which show marked dissimilarity from the *cal1* cDNA, have been characterized. Genomic clones will be obtained to determine if these cDNA clones represent multiple loci. **Tubulins** are the major components of microtubules, structures that play an integral role in eukaryotic cell division and growth, and as such are good candidates for genes that respond to dedifferentiation signals. We are in the process of isolating and characterizing ß-tubulin cDNAs from an *M. sativa* library. These clones will be used to determine whether nodule specific and/or meristem specific expression occurs. Studies with *Medicago truncatula*, a diploid relative of *M. sativa* more amenable to genetic analysis, are also underway.

**A 102** CHARACTERIZATION OF AN *A. TUMEFACIENS* GENE RESPONSIBLE FOR PLANT PHENOLIC SIGNAL MODIFICATION, Linda A. Castle, John W. Morris, Kristin Smith, Richard Meilan and Roy O. Morris, Biochemistry Department, University of Missouri-Columbia, Columbia, MO 65211

Activation of the virulence genes of *Agrobacterium tumefaciens* by plant phenolic compounds, such as acetosyringone, is essential for T-DNA transfer. Coniferin, isolated from Douglas-fir needles, is able to induce *vir* genes in some *A. tumefaciens* strains but not in others (Morris and Morris 1990, PNAS 87:3614). Strains which are induced have a ß-glucosidase activity that hydrolyzes coniferin to coniferyl alcohol. A much larger group of strains is induced by coniferyl alcohol. Coniferin induction and ß-glucosidase activity correlate with strain infectivity on Douglas-fir. A ß-glucosidase gene from *A. tumefaciens* encoding an 88 KDa protein that is able to hydrolyze coniferin was cloned and sequenced. The deduced amino acid sequence is similar to several fungal ß-glucosidases. The biological role of this gene in signal modification and host range is being verified by 1) deletion of the gene from the parental strain, 2) introduction of the gene into strains lacking activity and showing low virulence on Douglas-fir, 3) *vir* gene induction analysis of the modified strains, 4) tumorigenesis assays on Douglas-fir and 5) characterization of the region upstream of the ß-glucosidase gene believed to be involved in regulation of the signal modification process. This work was supported by USDA grant #85-FSTY-9-0146.

**A 103** **MATING IN TETRAHYMENA THERMOPHILA: CILIARY PROTEINS OF THE WILD TYPE AND A MATING MUTANT,** Lee-ju Cheng and Linda A. Hufnagel, Department of Microbiology, University of Rhode Island, Kingston, RI 02881
Pair formation between complementary mating types of T. thermophila appears to be mediated first by inductive interactions ("costimulation") which likely occur via direct ciliary contact. Our laboratory has found that early interactions are blocked by the lectin, Con A, which binds to mannose-rich glycoproteins. A mating mutant of T. thermophila, strain RH179E1, isolated by R.L. Conner, is unable to participate in pair formation during mating, although it appears to costimulate cells of complementary mating type. Whole ciliary proteins were isolated from axenically grown cells of the mutant strain and from wild type strains Cu427 (mt VI) and Cu 428 (mt VII). Proteins from non-initiated (NI, non-starved), initiated (I, starved overnight in 10mM Tris buffer, pH 7.2) and 2 1/2 hr mating cells were separated on a modified Laemmli, one-dimensional LDS continuous (11-16%) polyacrylamide gel system. A major 43 kD protein was present in NI cells but absent in I cells, of both WT and mutant strains. Other minor protein differences were seen. Between proteins of I cells and mating cells, no differences were seen. Between WT and mutant there were some minor protein differences. No differences were found between the two mating types, either NI or I. Further studies are in progress to determine whether any of the WT or mating mutant ciliary proteins, in particular membrane proteins, bind Con A, and, if so, whether there are any changes in Con A binding proteins of cilia during pairing and co-stimulation of mating-competent cells.

**A 104** **Tn5 MUTAGENESIS OF Rhizobium sp. STRAIN G-20 AND CHARACTERIZATION OF SYMBIOTIC MUTANTS,** B.N. Dahiya, Sangeeta Mand and K. Lakshminarayana, Department of Plant Breeding & Microbiology, Haryana Agricultural University, Hisar 125004. India.

A cowpea Rhizobium strain G-20 exhibits high levels of ex planta nitrogenase activity in yeast extract mannitol agar medium and also nitrogen free medium under air, requiring no specific regulatory conditions. Tn5 mutagenesis of this strain with suicide plasmid PGS9 led to the isolation of four categories of symbiotic mutants. The properties of these mutants indicate that the nitrogen fixation and nodulation genes are plasmid borne and the control of nitrogenase activity in planta and in vitro could be different.

**A 105** EXPRESSION OF A BEAN CHALCONE SYNTHASE PROMOTER IN TRANSGENIC ALFALFA.
Karen Dalkin, Ian Dubery[1], Christopher J. Lamb[2] and Richard A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. [1]Department of Chemistry and Biochemistry, Randse Afrikaanse University, Auckland Park, Johannesburg, Republic of South Africa. [2]Plant Biology Laboratory, The Salk Institute for Biological Studies, 11010 N. Torrey Pines Road, La Jolla, CA 92037.
A number of plant defense response genes are under complex developmental and environmental regulation. In legumes, such as alfalfa (Medicago sativa L.) and bean (Phaseolus vulgaris L.) chalcone synthase (CHS) genes are involved in the biosynthesis of antimicrobial isoflavonoids during induced defense against pathogenic fungi and bacteria, in the formation of flavonoid compounds involved in flower pigmentation, UV-protection, hormone transport, and as rhizosphere signals for root nodulating bacteria. These multiple functions suggest that the regulation of CHS gene expression is likely to be multi-faceted. We have recently initiated a program to study the expression of defense and stress response genes in alfalfa, a species which is readily amenable to stable genetic transformation. Alfalfa cell suspension cultures, and protoplasts derived from them, respond to fungal elicitor with rapid increases in the activities of a number of enzymes, including CHS, involved in the synthesis of isoflavonoid phytoalexins. We describe a system for functional analysis of an elicitor-inducible bean CHS promoter in transgenic alfalfa plants. We compare expression of the transgene to the expression patterns of the endogenous alfalfa CHS genes, and describe the effects of 5'-deletions in the CHS promoter on developmental and environmental control of transgene expression.

**A 106** REGULATION OF *AGROBACTERIUM TUMEFACIENS* VIRULENCE GENE EXPRESSION. Anath Das, Gregory J. Pazour and Christopher N. Ta , Department of Biochemistry and Plant Molecular Genetics Institute, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108

The *vir*A and *vir*G genes of *Agrobacterium tumefaciens*, in conjunction with plant phenolics like acetosyringone, regulate expression of the virulence ( *vir*) genes. VirG, the transcriptional activator, interacts with a conserved vir box sequence to activate transcription. Several *vir*genes contain multiple vir box sequences in their 5' nontranscribed regions. To identify which of these vir boxes is required for transcriptional activation site-specific mutagenesis procedures were used. Analyses of the mutants indicated that only one of the five vir boxes of *vir*D is required for its expression. Both *vir*B and *vir*C require two vir boxes, only one of these is essential for expression. Mutational analyses indicated that the vir box is a tetradecameric sequence with a dyad symmetry.
To elucidate the mechanism of *vir*gene expression mutational approaches were used. We isolated nine mutations in *vir*A that support *vir*gene expression in the absence of an inducing agent. One of the mutations mapped to the putative first transmembrane domain. Further analyses of this mutant and of other mutants created within this transmembrane domain indicate that this segment is probably not a transmembrane domain as presumed earlier.

**A 107** TRANSFORMATION STUDIES ON *LATHYRUS ODORATUS* L. (SWEET PEA)
L. Fraser, J. Kent and C. Harvey. DSIR Fruit and Trees, Auckland, NEW ZEALAND.
The introduction of yellow into the colour range of sweet peas would be a desirable commercial attribute. Experiments to define a method of transformation for *L. odoratus* are therefore being carried out. An *Agrobacterium*-mediated transformation system was considered appropriate as preliminary studies showed gall formation with several virulent *Agrobacterium* strains. The cultivars Mrs Collier and Dorothy Eckford were co-cultivated *in vitro* with four *Agrobacterium* strains (A722, A281, C58, LBA4404) carrying the binary plasmid pKIWI110. pKIWI110 carries a gene conferring kanamycin resistance and a GUS gene expressed only upon transfer to plant cells and not in *Agrobacterium*. Expression of the GUS reporter gene was observed primarily in the terminal 2-3 cm of the growing tips of seedlings 4 days after co-cultivation.
Mrs Collier gave a higher incidence of GUS expression than Dorothy Eckford. Two parameters improved transformation efficiencies; preculture of tissue for 2 days, and use of acetosyringone (20μM) in bacterial inoculum and co-cultivation plates. A range of media and hormone conditions are currently being investigated to obtain regeneration of shoots from stem and leaf explants.

**A 108** DEVELOPMENTAL REGULATION OF CALMODULIN AND *cdc2* GENE EXPRESSION DURING SYMBIOTIC ROOT NODULE FORMATION, Margaret Gawienowski and Thomas Jacobs, Department of Plant Biology, University of Illinois, Urbana, IL 61801.

Symbiotic root nodulation of legumes is initiated by a progression of plant-bacterial signal exchanges which culminate in the formation of the new plant organ. We have used the early development of root nodules on alfalfa as a model system for the study of the expression of genes potentially involved in signal transduction, mitotic induction, and meristem formation. Two regulatory genes, calmodulin (CAM) and *cdc2* (see poster by Feiler, Prewett and Jacobs) have been selected, based on their hypothesized roles in signal transduction and mitotic induction, respectively. We have cloned and sequenced a calmodulin gene from a nodulated alfalfa root cDNA library. A pea *cdc2* gene was obtained using pea epicotyl hook cDNA as a template for PCR synthesis. The pea *cdc2* probe detects a unique mobility species of the expected molecular weight on northern blots of alfalfa RNA. Levels of CAM and *cdc2* transcripts in *Rhizobium*-inoculated roots will be quantified by slot blot analysis. Total RNA was purified from alfalfa root segments 1, 3, 7, and 23 days following inoculation with *Rhizobium meliloti* strain 1021. The time course of expression of two nodule specific genes, ENOD2 and leghemoglobin, an early and a late nodulin, respectively, are also monitored in order to place CAM and *cdc2* expression on the nodulation time line.

**A 109** TURNIP CRINKLE VIRUS GENE PRODUCTS REQUIRED FOR VIRAL REPLICATION, MOVEMENT, AND ASSEMBLY. David Hacker, Ian T. D. Petty, Ning Wei, and Thomas J. Morris. Department of Plant Pathology, University of California, Berkeley, CA 94720.
Turnip crinkle virus is a member of the carmovirus group and has a single-component, positive-sense RNA genome of 4.0 kb. Sequence analysis has revealed the presence of 5 ORFs which could encode polypeptides of >7kd. Mutational analyses have shown that ORF1 (p27) and ORF2 (p88), the readthrough ORF, are the only viral gene products required for replication of the viral genome and for synthesis of the two subgenomic RNAs in protoplasts. Mutations in either ORF3 or ORF4, each of which could encode a protein of 7 kd, demonstrate that these two proteins are involved in cell-to-cell movement of the virus in plants. Neither of these proteins, however, has a role in viral replication or assembly as determined by the production of wild-type levels of viral RNA and virions in protoplasts. The coat protein (p37) is the product of ORF5 and is involved in virus assembly, but mutations in this gene also prevent systemic movement of the virus in plants.

**A 110** THE 17 KD PRODUCT OF THE PEA DISEASE RESISTANCE RESPONSE GENE 49 ACCUMULATES IN THE HETEROCHROMATIC REGIONS OF HOST NUCLEI ADJACENT TO THE FUNGAL CHALLENGE, Lee A. Hadwiger, Becky S. Allaire, and Chin C. Chiang, Department of Plant Pathology, Washington State University, Pullman, Washington 99164-6430.
Gene 49 in peas is a major disease resistance response gene with homologs in parsley, potato, birch, alder and soybeans. mRNA and protein products of this gene accumulate in temporal correlation with the expression of either the specific resistance of peas to races of Pseudomonas syringae pv. pisi or with non-host resistance to Fusarium solani f. sp. phaseoli (Fsph) the function of this gene is unknown. For additional insight into function we have used anti-gene 49 antisera in immunocytological procedures designed to follow the localization of the gene 49 product. The 17 Kd product accumulates in pea endocarp cells adjacent to the challenges of individual Fsph spores. Intracellular accumulations occur in the heterochromatin of nuclei and in secondary wall thickenings of xylem cells. Accumulations were also evident in the heterochromatin of cells near vascular elements in non-treated tissue. The effect of selective accumulation of gene 49 product on nuclear structure will be discussed in relationship to the hypothesis that disease resistance is assisted by maintaining host cell viability.

**A 111** REGULATION OF TRANSCRIPTION FACTORS FOR A BEAN CHALCONE SYNTHASE GENE. Maria J. Harrison, Christopher J. Lamb[1], Richard A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. [1]Plant Biology Laboratory, The Salk Institute for Biological Studies, 11010 N. Torrey Pines Road, La Jolla, CA 92037.
Chalcone synthase (CHS) catalyzes the first committed step in the branch of the phenylpropanoid pathway specific for flavonoid and isoflavonoid compounds. In order to understand the complex developmental and environmental control of the CHS gene, transcription factors specific for cis-elements which show functional significance are being characterized and cloned. A factor which binds to 3 sequences in the distal portion of the λ CHS15 promoter (-326 bp to -170 bp) has been implicated in modulating the overall levels of expression. This factor, SBF-1, has been purified and the production of antibodies is currently in progress. In vitro treatment of SBF-1 with alkaline phosphatase results in loss of binding activity. Studies on the rephosphorylation of SBF-1 to establish the significance of this potential control mechanism are being pursued. Functional analysis in an electroporated protoplast system has implicated the region between -173 bp and -130 bp in the upregulation of the promoter by the pathway intermediate cinnamic acid. A nuclear DNA binding activity which specifically recognizes a sequence in this region has been partially characterized and the recognition sequence is being used to screen expression libraries to directly clone this factor.

**A 112** TOWARDS TRANSPOSON TAGGING OF THE TMV RESISTANCE GENE *N* USING THE MAIZE CONTROLLING ELEMENTS *Ac* AND *Ds* IN TRANSGENIC TOBACCO, Reinhard Hehl[a] and Barbara Baker[a,b], [a]Plant Gene Expression Center, USDA-ARS/UC Berkeley, 800 Buchanan St, Albany CA 94710. [a,b]Department of Plant Pathology, UC Berkeley, Berkeley CA 94720

In tobacco cultivars that harbor a dominant resistance gene *N*, challenge by tobacco mosaic virus (TMV) infection produces necrosis at and around each point at which the tissue was infected. This hypersensitive response has been well studied in genetic and biochemical terms, yet the HSR remains a poorly understood process in many respects. Our work has focused on the isolation of the *N* gene using the maize transposable elements *Ac* and *Ds* for transposon tagging in transgenic tobacco. Transposon tagging requires that a large number of heterozygous seedlings can be screened effectively for loss of *N* gene function caused by insertion of a transposable element. We have developed a very efficient positive selection method for loss of *N* gene function. We employ conditions under which seedlings harboring a functional *N* gene die after TMV infection and temperature treatment. TMV infected seedlings void of *N* or seedlings that lost *N* gene function survive. During development of this screen we determined the degree of *N* gene instability to be one in $10^{-3}$. Loss of *N* gene function is stable since no reversion is observed in large F1 populations derived by selfing and out-crossing of plants that lost *N* gene function. This *N* gene instability has to be taken into account for transposon tagging strategies. To increase the probability for tagging *N* we attempt to link a nonautonomous *Ds* element that harbors a selectable marker gene (NPTII) to *N*. This element ($Ds^{neo}$) is inserted into the dominant selectable marker gene HPT. We have transformed heterozygous Nn tobacco with a T-DNA construct harboring $Ds^{neo}$ and are currently in the process of identifying transgenic tobacco plants in which the *Agrobacterium* T-DNA cosegregates with the resistance gene *N*. More than 300 independent T-DNA transformants are under investigation.

**A 113** MECHANISM OF PHENOLIC ACTIVATION OF *VIR* EXPRESSION IN A. TUMEFACIENS: DEVELOPMENT OF SPECIFIC INHIBITORS, Kathleen M. Hess, Matthew W. Dudley, David G. Lynn, Searle Chemistry Laboratory, 5735 Ellis Ave, The University of Chicago, Chicago IL 60637; Rolf D. Joerger, Andrew N. Binns, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6017.

An unusual series of phenolic growth factors, the DCGs, have been discovered in crown gall tumors. These factors are able to substitute for some of the hormone requirements of tobacco cell division. While these factors do not conform with the previously established structural requirements necessary for the activation of *vir* expression in *Agrobacterium tumefaciens*, they are potent inducers of *vir* expression. A systematic evaluation of the structural requirements of these inducers has led to a theory detailing the role of the phenolics in the induction process. Using this theory, the first specific inhibitors of *vir* induction have been developed. These inhibitors do not effect the induction of other genes on the Ti plasmid, but irreversibly block *vir* expression. This poster will discuss the development of these inhibitors, the perspective that they provide on the mechanism of the signal transduction events, and how they may be used to understand plant cell growth.

**A 114** RFLP MARKERS LINKED TO A NEMATODE-RESISTANCE GENE, *Mi*, IN TOMATO, Jeong-Yau Ho and Valerie M. Williamson, Department of Nematology, University of California, Davis, CA 95616

A single, dominant gene, *Mi*, conferring resistance to root-knot nematodes, was introgressed from the wild spieces *Lycopersicon peruvianum* to the cultivated tomato *L. esculentum*. This gene is tightly linked to one of the acid phosphatase isozymes, *Aps1*. The gene product of the $Aps1^1$ allele, which was introduced from *L. peruvianum* along with the *Mi* gene, shows different eletrophoretic mobility from that of *Aps1*+ allele, which originally exists in the cultivated tomato. *Aps1* thus serves as a useful marker in breeding tomatoes for nematode resistance. However, more genetic markers are needed to facilitate breeding programs and for chromosome walking toward the *Mi* gene. To identify RFLP markers surrounding the *Mi* gene, tomato DNA inserts were isolated from cDNA clones and screened against the restriction digestion pattern of the genomic DNA of nearly-isogenic tomato lines which differ in resistance to nematodes. The cDNA clones, which exibit RFLPs between resistant and susceptible lines, were further tested for linkage to the *Aps1* gene in a segregating F2 population. Among 397 screened cDNA clones, four RFLP markers were linked to *Aps1*. Three of them were only present in resistant cultivars. Detailed mapping is in process.

**A 115** A PUTATIVE AVIRULENCE GENE FROM XANTHOMONAS ORYZAE PV. ORYZAE IS A HOMOLOG OF THE ESCHERICHIA COLI GENE FOR A PHOSPHATE-BINDING PROTEIN, Christopher M. Hopkins, Frank F. White, and Jan E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506

A 2.5 kb DNA fragment cloned from Xanthomonas oryzae pv. oryzae (Xoo) contains a functional avirulence gene (avr10) that corresponds to the resistance gene Xa-10 in rice. The nucleotide sequence of an open reading frame (ORF) within the 2.5 kb fragment was found to be related to the phoS gene of Escherichia coli which encodes a periplasmic phosphate-binding protein. The ORF was cloned into the T7 expression vector pT7-7 and protein was produced in E. coli by induction of the T7 RNA polymerase gene. Polyclonal antisera were raised in rabbits to a protein of MW 36 kd found in the periplasmic cell fraction of the E. coli expression strain. The antibodies have been shown by western blot analysis to react with a periplasmic protein of Xoo with a MW corresponding to the putative avr10 protein. Studies are in progress to determine if this protein binds phosphate and whether expression is regulated by phosphate (similar to E. coli) and in a race-specific manner.

**A 116** FUNCTION OF THE *lemA* LOCUS IN TWO PATHOVARS OF *Pseudomonas syringae*, Estelle M. Hrabak[1], Terese M. Barta[1] and David K Willis[1,2], [1]Dept. of Plant Pathology and [2]USDA/ARS, University of Wisconsin, Madison, WI 53706

All pathovars of *P. syringae* tested to date contain DNA which hybridizes to the *lemA* locus. In *P. s.* pv. *syringae*, the *lemA* locus is required for lesion formation on bean (*Phaseolus vulgaris*) and production of syringomycin (SR) and protease (PR) *in vitro*. Mutations in *lemA* do not affect plant-associated growth or the hypersensitive reaction on nonhost plants. The *lemA* locus is approximately 3 kb in length, as determined by subcloning and Tn3-HoHo1 mutagenesis. In pv. *coronofaciens*, a tabtoxin-producing pathogen of oats, mutagenesis of *lemA* abolishes the ability to make tabtoxin, but does not affect lesion formation. A clone containing the *lemA* locus from pv. *syringae* restores tabtoxin production. Conversely, the *lemA* locus from pv. *coronofaciens* restores lesion formation and SR and PR production to a *lemA* mutant of pv. *syringae*. Using a probe containing part of the tabtoxin biosynthetic genes, we found that *lemA* is required for at least one toxin mRNA to be detected. Thus, *lemA* may function as a positive transcriptional regulator.

**A 117** IDENTIFICATION AND CHARACTERIZATION OF A GENE FROM *PSEUDOMONAS SYRINGAE* PV. *TOMATO* THAT DETERMINES AVIRULENCE ON BOTH *ARABIDOPSIS* AND SOYBEAN, Roger W. Innes, Andrew F. Bent, and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720

We are using *A. thaliana* to study the molecular genetic basis of "gene-for-gene" disease resistance. In a gene-for-gene interaction, resistance is determined by a single plant "resistance gene" that is in some way specific to a single pathogen "avirulence" gene; loss of either member of this gene pair results in plant disease. We have found that *A. thaliana* ecotype Col-0 is resistant to the bacterial pathogen *P. syringae* pv. *tomato* (*Pst*) strain 1065, but is susceptible to *Pst* strain DC3000. We therefore tested whether an avirulence gene(s) in strain 1065 controlled induction of resistance in *A. thaliana*. A cosmid that determined avirulence was isolated from a genomic library of strain 1065 by mobilization of the library into strain DC3000 and assaying for conversion to avirulence on *A. thaliana* Col-0. The avirulence activity of this cosmid was subcloned to 1.4 kb and sequenced. The sequence revealed a single open reading frame, which we have designated *avrRpt2*. Cloning of *avrRpt2* predicts a corresponding resistance gene (*Rpt2*) in *A. thaliana* Col-0. As a first step towards genetically mapping *Rpt2*, we have identified *A. thaliana* ecotypes that are susceptible to strain DC3000 expressing *avrRpt2*. These susceptible ecotypes, Hs-0 and Po-1, were crossed to the resistant ecotype Col-0. Segregation of resistance to DC3000(*avrRpt2*) in the F2 generation is now being analyzed

We also moved the *avrRpt2* locus from *Pst* strain 1065 into the soybean pathogen *P. syringae* pv. *glycinea* to test whether this locus could determine avirulence on soybean. The resulting strain induced a resistant response in a cultivar-specific manner, suggesting that similar resistance mechanisms may function in *A. thaliana* and soybean.

**A 118** TOWARDS CLONING PATHOGEN GENES DETERMINING PLANT RESISTANCE: CHROMOSOME WALKING TO AVIRULENCE LOCI IN *BREMIA LACTUCAE* (LETTUCE DOWNY MILDEW) AND GENE TRANSFER IN *B. LACTUCAE* AND *PHYTOPHTHORA INFESTANS*. Howard S. Judelson and Richard W. Michelmore, Dept. of Vegetable Crops, University of California, Davis, CA 95616.

In lettuce downy mildew host resistance is determined by interacting pairs of resistance loci in lettuce and genes for avirulence *(Avr)* in the oomycete *B. lactucae*. Five *Avr* genes have been placed on the genetic (RFLP) map of *B. lactucae*. *Avr* genes will be cloned by walking from linked RFLP loci using cosmid, phage, and YAC libraries. Ultimately, clones will be introduced into virulent isolates of the fungus to test for functional *Avr* genes. Procedures for transformation are therefore being developed. Initially, vectors were constructed containing fusions between 5' and 3' sequences from the highly expressed *hsp70* and *ham34* genes of *B. lactucae* and resistance (NPTII, HPT) or reporter genes (GUS). Since *B. lactucae* can not be grown in artificial media, traditional methods for selection and for transformation using protoplasts are not feasible. Instead, DNA is being introduced by microparticle bombardment and transformants will be selected on drug-imbibed cotyledons.

The *ham34* and *hsp70*-derived vectors are also being used in an attempt to transform another oomycete, *Phytophthora infestans*. These experiments will verify the function of our vectors and facilitate the molecular analysis of the late blight diseases. Vectors containing fusions between GUS and the *hsp70* or *ham34* regulatory elements were introduced into protoplasts using electroporation, polyethylene glycol and $CaCl_2$, or cationic liposomes. The transient expression of GUS was detected using both the *ham34* and *hsp70*- derived vectors, and with each method for DNA uptake. Transient assays were used to identify the superior vectors for transformation and optimal methods for introducing DNA.

**A 119** GENETIC AND MOLECULAR ANALYSIS OF XANTHOMONAS CAMPESTRIS PATHOGENESIS, Sophien Kamoun, Haresh Kamdar, and Clarence I. Kado, Department of Plant Pathology, University of California, Davis, CA 95616

Xanthomonas campestris pv. campestris (Xcc) causes the black rot disease on crucifers, and elicits a hypersensitive response (HR) on nonhost plants. The hrpXc gene, a member of the hrp family, is required for pathogenicity as well as for HR induction. HrpXc mutants of Xcc were shown to be complemented by coinoculation with wild-type strains, suggesting that this gene may encode for an exocellular component. The molecular analysis of hrpXc has been conducted and the gene was localized to a 1.5 kb. DNA fragment. Subcloning of hrpXc into promoter probe vectors allowed the isolation of the hrpXc promoter. Transcriptional fusions of hrpXc to lux, cat, and uidA (GUS) reporters were constructed and used to demonstrate that the expression of hrpXc is strongly induced when the bacteria is present in crucifer xylem. Southern blot hybridizations indicated that the hrpXc locus is conserved among all the examined X. campestris pathovars including pv. armoracae, vesicatoria, vitians, and oryzae. The hrpXc homologs of Xcv and Xco have been cloned and their role in pathogenicity and HR induction is being determined. We have also developped a marker-insertion system for the mutagenesis of hrpXc in several Xc strains. Preliminary evidence suggests that hrpXc is a basic pathogenicity gene required for the survival and growth of X. campestris in plant tissue.

**A 120** GENETIC AND MOLECULAR ANALYSIS OF RESISTANCE OF ARABIDOPSIS TO *PSEUDOMONAS SYRINGAE*.

**Barbara N. Kunkel, Andrew F. Bent, Roger W. Innes and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720.**

***Arabidopsis thaliana*** **has recently been established as a model host in which to study the molecular genetic basis of disease resistance to the bacterial pathogen *Pseudomonas syringae*. Two approaches are being taken to identify and characterize plant genes that control the specificity of disease resistance and the subsequent expression of defense genes. First, we are in the process of isolating *Arabidopsis* mutants that are altered in their ability to express disease resistance to *P. syringae* pv. *tomato* (*Pst*) strain DC3000 bearing the avirulence gene *avrRpt2*. We expect to uncover mutations both in the corresponding plant resistance gene *Rpt2* and in genes that are required for expression of resistance subsequent to recognition of the pathogen.**

**The second approach involves the direct differential screening of an *Arabidopsis* genomic library using mixed cDNA probes synthesized from RNA isolated either from uninoculated *Arabidopsis*, or from plants that have been inoculated with isogenic virulent (DC3000) or avirulent (DC3000 containing *avrRpt2*) *Pst* strains, and are thus exhibiting susceptible or resistant responses, respectively. Comparison of the hybridization patterns should allow the identification of genes that are expressed specifically in each interaction. Our progress towards identifying genes that regulate expression of disease resistance by both of these methods will be presented.**

**A 121** PHENYLAPROPANOID PATHWAY INTERMEDIATES REGULATE TRANSIENT EXPRESSION OF A CHALCONE SYNTHASE GENE PROMOTER IN ELECTROPORATED PROTOPLASTS. Gary Loake[1], Arvind Choudhary[1,3], Maria Harrison[1], Ouriel Faktor[2], Christopher J. Lamb[2] and Richard A. Dixon[1]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. [2]Plant Biology Laboratory, The Salk Institute for Biological Studies, 11010 N. Torrey Pines Road, La Jolla, CA 92037. [3]Department of Botany, Nagpur University Campus, Nagpur 440010, INDIA.
Chimeric constructs containing promoter sequences from the bean CHS15 gene fused to a CAT reporter gene were introduced into alfalfa protoplasts by electroporation. CAT activity was determined for a series of 5' and 3' deletions and specific mutations of the CHS15 promoter following treatment of the protoplasts with fungal elicitor and a range of concentrations of a specific phenylpropanoid pathway intermediate. Regions of the CHS promoter have been identified which affect quantitative expression in alfalfa cells, but do not alter elicitor inducability. Using co-electroporation and in vitro footprinting techniques, elements within these regions have been identified which mediate down-regulation by cinnamic acid, the product of the PAL reaction, and up-regulation by p-coumaric acid, the second intermediate in the phenylpropanoid pathway.

**A 122** THE DCG GROWTH FACTORS: THE CHARACTERIZATION OF THE ENZYMES INVOLVED IN THEIR BIOSYNTHESIS, David G. Lynn, John D. Orr, 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 dehydrodiconiferyl alcohol glycosides were discovered in Agrobacterium-transformed Vinca tissue as compounds that could replace some of the requirements for plant hormones in tobacco cell growth. This discovery has provided a system where the biochemical controls on the production of these phenolic signal compounds might be investigated. This poster will describe studies that have allowed for the definition of the individual enzymatic steps involved in the synthesis of the DCGs. The implications of this pathway in the control of plant cell growth and Agrobacterium transformation will also be discussed.

**A 123** EXPRESSION OF DEFENSE-RELATED HYDROLASES IN ALFALFA. Eileen A. Maher[1,2], Christopher J. Lamb[2] and Richard A. Dixon [1]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. [2]Plant Biology Laboratory, The Salk Institute for Biological Studies, 11010 N. Torrey Pines Road, La Jolla, CA 92037.
Chitinase and glucanase are expressed in plants in response to infection by plant pathogens. To determine whether chitinases and glucanases have a role in plant defense, we plan to evaluate transgenic plants that have altered expression of these hydrolases. In alfalfa, both chitinases and glucanases constitute small gene families and several enzyme forms have been detected in plant extracts. Alfalfa cDNA libraries from inoculated plants and elicited cell suspension cultures have been probed with an acidic glucanase cDNA and a putative chitinase cDNA from alfalfa to isolate additional members of these gene families. Gene-specific probes for chitinases and glucanases will be designed to evaluate gene expression in plant tissues from susceptible and resistant cultivars.

**A 124** PROGRESS IN MAP BASED CLONING OF MATCHING RESISTANCE AND AVIRULENCE GENES FROM LETTUCE DOWNY MILDEW., Richard W. Michelmore, Richard V. Kesseli, Howard S. Judelson, Ilan Paran, David M. Francis, Marc Fortin and Lore Westphal. Department of Vegetable Crops, University of California, Davis, CA 95616.
The genetic bases of specificity in the gene-for-gene interaction between lettuce (*Lactuca sativa*) and its obligate fungal pathogen (*Bremia lactucae*) is being determined using parallel studies of both organisms. Classical genetics has defined the numbers of interacting genes. Thirteen single matching resistance genes (*Dm*) are clustered in four linkage groups. Detailed genetic maps have been generated for both organisms predominantly using RFLP markers. Regions containing *Dm* genes are being saturated with markers using a variety of techniques including screening near-isogenic lines with bulks of random probes and with RAPD primers. The relationship between genetic and physical distance in the target regions is being determined by developing long-range restriction maps and analyzing the segregation of polymorphisms in these maps relative to recombination between *Dm* genes. Chromosome walking will be conducted using a variety of genomic libraries; partial YAC libraries have been constructed for both organisms and the inserts are currently being characterized. Progress of the walks will be monitored using segregation analysis. Ultimately, sequences which absolutely cosegregate with resistance or avirulence will be tested for function in transgenics. Transformation of lettuce mediated by *Agrobacterium tumefaciens* is routine; transformation for *B. lactucae* is under development.

**A 125** REGULATION OF L-PAL BY CINNAMIC ACID IN LEGUMES. John D. Orr, Robert Edwards and Richard A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402.
Addition of trans-cinnamic acid to alfalfa cell suspension cultures after their treatment with fungal elicitor resulted in the loss of induced phenylalanine ammonia-lyase (PAL) enzymatic activity and a decrease in the levels of PAL mRNA. Treatment of elicited cultures with L- α-aminooxy-phenylpropionic acid (AOPP) (a potent and specific inhibitor of PAL activity in vitro, and therefore an inhibitor of cinnamic acid accumulation) resulted in superinduction of PAL enzymatic activity and increased levels of PAL mRNA. These data have led to the hypothesis that cinnamic acid or a related metabolite acts as an endogenous regulator of flux through the phenylpropanoid biosynthetic pathway. To test this hypothesis, the levels of free cinnamic, p-coumaric, caffeic, and ferulic acids were quantified by high resolution gas chromatography during elicitation in the presence or absence of AOPP. Variations in the concentrations of phenylpropanoid intermediates were correlated with changes in the levels of PAL mRNA and PAL enzyme activity. The mechanism of the regulation of PAL mRNA species by cinnamic acid was examined. Specific inhibitors of transcription and translation were used to determine if exogenous treatments of cinnamic acid effected a blockage of PAL transcription or if cinnamic acid activated the selective turnover of PAL mRNA.

**A 126** MICROBIAL GENES FOR FUSARIC ACID DEGRADATION AND THEIR USE FOR THE PRODUCTION OF DISEASE RESISTANT PLANTS, Seiji Ouchi, Hideyoshi Toyoda, Yoshinori Katsuragi and Ryutaro Utsumi, Faculty of Agriculture, Kinki University, Nara 631, Japan
Fungal pathogens produce an array of compounds for their establishment in plant tissues, including toxins which are,in one way or the other,involved in the expression of symptoms in host plants. Fusaric acid, 5-n-butylpicolinic acid(FA), is one of the common metabolites of Fusarium spp. which invade,in many cases, xylem vessel through root system causing epinasty, plugging and browning of xylem, wilting and other symptoms in various plants, hence has been implicated in the expression of these symptoms. One of bacterial isolates from soil, subsequently identified as Klebsiella oxytoca, was capable of catabolizing FA. The genes for FA degradation of this bacterium was cloned into E. coli by the use of pUC19. Subcloning and deletion experiments indicated that DNA fragment of 3.6kb derived from Bam HI and Hind III digestion(BH fragment) was responsible for the degradation. The BH fragments reciprocally inserted into pUC19(pFAR-1) and pUC18(pFAR-2) were equally functional and did not require isopropyl- -D-galactopyranoside, suggesting that the fragment contains an intrinsic promotor. Base sequence analysis and amino acid assignment to the sequence suggested that this sequence contained at least three open reading frames(ORF). Souther blots with probes containing respective ORF sequence suggested that the 3.6kb BH fragment is transcribed to a polycistronic mRNA. Isolation and characterization of the gene products are underway. On the basis of our previous finding that preliminary application of FA-detoxifying bacteria protected tomato plants from Fusarium wilt, the use of this DNA fragment for the production of FA-resistant and most probably Fusarium-resistant plants will be discussed.

**A 127** MULTIPLE LOCI REQUIRED FOR LESION FORMATION IN *Pseudomonas syringae* pv. *syringae*, Jessica J. Rich[1,2] and David K. Willis[1,3], [1]Departments of Plant Pathology and [2]Molecular Biology and [3]ARS/USDA, University of Wisconsin, Madison, WI 53706

We are studying genes involved in disease lesion formation on host plants by *Pseudomonas syringae* pathovars. The *lemA* locus of *P. s. syringae*, the causal agent of bacterial brown spot disease of bean, is required for lesion formation as well as production of the toxin, syringomycin; the ability to induce the hypersensitive reaction on the nonhost tobacco is not altered in a *lemA* mutant. Clones from both *P. s. syringae* and *P. s. phaseolicola* genomic libraries restored the defects of the *P. s. syringae lemA* mutant. Introduction of the *lemA1*::Tn*5* mutation into the *P. s. phaseolicola* chromosome, however, did not disrupt production of disease symptoms or of the toxin, phaseolotoxin. Sixteen additional Tn*5* mutants of *P. s. syringae* were identified that did not form lesions on bean, yet retained the ability to induce the hypersensitive response on tobacco. Nine mutants failed to produce syringomycin; none of the mutants were closely linked to the *lemA* locus. DNA sequences flanking the Tn*5* insertion within one mutant were isolated by inverse PCR amplification using a primer extending outward from the inverted repeats of Tn*5*. Complementing clones were isolated from a wild-type genomic library using the obtained PCR fragment as a hybridization probe. We are investigating potential regulatory and linkage relationships between these loci required for lesion formation.

**A 128** AN ALTERED LEVEL OF DEFENSE GENE EXPRESSION IN VERTICILLIUM RESISTANT TOMATO, Jane Robb, Shin-Woo Lee, Douglas A. Powell, and Ross N. Nazar, Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Enzyme activities and mRNA levels for phenylalanine ammonia-lyase (EC 4.3.1.5) were compared in two nearly isogenic lines of tomato which differed significantly in their resistance to colonization by the wilt fungus, *Verticillium albo-atrum*, a common destructive pathogen of many economically-important plants. The degree of vascular coating which is an early defense response correlated closely with increases in levels of phenylalanine ammonia-lyase activity, essential to the biosynthesis of vascular coats. In contrast, levels of mRNA did not increase proportionally; rather, there was a substantial suppression of mRNA levels in the susceptible tomato line, consistent with a much lower elevation of phenylalanine ammonia-lyase activity and significantly less vascular coating. The results raise the possibility that the pathogen can suppress defense gene expression in susceptible plants and suggest that post-transcriptional regulation plays a significant role in defense gene expression.

**A 129** ACTIVITY OF A PLANT ENCODED DSRNA DEPENDENT PROTEIN KINASE IS TEMPORALLY REGULATED BY TMV INFECTION, Don Roth and Jie Hu, Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071-3354

Nucleotide binding and protein phosphorylation are fundamental events in the regulation of cell metabolism. Post-translational protein phosphorylation offers an effective mechanism by which plant pathogens can regulate host metabolism during pathogenesis. We have previously identified and characterized a host encoded dsRNA dependent protein kinase (p68) which is autophosphorylated in response to plant virus and viroid infection. In order to determine if the activity of p68 is temporally regulated by virus infection, protoplasts were synchronously infected with TMV. The enzymatic activity of p68 is elevated 6-8-fold over basal levels observed in mock inoculated protoplasts at 3-4 hr. post-inoculation. Activity reaches a maximum 6-8 hr. post inoculation and then slightly declines to steady state levels 2-fold higher than in comparable mock inoculated protoplasts. Similar activity response curves are observed when TMV RNA or TMV dsRNA (purified from infected leaf tissue) were electroporated into protoplasts. However, no induction of activity is observed in protoplasts electroporated with total RNA or mRNA from uninoculated plants. The induction response of p68 to TMV infection correlates with virus replication and decreased host protein synthesis.

**A 130** THE PARASITIC PLANTS: INSIGHTS INTO THE MECHANISMS FOR THE ACTIVATION OF CELLULAR DIFFERENTIATION, Tom Ruttledge, Zhaoxian Zeng, David G. Lynn, Department of Chemistry, 5735 Ellis Avenue, The University of Chicago, Chicago, IL 60637.

A group of plants that have been found across 20 different families have evolved the capability of parasitizing other plant species. These parasitic angiosperms respond to phenolic signals from the host plant and initiate the development of a unique organ, the haustorium, that functions to physically attach the parasite to the host. This signal transduction system presents an opportunity to dissect the biochemical mechanisms for eucaryotic development as well as to develop a better understanding of the details of the host/parasite interaction. The definition of the initial signal transduction molecules has led to the development of a biochemical mechanism for signal detection. This poster will discuss the use of specific inhibitors of haustorial development based on this mechanism to delineate some of the details of this developmental process.

**A 131** MOLECULAR GENETICS OF RESISTANCE OF TOMATO TO PSEUDOMONAS SYRINGAE. John Salmeron, Francine Carland, Brian Kearney, Doug Dahlbeck and Brian Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA, USA 94720.

Resistance of tomato to bacterial speck disease is determined by the host resistance *Pto* and the corresponding pathogen avirulence gene *avrPto*, in a classic "gene-for-gene" relationship. We are investigating the basis of bacterial speck resistance by cloning and characterizing these genes. The *avrPto* gene has been cloned and found to encode a 164 amino acid protein of 18.3 kilodaltons. We report here that *avrPto* belongs to a subset of avirulence genes which are dependent upon specific products of the *P. syringae hrp* gene cluster for transcription. *P. syringae hrp* genes are required both for virulence on host plants and induction of resistance responses on non-host plants. The *avrPto* promoter contains a motif found upstream of other *hrp* regulated genes, and this motif appears to be required for *avrPto* transcription.

We are taking two approaches towards cloning the corresponding tomato resistance gene *Pto*. First, we have generated 25 transformed *Pto/Pto* lines containing the maize transposable element *Ac* that we will use in a transposon tagging strategy. We have developed a rapid and facile method of plant inoculation that permits screening the large numbers of individuals required for transposon tagging. Second, we are attempting to isolate DNA clones at or near the *Pto* locus by subtracting a genomic library of a *Pto/Pto* line against one of a near-isogenic line lacking the *Pto* gene. Our progress toward cloning *Pto* by each of these methods will be reported.

**A 132** MOLECULAR CHARACTERISATION OF BEHAVIOURAL GENES FROM *AGROBACTERIUM TUMEFACIENS*, Charles H. Shaw, Adrian P. Brown, Gary J. Loake, and Christine S. Garrett, Department of Biological Sciences, University of Durham, Durham, DH1 3LE, UK.

Chemotaxis is now established as the initial step in crown gall tumourigenesis. Thus *virA* & *G* are required to guide rhizosphere *Agrobacterium tumefaciens* towards wounded plant cells in response to chemoattractant *vir*-inducers. We are investigating the molecular basis of this attraction.

20 Tn5-induced mutants have been used to define the basic features of motility in this organism. A polar tuft, of 2 flagella, plus 2-4 lateral flagella rotate unidirectionally to drive the bacterium at speeds of up to 60μm. $s^{-1}$. Non-motile mutants are impaired in root colonisation. Using Tn5-flanking sequences, we have mapped 13 of the mutants to 4 non-overlapping cosmids, one of which carries at least 8 separate loci concerned with motility. None of the identified loci shows any hybridisation to *E.coli* DNA, but variable degrees of similarity to *Rhizobium*. These loci are being subcloned and sequenced.

Despite considerable differences to the Enterobacteriaceae, similarities are evident in the signal transduction pathway. Thus, methionine is required for chemotaxis, methyl groups being transferred *via* S-adenosyl methionine to proteins which cross react with antiserum to the *E.coli* methyl-accepting chemotaxis protein (MCP) Tar. Methylation is reduced in a non-chemotactic "tumbly" mutant. A synthetic oligonucleotide complementary to MCP C-terminal domains hybridises at high stringency to 4 major and 4 minor bands in *A.tumefaciens* DNA. These results are interpreted as evidence in favour of the existence of MCP-like proteins in *A.tumefaciens*. PCR amplification is being used to isolate the corresponding genes.

**A 133** AUXIN DEFICIENCY OF A TUMOR-INDUCING MEGAPLASMID LIMITS THE HOST RANGE OF AGROBACTERIUM TUMEFACIENS, L. UNGER[1], G.L. Cao[1], M Nguyen[1], E.W. Nester[2], and D. Raineri[2], Colleges of Medicine, Universities of Oklahoma[1] and Washington[1], Oklahoma City, OK 73190 and Seattle, WA 98195

We are studying the molecular basis of the interaction of a bacterial phytopathogen, Agrobacterium tumefaciens, AB2/73, with its host plants. This strain has a limited host range, inducing tumors on Lippia canescens, Nicotiana glauca and members of the cucurbit family. It harbors a 500 kb tumor-inducing megaplasmid (pTiAB2/73) which shares homology with the auxin biosynthetic (tms) genes from Pseudomonas savastanoi. Despite this homology, the tms-related loci in pTiAB2/73 are weak or inactive since: 1) tumorigenesis is dependent on plant-supplied auxin in as much as pTiAB2/73 incites tumors on the auxin-enriched apical surface, but not on the auxin-depleted basal surface, of carrot discs; 2) pTiAB2/73 is avirulent on plants (tomato, Kalanchöe) on which active tms genes would normally be sufficient for oncogenesis; 3) the introduction of functional tms genes complements pTiAB2/73 and expands host range to include Kalanchöe and tomato; as expected, the root proliferating morphology of tumors induced by the tms-complemented strains is similar to that of tumors incited by WHR cytokinin (tmr) mutants indicating a high auxin/cytokinin ratio. We conclude that the auxin-related sequences in pTiAB2/73 are weak or non-functional and that the auxin deficiency limits host range.

**A 134** BIOCHEMICAL CHARACTERISATION OF POLLEN-SPECIFIC PROTEINS.

Guy Vancanneyt, Judy Yamaguchi and Sheila McCormick. Plant Gene Expression Center USDA/ARS, 800 Buchanan Street, Albany CA 94710

A set of tomato genes (LAT52, LAT56, LAT59) which are coordinately expressed in the male gametophyte have been isolated. The specific role of these proteins during pollen formation and/or fertilisation remains largely unknown. Although the LAT56 and LAT59 cDNAs do not cross-hybridize, their deduced protein sequences share 54% amino acid identity. They also reveal a significant sequence similarity to both prokaryotic (*Erwinia*) pectate lyases and a eukaryotic (*Aspergillus*) pectin lyase. Experiments are currently being undertaken to analyze similar enzymatic activities in pollen extracts. The LAT52 gene encodes a putative 18 kD cysteine-rich protein which shows significant sequence homology to a maize pollen-specific cDNA and has partial similarity to several Kunitz trypsin inhibitors.

In order to characterize the three pollen-specific proteins, the coding region (or part of them) were fused to truncated *trp*E genes and overexpressed in *E.coli*. These fusion proteins were used as antigens for raising polyclonal antisera in both mice and rabbits. The antisera are currently being tested for their affinity and specificity to plant extracts. A biochemical characterization of the LAT52, LAT56 and LAT59 proteins using these antisera will be presented.

**A 135** ANTIFUNGAL ACTIVITY OF CHITINASES EXPRESSED IN TRANSGENIC TOBACCO,

Peter J.M. van den Elzen, Charles P. Woloshuk, and Theo C. Verwoerd, MOGEN international nv, Einsteinweg 97, 2333 CB Leiden, The Netherlands

Nicotiana tabacum SR1 was transformed with a vector containing both an acidic chitinase gene from petunia and a basic chitinase gene from tobacco. The genes were expressed constitutively using the 35S promoter of Cauliflower Mosaic Virus. Two of the resulting transgenic plants showed high levels of expression of both genes in Northern analysis. This elevated expression was also observed at the protein level by Western analysis. Chitinase activity in these plants was 4 and 7 times that of the nontransformed tobacco, respectively. Total protein extracts were used to measure in vitro antifungal activity against Trichoderma viride and Fusarium solani. Extracts caused lysis of hyphal tips and inhibition of growth. In these assays the transgenic plants were 5 and 10 times, respectively, more active against these fungi than the controls. These data demonstrate the expression of active chitinases in transgenic tobacco and indicate that antifungal activity correlates with the level of enzyme activity.

**A 136** CLONING OF THE GENES FOR INDOLEACETIC ACID SYNTHESIS FROM PSEUDOMONAS SYRINGAE PV. SYRINGAE, **Frank F. White and Steven F. Ziegler,** Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, and Immunex Corp., 51 University Street, Seattle, WA 98101

Indole acetic acid (IAA) is synthesized by a number of plant pathogenic bacteria. The function of IAA in a disease complex that does not involve tissue hyperplasia is unknown, and, therefore, a genetic study of IAA biosynthesis in *Pseudomonas syringae* pv. *syringae*, a non-gall-forming pathogen and the causal agent of brown spot disease in bean, was initiated. Hybridization signal was observed at a 2.8 kb *Eco*RI fragment when DNA from six strains of *P. s.* pv. *syringae* was probed by DNA filter hybridization using the *iaa* genes of *Pseudomonas syringae* pv. *savastanoi*. Hybridization with a genomic clone of the *iaa* region of *P. s.* pv. *syringae* indicated that the six strains, originally isolated from bean and pear, were closely related. Hybridization was not observed in strains of *P. s.* pv. *syringae* from wheat, corn, or tomato, or in two strains of *Pseudomonas syringae* pv. *pisi*. The *iaa* region from strain Y30 of *P. s.* pv. *syringae* was found to direct IAA synthesis in the Iaa⁻ strain EW2009-3rif of *P. s.* pv. *savastanoi*, and mutations with the 2.8 kb *Eco*RI fragment eliminated IAA synthesis. Partial nucleotide sequence analysis of the *iaaM* region confirmed that a homolog to the *iaaM* gene of *P. s.* pv. *savastanoi*

**A 137** TISSUE-SPECIFIC AND WOUND-RESPONSIVE EXPRESSION OF A HYDROXYPROLINE-RICH GLYCOPROTEIN GENE PROMOTER IN TRANSGENIC TOBACCO. Keith L. Wycoff[§], Patricia A. Powell, Richard A. Dixon[§], and Christopher J. Lamb, [§]Plant Biology Division, Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and the Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037.

Hydroxyproline-rich glycoproteins (HRGPs) are the major structural proteins of plant cell walls and also accumulate in response to pathogen infection, wounding and exposure to fungal elicitor. Using an HRGP gene from bean, HRGP4.1, we have constructed promoter-GUS fusions and expressed them in transgenic tobacco. The pattern of expression in response to wounding and infection in tobacco is similar to that in bean, while the tissue-specific pattern of GUS enzyme expression is somewhat different. In transgenic tobacco the presence of GUS activity was highly localized to root tips, stem-petiole junctions and stigmas. Functional analysis of promoter deletions in transgenic tobacco have identified sequences required for tissue-specificity and wound responsiveness. Sequences responsible for stigma-specific expression are located between -927 and -913 from the transcription start site, while genes responsible for wound responsiveness and other aspects of tissue specificity are located between nucleotides -250 and -93. Gel mobility shift assays and footprinting analysis are being used to identify DNA binding factors which interact with the functionally important sequences.

**A 138** MOLECULAR ANALYSIS OF THE *atp6* LOCUS FROM MALE FERTILE AND CYTOPLASMIC MALE STERILE SUGAR BEET AND AN OPEN READING FRAME UNIQUELY TRANSCRIBED IN CMS MITOCHONDRIA, Yongbiao Xue*, Colwyn M. Thomas and D. Roy Davies, John Innes Centre for Plant Science Research, Colney Lane, Noriwch, NR4 7UH,U.K. and * Present address, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA, U.K.

Cytoplasmic male sterility (CMS) is a maternally inherited trait which is characterized by the failure of a plant to produce functional pollen. The detailed analysis of CMS in *Zea mays* CMS-T and *Petunia hybrida* has led to the identification of CMS-inducing genes, which were shown to have arisen as a result of multiple recombination events. In an attempt to define differences in genome organization and expression between CMS and MF sugar beet mitochondria, cDNA libraries from both mitochondrial genotypes were constructed and screened in reciprocal hybridization experiments. One cDNA sequence (pYC700) is unique to CMS mitochondria. At the corresponding genomic locus an open reading frame of 525 bp capable of encoding a hydrophobic polypeptide of 20 Kd was detected. This open reading frame (CMSorf1) is co-transcribed with the Fo-ATPase subunit 6 gene (*atp6*) located 223 bp downstream. The *atp6* locus from MF mitochondria was also cloned and the genomic organization, DNA sequence and transcription of each locus was studied. A sequence upstream of CMSorf1 (repeat II) is repeated in CMS mtDNA but is present as a single copy in MF mtDNA though not at the *atp6* locus. The sugar beet *atp6* gene sequence shares extensive homology to other plant *atp6* genes. The MF and CMS genes encode an identical protein of 250 aa, the shortest plant ATP6 reported to date. High resolution S1 mapping demonstrated that DNA sequences at the 5' terminus of both CMS and MF *atp6* genes show extensive sequence homology to other sugar beet transcripts and mitochondrial genes from other dicotyledonous spp.

**A 139** CLONING OF PHENYLALANINE AMMONIA-LYASE GENE FROM *PISUM SATIVUM*: REGULATION BY FUNGAL SIGNAL MOLECULES, Tetsuji Yamada[1], Shinji Kawamata[2], Hirofumi Yoshioka[1], Tomonori Shiraishi[1], Yoshikazu Tanaka[1], Hidenori Kato[1], Yuki Ichinose[1] and Hachiro Oku[1], [1]College of Agriculture, Okayama University, Tsushima, Okayama, 700, Japan, [2]Takasago perfumery R & D Institute, Kamata, Minato, Tokyo, 144, Japan

A nearly full-length cDNA encoding phenylalanine ammonia-lyase (PAL) of pea (*Pisum sativum*) was cloned and the complete nucleotide sequence was determined. The deduced amino acid sequence is highly homologous to that of PAL from *Phaseolus vulgaris*, *Oryza sativa*, or *Solanum tuberosum*. Southern blot analysis showed that the PAL gene of pea plant also exists as a small multigene family. Using this PAL-cDNA clone, we show that fungal elicitor from *Mycosphaerella pinodes* and abiotic elicitors such as glutathione rapidly induce the accumulation of PAL-mRNA in elicitor-treated pea epicotyl tissues, whereas fungal suppressor from *M. pinodes* delays the induction. We have recently shown that fungal suppressor inhibits plasma membrane ATPase in pea (H. Yoshioka et al. *Plant Cell Physiology* in press). An inhibitor for plasma membrane ATPase, orthovanadate, also delays the accumulation of PAL-mRNA in elicitor-treated pea epicotyl tissues in the same manner as fungal suppressor. These results may suggest that the inhibition of plasma membrane ATPase is associated with the suppression of plant defense reactions in pea.

**A 140** PURIFICATION OF FACTORS INVOLVED IN THE ACTIVATION OF CHALCONE SYNTHASE (CHS) TRANSCRIPTION IN CELL CULTURES OF PHASEOLUS VULGARIS (P.V.), L.M. Yu*, R.A. Dixon* and C.J. Lamb**, * Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and **The Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, Ca 92037.

Phenylpropanoid metabolism produces compounds necessary for plant development and defense. CHS is a transcriptionally regulated enzyme located at the base of a branch path, off the central phenylpropanoid pathway, whose end products are the (iso)flavonoids found in legumes such as P. v. A variety of treatments similarly activate the transcription of certain members of the P. v. CHS gene family. The 5' region of CHS15 contains cis elements necessary for transcriptional activation as it confers responsiveness of expression to a linked reporter gene in soybean cells. The nucleotide sequence $CCTACC(N)_7CT$ occurs three times in this region. The 15mer or its 5' most hexanucleotide is found in other transcriptionally activatable defense genes and has been functionally implicated in the expression of genes from snapdragon and parsley. We are purifying factors that bind to this sequence. The core 15mer was embedded in a ds28mer without other relevant sequences such as SBF-1 or G boxes and binding to cell extracts was analyzed by a gel retardation assay. Assay validity was established by the success and failure of certain mutations within the embedded 15mer to compete for binding in the presence of at least 150 fold weight excess of poly (dI-dc)·poly (dI-dc). Purification schedules for several binding activities, similar in both elicited and control cultures, will be presented.

*Genetic Dissection of Stress-Related Functions*

**A 200** CHARACTERIZATION OF ROOT SURFACE PROTEINS ASSOCIATED WITH PLANT-MICROBE INTERACTIONS. Anne J. Anderson, Chris Heck and Rob Zdor, Department of Biology, Utah State University, Logan UT 84322-5305

Root surface proteins are an integral part of interactions with both beneficial and deleterious bacteria. Certain root surface colonizing bacteria stimulate the plant root to produce activated oxygen species and root surface peroxidases may play a role in this possible plant defense response. Binding of pseudomonads to the plant root may involve interaction with a root surface glycoprotein, an agglutinin, which causes the bacterial cells to agglutinate. We are purifying the root surface peroxidases and the agglutinin to further study their roles. Components washed from sterile-grown bean root surfaces are fractionated through Amicon cartridges with cut off of 10 kd. The higher molecular weight materials are passaged through ConA Sepharose. The agglutinin elutes just prior to the peroxidase with 10mM $\alpha$-methyl mannoside. Further purification involves DEAE-Sephadex chromatography. The agglutinin travels as a broad band on cationic PAGE. The peroxidase yields two fast moving bands on nondenaturing PAGE.

**A 201** THE COAT PROTEIN GENE AND ASSOCIATED SEQUENCES OF POTATO VIRUS X David Baulcombe, Sean Chapman, Simon Santa Cruz, Matthew Goulden and Tony Kavanagh, The Sainsbury Laboratory, Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH UK and Trinity College, Dublin, Eire.

Experiments with intact and modified genomes of potato virus X have demonstrated that coat protein expression is essential for infection of intact plants. However, the requirement for coat protein is not the same as with alfalfa mosaic virus as infection by naked RNA occurs in the absence of added coat protein. In one instance it was possible to infect plants with a mutant form of the viral genome in which the coat protein gene was shifted out of frame. The progeny RNA in this experiment showed a secondary mutation which shifted the coding sequence back into the coat protein reacting frame. It is concluded therefore that coat protein is required either for post-replicative stages of the infection cycle, as a quantitative co-factor of viral replication or as an essential component for replication acting later than negative strand synthesis, but not affecting production of sub-genomic mRNA for coat protein.

Analysis of resistance breaking strains of potato virus X have implicated sequence within, or close to, the coat protein gene as affecting the interaction of the virus with resistance gene genes $N_x$ and $R_x$ in potato. Experiments will be described that will determine whether the resistance gene interaction is mediated by coat protein and, if so, whether the effect is related to the requirement of coat protein for infection of intact plants, as described above.

**A 202** SHOOT REGENERATION SYSTEM FOR DED SUSCEPTIBLE AND PUTATIVELY RESISTANT AMERICAN ELMS, Mark G. Bolyard, C. Srinivasan and Mariam B. Sticklen, Pesticide Research Center, Michigan State University, East Lansing, MI 48824-1311.

The genetic engineering of various tree species has been hampered by the lack of a facile plant regeneration system. In this report we describe a leaf disc regeneration system for the Dutch elm disease (DED) susceptible American elm. Surface sterilized leaf strips were cultured on Murashige and Skoog salts and vitamins supplemented with various concentrations of the cytokinins pyranyl-benzyladenine (PBA) or thidiazuron (THD). The most efficient treatment was 0.1 $\mu$M THD, which produced primordial shoots from 77% of the explants tested. Although the percentage of shoots from the optimal concentration of PBA (1 $\mu$M) was much lower (11%), certain explants produced high numbers of shoots (up to 12). A combination of these treatments did not produce a synergistic effect, as the percentage of explants producing shoots was 69%. This technique was also used to test the regeneration of several putatively DED resistant American elms, which were selected following repeated inoculations with the fungal pathogen. There seems to be an inverse correlation between the percentage of explants producing shoots on 0.1 $\mu$M THD and the observed resistance to DED. This system will certainly be useful for introducing DED resistance-associtaed genes, as well as other beneficial genes, into American elms. Experiments have been initiated to introduce reporter genes into susceptible American elms using the Agrobacterium tumefaciens method. It will also be useful for propagating putatively DED resistant American elms, and it may also serve as a screening technique for resistance to DED.

**A 203** **A BYDV SEQUENCE CAUSES TRANSLATIONAL FRAMESHIFTING IN OAT CELLS**, Veronique Brault, Di Rong and Allen Miller, Department of Plant Pathology, Iowa State University, Ames, IA 50010
Based on the nucleotide sequence of Barley Yellow Dwarf virus (BYDV), the polymerase gene was proposed to be translated via a frameshifting event. We have now demonstrated that a BYDV sequence can cause frameshifting in vitro, in E.coli and in oat cells. The region of the BYDV genome expected to be responsible for frameshifting was fused upstream to the GUS reporter gene and introduced into oat protoplasts by electroporation. Translational frameshifting was demonstrated as mesured by glucuronidase activity expressed from a construct in which the GUS gene was inserted in a -1 frame relative to the start codon. Mutagenesis is underway to determine the sequences and RNA structures which contribute to this unusual translational event.

**A 204** STUDIES ON THE MECHANISM OF VIRUS RESISTANCE IN TRANSGENIC TOBACCO TRANSFORMED WITH THE NONSTRUCTURAL 54 kDa PROTEIN GENE OF TOBACCO MOSAIC VIRUS, John P. Carr and Milton Zaitlin, Department of Plant Pathology, Cornell University, Ithaca, NY 14853
*Nicotiana tabacum* cv. Xanthi (nn) plants are rendered completely resistant to tobacco mosaic virus (TMV) when transformed with DNA sequences corresponding to the region of the virus genome encoding the 54 kDa putative nonstructural protein (1). Although a transcript of the expected size is observed in these plants so far no 54 kDa protein has been detected and it is unclear whether resistance is mediated by small amounts of the 54 kDa protein itself or by its transcript. In an attempt to dissect the mechanism(s) underlying this resistance phenomenon, studies with protoplasts have been initiated. Resistance to the $U_1$ strain of TMV is expressed in protoplasts derived from transformed plants. Like the plants they are isolated from, these protoplasts were resistant to the virus strain ($U_1$) from which the 54 kDa protein sequence was obtained but not to a different strain ($U_2$) of TMV. Resistant protoplasts did not synthesize detectable levels of viral RNA (+ or - sense) or 126 kDa (replicase) protein. However there was no apparent difference in stability of the input (infecting) viral RNA in the transformed versus untransformed protoplasts. Current results indicate that the resistance mechanism does not affect viral cell-to-cell transport but blocks an extremely early step in replication.

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This work was supported by Grant 90-37262-5293 from the USDA Competitive Grants Program.

**A 205** THE GAPC GENE FAMILY OF MAIZE: STRUCTURE OF GENES AND THEIR DIFFERENTIAL ANAEROBIC EXPRESSION, R. Cerff, A. Peters and M.-F. Liaud, Institut für Genetik, Biozentrum, Technische Universität Braunschweig, D-3300 Braunschweig, FRG.
Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC) of maize is encoded by at least 3 different functional genes which are regulated differentially under anaerobic conditions (1, 2). We have isolated 3 GAPC genes, two of which (genes *Gpc1* and *Gpc2* ) are repressed and one of which (gene *Gpc3* ) is strongly induced by anaerobic conditions. Surprisingly, the constitutive-aerobic *Gpc1* gene has an anaerobic promoter with a typical ARE-element (containing the core sequence TGGTTT) 158 nucleotides upstream the transcription start site. Experiments are in progress in our laboratory to characterize genes *Gpc2* and *Gpc3* and to compare their promoter structures with that of the *Gpc1* gene.

1. Martinez, P., Martin, W. and Cerff, R. (1989) J. Mol. Biol. 208, 551-565.
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**A 206** GENE STRUCTURE ANALYSIS OF A 3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE ISOGENE ASSOCIATED WITH DEFENSE RESPONSES IN TOMATO, Carole L. Cramer, H. Park and C.J. Denbow, 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. In higher plants, HMGR is involved in synthesis of sterols, carotenoids, electron transport components, rubber, gibberellins, and abscisic acid. In the Solanaceae, HMGR also directs production of sesquiterpenoid phytoalexins associated with disease resistance. We cloned and sequenced a tomato HMGR gene. Gene structure was determined by RNase protection analyses and sequence comparisons with PCR-generated cDNA sequences and identified a coding region of 602 amino acids interrupted by three introns. Comparison of derived amino acid sequences indicates that the C-terminus region of tomato HMGR (residues 188-602) is highly conserved with Arabidopsis, yeast, and human HMGRs (91%, 75%, and 74% sequence similarity, respectively). In contrast, the N-terminus shows no sequence identity to human or yeast HMGRs. Identity to Arabidopsis HMGR is limited to two presumptive membrane spanning regions. We monitored HMGR mRNA levels in various tomato tissues and in cultured cells using conserved-region or gene-specific probes. HMGR mRNAs observed early in fruit development (presumably associated with sterol synthesis) are not due to expression of the HMGR gene that we have cloned. However, the HMGR mRNAs rapidly induced in response to fungal-elicitor treatment or wounding are derived from the cloned gene suggesting this isogene functions as a defense-related gene in tomato.

**A 207** MOLECULAR CLONING AND ANALYSIS OF cDNAS MODULATED DURING WATER DEFICIT IN SOYBEAN SEEDLINGS, Robert A. Creelman and John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

Transfer of soybean seedlings to low water potential vermiculite (-0.3 MPa) caused the modulation of several polysomal mRNAs [Creelman et al. Plant Physiol. 92:205]. We constructed cDNA libraries from the hypocotyl elongating region of well-watered, water-deficient, and ABA-treated soybean seedlings. We report here the isolation of cDNA clones corresponding to three genes whose expression is modified by water deficit. Two clones (pGE16 and pGE95) correspond to genes whose mRNA levels increase, and one cDNA (pGE23) to a gene whose mRNA is decreased by water deficit. One of the induced clones (pGE16) hybridizes to multiple transcripts (0.53, 1.03, 1.5 Kb), while the other hybridizes to a unique mRNA (pGE95, 1.25 Kb) and is a single copy gene. While expression of pGE16 and pGE95 in well-watered seedlings occurs primarily in mature regions of the seedling, in water deficient seedlings expression is reduced in mature regions and is enhanced in elongating regions. Of the plant growth substances tested, only methyl jasmonate (and to a slight extent ABA) affected mRNA levels of pGE16. Growth substances did not modulate pGE95 mRNA levels. Expression of pGE23 (1.7 Kb) is slowly repressed in response to water deficit in tissues of the soybean seedling having cells undergoing division and expansion, similar to the expression of JCW1, a clone corresponding to an auxin induced mRNA. However, no effect of auxin was observed on the expression of pGE23. Sequence analysis reveals that pGE23 has a high homology with $\beta$-tubulin. Studies are currently underway to determine if transcription or mRNA stability is involved in regulating water deficit induced changes in mRNA levels of these genes.

**A 208** CLONING OF THE POLYGALACTURONASE-INHIBITING PROTEIN (PGIP) OF *PHASEOLUS VULGARIS* L., G. De Lorenzo, P. Toubart, G. Salvi, A. Desiderio and F. Cervone, Dipartimento di Biologia Vegetale, Universita' di Roma "La Sapienza" and ENEA, Rome, ITALY; C. Bergmann, A. Darvill and P. Albersheim, Complex Carbohydrate Research Center, The University of Georgia, Athens, Georgia 30602.

The N-terminus and several trypsin-released peptides of PGIP from *P. vulgaris* L. were sequenced by automated Edman degradation. The amino acid sequences of the N-terminus and of one of the trypsin-released peptides were used to synthesize oligonucleotide primers for a polymerase chain reaction (PCR). Total DNA of *P. vulgaris* L. was used as a template. A DNA band of 0.74 kb, an amplification product of the gene encoding PGIP, was isolated, cloned and sequenced. It consisted of an uninterrupted open reading frame coding for an estimated 70% of the entire PGIP gene. The deduced amino acid sequence contained the expected terminal regions corresponding to the primers used, as well as regions matching the amino acid sequences of three previously characterized trypsin-released peptides. An effort is being made to obtain to obtain the entire gene encoding PGIP by screening a genomic library of *P. vulgaris* L. Overexpression of PGIP in transgenic plants will make it possible to determine whether increased levels of PGIP enhance the resistance of plants to fungal pathogens. Acknowledgments: This work is supported in part by an MPI grant, by U.S. Department of Energy grant DE-FG09-85ER13425, and by DE-FG09-87ER13810 as part of the DOE/NSF/USDA Plant Science Centers Program.

**A 209** LOCAL AND SYSTEMIC GENE EXPRESSION IN TOMATO PLANTS INDUCED BY WOUNDING
Helen M. Doherty and Dianna J. Bowles, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds, LS2 9JT, U.K.

Damage to the leaves of tomato plants caused by wounding results in transient changes in gene expression in different parts of the plant. The diversity of signalling mechanisms involved can be investigated by following the pattern of expression of a number of genes occuring either locally or systemically with respect to the wound site. The induction of mRNAs homologous to tomato proteinase inhibitors PrI I and PrI II occurs after a short lag in both wounded and unwounded leaves; in this case the ability of endogenous oligosaccharide elicitor fractions to mimic the effects of wounding has led to the proposal that oligosaccharides constitute a signal that is released from the wound site[1]. The appearance of an mRNA homologous to the ripening-related gene, pTom13[2], occurs more rapidly, and its induction is restricted to the area in the immediate vicinity of the wound. It has been proposed that this gene product may be involved in ethylene synthesis[2]. Unlike PrI gene expression, pTom13 does not appear to be induced in the leaves in response to pectic oligosaccharides. The differences in the patterns of expression of the PrI genes and of pTom13 have been investigated by Northern blot analysis and *in situ* hybridisation. The results are used to determine those regions which may be defined as local and systemic with respect to the wound site, and hence the source and target of any signal may be established. This classification will be applied to the pattern of induction of mRNAs hybridising to cDNA clones derived from a cDNA library specific for the local wound site.

(1) Bishop *et al.* (1984) Journal of Biological Chemistry *259*, 13171-13177
(2) Holdsworth *et al.* (1987) Nucleic Acids Research *15*, 731-738

**A 210** BIOCHEMICAL AND ULTRASTRUCTURAL LOCALIZATION OF TMV - MP IN TRANSGENIC TOBACCO PLANTS,
Csilla A. Fenczik, Patricia J. Moore, and Roger N. Beachy, Biology Department, Washington University at St. Louis, St. Louis, MO 63130

The tobacco mosaic virus movement protein (TMV-MP) is required for cell-to-cell movement during virus infection. The TMV-MP is proposed to potentiate virus movement by altering the size exclusion limits of the plasmodesmata. Transgenic tobacco plants expressing the movement protein have been shown to complement a virus that is deficient in the MP. These MP(+) plant lines have altered plasmodesmata which allow the movement of larger dye probes from cell to cell. We have examined both the biochemical and ultrastructural localization of TMV-MP in these transgenic plants. Immunocytochemical studies have shown that TMV-MP is localized in plasmodesmata in middle - aged and older leaves. In young leaves, TMV-MP is seen in the plasmodesmata only near the oldest region of the leaf, i.e., the tip. Occassionally gold particles were also seen along the plasma membrane near the plasmodesmata. In younger leaves, no antibody staining could be detected. A plasma membrane enriched fraction has been isolated from these transgenic tobacco plants, by aqueous phase partioning. High purity of the membranes was confirmed by marker enzyme assays. In younger leaves the MP is enriched in the plasma membrane fraction. As the leaf ages more MP is seen in the cell wall fraction. Sodium carbonate (pH 11.5) and Triton X-114 washes have been performed to determine the interaction of the TMV-MP with the membrane. Protease sensitivity experiments are also in progress to determine which parts of the protein interact with the membrane. Results of these experiments will be presented and discussed.

**A 211** CLONING OF A NOVEL DNA BINDING PROTIEN WITH SPECIFICITY FOR THE ARE REGION OF THE MAIZE *Adh1* 5' FLANKING REGION,
Robert J. Ferl and Beth J. Laughner, Plant Molecular and Cellular Biology Working Group and Department of Vegetable Crops, University of Florida, Gainesville, FL 32611

The maize alcohol dehydrogenase-1 *(Adh1)* gene is induced by hypoxic conditions. A region of the promoter, from approximately -90 to -140 has previously been shown to be involved in the regulation of this transcriptional induction by both deletion/mutation analysis and *in vivo* footprinting. We describe here a cDNA clone that encodes a protein that specifically recognizes a 17bp segment within that region of the promoter. The target DNA segment lies within the functionally defined Anaerobic Response Element (ARE) and covers a strong *in vivo* footprint centered at -130. Sequence analysis of the cDNA shows that the protein represents a novel eukaryotic DNA binding protein that is, in part, loosely similar in structure to a class of bacterial plasmid replication origin binding proteins. Based on its DNA binding specificity, the protein produced from this cDNA appears to be a likely candidate for the factor mainly responsible for transcriptional activation of *Adh1* by hypoxic stress.

**A 212** A NUCLEASE FROM FUSARIUM SOLANI IS HOST-INDUCED, Dave Gerhold, Andrew Pettinger, and Lee Hadwiger, Department of Plant Pathology, Washington State University, Pullman, Washington 99164-6430.
Fusarium solani f. sp. pisi and phaseoli isolates were found to secrete a nuclease from germinating microconidia. This nuclease is primarily a nicking enzyme, but will, in large quantities, linearize and degrade double-stranded DNA. Thus, the activities resemble those of endo-exonuclease from Neurospora crassa and alpha nuclease from yeast. The Fusarium solani nuclease is uniquely produced when cultured on pea pods or pod extracts but not on media lacking plant extracts. The enzyme's presence in host tissue is correlated temporally with major alterations in host nuclear structure and enhancements in the transcription of defense genes. Thus, we are further investigating the effect this nuclease may have on the host defense response.

**A 213** SOME GENES REGULATED BY OXIDATIVE STRESS ARE ALSO EXPRESSED DURING AGING IN SOYBEAN SEEDS, Xavier Gidrol, Norbert Dégousée and Abdelmajid Noubhani, Station de Physiologie Végétale, INRA, BP 81, 33883 Villenave D'ornon Cedex, France.
As a first step toward understanding the molecular mechanisms of plant senescence and most particularly of seed aging, we present here a biochemical analysis of genes expressed during both oxidative stress and the aging process. When soybean seeds were either exposed to hydrogen peroxide for 2 h or acceleratedly aged for several days at 42°C and 100% relative humidity, many common proteins were induced. As demonstrated by Matters and Scandalios (1986), superoxide dismutase and catalase are induced by oxidative stress. Similarly a dramatic increase in superoxide dismutase activity was observed during seed aging, while catalase activity remained very low. The detection of activity on polyacrylamide gel allowed us to determine that Cu, Zn SOD was responsible for the increase of activity. Further analyses demonstrated that the activity increase resulted from an induction of Cu, Zn superoxide dismutase.These results suggest that seed aging could be regarded as an oxidative process.

Matters G. L. and Scandalios J. G. 1986, B.B.A 882:29-38

**A 214** **IDENTIFICATION OF PLANT GENES EXPRESSED AT THE FEEDING SITE OF THE POTATO CYST NEMATODE.** **Sarah J Gurr, Michael J McPherson, Howard J Atkinson* & Dianna J Bowles. Department of Biochemistry and Molecular Biology and *Department of Pure and Applied Biology University of Leeds, Leeds, LS2 9JT, UK.**

**The interface between plant and pathogen is a site of key importance in the determination of the plants susceptibility or resistance to the invasion. In some plant pathogen interactions, the interface increases in area as colonization proceeds. In others, particularly those involving obligate biotrophs the direct contact between the parasite and plant is kept to a minimum throughout the life cycle of the pathogen often constituting only a few cells.**

**The potato cyst nematode (PCN) *Globodera rostochiensis* causes major annual losses to the potato harvest. Current control relies upon crop rotation and toxic nematicides or by the laborious breeding of resistance genes into agronomically favoured cultivars. Genetically engineered resistance strategies therefore offer an attractive alternative. Towards this end we are studying patterns of gene expression within the plant syncitia, the specialised feeding sites of the PCN. We have used a polymerase chain reaction method to construct cDNA libraries from root tissue enriched in syncitia. Differential screening strategies have led to the identification of a number of interesting clones. Temporal and spatial expression studies of the corresponding genes will be presented.**

**A 215 TRANSFORMATION OF RICE USING A TUNGSTEN MEDIATED MICROPROJECTILE METHOD,** Hajela, R., Hajela, N., Wu, R.*, and Sticklen, M. B., Plant Tissue Culture and Genetic Engineering Laboratory, Pesticide Research Center, Michigan State University, East Lansing, MI 48824 and *Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Two plasmids were used to bombard rice root sections using a Biolistic™ microprojectile device. These include pAct1-F (McElroy et. al. 1990, The Plant Cell, 2:163-171), containing the *E. coli* β-glucuronidase (Gus) gene driven by a rice actin promoter, and pDM102 (McElroy et. al., 1990, unpublished) containing a mutated *Salmonella* EPSP synthase gene, encoding resistance to the herbicide glyphosate, also driven by a rice actin promoter. Transient expression of GUS activity was seen in the material within 24 hours after bombardment. Southern analysis of total DNA extracted after two months in culture of root derived somatic embryos (Sticklen, 1990, Plant Cell Re., submitted) revealed sequences homologous to both the Gus gene coding region as well as the glyphosate resistance gene coding sequences. Work is currently underway to analyze the regenerated plants to test for stable integration and expression of these genes. Our goal is to develop insect and herbicide resistant transgenic rice plants, and eventually to develop transgenic corn, wheat and other cereals carrying beneficial genes.

**A 216** AMINO ACID SUBSTITUTIONS IN THE TURNIP CRINKLE VIRUS CAPSID PROTEIN MODIFY PATHOGENICITY, L.A. Heaton*, T.L. Hsiao, N. Wei, and T.J. Morris, Depts. of Plant Pathology, *Kansas State University, Manhattan, KS 66502, USA, and University of California, Berkeley, CA 94720, USA.

The turnip crinkle virus (TCV-B) nucleotide sequence has been determined (Carrington *et al.* 1989. Virol. 170, 219-226), infectious RNAs have been synthesized *in vitro* (Heaton *et al.* 1989. Virol. 170, 214-218), and several site-directed mutants have been constructed (Heaton *et al.*, unpublished). One of our "P domainless" capsid protein (CP) mutants replicated in inoculated leaves and protoplasts, there was no detectable accumlation of mutant capsid protein in either leaves or protoplasts, and the mutant failed to move systemically. After inoculated plants grew for several weeks under greenhouse conditions, we purified virions in which the introduced translation terminators had reverted to non-wild-type sense codons. One of the revertants, with amino acid substitutions in the hinge, elicited milder symptoms than those elicited by TCV-B, and another elicited more severe symptoms. Oligonucleotide-directed mutagenesis was used to show that single-amino acid substitutions in the CP are sufficient to elicited the milder, but not the more severe, symptom syndrome.

**A 217** "CHARACTERIZATION OF PISUM SATIVUM DISEASE RESISTANCE RESPONSE GENE" 206. Daniel A. Horovitz and Lee A. Hadwiger. Department of Plant Pathology. Pullman, WA 99163.
A partial cDNA from pea gene DRRG 206, whose homologous RNA accumulation is temporally correlated with non-host disease resistance and elicited by chitosan has been sequenced. Its sequence is not homologous with any data bank gene. Southern blot analysis reveals DRRG 206 may belong to a multigenic family containing 4-5 members. Eleven genomic clones were obtained and two were sequenced. A 1 Kb 5' promoter fragment of one clone was fused to the chloramphenicol acetyl transferase (CAT) gene and assayed for its ability to drive transcription in tobacco protoplasts and transgenic plants. This 5' region has several interesting consensus sequence "boxes" including an octamer sequence found in several plant defense genes, a hexameric site found in nearly all plant histone genes, and a nonamer found in pea DRRG 49. In addition there are large A-T rich tracts, such as those found in animal systems which complex with HMG-I proteins. Preliminary gel retardation experiments show that purified mouse HMG-Y can bind to DRRG 206 promoter fragments containing A-T rich regions. Furthermore distamycin, which has been shown to bind to A-T rich DNA in a manner comparable to HMG-I proteins, activates DRRG 206 in vivo.

**A 218** CLONING AND CHARACTERIZATION OF THE GENES WHICH EXPRESS UNDER DRIED CONDITION OF RICE CALLUS, Shoshi Kikuchi, Mohamed Soliman, Dong-Hyun Shin and Kiyoharu Oono, Department of Cell Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305 JAPAN

We have established the method to preserve rice callus under dried condition. To know how the plant cell obtains the protective mechanism against drying, a cDNA library was constructed from the mRNA of dried callus. Two methods were used to fish out cDNA clones which express specifically under dried condition. One was using *rab16A* DNA (Mundy and Chua 1988, EMBO J. 7, 2279) as a probe. The other was a differential hybridization screening between dried and fresh callus cDNA libraries. Four independent cDNA clones were isolated. One is an analogue of *rab16A*. The other three are named pDRC1, 2, 3. Northern hybridization revealed that the corresponding transcripts were observed only in dried callus RNA and not in fresh callus RNA. The size of them were 0.8-1.1kb. Further analysis with several physiological conditions indicated that these transcripts were induced when callus was desiccated, slightly induced by the addition of abscisic acid (ABA) to the medium and that they exist in dried mature seed but disappear after imbibition. Rescreening of the cDNA library with the isolated cDNA clones was made. Around half of the cDNA clones hybridized with one or some of the isolated clones. One cDNA clone which hybridized with *rab16A*, pDRC1 and pDRC2 has a long insert of 3.8kbp DNA. Restriction analysis followed by Southern hybridization analysis revealed the tandem alignment of pDCR1 and pDCR2 sequences on the insert.

**A 219** SPECIES-SPECIFIC RESPONSES OF *OROBANCHE* SEEDS TO HOST-DERIVED AND SYNTHETIC GERMINATION STIMULANTS, David E. Matthews and John C. Steffens, Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853

Seeds of the root parasite *Orobanche* (broomrape) can remain dormant in soil for many years until stimulated to germinate by as-yet-unidentified compounds present in root exudates from its hosts. Strigol, a sesquiterpene lactone from cotton root exudate, and some of its synthetic analogs (GR compounds) are also potent germination stimulants for *Orobanche*. Reactions to these stimulants were compared in two *Orobanche* species attacking crop plants in Israel. *O. aegyptiaca* has a broad host range including solanaceae, legumes, cucurbits and composites, while *O. cumana* is relatively specific for sunflower. Germination of *O. cumana* but not *O. aegyptiaca* was found to be strongly affected by the incubation medium, glass-fiber filters vs. agarose. *O. aegyptiaca* germinated in response to GR-7, GR-24, and exudates from flax or tomato but not sunflower, while *O. cumana* responded only to GR-24 and sunflower exudate. The insensitivity of *O. aegyptiaca* to sunflower exudate was due to lack of an effective stimulant, rather than presence of a germination inhibitor.

**A 220** EXPRESSION AND ANALYSIS OF BETAINE ALDEHYDE DEHYDROGENASE, A SALT INDUCIBLE ENZYME CLONED FROM SUGAR BEET (*Beta vulgaris*), Kent F. McCue & Andrew D. Hanson, MSU-DOE Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

Betaine accumulates and serves as a compatible osmolyte in some plants subjected to drought or salinity stress. The last enzyme in the betaine biosynthetic pathway is betaine aldehyde dehydrogenase (BADH). The activity of BADH increases in response to increasing salinity levels. This increase in activity corresponds to an increase in protein detectable by immunoblotting, and to an increase in the translatable BADH mRNA. BADH was cloned from a cDNA library constructed in \gt10 using poly(A)$^+$ RNA isolated from leaves of sugar beets salinized to 500 mM NaCl. cDNAs were size selected (>1kb) before ligation into the vector, and the library was screened with a spinach BADH cDNA probe. Three nearly full length clones obtained were confirmed as BADH by their nucleotide and deduced amino acid homology to spinach BADH. Clones averaged 1.75 kb and contained open reading frames of 500 amino acids at 80% identity with spinach BADH. RNA gel blot analysis of total and poly(A)$^+$ RNA isolated from both leaves and roots indicated that salinization to 500 mM NaCl resulted in a several-fold increase of BADH mRNA levels in both organs. Genomic reconstructions indicate that BADH is present as a single copy in the haploid sugar beet genome. The three clones obtained showed minor sequence differences dividing them into two groups. The small sequence differences are suggestive of allelic variation which was corroborated by the existence of restriction fragment length polymorphisms in DNA isolated from individual plants.

**A 221** TRANSCRIPT AND PROMOTER ANALYSIS OF THE DOUBLE STRANDED DNA VIRUS COMMELINA YELLOW MOTTLE VIRUS (COYMV), Scott Medberry, Ben Lockhart, and Neil Olszewski, University of Minnesota, St. Paul, MN 55108

Commelina yellow mottle virus (CoYMV) is the type member of a group of bacilliform double-stranded DNA viruses (badnaviruses). Using strand-specific RNA probes generated from the cloned genome one major transcript, a greater than genome length RNA was detected in infected *Commelina* plants and not in healthy plants. A transcript with homology to the opposite strand was also identified. It is host encoded since it is located in both infected and healthy tissue. Poly A+ purification followed by northern blotting and probing indicates that a significant portion of the full length viral transcripts are polyadenylated, although the majority of transcripts detected appear to be poly A-. The host encoded transcript appears to be poly A-. Based on 3' end sequencing of cDNA clones derived from viral transcripts, the location of the 3' terminus of the transcripts was determined. The 5' end of the viral transcript has been identified by primer extension and RNase protection. These results indicate a viral transcript of genome length plus 120 nucleotides and is consistent with replication via reverse transcriptase. A promoter cassette containing sequence presumed necessary for viral transcription has been made. Preliminary results in transgenic tobacco plants ($R_0$) indicate high levels of promoter activity in the phloem or phloem associated cells of leaves, petioles, and roots. Dissection of the promoter to determine elements responsible for cell-specific expression will be discussed.

**A 222** MOLECULAR ELEMENTS CONTROLLING THE DEVELOPMENTALLY- AND ENVIRONMENTALLY-INDUCED EXPRESSION OF THE *Nicotiana tabacum* CHITINASE GENE , Alan D. Neale[1] and Elizabeth S. Dennis[2], Department of Genetics and Developmental Biology, Monash University, Clayton, Victoria, Australia[1], CSIRO, Division of Plant Industry, Black Mountain, ACT, Australia[2].

The tobacco gene encoding the basic isoform of chitinase is expressed at high levels in response to wounding and pathogen attack as well as exhibiting root-specific and floral organ-specific expression at various developmental stages in healthy plants. An understanding of the molecular factors controlling the expression of this gene will provide insight into plant defence mechanisms and the interrelation of these mechanisms with those directing normal plant development. Results obtained with a deletion series of the 2kb promoter region of the chitinase gene will be presented. This deletion series has been coupled to a GUS reporter gene in transformed plants to enable localization of TMV-inducible and wound-inducible cis elements, as well as those promoter elements responsible for tissue-specific developmental expression in healthy plants.

**A 223** PROTECTION AGAINST TOBACCO MOSAIC VIRUS CHALLENGE THROUGH EXPRESSION OF A RIBOZYME. Richard S. Nelson, Brent V. Edington, Arvind D. Choudhary and Richard A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402.

RNA molecules capable of endoribonuclease activity have been identified and are often referred to as ribozymes. A ribozyme, targeted against the putative RNA-dependent RNA polymerase (RdRp) coding region of tobacco mosaic virus (TMV), was chemically synthesized. The nucleotide sequence of the catalytic region is identical to that found in the positive strand RNA of the satellite of tobacco ringspot virus. The 5' and 3' flanking regions each consist of 20 nucleotides complementary to the RdRp coding region. The cleavage site on the TMV genome is at nucleotide position 2467. In <u>in vitro</u> studies, transcripts containing the ribozyme sequence have cleaved the genomic RNA of two TMV strains ($U_1$ and PV42, American Type Culture Collection designation) producing fragments of the expected size. Protoplasts were subjected to simultaneous PEG-mediated uptake of virus (16 ug/ml) and binary vectors containing ribozyme constructs driven by the 35S promoter of CaMV. TMV coat protein accumulation in these cells was 10-40% that of the levels found in protoplasts containing vectors with antisense constructs, vectors without constructs, or no vectors. Increasing the challenge virus inoculum to 160 ug TMV/ml did not overcome the protection. Results will be presented describing the level of protection against challenge viral RNA as well as the accumulation of viral RNA after challenge.

**A 224** STUDIES ON THE MECHANISM OF VIRUS RESISTANCE IN Solanum brevidens AND ITS TRANSFER TO S. tuberosum GERMPLASM THROUGH ASYMMETRIC HYBRIDIZATION Pehu, E., Xu, Y-S, Poutala, T., Murto, M., Jones*, M.G.K., Valkonen, J., Pehu, T. and Lehto, K. Dept. of Crop Husbandry, University of Helsinki, Finland; *Dept. of Biochemistry and Physiology, Rothamsted Experimental Station, Harpenden, U.K.

Solanum brevidens is a wild potato species which is resistant to PLRV, PVY and PVX. For all these viruses the resistance is manifested by prevention of build up of virus titre. Studies on the replication rates of viruses introduced into isolated protoplasts of S. brevidens and a susceptible dihaploid potato line showed that these were similar. This suggests that the resistance mechanism in S. brevidens plants is an inhibition of cell-to-cell spread of the viruses, and this possibility is being investigated. To study this aspect we are cloning the sequence coding for the putative movement protein of PVY. In addition the donor-recipient protoplast fusion approach has been applied to transfer resistance from S. brevidens to S. tuberosum. Following fusion experiments, ten thousand resultant calli are being analysed to identify asymmetric hybrids using species-specific and linkage group specific probes.

**A 225** BIOSYNTHESIS OF THE PHYTOALEXIN PISATIN: STUDIES WITH ANTISERA AGAINST THE 6a-HYDROXYMAACKIAIN 3-*O*-METHYLTRANSFERASE, C. L. Preisig[1], H. D. VanEtten[2], and R. A. Moreau[1], [1] USDA Agricultural Research Service ERRC, 600 E. Mermaid Ln., Philadelphia, PA 19118 [2] Department Plant Pathology, 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 that catalyzes the terminal step, synthesizing pisatin by methylating the 3-hydroxyl of (+)6a-hydroxymaackiain, was purified over 370-fold from $CuCl_2$-stressed pea seedlings and identified as a 43 kD protein by photoaffinity labelling and SDS-PAGE, although enzyme activity eluted in the same volume as BSA (66 kD) on nondenaturing gel filtration columns (Preisig et al. 1989 Plant Physiology 91:559-566).

Polyclonal antiserum against the purified enzyme inhibited (+)6a-hydroxymaackiain methyltransferase (HMKMT) enzyme activity and had high specificity for a 43 kd protein on Western blots. This protein was induced in garden pea by $CuCl_2$. Protein of the same size is specifically immunoprecipitated from *in vitro* translation products of total RNA from stressed pea; $CuCl_2$-treatment induced an increase in the HMKMT mRNA translational activity. Further results on the timing of this induction and progress on localization of the HMKMT protein in treated roots will be presented.

**A 226** A TISSUE CULTURE SYSTEM FOR MOLECULAR ANALYSIS OF PLANT RESPONSES TO ROOT-KNOT NEMATODE ATTACK, D. Radin, X. Zhang, D. Weissenborn, J. Eisenback and C. Cramer, Departments of Crop and Soil Environmental Sciences and Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute & State University, Blacksburg VA 24061.

A root culture system has been developed for achieving high frequency infections of tomato by Meloidogyne incognita. Epidermal thin-cell-layer explants from floral stems of Lycopersicon esculentum produce up to 40 adventitious roots per culture within 7 to 10 days on Murashige and Skoog medium. When rooted cultures are transferred to Gamborg's B-5 medium and exposed to infective juvenile nematodes, gall formation is apparent within five days in genetically susceptible (mi-/mi-) tomato cultivars, Rutgers, Red Alert and Pearson. Root-knot resistant genotypes (Mi+/Mi+), LA655, LA656, LA1022, exhibit a characteristic hypersensitive resistance response in thin-cell-layer roots. A gene transfer method mediated by Agrobacterium tumefaciens is being developed for tomato thin-cell-layers to analyze the effects of specific introduced genes on the response of roots to nematode attack. Preliminary experiments utilizing a disarmed Agrobacterium binary GUS plasmid system has demonstrated expression of the beta-glucuronidase (GUS) reporter gene driven by the CaMV 35S promoter in tomato thin-cell-layers. The expression of specific genes occurring as part of the host defense response to nematode attack is also being investigated using in situ hybridization of nematode infected thin-cell-layers with RNA probes.

**A 227** AN STS-RFLP MAP IN *ARABIDOPSIS THALIANA* FOR MAPPING AND CHROMOSOME WALKING USING A RECOMBINANT INBRED POPULATION, Robert Reiter, Kenneth Feldmann, Andrew Paterson, Antoni Rafalski, Scott Tingey, John Williams, and Pablo Scolnik, CR&DD, The Du Pont Company, Wilmington, DE 19880-0402

A recombinant inbred population was developed in *Arabidopsis thaliana* by crossing the Wassilewskija (WS) ecotype with W-100, a line containing 10 phenotypic marker loci (1). The lines have been inbred to the F8 generation, and are being used to generate a high-density RFLP linkage map with an average marker distance of 2 cM. A majority of the markers are based on the random amplification of polymorphic DNA (RAPD mapping). This approach uses arbitrarily-defined 10mer oligonucleotides as primers in genomic DNA amplification reactions, with each 10mer thus defining a sequence-tagged site (STS) in the *Arabidopsis* genome These RAPD markers have been mapped by scoring unique bands in a population of 46 recombinant inbred lines. We have included a number of RFLP markers provided kindly by E. Meyerowitz (2) to integrate the two Arabidopsis RFLP maps. In addition, we have used PCR to rescue plant DNA flanking T-DNA insertion events (3). These PCR products are being used to detect RFLP's in order to map the location of T-DNA insertion events via hybridization. Our goal is to generate a high-density genetic map of *Arabidopsis* to support physical mapping and chromosome walking strategies.

1. Koornneef et al., 1987, A.I.S. 23:46-50
2. Chang et al., 1988, Proc. Natl. Acad. Sci. USA 85:6856-6860
3. Feldmann and Marks, 1987, Mol. Gen. Genet. 208:1-9

**A 228** GENETIC MAPPING OF FUNCTIONAL DOMAINS OF CUCUMBER MOSAIC VIRUS RNA 1, Marilyn J. Roossinck and Peter Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Cucumber mosaic virus (CMV) is a broad host range, tripartite, plus-stranded RNA virus. Different strains of CMV induce widely different symptoms in host plants. Two strains of CMV, Fny-CMV and Sny-CMV, have several phenotypic differences: time of appearance and severity of symptoms when inoculated onto squash; temperature-sensitive replication in muskmelon; and efficiency of replication of satellite RNAs. Using pseudorecombinant viruses, we have mapped these differences to RNA 1. RNase protection assays using antisense RNA probes, and partial sequencing of viral RNAs, indicated that there were only minor differences in the RNAs 1 from these two strains. Using full length cDNA clones of Fny-CMV RNAs, and cDNA clones of RNA 1 of Sny-CMV, we are constructing a series of recombinant viruses. These recombinant viruses are being used to map precise domains of RNA 1 which are responsible for the phenotypic differences between these strains. RNA 1 is required for viral replication, and its single open reading frame contains the conserved motifs found in all known helicases, as well as sequences consistent with NTP-binding properties, although little is known about the precise role of RNA 1 in viral replication, and/or virus-host interactions. Results will be presented which delineate some of the functional domains of RNA 1.

**A 229** A NOVEL PLASMA-MEMBRANE PROTEIN IS INDUCED BY SALT IN THE HALOTOLERANT ALGA ***Dunaliella salina***, Avi Sadka and Ada Zamir, Biochemistry Dept. Weizmann Institue of Science, Rehovot 76100, Israel

*Dunaliella*, a unicellular alga lacking a rigid cell wall, is capable of growth in extremely varied salinities, e.g., from 0.05 M to 5.5 M NaCl, and responds to hypo or hyper-osmotic shock by rapid synthesis or elimination of glycerol to a level osmotically compatible with the external salinity. To unravel additional functions involved in salt tolerance, we have compared the protein profiles of *D. salina* continuously grown under different salinities. The level of a 150 kDa protein (p150) was dramatically elevated with the salinity of the medium. After purification to homogeneity p150 was shown to be a glycoprotein. Polyclonal anti-p150 antibodies were used to monitor the protein following drastic hyper-osmotic shocks. The cycloheximide-sensitive appearance of p150 coincided with the resumption of cell division and the length of the growth lag period was inversely related to the initial level of the protein, implicating a role for p150 in the salt adaptation of *D. salina* . Immunoelectron microscopy indicated that the protein was concentrated in the outer cell membrane. Immunological cross reaction suggested that p150 belonged to a family of conserved surface glycoproteins.

**A 230** PRODUCTION OF SYNTHETIC MAIZE CHLOROTIC MOTTLE VIRUS (MCMV) RNA WHICH IS INFECTIOUS IN BLACK MEXICAN SWEET (BMS) MAIZE PROTOPLASTS, Kay Scheets[1], Robert C. Nutter[2] and Roya Khosravi-Far[3], [1]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078, [2]Pioneer Hi-Bred International, Leiden, Netherlands, [3]La Jolla Cancer Research Institute, La Jolla, CA 92037.

A full length cDNA clone of MCMV RNA was constructed in the vector pT7E19. Using T7 RNA polymerase, capped RNA was made that contained one additional G residue at the 5' end. The cDNA clone has a unique Hind III site at the 3' end. RNA produced from the template had either one or five additional 3' nucleotides depending on whether the Hind III cut template was treated with mung bean nuclease. BMS protoplasts inoculated with the RNA transcript produced genomic and subgenomic RNAs and coat protein at similar times after inoculation as protoplasts inoculated with viral RNA, but the levels of RNA and protein were lower. Capped synthetic RNA with one additional 3' nucleotide was 25-50 times less infectious than viral RNA. Synthetic RNA with no cap was much less infectious than capped RNA. The addition of five extra nucleotides at the 3' end lowered the infectivity of the RNA compared to RNA with only one additional 3' nucleotide. RNase A protection analysis of the synthetic RNA indicated an error of at least one base in the replicase coding region. The correction of this error should lead to the production of a transcript capable of infecting maize plants.

**A 231** DNA PROBES THAT DIFFERENTIATE BETWEEN SPECIES AND PATHOTYPES OF POTATO CYST NEMATODE, Robert Shields, Rebecca Stratford and Andrew P. Goldsbrough, Plant Breeding International, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

Potato cyst nematode (PCN) is a major pathogen of the potato crop and two closely related species have been identified (Globodera rostochiensis and Globodera pallida). A number of pathotypes within these species have been distinguished on the basis of their ability to multiply on different species of wild potato. The resistance and susceptibility to some pathotypes appears to be governed by a gene-for-gene relationship, while in other cases the situation is less clear.

We have identified extensive RFLP differences between the two species of PCN using random genomic clones of low or single copy sequences as probes. In addition, we can readily distinguish the Pal pathotype of G. pallida from the Pa2 and Pa3 pathotypes. This suggests that it might be feasible to locate PCN avirulance genes by RFLP mapping.

A number of highly-repetitive clones that are diagnostic for either G. rostochiensis or G. pallida have been obtained by a differential screening procedure. These clones have been used to reliably identify PCN species by a simple dot blot technique using single cysts or less. The genomic organisation of these clones has been investigated. Some were found to exist in very long tandem arrays which are likely to have arisen by unequal crossing-over.

**A 232** ENDOPHYTE EFFECTS ON CORN RESIDUE DECOMPOSITION, C. F. Tester, and D. D. Kaufman. USDA, ARS, Soil-Microbial Systems Laboratory, Beltsville, MD 20705

Dekalb var 'T1100' corn (*Zea mays* L.) was grown in a greenhouse. When the plants were 18 d old they were inoculated with either the endophytic bacterium *Clavibacter xyli* subsp. *cynodontis* or a genetically engineered construction which was transformed by inserting a gene encoding for production of a Δ-endotoxin insecticidal protein from the bacterium *Bacillus thuringiensis*. Plants were harvested at maturity and appropriate plant parts were incorporated into soil to determine the extent of their decomposition in a 63 d laboratory incubation study. Physical and chemical properties of the residues will be presented and correlated with the rate and extent of residue decomposition. The leaves of the plants which had been inoculated with the recombinant endophyte retained significantly more water than did those from the control or wild type inoculated plants. The leaves of the inoculated plants contained more carbon than did those of the control plants, whereas the stalks contained less carbon. Inoculation with the endophyte increased the quantity of N present in all plant parts when compared with the control plants. The leaf residues contained more readily decomposable substrates than did the stalk residues. The leaves from control plants decomposed significantly but only slightly more during 63 d than did those from the wild type or recombinant. On the other hand the corn stalks from the recombinant decomposed significantly more than did those from the control and wild type. On the average the residues in this study decomposed approximately 33% within 63 days. This is the first study conducted to determine whether the presence of the endophyte or a geneticaly modified counterpart in plant residues influences subsequent decomposition of the residues in soil.

**A 233 DNA POLYMORPHISMS AMPLIFIED BY ARBITRARY PRIMERS ARE USEFUL AS GENETIC MARKERS.** **Scott V. Tingey, John G.K. Williams, and Antoni Rafalski,** E.I. du Pont de Nemours & Co., Inc., Experimental Station, Wilmington, DE 19880. Molecular genetic maps are commonly constructed by analyzing the segregation of restriction fragment length polymorphisms (RFLPs) among the progeny of a sexual cross. RFLP markers have shown great utility for determining genotype in a variety of applications. The major drawback to using RFLP markers is that the technology is difficult to automate. Here we describe a new DNA polymorphism assay based on the observation that single oligonucleotide primers of arbitrary sequence function to amplify discrete loci in a complex genome. This new assay, which we have named the RAPD assay after random amplified polymorphic DNA, provides a means for automating the tedious process of genotype determination. Data will be presented to show that RAPD markers faithfully assay DNA based polymorphism, and provide technology for automated genotype determination.

**A 234 A NEW CLASS OF ANTIFUNGAL PROTEINS IN PLANT SEEDS,** Alison J. Vigers, Walden K. Roberts and Claude P. Selitrennikoff, Departments of Cellular & Structural Biology (AJV & CPS) and Microbiology & Immunology (WKR), University of Colorado Health Sciences Center, Denver, CO 80262.

Plants defend themselves against pathogen attack with a variety of antimicrobial compounds, including proteins such as chitinases, $\beta$-1,3-glucanases, ribosome-inactivating proteins and proteinase inhibitors. We have isolated a protein, zeamatin, from corn (*Zea mays*) seeds that has potent antifungal activity against a variety of fungi, including human and plant pathogens. Zeamatin has no chitinase or $\beta$-1,3-glucanase activity nor is it a proteinase inhibitor. It appears to kill fungi by causing membrane permeabilization, resulting in leakage of fungal-cell components. Zeamatin has the interesting property of showing synergy with several antifungal drugs. This synergy is especially marked with the chitin synthase inhibitor, nikkomycin, and this has formed the basis for a sensitive assay for zeamatin-like proteins. Using this assay, we have detected proteins in soluble extracts from barley, flax, oats, sorghum and wheat that act synergistically with nikkomycin to kill *Candida albicans*, are approximately 22 kDa and cross-react with anti-zeamatin antibodies. This demonstrates that zeamatin is not a unique protein, but is a member of a novel family of antifungal proteins present in several plant seeds.

**A 235 INF56, A GENE EXPRESSED DURING SPORE DIFFERENTIATION OF THE BEAN RUST FUNGUS, BELONGS TO A MULTIGENE FAMILY.** X.-Ling Xuei, S. Bhairi, R. C. Staples and O. C. Yoder. Dept. of Plant Pathology and Boyce Thompson Institute, Cornell Univ., Ithaca, NY 14853.
Uredospores of the bean rust fungus, *Uromyces appendiculatus*, differentiate to form infection structures in response to the topography of stomatal guard cells or to 0.5um ridges on a plastic surface. One of the six differentiation-specific clones, INF56, was molecularly characterized. It encodes a 1.0kb transcript, which contains two open reading frames, ORF1 and ORF2. ORF1 codes for a 14kD polypeptide and has high level of similarities to the C-terminal domain of mammalian cytokeratin typeII proteins; while ORF2 codes for a 10kD polypeptide and has high level of similarities to the functional domain of chicken fibronectin. Sequence comparison between the genomic DNA and the cDNA and a series of Southern analyses suggested that INF56 is a multigene family with at least five copies in the fungal genome and that the copy characterized is actively expressed during the differentiation of the bean rust germlings.

*Genetic Dissection of Plant Cell Biology*

**A 300** **ANALYSIS OF PHOTOSYNTHETIC MUTANTS USING AN IMPROVED NATIVE GREEN GEL SYSTEM.** Allen, K.D., Staehelin, L.A., Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

We have developed an improved native green gel system that resolves approximately twenty chlorophyll-protein complexes from the thylakoid membrane of higher plants and green algae, with very little release of free pigment, and a very high degree of preservation of subunit-subunit interactions. Thus, we resolve each of the three major chorophyll-containing components of the thylakoid membrane into multiple subclasses. Multiple PSI-LHCI complexes, differing in antenna size, are resolved. PSII is largely preserved intact, and is resolved as multiple PSII-LHCII complexes. LHCII, the major light harvesting antenna for PSII, is resolved into at least three trimeric complexes in every species examined to date. In addition, a number of small complexes, some of which may arise from photosystem dissociation, are resolved. Our characterization of this system indicates that the multiplicity of complexes resolved here is not a product of detergent modification of a small number of *in vivo* complexes, but actually is providing a more accurate description of the diversity of chlorophyll-protein complexes in the native membrane. Work to date indicates that this system can be used as a 'fingerprinting' technique to 1) analyze subtle differences between species, 2) to pinpoint alterations in chlorophyll-protein complexes in photosynthetic mutants, and 3) to study the assembly of chlorophyll-protein complexes during chloroplast development. We will present data illustrating how this system can be employed to elucidate changes in chlorophyll-protein complex composition in chlorophyll b-deficient mutants of *Chlamydomonas* and several higher plant species. This work has been supported by NSF grant GM22912 to LAS.

**A 301** ISOLATION OF NUCLEAR GENES WITH NOVEL ROLES IN CHLOROPLAST BIOGENESIS BY TRANSPOSON TAGGING IN MAIZE. Alice Barkan, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

With the goal of elucidating mechanisms by which nuclear gene products dictate the timing and cell-type specificity of chloroplast development, studies are being conducted with a collection of maize nuclear mutants whose phenotypes suggest lesions in regulatory or assembly functions involved in chloroplast biogenesis. Fourteen *hcf* mutants have been isolated from a line of maize harboring an active *Mu* transposon system. These mutants are slightly pale green, non-photosynthetic, and seedling lethal. Many exhibit the late somatic reversion events typical of *Mu*-induced mutations. Results of Western analyses indicate that six mutants are deficient specifically in either the PSII, PSI, cytochrome f/b6 or ATPase protein complex; these phenotypes may result from lesions in genes encoding a structural component of the affected complex. The remaining eight mutants are more likely to define regulatory or assembly functions: four are defective in multiple membrane complexes, one lacks only RUBPCase and three are deficient in all plastid-encoded photosynthetic complexes. These mutations may define genes that regulate the expression of sets of nuclear or chloroplast genes, that are involved in protein uptake into the chloroplast, or that mediate the assembly of multimeric complexes. The results of experiments to further define the step at which each mutation acts will be reported. Based upon the results of these studies, several of the mutations will be selected for cloning by using the *Mu* tag, as we have done previously for mutant *hcf*106 (Martienssen, Barkan, Freeling, and Taylor, EMBO J., 1989). To provide a context for understanding the phenotypes of newly isolated mutants, the properties of several EMS-induced mutants (provided by Dr. Don Miles) have been analyzed in detail. One mutant, *hcf1*, is defective in plastid translation initiation and in the processing of the 16S rRNA. The pigmentation and degree of rRNA processing in this mutant are dependent both upon the temperature and upon the dosage of the mutant allele. The implications of these results will be discussed.

**A 302** ISOLATION AND CHARACTERISATION OF cDNA CLONES FOR THE CYTOSOLIC AND PLASTID ISOZYMES OF PYRUVATE KINASE FROM POTATO AND CASTOR BEAN ENDOSPERM. Stephen D. Blakeley and David T. Dennis, Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, CANADA

A full length cDNA clone for the cytosolic isozyme of pyruvate kinase has been isolated from a potato tuber cDNA expression library. The derived amino acid sequence of this, and cDNA clones for the same enzyme from developing castor seed endosperm, are very similar to the sequence of pyruvate kinases from both yeast and mammals. All amino acid residues which are involved in binding of ATP/ADP and phosphoenolpyruvate (PEP) in the mammalian protein are conserved in the plant cytosolic isozyme. The 3'-untranslated regions of the potato cDNA clones are characterised by multiple polyadenylation signals and the corresponding region of the castor clones are unusually long and variable in length. Northern analysis demonstrates the existence of several transcripts for castor cytosolic pyruvate kinase. cDNA clones for the plastid isozyme of pyruvate kinase have been isolated from a castor endosperm expression library. The derived sequences of these closely resemble both the plant cytosolic and non-plant enzymes. ATP/ADP and PEP binding sites are highly conserved and the $NH_2$ terminal of these proteins have extremely hydrophobic regions which may represent transit peptides. Two different hydrophobic $NH_2$ terminal regions have been identified, attached to otherwise identical proteins, and we are investigating the possibility that expression of the plastid pyruvate kinase gene involves differential splicing of exons from a single gene. Neither plant pyruvate kinase isozyme has a phosphorylation site comparable to the yeast or mammalian enzyme.

**A 303** EXPRESSION OF GENES CONTROLLING SESQUITERPENE BIOSYNTHESIS IN LYCOPERSICON TRICHOMES, C.D. Carter, F.R. Rahimi, J.N. Sacalis and T.J. Gianfagna, Department of Plant Science, South Dakota State University, Brookings, SD, 57005, and Department of Horticulture, Rutgers University, New Brunswick, NJ 08903

The sesquiterpene zingiberene accumulates at high levels exclusively in specifically differentiated cells (terminal cells of glandular trichomes) of the wild tomato species Lycopersicon hirsutum f. hirsutum (hir) (Carter et al., J Agric Food Chem 37: 1425). Labelled precursor experiments demonstrate $^{14}C$ accumulation in a potential intermediate and suggest that biosynthesis occurs in stalk or leaf matrix cells. Zingiberene production is controlled by multiple alleles at a single gene locus that we have designated Z, where $Z^1$ codes for presence of zingiberene and is codominant to $Z^3$ and recessive to $Z^2$.

Sesquiterpenes are derived from the isoprene pathway. Hydroxymethyl-glutaryl Coenzyme A reductase (HMGR) is a key enzyme in this pathway, and is regulated by GTP-binding proteins. Poly(A+) RNA was extracted from leaves and trichomes and hybridized to $^{32}P$-labelled cDNA probes for HMGR and GTP-binding protein genes to identify homologous mRNAs. HMGR and associated gene expression in relation to the Z alleles and differential sesquiterpene biosynthetic capacity will be presented.

**A 304** RECOMBIGENIC STRAND-TRANSFER ACTIVITY IN CHLOROPLAST EXTRACT FROM PEA, Heriberto D. Cerutti and Andrè T. Jagendorf, Section of Plant Biology, Cornell University, Ithaca, NY 14853

The endosymbiotic origin of plastids and the key role played by RecA proteins in DNA recombination and DNA repair processes in bacteria (including cyanobacteria) made a search for a RecA-like protein and/or activity a reasonable first step in understanding the function of the plastid recombination and DNA repair systems. We found a strand transfer activity in Triton X-100 permeabilized chloroplasts and crude stromal extracts. Radioactively labeled ssDNA chloroplast 16SrDNA invades the resident gene in the dsDNA plastome according to the pattern obtained by gel autoradiography. Purified DNA from the putative recombinant band contains the expected D-loop structures when analyzed by electron microscopy. The strand transfer activity present in crude stromal extracts is similar to that of the purified E. coli RecA protein as detected by exogenously added DNA substrates. This activity appears to require MgATP and seems stable in the presence of 0.6% SDS under our experimental conditions. A chloroplast protein of 36 KDa (slightly smaller than RecA) crossreacted with two polyclonal antibodies raised against E. coli k12 RecA protein. The steady-state level of this protein was increased by DNA-damaging agents like mitomycin C, nalidixic acid and UV irradiation suggesting that it may be involved in DNA repair/recombination processes. Understanding the enzymatic machinery involved in these processes may provide ways of designing a better system of chloroplast transformation by recombination.

**A 305 EXPRESSION OF A PETUNIA GLYCINE-RICH PROTEIN-ß-GLUCURONIDASE GENE FUSION IN TRANSGENIC PETUNIA** , Shu-Hua Cheng, and Carol M. Condit, Departments of Plant Science and Biochemistry, University of Nevada, Reno, NV 89557, U.S.A.

Glycine-rich proteins (GRPs) represent a newly discovered class of plant cell wall structural protein. In petunia, the expression of GRP1 at the mRNA and protein level has been shown to be developmentally regulated, being high in young tissue and very little or none in old tissue. The steady-state levels of petunia GRP1 transcripts were highest in stems and leaves, and lowest in flowers. No petunia GRP1 transcripts were found in roots. This gene is also wound activated and increased levels of the transcripts were detected within 5 min after wounding. We are interested in the cis-acting elements responsible for the developmental expression, organ specificity and wound induction. A 2100 bp upstream promoter fragment of the petunia GRP1 gene was fused to the ß-glucuronidase (GUS) reporter gene and the histochemical localization of GUS was performed in transgenic petunia. Our preliminary results indicated that GUS activities were present in stems, leaves and flowers. In leaves, GUS activities were particularly pronounced in vascular tissue. Upon wounding of leaves, this gene fusion was also found to be highly expressed in trichomes. Further characterization of the regulatory properties of the promoter will be presented.

**A 306** AN ULTRASTRUCTURAL AND BIOCHEMICAL ANALYSIS OF SOYBEAN VARIEGATED LEAF MUTANTS, Tai-Sheng Cheng and Joel M. Chandlee, Department of Plant Sciences, University of Rhode Island, Kingston, RI 02881

The Y18 gene of soybean (*Glycine max*) contributes toward chloroplast development. A variant allele (Y18-m) has been identified which normally gives rise to green leaf tissue, however, frequent somatic mutations produce yellow sectors on the leaves resulting in a variegated leaf pattern. The mutations can also occur germinally such that homozygous recessive yellow plants can be derived from a mutable plant. LDS-PAGE gel analysis of thylakoid membrane proteins from chloroplasts isolated from green or yellow tissue revealed the absence of four polypeptides (51, 35, 25, and 22 KD) in the mutant chloroplasts. Northern blots have been examined to determine the affect of the mutation on mRNA populations. An experiment involving *in vitro* translation coupled with an *in vitro* reconstitution system is being carried out to identify the primary lesion in the Y18 mutant. A second variegated leaf mutant (E25-10), phenotypically similar to T225M, has been produced by exposure of seed to gamma radiation. EM studies of chloroplasts in yellow leaves revealed fewer grana stacks and a distortion of the membrane structure. Several polypeptides of the thylakoid membranes are missing, and many are reduced. A spontaneously occurring variegated leaf mutant of soybean (Ogemaw) has been recovered recently. This mutant shows a different variegated leaf pattern to those produced either by T225M or E25-10. Instead of yellow leaf sectors, white sectors have been found in the mutant. A genetic analysis is underway.

**A 307** GENETIC ANALYSIS OF FRUIT SUCROSE ACCUMULATION IN *LYCOPERSICON CHMIELEWSKII*, Roger Chetelat[1], Serge Yelle[1], Joseph DeVerna[2] and Alan Bennett[1], [1]Department of Vegetable Crops, University of California, Davis, CA 95616, [2]Campbell Institute for Research and Technology, Route 1 Box 1314, Davis, CA 95616

Fruit of the domestic tomato (*Lycopersicon esculentum*) accumulate sugars as glucose and fructose, while fruit of the wild species *L. chmielewskii* accumulate predominantly sucrose. This difference in fruit sugar accumulation is due primarily to low levels of acid invertase in the wild species, and may contribute to the greater total sugar content of its fruit. An interspecific BC1F2 population, segregating for hexose vs sucrose accumulation, was scored for 100 isozyme loci and RFLP markers, the latter consisting of random genomic clones provided by S. Tanksley at Cornell University. Sucrose accumulation was inherited as a monogenic recessive trait, and cosegregated only with markers on chromosome 3, particularly TG102. In a BC2F2 population, the map distance between TG102 and the sucrose locus was less than 1.5cM, suggesting the RFLP marker can be used to facilitate rapid introgression of the trait. Sucrose accumulation was also associated with yellow fruit flesh and reduced fruit set, traits most likely encoded by linked genes. Crosses to *L. chmielewskii* confirmed both the monogenic inheritance of sucrose accumulation and the tight linkage to TG102.

**A 308** ANALYSIS OF T-DNA TAGGED PHOTOSYTHETIC MUTANTS, Alice Cheung and Beatrice Howard, Department of Biology, Yale University, New Haven, CT 06511

Using T-DNA as an insertional mutagen in *Nicotiana plumbaginafolia* and *Arabidopsis thaliana*, we have isolated three photosynthetic mutants: BH102, BH152, and 2N-29. BH102 is a pale-yellow mutant *Arabidopsis* ; BH152 is an albino *Arabidopsis;* and 2N-29 is an albino *N. plumbaginafolia* mutant. Both BH102 and BH152 harbor a single T-DNA insert carrying a nosNPTII gene while 2N-29 has two T-DNA inserts carrying a promoterless NPTII gene. Molecular and biochemical analyses of the mutants to elucidate the functional roles for the tagged genes will be presented. On-going efforts on isolating the tagged genes, their wild-type counterparts and the complementation of the mutations by the isolated genes will be discussed.

**A 309** CELL-SPECIFIC ACCUMULATION OF SUCROSE SYNTHASE (SS) IN SOYBEAN NODULES AND MAIZE ENDOSPERM AND TAPETAL CELLS IN ANTHERS, Prem S. Chourey, Michael E. Miller, Yen-C. Chen and Larry C. Purcell, USDA/ARS and University of Florida, Gainesville, FL 32611

Antibodies directed against maize SS have been used to analyze cell-specific accumulation of the SS protein in soybean nodules and in developing endosperm and anthers of maize. Immunohistological analyses show much SS localization in phloem cells of the soybean root. The nodule sections show significantly higher levels of the SS protein in the infected cells of the central tissue (CT). No SS protein was detected in the uninfected cells which showed low levels of starch deposition. Infected cells showed a marked spatial gradient in the CT. The SS protein in the infected cells appeared to be preferentially localized to peribacteriodal membrane, known to originate from the host cell plasmalemma. In maize endosperm, the storage cells in the central part of the endosperm were characterized by one of the two SS isozymes, SS1. The loss of the SS1, as in the case of shrunken mutation, led to an early cell degeneration in the endosperm. Cell wall degeneration was specific to the sh endosperm. In the young sporocytes of the tassel, a great abundance of the SS2 was seen in the tapetal cell layer, coinciding with callose wall biosynthesis in pollen. The role of the SS enzyme in generating UDPG pools for: (a) peribacteriodal membrane in nodule (b) stabilization of cell wall in endosperm and (c) callose wall biosynthesis in developing pollen will be discusses.

**A 310** GENOMIC ANALYSIS OF CYTOSOLIC AND PLASTID PYRUVATE KINASE FROM POTATO AND CASTOR BEAN. Kelvin P. Cole, Stephen D. Blakeley, and David T. Dennis, Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Genomic clones of cytosolic pyruvate kinase (PKc) from potato have been generated using the polymerase chain reaction (PCR). Primers were designed based on the sequence of a full length PKc cDNA clone isolated and sequenced in this laboratory and used to prime potato genomic DNA. Overlapping fragments generated by the PCR reaction were cloned and sequenced. Southern analysis and sequence data show that PKc is encoded by three genes. Within the coding regions the nucleotide sequence is highly conserved between the different genes. Of the eleven single base pair changes so far detected, all occur in the third position of the codon and do not alter the encoded amino acid residue. Sequence within introns is also highly conserved. Two of the genes carry an identical 259 bp intron, the third gene while conserving all 259 bp has an additional 57 bp inserted in the 3' region of this intron. The high degree of homology between the genes suggests their divergence has been a relatively recent evolutionary event. The 1.53 kb of coding sequence is dispersed in four exons and three introns covering approximately 5 kb on the genome. This contrasts the organization of mammalian PK's which have 12 exons and 11 introns dispersed over 9.3 kb (L and R isozymes) or 20 kb (M1 and M2 isozymes). We are now applying the same rationale to the study of the plastid isozyme of PK from castor bean.

**A 311** **THE ptGRP1 GENE PRODUCT IS A SPECFIC MERISTIMATIC AND VASCULAR TISSUE ASSOCIATED PROTEIN**, Carol M. Condit, Departments of Plant Science and Biochemistry, University of Nevada, Reno, Reno, NV. 89557.

The petunia GRP1 gene codes for a presumptive glycine-rich cell wall associated protein. Two anti-ptGRP1 antibodies were used for biotin-streptaviden-alkaline phosphatase localization of this protein. The first antibody (3517), previously characterized (Condit *et al.*, 1990, Plant Physiol. **93**, 596-602), reacts against amino acids 22 thru 36 of the mature ptGRP1 protein. The second (3127) was made against a peptide comprising amino acids 157 thru 172 (this peptide sequence is repeated three times at positions 199 thru 214, 241 thru 256 and 279 thru 294) of the mature ptGRP1 protein. Both anti-ptGRP1 antibodies reacted identically immunocytochemically, with the exception that 3127 gives a much stronger signal.

In young petunia stem the ptGRP1 protein was found to be expressed at high levels in meristematic tissue of the apex and internodal bud primordium. In addition, in developing leaves surrounding the apical bud, this protein is specifically and highly expressed only in cells of the procambium. In older stem tissue (> 2mm and extending to 8 cm below the apical bud) ptGRP1 is also detected at very low levels in all cell types with the exception of the protopholem, where it is again highly expressed. In addition, some accumulation of ptGRP1 was seen in some of the younger cells of the protoxylem, the cell type to which french bean GRP1.8 localizes in that species (Keller, *et al.*, 1989; P.N.A.S. **86**, 1529-1533). These results indicate that ptGRP1, like fbGRP1.8 is associated with the development of the vascular tissue but that ptGRP1, in addition, is associated with early meristimatic tissue of leaves and stems.

**A 312** THE ORANGE PERICARP MUTANT OF MAIZE IS A TRYPTOPHAN AUXOTROPH, Karen C. Cone, Allen D. Wright, Cynthia A. Moehlenkamp, and M.G. Neuffer, Division of Biological Sciences and Agronomy Department, University of Missouri, Columbia, MO 65211.

Orange pericarp is a seedling lethal mutant of maize caused by the duplicate unlinked recessive loci *orp1* and *orp2*. Several lines of biochemical evidence suggest that the mutant is a tryptophan auxotroph and that the specific defect is in the B subunit of tryptophan synthase (*trpB*). Furthermore, genetic evidence indicates that there are probably two *trpB* genes in maize, as is the case in Arabidopsis. To determine if there are indeed two genes in maize, a kernel-specific cDNA library was screened with a portion of the *trpB* gene from Arabidopsis. Clones representing two different *trpB* genes were isolated and partially sequenced. Both genes potentially encode polypeptides that contain blocks of conserved amino acids that correspond to regions of tryptophan synthase known to be important for enzymatic activity. To determine if the *trpB* genes represent *orp1* and *orp2*, the map locations of the *trpB* genes were determined by RFLP mapping in a recombinant inbred family. *trpB1* mapped to chromosome 4L and *trpB2* mapped to 10S. These locations are in agreement with the positions of *orp1* and *orp2* determined by traditional mapping methods. Taken together, the data verify the identity of *orp1* and *orp2* as duplicate structural genes for the B subunit of trytptophan synthase and confirm the orange pericarp mutant as a tryptophan auxotroph of maize.

**A 313** **WALL-ASSOCIATED PROTEIN INDUCES EXTENSION OF ISOLATED PLANT CELL WALLS,** Daniel J. Cosgrove, Simon McQueen-Mason, Daniel M. Durachko, Department of Biology, Penn State University, University Park, PA 16802

By reconstitution experiments we have attempted to identify the hypothetical wall-loosening enzymes that mediate extension of frozen/thawed cucumber hypocotyl walls held in tension at acid pH. Endogenous extension activity was inactivated by protease or heat. Proteins were extracted from "active" walls with 3-M LiCl and, upon crude fractionation, were found to induce extension in inactivated walls. Activity was localized to the growing region of the hypocotyl (no activity in wall extracts from nongrowing tissues). The cucumber wall extract could induce extension in walls from the growing regions of various dicots (cucumber, tomato, pea and radish seedlings), but not maize and barley coleoptiles. Extension was not induced in basal (nongrowing) walls. The wall-bound activity was readily renatured after boiling in methanol, but not in water. Fractionations indicate that the activity belongs to a single, small protein that is ionically bound to the wall. Analysis of sugars released by the walls indicates that the active protein contains little/no glycanase activity.

**A 314** THE REGULATION OF PROLINE ACCUMULATION DURING DEVELOPMENT AND AT LOW WATER POTENTIALS IN *Arabidopsis thaliana*, Abhaya M. Dandekar and Hui-Hwa Chiang, Department of Pomology, University of California, Davis, CA 95616

Desiccation induced by decreasing water potential is perhaps one of the most profound environmental factors that influences the growth, development, productivity and dispersion of plants in the biosphere. In contrast, certain plant tissues such as embryo and pollen will undergo a developmentally induced process of desiccation to extremely low water contents. Several studies suggest that this programmed desiccation could play an important role in switching cellular activities from one developmental stage to another. We are interested in studying the regulation of metabolic processes induced in plant tissues subjected to such extreme desiccation. Spatial differences were found in amino acid content of reproductive structures that had relatively lower water content as compared to vegetative structures. The most significant difference was in the level of the amino acid proline which was 11-26 % of the total free amino acids in reproductive tissues (floret, silique, peduncle and seed), while only 1-3 % of the total in vegetative tissues (rosette leaf or root). Significant proline accumulation occurred upon desiccation of vegetative tissues when the plants were subjected to experimentally induced decreasing water potentials simulated by the addition of salt or polyethylene glycol (PEG) in the plant growth medium. For instance, a 8-10 fold increase in proline accumulation was observed in the presence of 120 mM NaCl or KCl; and a 20 fold increase was stimulated by 60 mM PEG. We have found that the proline accumulation in response to PEG occurs through increased biosynthesis and through $^{14}C$ isotopic tracer experiments, we have established that glutamic acid is the major precursor of proline biosynthesis in response to desiccation in *Arabidopsis thaliana*. We have detected for the first time in plants, activity of the first enzyme in the pathway, gamma-glutamyl kinase (GK), which catalyzes the formation of the intermediate gamma-glutamyl phosphate from glutamic acid. We would like to identify and study the regulation of the gene(s) encoding GK through cDNA cloning and mutant isolation. We shall also report on the progress we have made in these two areas.

**A 315** **The putative plant homologue of the mammalian luminal binding protein (BiP) is encoded by a multigene family in tobacco,** Jürgen Denecke[1], Maria Helena S. Goldman, Jef Seurinck and Johan Botterman, Plant Genetic Systems N.V., J. Plateaustraat 22, 9000 Gent, Belgium. (1) Present address: Dep. Molecular Genetics, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

We isolated tobacco cDNAs that are highly homologous to the mammalian luminal binding protein (BiP). The sequences of the cDNAs and genomic Southern analyses indicate that in tobacco, putative BiP is encoded by a gene family of at least 4 independent members. The organ specific and stress related expression patterns of the different members of the gene family were analyzed at the mRNA level using gene specific probes. The coding sequences start with a typical signal sequence for translocation through the endoplasmic reticulum (ER) membrane and end with a HDEL retention signal. This sequence has been shown to be both necessary and sufficient to cause retention of proteins in the plant ER (Denecke et al. unpublished results). The deduced amino acid sequences downstream the signal peptide show highest similarity with the BiP sequences from yeast and animals. Antibodies raised against the C-terminal part of the putative tobacco BiP recognize a 75kD protein in microsomal fractions of tobacco leaf protoplasts. This protein is more abundant in cells inhibited for N-glycosylation of proteins which results in the accumulation of malfolded proteins in the ER.

**A 316** THE PROMOTER FOR A GENE ENCODING THE *ARABIDOPSIS THALIANA* PLASMA MEMBRANE PROTON PUMP (H+- ATPase) DIRECTS REPORTER GENE EXPRESSION PREDOMINANTLY TO PHLOEM CELLS IN TRANSGENIC PLANTS,
Natalie D. DeWitt, Jeffrey Harper, and Michael R. Sussman, Department of Horticulture, University of Wisconsin, Madison, WI 53706

Sucrose is photosynthesized in the leaf mesophyll cells and transported throughout the plant via the phloem. Sucrose loading into the phloem is an active process: a plasma membrane H+-ATPase generates a proton gradient which drives a plasma membrane sucrose/H+ cotransporter. In *Arabidopsis*, there is evidence for at least nine isoforms of the plasma membrane H+- ATPase. The 5' flanking region of one of these isoforms, AHA3, has been fused to the reporter gene, beta-glucuronidase (GUS) and transformed into tobacco and *Arabidopsis*. In histochemical assays, transgenic plants of both species express GUS predominantly in phloem cells of leaves, stems, roots, and flowers; and also in pollen. MUG assays confirm these results biochemically: vascular tissue was dissected away from pith in tobacco stems and both tissue types were assayed: GUS activity was detected in the vascular tissue, but not in the histochemically negative pith cells. 600 base pairs upstream of the start codon was sequenced and its transcription start site mapped. It did not have significant homology to the promoter sequences of isoforms AHA1 and 2, which have different tissue specificities. These data suggest that the AHA3 isoform may be the major phloem H+- ATPase which provides the driving force for sucrose transport.

**A 317** **High Chlorophyll Fluorescence Mutants of *Arabidopsis thaliana*.**
**R.D. Dinkins, B.R. Green, A.J.F. Griffiths**
**Department of Botany, University of British Columbia, Vancouver, BC, Canada**

Several photosynthetic mutants of *Arabidopsis thaliana* have been successfully isolated using high chlorophyll fluorescence (hcf) as a screen. The hcf phenotype arises from the reemission of absorbed light which cannot be utilized due to a defective electron transport system. Hcf lines which segregated as a single nuclear recessive in the $M_3$ generation were each derived from a separate EMS mutagenized $M_1$ bulk. The hcf mutants are seedling lethal in soil and must be maintained as heterozygotes, thus analysis of the mutant lines was done on plants grown on 1/2 MS salts supplemented with 5% sucrose. Characterization of the mutants has included fluorescence induction kinetics, fluorescence quenching, electron transport activity, SDS-PAGE and 'green' gel profiles. Analysis of ten hcf lines reveals that each exhibit a unique fluorescence induction kinetics, except *hcf1* and *hcf8* which were similar. Both *hcf1* and *hcf8* appear to have a disrupted photosystem I (PSI) reaction center, having reduced PSI reaction center complex (CPI), PSI activity and amounts of several PSI polypeptides as determined by immunoblotting. The phenotype of *hcf1* is observable upon germination, whereas *hcf8* appears normal and gradually (2-3 weeks) becomes hcf, thus the mutation may be developmental. *hcf2* and *hcf6* are also depleted in PSI (CPI) as well as PSII (CP47 and CP43) chlorophyll protein complexes. In addition, *hcf2* appears to be reduced in cytochrome *f* and $b_6$ (immunoblotting). Mutant lines examined thus far display a diversity of phenotypes. Some mutants have affected only one pigment-protein complex (*hcf1* and *hcf8*), while others (*hcf2* and *hcf6*) appear to have reduced amounts of several components of the electron transport system. Continued analysis of these and additional mutants is in progress and will be presented. (Research supported by NSERC).

**A 318** CHARACTERIZATION OF THE FAMILY OF GENES ENCODING P-TYPE ION-TRANSLOCATING ATPases IN TOMATO, Nicholas N. Ewing and Alan B. Bennett, Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616
We have reported the isolation of cDNAs encoding two isoforms of the plasma membrane $H^+$-ATPase in tomato [N.N. Ewing, L.E. Wimmers, D.J. Meyer and A.B. Bennett (1990) Plant Physiol., in press]. Southern blot analysis of tomato genomic DNA indicates that the genes encoding these two isoforms may be members of a subfamily of P-type ATPase genes consisting of as many as six to eight genes. Experiments to define the overall complexity of the P-type ATPase gene family will be presented. In order to determine which members of this gene family encode P-type $H^+$-ATPases and to determine whether other members of this family represent pseudogenes or possibly other P-type ion-translocating ATPases we have utilized our two cDNAs to isolate corresponding genomic clones. These genomic clones are currently being characterized and their tissue specific and developmental expression will be presented.

**A 319** **COMMON FEATURES IN SEVERAL LIGHT-INTENSITY DEPENDENT CHLOROPHYLL-DEFICIENT MUTANTS IN HIGHER PLANTS.** Falbel, Tanya G, and Staehelin, L. Andrew, MCD Biology, University of Colorado, Boulder, Colorado 80309
During thylakoid membrane biogenesis, chlorophyll synthesis and the accumulation of chlorophyll binding proteins are tightly linked, light regulated processes. We are investigating the consequences faced by plants defective in these processes using a collection of mutant plants in a variety of species which have abnormal thylakoid membrane systems. The following characteristics are expressed in a light-intensity dependent manner in all of these mutants: (1) a reduced content of chlorophyll, (2) a preferential loss of chlorophyll *b*, (3) a significant reduction in light-harvesting chlorophyll-protein complexes, and (4) the formation of grana-deficient thylakoid membranes. These maize, tobacco, soybean, and tomato mutants segregate 1:2:1 (green, yellow-green, lethal yellow) for the lesion. The yellow-green heterozygous mutant plants have partial blocks in chlorophyll synthesis, while the seedling lethal yellow homozygous mutants have complete blocks in chlorophyll synthesis. Several dosage dependent wheat mutants with similar characteristics have also been analyzed. Analysis of precursor pools in the chlorophyll synthesis pathway indicates that the wheat, maize, and tobacco mutants could be blocked in the same place in the pathway. We are now studying the chlorophyll-protein complexes, the thylakoid membrane apoproteins and ultrastructure of these mutants to determine if the postulated common defect in the chlorophyll synthesis pathway leads to the same alterations in the thylakoid organization at the molecular level. Supported by NIH grant GM 22912

**A 320** CLONING AND REGULATION OF THE TRYPTOPHAN DECARBOXYLASE GENE FROM CATHARANTHUS ROSEUS.
O.J.M. GODDIJN, P.J.L. VAN DER HELM, R.J. DE KAM, R.A. SCHILPEROORT and J.H.C. HOGE. Biotechnology Delft-Leiden, Project Group Plant Cell Biotechnology, Department of Plant Molecular Biology, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

One of the key enzymes in the biosynthesis of secondary metabolites in Catharanthus roseus is tryptophan decarboxylase (TDC:EC 4.1.1.28) as it links primary metabolism to terpenoid indole alkaloid (TIA) production by converting tryptophan into tryptamine. We cloned the gene after screening an expression library with antibodies against TDC. Overexpression of this cDNA clone in Nicotiana tabacum SR1 resulted in high levels of tryptamine. Currently we are studying the effects of sense and antisense overexpression of TDC cDNA in C. roseus on TDC mRNA levels, cell division and TIA production.
In suspension cultures TDC mRNA levels increase 5 to 10-fold upon transfer to auxin-free medium. Addition of $>10^{-7}$M NAA to auxin-starved suspension cultures causes a rapid decrease (within 6 hours) of the TDC mRNA level. In hairy root cultures, which generally display high TDC mRNA levels, auxin addition also causes a 10-fold, although much slower, reduction of the mRNA level. Currently we are studying the negative regulation by auxins by in vivo and in vitro promoter studies.

**A 321** THE DEVELOPMENT OF AN ACYL CARRIER PROTEIN HOMOLOGOUS RECOMBINATION/GENE REPLACEMENT IN ARABIDOPSIS. Dan Guerra*, Chengbin Xiang*, Phil Beremand** and László Mártón***, *University of Idaho, Department of Bacteriology and Biochemistry, Moscow, ID 83843, **USDA/ARS, Peoria, IL 61604, ***University of South Carolina, Department of Biological Sciences, Columbia, SC 29208.

We are developing a strategy to cause homologous recombination and gene replacement of acyl carrier protein (ACP) in Arabidopsis. The deletion of the coding sequence of ACP from a 4.3 Kb fragment of a genomic clone followed by the introduction of the protein A:ACPI gene fusion are being conducted in pGEM. The subsequent introduction of multiple antibiotic resistance markers to allow selection and screening for putative homologous recombinants should provide a basis for identifying transgenic Arabidopsis harboring a replaced ACP gene. We will describe the design of our cloning strategy and discuss the possible biochemical and physiological consequence of our use of the protein A:ACPI gene.

**A 322** ISOLATION AND CHARACTERIZATION OF MUTANTS OF Arabidopsis thaliana THAT ARE SENSITIVE TO SHORT WAVELENGTH ULTRAVIOLET LIGHT. Greg R. Harlow and David W. Mount, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

We have developed a genetic screen to help identify mutant Arabidopsis plants that are hypersensitive to the damaging effects of UV light. The goal of this work is to determine the mechanisms of UV resistance in higher plants. A combination of genetic and biochemical techniques has helped to identify many genes involved with DNA repair in bacteria, lower eukaryotes, and animal cells. By comparison, very little is known about the genetics or biochemistry of DNA repair in plants.

EMS-mutagenized M2 plants were screened by first applying a UV-opaque foam solution to the center of the plants at the 6-10 leaf stage. This covered the apical meristem containing the germ cells but left the mature outer leaves exposed. The plants were then irradiated with a dose of UV light that is below the fluence that causes detectable damage in the wild type plant. Twenty-six plants with differing degrees of increased UV sensitivity were isolated from a screen of approximately 36,000 M2 plants. Leaf tissues of these plants were all severely damaged by small doses of short wavelength UV light that have little effect on normal plants. These plants are likely to have either a defect in a DNA repair process or in synthesis of one or more UV-filtering compounds. Present efforts are directed towards a) genetic analysis of the mutants, (backcrossing, mapping, and sorting into complementation groups), and b) biochemical assays, (incidence and rate of removal of pyrimidine dimers, presence of DNA damage-binding proteins, and pigment chromatography) to identify the DNA repair or UV protection defect.

**A 323** PLASMA MEMBRANE H+ATPase: EXPRESSION AND BIOGENESIS, Jeff Harper, Natalie D. DeWitt, Michael Sussman, Plant Sci., 1575 Linden Dr., U of WI, Madison WI 53706

The active transport of ions across the plasma membrane is a fundamental property of living cells. In plant cells as much as 1/4 to 1/2 of the cellular ATP is utilized by a H+ATPase to generate a pH and electrical gradient across the plasma membrane. Our long range objective is to understand the structure and function of the plasma membrane and the role of the proton pump in development.

In *Arabidopsis* we have identified 9 H+ATPase genes by a PCR method that utilizes degenerate DNA primers corresponding to regions conserved in all P-type ATPases. This approach has also permitted the identification of 4 new P-type ATPases with unknown ion specificites. The presence of a large H+ATPase multigene family suggests that individual genes may be differentially regulated. Northern blot analysis of two H+ATPase genes, AHA1 and AHA2, indicates that these genes are expressed predominantly in root tissue. The two genes also share similar regulatory sequences (e.g. the region surrounding their putative TATA boxes share 38 of 43 bp). GUS fusions with 5' and 3' regulatory regions of AHA2 resulted in strong expression in roots of transgenic *Arabidopsis* plants. In contrast, the promotor region for a third gene, AHA3, did not show any significant similarity to AHA1 or 2, and directed a different pattern of expression when fused to the GUS reporter.

AHA1 was cloned as a full length cDNA and provided the first gene encoding a plasma membrane protein to be cloned from higher plants. We have constructed GUS fusions with portions of the AHA1 cDNA coding sequence in order to investigate protein targeting to the plasma membrane of plant cells. Evidence indicates that the region including the first two putative transmembrane domains of AHA1 is sufficient to result in an association of the fusion protein with a microsomal fraction.

**A 324** EXPRESSION OF A WOUND-INDUCIBLE PROMOTER IN TRANSGENIC POTATO. John M. Henstrand[1], José E.B.P. Pinto[2], Lisa M. Weaver[2], Klaus M. Herrmann[2], and Avtar K. Handa[1], Departments of Horticulture[1] and Biochemistry[2], Purdue University, West Lafayette, IN 47907
3-Deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase catalyzes the first committed step in the biosynthesis of aromatic amino acids. In Solanaceae, the enzyme is induced by mechanical wounding that causes de novo synthesis of the polypeptide (Dyer et al. (1989) Proc. Natl. Acad. Sci. USA **86**, 7370). A cDNA encoding DAHP synthase from suspension culture cells of potato (Solanum tuberosum L.) served as a probe to isolate a genomic clone encoding the 5′-end of the shkA gene encoding a potato DAHP synthase isoenzyme (Dyer et al. (1990) J. Biol. Chem. **265**, 1608). To initiate a structure-function analysis of the genomic nucleotide sequence preceding the coding region of this gene, the shkA regulatory region and various portions of the coding region were translationally fused to the coding region of the reporter gene ß-glucoronidase (GUS), followed by the nos terminator. The constructs were introduced into the potato line FL-1607 by Agrobacterium-mediated transformation. In the resulting transgenic plants, mechanical wounding of leaf tissue induced GUS four-fold within 18 hours when GUS expression was driven by a 0.9 kb DNA fragment containing the coding region for the amino terminal 111 amino acid residues of DAHP synthase and about 600 bp of the 5′ upstream regulatory region. GUS expression was cell specific.

**A 325** STRUCTURE AND FUNCTION OF THE *cdc2* GENE OF *Arabidopsis thaliana*.

**Takashi Hirayama, Yoshiro Imajuku, Minami Matsui[1] and Atsuhiro Oka, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, and [1]Cancer Research Laboratory, Institute of Gerontology, Nippon Medical School, Kawasaki, Kanagawa 211, Japan**

p34$^{cdc2}$ is one of most important proteins controlling an entry timing into mitosis, by modulating its protein serine/threonine kinase activity. Both structure and function of p34$^{cdc2}$ are highly conserved from human to yeast. However, there has been almost no information on plant *cdc2* genes and their products. In this report, using the *Schizosaccharomyces pombe cdc2* gene as a probe, we isolated *cdc2* overlapping cDNA clones from a cDNA library of *Arabidopsis thaliana* Columbia ecotype, and sequenced them. The sequence was capable of coding for a polypeptide of 34 kilodaltones, and the predicted amino acid sequence contained a putative kinase domain and was entirely homologous to the *cdc2* genes of human and yeast. The functional similarity between *A. thaliana* p34$^{cdc2}$ and *S. pombe* p34$^{cdc2}$ has been tested by complementation analysis.

**A 326** DEVELOPMENTAL AND TISSUE SPECIFIC EXPRESSION OF THE LARGE ATP SYNTHASE GENE CLUSTER IN SPINACH CHLOROPLASTS Margaret J. Hollingsworth and Cynthia D. Green, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260

The ATP synthase complex of thylakoid membranes is composed of nine individual subunits. Four of the six chloroplast-encoded subunits are found in a single multigenic cluster on the chloroplast genome. The genes, in order of transcription, encode $CF_o$-IV (atpI), $CF_o$-III (atpH), $CF_o$-I (atpF), and $CF_1$-alpha (atpA). Cotranscription of the genes is followed by splicing and numerous endo- and exo-nucleolytic cleavages. Experiments were performed to determine the expression of this gene cluster during four leaf developmental stages and in four different tissues from spinach.

Plastids for developmental studies were isolated from etiolated seedlings with or without 24 hours of illumination, young leaves, and mature leaves. The overall level of the RNAs from this gene cluster was greatly increased in young leaves as compared to the other three stages. The gene dosage was a significant factor in this observed change in transcript levels, but was not the sole influence. The relative amount of processing also varied during development, with a smaller proportion of uncleaved RNAs found as the leaves matured.

Examination of tissue specific expression was carried out in root, stem, leaf, and flower plastids. An interesting finding was that the relative amount of expression of this cluster is much higher in stem chloroplasts as compared to other tissues. This is not a gene dosage effect, but appears to reflect an overall higher concentration of all chloroplast RNAs in the stem. In contrast, root amyloplasts have very few copies of these transcripts present.

**A 327** EXPRESSION OF MAIZE PHOSPHOENOLPYRUVATE CARBOXYLASE IN TRANSGENIC TOBACCO PLANTS CONFERS AN INCREASED MALIC ACID LEVEL IN LEAVES, Richard L. Hudspeth[1], John W. Grula[1], Ziyu Dai[2], G.E. Edwards[2] and M.S.B. Ku[2]. [1]Phytogen Inc. 101 Waverly Dr. Pasadena, California 91105, [2]Department of Botany, Washington State University, Pullman, Washington 99164

The enzyme phosphoenolpyruvate (PEP) carboxylase plays a key role in $C_4$ photosynthesis, which is the fixation of atmospheric carbon dioxide in leaf mesophyll cells. To study the effect of elevated levels of a $C_4$ plant PEP carboxylase in a $C_3$ plant, tobacco was transformed via Agrobacterium tumefaciens with different plasmids containing maize PEP carboxylase sequence. Tobacco plants transformed with the unaltered PEP carboxylase gene had low levels of PEP carboxylase mRNA and the transcript size was larger than expected. Plants transformed with an intron-less PEP carboxylase gene gave similar results. Leaf RNA from these transformants hybridizes with a 5'-flanking probe, indicating the occurance of aberrant transcription initiation in these plants. The level of PEP carboxylase mRNA was 30-fold greater in transgenic plants containing a chimeric gene consisting of 5'- and 3' flanking sequences from a light-regulated tobacco gene and maize PEP carboxylase cDNA sequences. Tobacco plants transformed with this construction exhibited higher (20-50%) PEP carboxylase activity than controls. In addition, two kinetic forms of PEP caboxylase were identified, one with a low $K_m$ for PEP and one with a high $K_m$(PEP) corresponding to the $C_3$ and $C_4$ forms, respectively. In general, transgenic plants had higher leaf acidity and malate content than controls.

**A 328** ARABIDOPSIS DAHP SYNTHASE GENES ARE REGULATED DIFFERENTLY IN RESPONSE TO WOUNDING AND PATHOGENIC ATTACK, Brian Keith*, Xinnian Dong', Frederick M. Ausubel', and Gerald R. Fink*, *Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, Massachusetts, 02142, and 'Department of Molecular Biology, Massauchsetts General Hospital, Boston, Massachusetts, 02114.

We have isolated two distinct cDNA clones encoding DAHP synthase (DS) from *Arabidopsis thaliana*. This enzyme catalyses the first committed step in the shikimate pathway and may be a principal target for the genetic and metabolic regulation of aromatic amino acid biosynthesis in plants. Both Arabidopsis cDNAs (DHS1 and DHS2) contain putative amino-terminal transit peptides and can complement mutations in a yeast strain lacking DS activity. Wounding has been shown to increase DS activity in several species and DS RNA levels in potato. When Arabidopsis leaves are wounded, DHS1 RNA levels are transiently induced 3-5 fold within 1.5 hours, while DHS2 RNA levels are unchanged. Similarly, infection of Arabidopsis leaves with avirulent *Pseudomonas syringae* strains induces DHS1 RNA levels within 6 hours, while DHS2 RNA levels remain unaltered. Infection with a virulent *P. syringae* strain also induces DHS1 RNA levels, but at later times (24 hrs). Our results suggest that DHS1 and DHS2 may fulfill distinct physiological roles in Arabidopsis.

**A 329** TOPOLOGY AND MEMBRANE INTEGRATION OF LHCP, B.D. Kohorn and A.H. Auchincloss, Botany Dept., Duke Univ., Durham, NC 27706.

Because the final topology of LHCP in the thylakiod remains ill defined, we have generated antisera to a number of peptides that correspond to LHCP sequences predicted to lie on different surfaces of the thylakoid membrane. These antibodies are being used to probe exposed areas of LHCP on the thylakoid membrane. LHCP inserts into the thylakoid membrane through a mechanism that appears to require the structural integrity of the mature portion of the protein. While defined regions of LHCP are required for thylakoid association and integration, extensive deletion and substitution mutagenesis combined with the analasis of fusion proteins has yet to define a discrete sequence that effects integration into the thylakoid. Moreover, as large deletions can have different affects that are dependent upon their neighboring sequence, it is likely that a larger tertiary structure rather than a short signal sequence is important. Consistent with this idea is the observation that proposed luminal targeting sequences are unable to either translocate LHCP through the membrane, or to eliminate the inhibitory effect (on integration) of introduced charged residues in different regions of LHCP.

**A 331** EXPRESSION OF MULTIPLE, RELATED CELLULASES IN RIPENING TOMATO FRUIT, Coralie C. Lashbrook and Alan B. Bennett, Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616

Fruit softening during ripening is thought to be dependent upon the degradation of cell wall polymers by specific cell wall hydrolases. Recently, polygalacturonase-catalyzed pectin degradation has been shown to be neither necessary nor sufficient for tomato fruit softening. We are investigating the possibility that non-pectolytic determinants of softening may contribute to fruit ripening and have cloned two cDNAs corresponding to two related members of the tomato fruit cellulase (endo-$\beta$-1,4-glucanase) gene family. Sequencing of the complete coding region of pTCEL1 and a 5' domain of pTCEL2 indicate that these clones differ significantly at the nucleotide and amino acid sequence levels. pTCEL1 exhibits approximately 50% sequence identity with endo-$\beta$-1,4-glucanases of avocado fruit and bean abscission zone. The developmental expression of pTCEL1 and pTCEL2 mRNAs in wild-type and ripening-impaired mutant tomato fruit will be discussed together with strategies designed to determine the number of cellulase gene family members present in tomato and expressed within tomato fruit.

**A 332** MOLECULAR CLONING AND IMPORT STUDIES OF CHLOROPLAST ENVELOPE PROTEINS, Hsou-min Li, Tom S. Moore and Kenneth Keegstra, Department of Botany, University of Wisconsin, Madison, WI 53706

Most chloroplastic proteins are synthesized in the cytosol and posttranslationally imported into the chloroplasts. They are subsequently processed by the stromal processing protease or further sorted to their proper locations in the chloroplasts. Despite the great importance of chloroplast envelope in protein translocation and biogenesis of carotenoids and galactolipids, its proteins represent the least understood group in chloroplastic protein biogenesis.

In order to acquire better knowledge about chloroplast envelope proteins, we isolated cDNA clones coding for chloroplast envelope proteins. A λgt11 cDNA expression library made from pea leaf mRNA was screened with antibodies raised against envelope proteins. Two clones have been identified to encode chloroplast envelope proteins. One of them encodes a 14kd outer membrane protein and does not possess a cleavable transit peptide. The other encodes a precursor protein of 37kd and is processed to a protein of 30kd after import. The nucleotide sequences of both clones have been determined. Import studies with proteins encoded by these clones are underway; details will be presented in the poster.

**A 333** STABLE TRANSFORMATION OF CHLOROPLASTS IN NICOTIANA TABACUM, Pal Maliga, Peter Hajdukiewicz and Zora Svab, Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ 08855-0759

Stable genetic transformation of the plastid genome is reported in a higher plant, *Nicotiana tabacum*. Plastid transformation was obtained after bombardment of leaves with tungsten particles coated with pZS148 plasmid DNA. Plasmid pZS148 (9.6 kb) contains a 3.7-kb plastid DNA fragment encoding the 16S rRNA. In the 16SrDNA gene a spectinomycin resistance mutation is flanked on the 5' side by a streptomycin resistance mutation, and on the 3' side by a *Pst I* site generated by ligating an oligonucleotide in the intergenic region. Transgenic lines were selected by spectinomycin resistance, and distinguished from spontaneous mutants by the flanking, co-transformed streptomycin resistance and *Pst I* markers. Regenerated plants are homoplasmic for the spectinomycin resistance and the *Pst I* markers and heteroplasmic for the unselected streptomycin resistance trait. Transgenic plastid traits are transmitted to the seed progeny. The transgenic plastid genomes are products of a multi-step process, involving DNA recombination, copy correction and sorting out of ptDNA copies.

**A 334** THE CYTOCHROME *C* OXIDASE SUBUNIT III GENE FROM THE MOSS *PHYSCOMITRELLA PATENS*: STRUCTURAL FEATURES AND TRANSCRIPTION PATTERN, Joachim R. Marienfeld, R. Reski and Wolfgang O. Abel, Department of Genetics, Institute of General Botany, University of Hamburg, Ohnhorststrasse 18, 2000 Hamburg 52, FRG

For the first time mitochondria and mitochondrial DNA from an archegoniate have been isolated and characterized. A mitochondrial coded gene of a moss has been identified, cloned and sequenced. The cytochrome oxidase III gene (*cox3*) of *Physcomitrella patens* (EMBL accession number X53679) consists of a 618 bp open reading frame showing high homology (around 72 %) to known *cox3* sequences of higher plants but is a quarter shorter than these. The *cox3* gene contains no introns and reveals a G+C-content of 41.3 %. The region containing the *cox3* gene exists as a single copy in the mitochondrial genome as shown by restriction mapping. In the 5' flanking sequence a putative ribosome binding site and a potential secondary structure can be found. Two main transcripts of 2400 bp and 2600 bp could be detectet indicating complex mitochondrial transcription pattern possibly due to cotranscription. In the 5' region as well as in the 3' region of the *cox3* gene additional open reading frames could be found.

**A 335** CHARACTERIZATION OF THE RICE ACTIN GENE FAMILY: IN-SITU LOCALIZATION OF ACTIN PROMOTER ACTIVITY IN TRANSGENIC RICE AND UTILIZATION OF ACTIN REGULATORY ELEMENTS IN CEREAL TRANSFORMATION. David McElroy[1], Wanggen Zhang[1], Alan Blowers[4], Madge Rothenberg[3], Jun Cao[2,4], Yixin Wang[2], Elizabeth D.Earle[3] and Ray Wu[2], ([1]Field of Botany, [2]Section of Biochemistry, [3]Department of Plant Breeding and Biometry and [4]Plant Science Center, Biotechnology Building, Cornell University, Ithaca, NY 14853, USA).

Four unique rice actin genes (designated RAc1, RAc2, RAc3 and RAc7) have been isolated and their respective pattern of transcript accumulation in developing rice plants has been determined. Both RAc1 and RAc7 display a constitutive pattern of transcript abundance in young rice seedlings while the level of RAc2 and RAc3 transcripts declines as the seedlings mature. Of the four rice actin genes analyzed RAc1 has the most abundant transcript in young rice seedlings. By transient assays of cells transformed with various RAc1 promoter-GUS fusions we found that the RAc1 5'-flanking region contains a relatively efficient promoter for foreign gene expression in transformed rice, maize, wheat and barley cells and that the stimulation of foreign gene expression is dependent on the presence of a 5'-intron from the transcribed region of the RAc1 gene. The RAc1 promoter has been used to for the expression of a number of agronomically important genes in transgenic rice The RAc1 5'-intron has been used to stimulate foreign gene expression, from constitutive and environmentally induced promoters, in transformed rice and maize cells. In-situ localization of GUS expression in transgenic rice plants has revealed that while the RAc1 promoter is active in all tissues examined it is most highly active in those cells undergoing rapid cell division.

**A 336** TARGETING OF A PROTEIN TO THE CELL WALL OF TRANSGENIC PLANTS Thomas D. McKnight and Daniel R. Bergey, Department of Biology, Texas A&M University, College Station, TX 77843

Proteins that migrate through the endomembrane system are subsequently sent either to the vacuole or to the cell surface, but it is not known how plant cells direct proteins to their appropriate final destination. We show, by immunogold localization at the electron microscope level, that the first 42 amino acids (including the 32 amino acid signal peptide and the first Ser-$Pro_4$ repeat) from the cell wall protein extensin are sufficient to target the prokaryotic protein beta-glucuronidase (GUS) to the endomembrane system and eventually to the cell wall and middle lamella in transgenic plants. No GUS was detected in the vacuole, indicating that secretion to the outside is not due to overloading of a vacuolar transport pathway. GUS is glycosylated during its passage through the endomembrane system. Inhibition of glycosylation has no effect on targeting to the outside, but unglycosylated GUS is confined to the cell wall and is absent from the middle lamella. This change probably reflects changes in the quarternary structure of GUS and indicates that the molecular weight cutoff for transport through the wall is between 67,000 and 280,000. We are using this system to locate vacuolar targeting signals, since any additional signals that redirect the extensin-GUS fusion protein to the vacuole must contain specific targeting information.

**A 337** GLUTAMATE DEHYDROGENASE AS A TARGET FOR IMPROVED AMMONIUM TOLERANCE IN PLANTS.

Michael J McPherson[1], Kerrie M Jones[1,2], Andrew C Cumming[2] and John C Wootton[2]
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Ammonium is a crucial metabolic intermediate, yet intracellular ammonium pools of even moderately raised concentration are toxic to most plants. Symptoms include stunted growth, distorted morphology, increased susceptibility to other stress and chlorosis by chloroplast degradation. Work at Leeds has demonstrated increased ammonium tolerance in a cyanobacterium carrying a foreign glutamate dehydrogenase (GDH) gene. This provides support for the hypothesis that increased capacity for ammonium assimilation into amino acids can cause ammonium tolerance. The route via the energy-efficient enzyme glutamate dehydrogenase rather than the normal assimilatory ATP-requiring glutamine synthetase provides a further metabolic advantage.

*Arabidopsis thaliana* is being used as a model to examine ammonium toxicity and tolerance in plants with a view to engineering imporoved tolerance. The programme involves (a) cloning of ammonium induced glutamate dehydrogenase genes (b) screening mutagenized samples for altered ammonium assimilation and tolerance characteristics and (c) biochemical analysis of ammonium induced proteins and mutational variants. The latest results of these complementary studies will be presented. Interestingly "universal" PCR primers for GDH genes have allowed amplification of not only the *Arabidopsis* GDH gene but also those from bacteria, fungi and a range of dicot and monocot plants.

**A 338** **REGULATION OF THE STRICTOSIDINE SYNTHASE GENE, ENCODING A KEY ENZYME IN ALKALOID BIOSYNTHESIS IN *CATHARANTHUS ROSEUS*,**

**J. Memelink, G. Pasquali, O.J.M. Goddijn, A. de Waal*, R.A. Schilperoort, J.H.C. Hoge. Department of Plant Molecular Biology, Wassenaarseweg 64, 2333 AL Leiden, and * Center for Bio-Pharmaceutical Sciences, Division of Pharmacognosy, P.O. Box, 9502, 2300 RA Leiden, Leiden University, The Netherlands.**

**We cloned two genes encoding enzymes acting early in a pathway of secondary metabolite biosynthesis in *Catharanthus roseus* (periwinkle). Tryptophan decarboxylase (TDC) converts tryptophan into tryptamine, and strictosidine synthase (SSS) subsequently couples tryptamine to secologanin to form strictosidine, which then serves as the precursor for numerous terpenoid indole alkaloids. SSS appears to be encoded by a gene present in one copy per haploid genome suggesting that the SSS isoenzymes, reported by others, are formed post-translationally from a single precursor. The *sss* and *tdc* genes appear to be coordinately regulated. In intact, healthy plants steady state mRNA levels for both genes are highest in roots. In cell suspension cultures, steady state mRNA levels were found to be reduced by auxin, and induced by salicylic acid or elicitors. Ethylene or cytokinin did not appear to play a role in *sss* or *tdc* expression. Currently, *in vivo* and *in vitro* promoter studies are underway to elucidate the regulatory pathways leading to expression of both genes, with emphasis on the negative regulation by auxins and the positive regulation by elicitors.**

**A 339** IDENTIFICATION AND CHARACTERIZATION OF A BiP-LIKE ENDOPLASMIC RETICULUM-LOCALIZED MOLECULAR CHAPERON IN TOMATO, David J. Meyer, Nicholas N. Ewing, and Alan B. Bennett, Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616

An understanding of the mechanisms of proper targeting and assembly of proteins in plants may be crucial to the implementation of genetic engineering techniques in an efficient and productive manner. Recent evidence from several non-plant systems implicates an ER-localized member of the HSP70 family in the precesses of ER targeting, protein folding and multimer assembly, and nuclear division. We have isolated cDNA and genomic clones corresponding to the ER-localized molecular chaperon, BiP, in tomato. The open reading frame encodes a 74 kD polypeptide which 1) bears a hydrophobic N-terminal extension, 2) contains the C-terminal ER-retention signal -H-D-E-L-COOH, and 3) lacks consensus N-glycosylation sites. Southern blot analysis of tomato genomic DNA indicates that this gene is present at low copy number. Polyclonal antisera raised against a fusion protein containing the C-terminal 72 amino acids of the tomato gene react strongly with a 74 kD protein in Western blot analysis; use of this antiserum to follow subcellular fractionation of suspension-cultured tomato cells indicates that this protein is found in vesicles of the endoplasmic reticulum. Expression of this gene in various plant tissues and the effects of various stresses on gene expression will be discussed.

**A 340** CHANGES IN GENE EXPRESSION ASSOCIATED WITH CHEMICAL INDUCTION OF CAROTENOGENESIS IN TOMATO, Thomas S. Moore and Alan B. Bennett, Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616
Although carotenoids constitute an important class of pigments required by all photosynthetic organisms, relatively little is known about the molecular biology of carotenogenesis. One model system for the study of carotenogenesis in higher plants is the induction of lycopene in tomato suspension culture cells treated with certain synthetic tertiary amines. We have observed that the induction of lycopene by CPOTA, one member of this class of tertiary amines, is inhibited by actinomycin D. This result is consistent with the previously forwarded hypothesis that these compounds act in part at the level of gene expression. Because plastids are thought to be autonomous for carotenoid biosynthesis, we expect that if CPOTA acts on gene expression, some of the proteins induced should be targeted to plastids. We have imported the translation products of mRNA isolated from control and induced cultured cells into isolated pea chloroplasts. We observed a modest increase in the translation products of a small number of plastid-targeted proteins upon CPOTA induction of carotenogenesis. These proteins will be further characterized and compared to the imported proteins synthesized from mRNA of ripening tomato fruit.

**A 341** RIBOSOMES PAUSE AT SPECIFIC SITES DURING SYNTHESIS OF MEMBRANE BOUND CHLOROPLAST REACTION CENTER PROTEIN D1, John E. Mullet, Jungmook Kim and Patricia Gamble Klein, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2128.
The reaction center of Photosystem II is composed of two chlorophyll-proteins, D1 and D2. D1 is encoded by the plastid *psb*A gene. *Psb*A is transcribed to produce a 1.2 kb RNA which lacks a consensus prokaryotic ribosome binding site. However, D1 is translated at high rates on membrane bound polysomes. Translation of D1 has been found to be discontinuous. D1 translation intermediates of 15 and 25 kd are observed in pulse-labeled plastids and toeprint assays revealed that ribosomes pause at discrete sites along the *psb*A mRNA. Recently it has been shown that co-translational binding of chlorophyll to D1 is required for D1 accumulation (Mullet *et al.*, (1990) PNAS 87:4038-42). In the absence of chlorophyll, D1 is degraded before release of full length D1 from ribosomes. Therefore, ribosome pausing during D1 translation may play a role in the binding of cofactors to D1 and in insertion of D1 into the thylakoid membrane.

**A 342** **Assembly of the chloroplast ATP synthase complex**
Johnathan A. Napier, Hakan K. Larsson and John C. Gray, Botany School, University of Cambridge, England, U.K.
The ATP synthase of the chloroplast thylakoid membrane is a typical $H^+$ translocating ATPase, sharing considerable homology with its prokaryotic and mitochondrial counterparts.In the case of the chloroplast ATP synthase, three of the nine component subunits are cytoplasmically synthesised and are transported across the chloroplast envelope, prior to assembly into the complex. The regulation of assembly of this complex is poorly understood, but it has been postulated that multimeric complexes like the ATPase are regulated by the abundance of the nuclear gene products. To test this idea, we have attempted to alter the levels of these polypeptides by either over-expression or antisense of the relevant gene in transgenic plants. To that end, we have isolated and characterised full-length cDNA clones from tobacco encoding the $\gamma$ and $\delta$ subunits, the two nuclear components of $CF_1$. Precursor proteins generated from these cDNAs *in vitro* are imported and integrated into the ATPase of isolated chloroplasts. These cDNAs have been cloned in both orientations into pBin 19, under two different promoters, and introduced into tobacco. The effects of this on the levels of $\gamma$ and $\delta$ subunits and the ATP synthase complex will be discussed.

**A 343** CHARACTERIZATION OF THE PHOTOSYSTEM I MUTANT *viridis-zb*$^{63}$ IN BARLEY, Nielsen, V.S., Okkels, J.S., Scheller, H.V., Kjær, B. and Møller, B.L., Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark.
The thylakoids of the barley mutant *viridis-zb*$^{63}$ contains no functionally active photosystem I (PSI) complexes as evidenced by the absence of a light induced $P700^+$ signal by EPR analysis and by the lack of PSI polypeptides as analyzed by SDS-PAGE. The mutant contains normal light-harvesting complexes and a normally functioning photosystem II. The mutation in *viridis-zb*$^{63}$ is located in the nuclear genome. Transcripts of the nuclear genes *PsaD, PsaE, PsaH* and *PsaL* and of the chloroplast gene *psaI* are present in the mutant in the same sizes and nearly the same amounts as in wild-type barley. *In vitro* translation and immunoprecipitation of these transcripts show identical sizes of the translated precursor polypeptides in the mutant and wild-type. Very low amounts of the photosystem I polypeptides are detected in mutant thylakoids by Western blotting analysis. The nuclear encoded subunits are processed to their mature sizes by the mutant chloroplasts. The lack of photosystem I in *viridis-zb*$^{63}$ may be associated with a translational defect, with a defect in uptake by chloroplasts, or with an increased turn-over of photosystem I components.

**A 344** TWO GENES FOR ANTHRANILATE SYNTHASE IN ARABIDOPSIS, Krishna K. Niyogi and Gerald R. Fink, Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142.
Anthranilate synthase (AS) catalyzes the first, committed step in tryptophan biosynthesis. The microbial enzyme is composed of two subunits, a large subunit (α) that binds chorismate and carries out its aromatization and a small subunit (β) that transfers an amino group from glutamine. In order to study the regulation of this key branchpoint enzyme in plants, we have cloned two genes encoding ASα from *Arabidopsis thaliana* using a conserved region of the corresponding yeast gene (*TRP2*) as a probe. These genes, designated *ASA1* and *ASA2*, have 63% nucleotide identity to each other, while the deduced proteins are 65% identical to each other and 31% identical to the yeast *TRP2* gene product. An *ASA1* cDNA complements deletion mutations in yeast *TRP2* and *E. coli trpE*, confirming that *ASA1* encodes a functional ASα and indicating that interactions between AS α and β subunits are conserved. The *ASA1* and *ASA2* deduced proteins both have putative amino-terminal chloroplast targeting sequences and conserved amino acids thought to be involved in feedback inhibition by tryptophan. RNA blot analysis shows that the steady-state level of *ASA1* mRNA is about 10-fold higher than that of *ASA2* in whole plants, and there appear to be differences in patterns of tissue expression and responses to wounding. We are making transgenic plants containing fusions of *ASA1* and *ASA2* promoters to the reporter gene β-glucuronidase (GUS) to study this further.

**A 345** THE MOLECULAR GENETICS OF TRYPTOPHAN BIOSYNTHESIS IN *ARABIDOPSIS*, Kim D. Pruitt, Alan B. Rose, Elaine R. Radwanski, and Robert L. Last, The Boyce Thompson Institute for Plant Research At Cornell University, Tower Rd., Ithaca, N. Y., 14853
Genetic and molecular biological approaches are being utilized to elucidate the steps in the tryptophan biosynthetic pathway, and to study the regulation of synthesis of this auxin precursor during plant growth and development. Genetic analysis of tryptophan auxotrophic mutants has identified three complementation groups. The *trp2-1* mutant is defective in tryptophan synthase β (TSB) subunit activity. Two tryptophan synthase β subunit genes have been identified[1]. *TSB1* (the gene mutated in the *trp2-1* mutant) and *TSB2* exhibit 95% identity at the amino acid level. The *TSB1* mRNA is approximately 10-fold more abundant than *TSB2* in leaf tissue; the relative abundance of *TSB1* and *TSB2* mRNAs in different tissues and genotypes is being investigated. The blue fluorescent *trp1-1* mutant is defective in anthranilate phosphoribosyl transferase (APT) activity, early in the pathway [2]. An *Arabidopsis* APT cDNA was isolated by complementation of an *E. coli trpD* mutation[3]. We have found that the cDNA-predicted protein sequence is 35% identical to both the *E. coli* and yeast APTs over 338 and 237 amino acids respectively. These proteins contain several clusters of homology, most notably a 40 amino acid stretch in which the *Arabidopsis* and *E. coli* APTs are identical at 30 residues, including a sequence of 9 consecutive amino acids that is also found in the yeast APT. Preliminary evidence indicates that the *trp3-1* mutant is blocked late in the pathway. 1. PNAS 86, p.4604-4608 (1989). 2. Science 240, p.257-364 (1988). 3. *TRP1* was isolated by J. Mulligan and R. Davis.

**A 346** IDENTIFICATION AND CHARACTERIZATION OF PHOTOSYSTEM I GENES AND SUBUNITS, Scheller, H.V., Okkels, J.S., Nielsen, V.S., Andersen, B., Møller, B.L. Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark.
The detailed analysis of photosystem I with the use of improved electrophoretic procedures have lead to the discovery of additional subunits with molecular masses below 9 kDa. Previously, five chloroplast genes and six nuclear genes encoding photosystem I subunits have been described. A seventh nuclear gene, *PsaL*, has now been identified and characterized in barley. The *PsaL* gene encodes a 22 kDa precursor polypeptide. The mature polypeptide is an integral membrane protein with a molecular mass of 18 kDa. The discovery of a hitherto overlooked subunit with a relative high molecular mass is surprising but is explained by the comigration of the *PsaL* and *PsaG* gene products in most SDS-polyacrylamide gel systems and by the fact that the *PsaL* as well as the barley *PsaG* gene products are N-terminally blocked. The *PsaL* genes as well as the other nuclear photosystem I genes have been localized to specific chromosomes by the use of addition lines of wheat. Analysis of RNA isolated during light-induction of barley plants shows that the expression of all the barley photosystem I genes, chloroplast genes as well as nuclear genes, have a similar pattern of induction in response to light.

**A 347** **GAP JUNCTION PROTEIN HOMOLOGUE FROM *ARABIDOPSIS THALIANA* -- A NEW KINGDOM FOR THE CONNEXIN FAMILY**, Melvin Schindler, Department of Biochemistry, Michigan State University, East Lansing, MI 48824
An *Arabidopsis thaliana* library constructed in the Bluescript expression vector Lambda ZAP II (Stratagene) was screened with affinity purified antibody raised against connexin 32 (rat liver gap junction polypeptide). The positive plaques were then rescreened with affinity purified antibody prepared against the 29 kDa polypeptide of a putative soybean connexin homologue (Meiners, S., and Schindler, M. (1989) Planta 179, 148-155), followed by another screen with antibody prepared against a synthetic peptide containing the amino acid sequence 98-124 found in connexin 32. A single clone was obtained whose gene product demonstrated immunological cross-reactivity with all three affinity purified antibody reagents. The cDNA from this clone contained 1,203 bp coding for a protein of 280 amino acids with a calculated molecular mass of 32,339 daltons and demonstrating approximatley 40% similarity with the sequence of connexin 32. Northern blot analysis utilizing the *Arabidopsis* cDNA as a probe demonstrated that soybean (*Glycine max* (L.) Merr. cv. Mandarin) root cells (SB-1 cell line) express an mRNA with similar size, while peptide maps resulting from both CNBr and *Staphylococcus aureus* V-8 protease treatment of the 29 kDa polypeptide derived from both soybean and *Arabidopsis* leaf tissue show identical cleavage patterns. The sequence homology observed between the 32.3 kDa polypeptide of *Arabidopsis* and connexin 32, in conjunction with observed similarities in predicted number and distribution of hydrophobic domains, channel-forming motifs, sites for post-translational modification, basic pI (10.4), and immunological relatedness, provide strong cumulative evidence that the biological range for connexin-type proteins may now be considered to include the plant kingdom.

**A 348** RECOMBINATION-ASSOCIATED FUNCTIONS IN CRUCIFERS, Ethan R. Signer, Joseph J. Kieber[1], Alain F. Tissier, and Mary F. Lopez[2]. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; Present addresses: [1]Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA; [2]Millipore Corporation, Bedford, MA 01730
Genetic recombination plays a major role in the life cycle of plants as of other organisms, and is likely to be important as well in the engineering of plant lines for practical agronomy. We have been concentrating on two enzymes likely to be involved, namely, DNA topoisomerase I and DNA recombinase (strand exchange protein). Both activities have been purified to near homogeneity from broccoli (*Brassica oleracea* var. *italica*). A gene for topoisomerase I has been cloned, and a gene for recombinase is now being sought, from *Arabidopsis thaliana*. The status of these and related studies will be described.

**A 349** DIRECT CLONING OF PROTEASES OF KNOWN SPECIFICITY, Tracy A. Smith and Bruce D. Kohorn, Department of Botany, Duke University, Durham, NC 27706
The degradation and cleavage of proteins is an essential feature of many biological regulatory mechanisms, yet while the site of cleavage within some proteins is known, relatively few proteolytic activities have been completely purified or their genes cloned. We have developed a rapid genetic selection to isolate eukaryotic cDNAs encoding proteases that cleave within a defined amino acid sequence. The assay was developed using the transcription factor gal4 from S. cerevisiae as a selectable marker, a cloned protease from Tobacco Etch Virus (TEVP), and the 7 amino acid TEVP target sequence. In yeast, TEV protease cleaves its target even when the target is fused to internal regions of the gal4 protein, resulting in an inactive gal4. DNA binding studies demonstrate that indeed protease decreases the activity of the gal4/target fusion protein. The cleavage can be detected phenotypically by the inability of the yeast cells to metabolize galactose. Cells expressing protease can also be selected on the suicide substrate 2DG. This selection scheme is being used to isolate a cDNA for the chloroplast transit peptidase using an active gal4/transit peptide fusion and an Arabidopsis cDNA library.

**A 350** DETERMINATION OF THE ACTIVATOR BINDING SITE IN THE SPINACH LEAF 54 kD SUBUNIT AND COMPARISON OF THE PRIMARY STRUCTURES OF ADPGLUCOSE PYROPHOSPHORYLASE FROM DIVERSE SOURCES, Brian Smith-White, Kathryn L. Ball and Jack Preiss, Biochem. Dept., Michigan State Univ., E. Lansing, MI 48824
ADPglucose pyrophosphorylase (EC2.7.7.27) is believed to act at a major regulatory site in the starch synthesis pathway of the leaf chloroplasts. ADPglucose pyrophosphorylase from spinach leaf is composed of two types of subunit with masses of 51 and 54 kD; which are probably the products of different genes. The enzyme is activated by 3-phosphoglycerate and inhibited by orthophosphate. The cDNA sequence of the small subunit has been isolated. Amino acid sequences of peptides generated from the 54 kD protein, by a variety of proteases, show a high degree of homology with the deduced amino acid sequence for the 51 kD protein and to the deduced amino acid sequence(s) of all other plant ADPglucose pyrophosphorylase proteins for which sequence data is available. Current efforts are directed toward obtaining cDNA sequence(s) for the 54 kD subunit as well as isolating the activator binding site sequence(s) for this protein. Thus far, data indicates that pyridoxal phosphate, an activator site probe, binds at more than one site on the large subunit; this is in contrast to the small subunit where pyridoxal phosphate is bound at a single site.

**A 351** CONTROL OF CHLOROPLAST mRNA STABILITY STUDIED USING CHLOROPLAST TRANSFORMATION IN *CHLAMYDOMONAS*, David B. Stern and Karen L. Kindle*, Boyce Thompson Institute for Plant Research and *Cornell NSF/DOE/USDA Plant Science Center, Cornell University, Ithaca, NY 14853
The control of mRNA stability is an important feature of plastid gene regulation during light-induced plant development. *In vitro* studies indicate that inverted repeats, which can form stem/loop structures in RNA and are found at the 3' termini of plant chloroplast mRNAs, stabilize upstream sequences and bind specific proteins that may regulate mRNA maturation and/or decay rates. To test these putative *cis*-acting regulatory elements *in vivo*, we have employed chloroplast transformation in the photosynthetic eukaryotic green alga *Chlamydomonas reinhardtii*. A series of deletions in the 3' non-coding region of the plastid *atp*B gene, which encodes the ß-subunit of the ATP synthase, have been introduced into *Chlamydomonas* chloroplasts. Partial or complete deletion of a complex secondary structure destabilizes *atp*B mRNA *in vivo*, although alternative secondary structures can also stabilize the mRNA. RNA instability leads to reduced production of *atp*B protein and consequently affects the growth rates of cells. Suppression of these effects can occur by retention of multiple copies of the *atp*B gene on extrachromosomal plasmids. The level of *atp*B protein also appears to be controlled post-translationally, since the reduction in *atp*B mRNA level is more drastic than the decrease in steady-state *atp*B protein levels.

**A 352** EXPRESSION OF HYDROXYPROLINE-RICH GLYCOPROTEINS IN SENSE AND ANTI-SENSE TRANSGENIC TOBACCO, Kathleen Swords [1], Johan Memelink [2] and L. Andrew Staehelin[1], (1) MCD Biology, University of Colorado, Boulder, CO 80309-0347, (2) Dept. Plant Mol. Biology, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Cell wall hydroxyproline-rich glycoproteins (HRGPs) or extensins are postulated to play a role in both pathogen resistance and normal tissue development. To test whether HRGPs are essential for plant development, an isolated tobacco extensin gene (Memelink, et al, 1987) was used to construct sense, anti-sense and vector-transformed tobacco plants. Northern analysis of non-transgenic plants has shown the extensin gene to be expressed strongly in root and stem tissues and weakly in the leaf. Sense transformants overexpress this gene in leaf, with detectable mRNA levels boosted in appropriate tissues, whereas anti-sense constructs give no detectable signal in any tissue. To determine if these changes in gene expression also affect synthesis and assembly of HRGPs, we have measured the hydroxyproline content, evaluated the pattern of extractable proteins by Western analysis and surveyed the accumulation of HRGPs using immunolocalization techniques. In agreement with the molecular behavior, Western analysis suggests a single HRGP is preferentially targeted in the sense and anti-sense plants. However, the expression of this protein appears to influence the pattern of other detected HRGPs, suggesting another level of coordinate regulation that varies tissue to tissue. Hydroxyproline content is higher in sense and lower in anti-sense tissues relative to vector controls. Immunolocalization of HRGPs in transgenic tissues will be discussed. Supported by NIH-GM18639 to L.A.S.

**A 353** INHIBITION OF PECTIN METHYLESTERASE (PME) GENE EXPRESSION IN TRANSGENIC TOMATO FRUIT BY ANTI-SENSE RNA, Denise M. Tieman, G. Ramamohan, Robert W. Harriman, and Avtar K. Handa, Department of Horticulture, Purdue University, West Lafayette, IN 47907

Demethoxylation of pectin by pectin methylesterase (PME)and subsequent degradation by polygalacturonase is suggested to contribute to textural changes as tomato fruits ripen. We have isolated and characterized three different genomic clones of tomato PME. To examine the role of PME in fruit development we have introduced a 1.3kb DNA fragment from one of these genomic clones under the control of the CaMV 35S promoter into 'Rutgers' tomato both in the sense and the anti-sense conformation using an Agrobacterium based binary vector. The 1.3kb DNA fragment includes part of the 5' untranslated region and a portion of the coding sequence with two introns. Two independent transgenic anti-sense plants have fruits with significantly reduced levels of PME activity and protein (less than 15% of control fruits), while fruits from transgenic sense plants show no significant changes. Northern analysis shows reduction of PME mRNA in fruits of these anti-sense plants and the presence of RNA transcribed from the introduced chimeric genes in leaves of sense and anti-sense plants. We are characterizing the effects of reduced PME expression on growth and development of tomato fruits and on the demethoxylation and solubilization of pectin in these fruits.

**A 354** ANALYSIS OF PROMOTER SEQUENCES NECESSARY FOR EXPRESSION OF $SbPRP_2$, A SOYBEAN PROLINE-RICH CELL WALL PROTEIN, Mary L. Tierney and Susan San Francisco, Agronomy Department and Biotechnology Center, Ohio State University, Columbus, OH 43210

Proline-rich cell wall proteins (PRP's) are a family of structural proteins which are differentially expressed in plants during normal development and after physical damage. These proteins are rich in proline and hydroxyproline, and contain the repetitive motif ProHypValXLys where X is often Tyr, His or Glu. In soybean seedlings, $SbPRP_2$ encodes a 1050 nucleotide transcript which accumulates in the hook and elongating region of the hypocotyl and appears to be regulated by auxin. In order to identify the DNA sequences responsible for $SbPRP_2$ expression and to investigate whether the developmental regulation of this cell wall protein is primarily at the transcriptional level we have used a genomic clone encoding $SbPRP_2$ to isolate 583 bp of DNA immediately 5' to the transcription initiation site. This promoter fragment was placed in the forward and reverse orientation in front of β-glucuronidase (GUS) and these constructs were introduced into *Arabidopsis* root explants using *Agrobacterium*-mediated transformation. Root tissue was transferred to new selective media every four days for 3 weeks to induce callus proliferation and callus tissue was assayed 5 weeks after the initial transformation for GUS expression. GUS activity was detected in callus tissue transformed with pBI121, containing the CaMV 35S promoter, as well as callus transformed with the $SbPRP_2$ promoter in the correct orientation. Callus tissue transformed with PBI101, a promoterless GUS construct, and callus transformed with the $SbPRP_2$ promoter in the reverse orientation contained no GUS activity. We are currently analyzing the pattern of GUS expression in transgenic *Arabidopsis* seedlings containing these $SbPRP_2$ promoter-GUS constructs to determine the pattern and tissue specificity of GUS expression during plant development.

**A 355** MOLECULAR REGULATION OF LYSINE BIOSYNTHESIS IN MAIZE, Andrew M. Tommey, Janita M. Sellner, Burle G. Gengenbach and David A. Somers, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

Two enzymes in the lysine biosynthesis pathway, aspartate kinase and dihydrodipicolinate synthase (DHPS), are feedback-inhibited by low concentrations of free lysine; consequently, altering one or both enzymes could change the synthesis and accumulation of lysine and/or related pathway amino acids, threonine, isoleucine and methionine.

To obtain molecular information about maize DHPS, cDNA clones were identified by transforming an Escherichia coli auxotrophic mutant lacking DHPS activity. The DHPS activity expressed in transformed E. coli cells had lysine inhibition characteristic of purified maize DHPS indicating that the cDNA coded for both catalytic and regulatory functions. The cDNA nucleotide sequence indicated a 54 amino acid transit peptide typical of enzymes localized in plastids plus 326 amino acids of the mature DHPS. A 1,400 bp transcript species was found in gel blots of embryo and endosperm total RNA; more than one homologous sequence was detected in gel blots of genomic DNA. Expression of wildtype DHPS is being examined in maize embryos and endosperm throughout kernel development.

Mutations in the maize DHPS cDNA were obtained by selecting the transformed E. coli auxotroph for growth in the presence of the lysine analog, S-(2-aminoethyl)-L-cysteine. The DHPS activity expressed by the mutants exhibited significantly less inhibition by lysine than the wildtype DHPS activity. The effect of altered lysine-feedback inhibition on lysine biosynthesis will be tested in maize cells transformed with the mutant DHPS genes.

**A 356** PHOTOGENE EXPRESSION IN CYANIDIUM CALDARIUM, Robert F. Troxler, Steven R. Rodermel and Lawrence Bogorad, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

A fundamental problem in plant molecular biology concerns the mechanism by which light regulates transcript levels of both plastid and nuclear encoded genes. Cells of the unicellular rhodophyte, Cyanidium caldarium, grown in the dark contain a small undeveloped plastid devoid of pigments but when placed in light cells develop a large cup-shaped plastid and synthesize chlorophyll-a, allophycocyanin (APC) and phycocyanin (PC). [Interestingly, heme substitutes for light in promoting the accumulation of APC and PC apoprotein mRNAs]. This developmental process is inhibited by rifampicin (rif) indicating the requirement for plastid RNA synthesis. Northern analyses performed with heterologous probes for APC (apc), PC (cpc), photosystem I and II proteins (psaA and psbA) and RUBISCO (rbcL and rbcS) revealed abundant transcripts in total RNA from illuminated cells but not from cells grown in the dark. A ca. 10-fold reduction in levels of APC and PC transcripts was observed on Northern blots of RNA from illuminated cells incubated with rif for 3 h in light. Message turnover was examined in cDNA from rif-treated cells using Polymerase Chain Reaction (PCR) and APC specific 5' and 3' oligonucleotides. A 465 bp APC PCR product was detected in both dark-grown and illuminated cells but decreased progressively following incubation with rif for 1-3 h. Assessment of mRNA turnover with labeled oligonucleotides for PCR will indicate whether elevated message levels in light result from changes in the rate of transcription, mRNA turnover, or both.

**A 357** THE CLONING AND CHARACTERISATION OF THE P SUBUNIT OF THE GLYCINE DECARBOXYLASE COMPLEX OF PEA, Simon R. Turner, Robert J. Ireland[1] and Stephen Rawsthorne. Dept. of Brassica and Oilseed Research, Cambridge Laboratory, John Innes Centre For Plant Science Research, Colney Lane, Norwich NR3 7UH, England. [1] Dept. of Biology, Mount Alison University, Sackville, NB, EO3A 3CO, Canada.

The differential distribution of glycine decarboxylase(GDC) in the leaves of C3-C4 intermediate type plant species, been has shown to be a key reason for their low compensation point (1). To further understand the molecular mechanisms that underlie the control of differential expression of GDC in the leaves of C3-C4 intermediates it is necessary to obtain probes for the GDC subunit genes. Using an antibody raised to the largest (P) subunit to screen a cDNA expression library we have identified several positive clones. One of these clones has been sequenced and contains the complete coding sequence for the P subunit together with short 3' and 5' untranslated regions. The open reading frame encodes a protein of 1057 amino acids. However, the N-terminal amino acid sequence, determined directly from the mature protein, is situated 84 amino acid downstream of the methionine start codon and matches exactly with the amino acid sequence derived from the cDNA. This not only confirms the correct identification of the clone, but is consistent with the presence of a N-terminal signal peptide required for import of the polypeptide into the mitochondria. Northern analysis suggests that the corresponding mRNA is located predominantly in mature leaves and its level of expression is light-regulated.

(1) Rawsthorne *et al.* Planta (1988) 173:298-308.

**A 358** CHARACTERIZATION OF MULTIPLE ISOFORMS OF THE SNOWDROP LECTIN AT THE MOLECULAR LEVEL , Els J.M. Van Damme, Ben Peeters and Willy J. Peumans, Laboratory for Phytopathology and Plant Protection and Department of Biochemistry, University of Leuven, Leuven, Belgium.

The snowdrop (Galanthus nivalis L.) lectin has previously been shown to occur as a very complex mixture of isolectins when analyzed by ion exchange chromatography and isoelectric focusing. Screening of a cDNA library constructed using polyA RNA isolated from young developing snowdrop ovaries resulted in the isolation of five lectin cDNA clones which definitely differ from each other for what concerns their nucleotide sequence and deduced amino acid sequence. The sequence homology for the total coding region at the amino acid level ranged from 69 to 94 % for the different lectin cDNA clones. The lectin is synthesized as a preproprotein which is post-translationally processed. However, whereas the signal peptide sequence of the different lectin clones is highly homologous the sequence of the C-terminal extension which is cleaved off during a second post-translational processing step shows some more heterogeneity.

The occurrence of closely related multiple isoforms of a lectin has previously been ascribed to differences in glycosylation (soybean lectin), combination of different protein subunits (Phaseolus lectin) or the simultaneous expression of lectin genes by each of the individual genomes of cereal species. Since all Galanthus nivalis isolectins have the same molecular structure and are not glycosylated, a possible explanation for the complexity of the isolectin pattern could be the occurrence of a family of closely related lectin genes.

**A 359** **MOLECULAR ANALYSIS OF UBIQUITIN CONJUGATING ENZYMES IN PLANTS**

Richard Vierstra, Pierre-Alain Girod, Peggy Hatfield, Michael Sullivan, and Steven van Nocker
Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706

Ubiquitin is a highly conserved, 76 amino acid eukaryotic protein that has a variety of important regulatory roles as a result of its covalent attachment to other cellular proteins. Best characterized of these roles is as a reusable recognition signal for proteolysis. Ubiquitin conjugation is accomplished by a multienzyme pathway involving at least three families of enzymes: ubiquitin activating enzyme (E1), ubiquitin carrier protein (E2) and ubiquitin protein ligase (E3). We have initiated a molecular characterization of these enzymes from both wheat and *Arabidopsis* in an effort to determine the specificity of the system and understand when the pathway is functional. Several different E1 genes have been identified all encoding ~ 120-kDa proteins. When these genes are expressed in *E. coli*, active E1s are synthesized. By site-directed mutagenesis, a cysteine residue potentially required for catalysis has been identified. Over 12 different E2 proteins have been isolated by "ubiquitin affinity" chromatography and DEAE-HPLC and the corresponding DNA clones obtained for 5 of them. Amino acid sequence comparisons have located conserved domains that may be important for function. Several of these domains have been confirmed as necessary by *in vitro* mutagenesis including a sequence of amino acids at the N-terminus, the active-site cysteine, and a domain at the C-terminus. The C-terminal domain appears to be required for target protein specificity such that transfer of this domain to heterologous E2s also transfers the ability to conjugate ubiquitin to specific substrate proteins. Work is now in progress to isolate E3s, which in combination with E2s are responsible for substrate specificity. Preliminary studies indicate that multiple forms of this enzyme with different kinetic properties exist in plants as well.

**A 360** CONSTITUTIVE EXPRESSION OF NR IS COMPATIBLE WITH NORMAL PLANT GROWTH AND DEVELOPMENT, M. Vincentz, L. Nussaume and M. Caboche, Laboratoire de Biologie cellulaire, INRA-Versailles F-78026 Versailles Cedex, FRANCE

A chimeric gene containing the cDNA corresponding to the *Nia2* gene of *Nicotiana tabacum* and the 3' end of this gene has been constructed and placed under the control of the 35 S promoter. NR nul mutants of *Nicotiana plubaginifolia* were transformed via agrobacterium gene transfer, and functional complementation was successfully selected by the ability of Kmr regenerated plantlets to grow on nitrate as sole nitrogen source. Genetic and molecular analysis confirmed that one to several copies of the chimeric gene have been integrated and are expressed. Analysis of the R1 progeny of several primary transformants showed a correlation between the steady-state level of NR mRNA, NR activity and growth on nitrate. It also appeared that steady-state level of NR mRNA does not show any of the WT described fluctuations. These latest results indicate that the regulations of NR expression in the WT are mainly transcriptional. Clearly, a deregulated NR activity can replace a regulated WT NR gene for plant viability and growth. The implications of these results will be discussed. We also will present results indicating that the chimeric NR gene described here is an efficient conter-selectable marquer both at the cellular and plant level.

**A 361** **Sucrose Phosphate Synthase, a Key Enzyme for Sucrose Biosynthesis in Plants: Protein Purification and Cloning of its cDNA**
Toni A. Voelker [1], Jean-Michel Bruneau[2], Ann C. Worrell[1], Bernard Cambou[2], Danielle Lando[2]. [1]Calgene Inc., 1920 Fifth Street, Davis, CA 95616, U.S.A, [2]Roussel Uclaf, 102 Route De Noisy, F 93230 Romainville, France.
We have purified to homogeneity the protein and isolated a complementary DNA sequence for the enzyme sucrose phosphate synthase (SPS) from corn (*Zea mays*). The 3509 bp long cDNA codes for a polypeptide of 1068 amino acids in length. The SPS cDNA and specific antibodies were used to monitor SPS mRNA and protein accumulation throughout the plant. The biological activity of the cDNA was confirmed by the ability of the cloned sequence to direct sucrose phosphate synthesis in *E. coli*. No plant specific factors were necessary for full enzymatic activity.

**A 362** CHARACTERIZATION OF TWO CHLORATE RESISTANT MUTANTS OF *ARABIDOPSIS THALIANA*, Jack Q. Wilkinson, Samuel T. LaBrie, and Nigel M. Crawford, Department of Biology, University of California, San Diego, La Jolla, CA 92093
Chlorate, the chlorine analog of nitrate, is toxic to plants and is used as a herbicide. Chlorate toxicity is thought to be due to chlorite, the product of chlorate reduction by nitrate reductase (NR). Eight complementation groups in *Arabidopsis* have been identified which when mutated confer chlorate resistance, including one putative uptake mutant (chl-1), two mutants with substantially reduced levels of NR activity (chl-2, chl-3), and several mutants with defects in the synthesis of an essential molybdo-pterin cofactor (Braaksma, 1982). We have selected additional chlorate resistant mutants following gamma irradiation of seed. Three independent mutants were found to be allelic to plants with lesions at the chl-3 locus. All three mutants had deletions of the nia-2 gene, one of two NR structural genes in *Arabidopsis*. Thus, chl-3 encodes a NR apoenzyme. The deletion mutants have about 10% of wildtype leaf NR activity and yet do not show any growth disadvantage under a variety of conditions compared to wildtype. A second chlorate resistant mutant, chl-2, also has low levels of nitrate reductase activity, but the locus does not map to any known NR structural gene. We have been investigating the nature of the defect in chl-2 and will report our findings.

**A 363** CLONING AND EXPRESSION OF THE TABTOXIN BIOSYNTHETIC REGION FROM Pseudomonas syringae, David K. Willis[1,2], Thomas G. Kinscherf[2], and Terese M. Barta[2], [1]ARS/USDA and [2]Department of Plant Pathology , University of Wisconsin, Madison, WI 53706
We have cloned the chromosomal region encoding tabtoxin biosynthesis from *Pseudomonas syringae* strain BR2. This region is approximately 30 kb in length and includes the gene or genes required for resistance to the glutamine synthetase inhibitor tabtoxinine-β-lactam (TβL), the biologically-active form of tabtoxin. Introduction of pRTBL823, a cosmid clone isolated from a BR2 genomic library, into the tabtoxin-naive *Pseudomonas syringae* epiphyte Cit7 resulted in both the biosynthesis of TβL and the expression of resistance to this antibiotic. The production of TβL did not cause Cit7 to become pathogenic on bean. The tabtoxin biosynthetic region was unstable, both in the laboratory and in the field, and excised at high frequency from the BR2 chromosome, possibly via a precise deletion event. Two *Pvu*II restriction fragments contained within pRTBL823 were found to be present in all tabtoxin producing *Pseudomonas syringae* strains examined. Deletion mutants were restored to tabtoxin production and resistance to TβL by the introduction of pRTBL823. Unlike the $Tox^-$ derivatives of other tabtoxin producing pathovars, tabtoxin-deficient mutants of BR2 were non-pathogenic.

**A 364** MOLECULAR CLONING OF A PUTATIVE PLANT $CA^{+2}$-ATPASE, Larry E. Wimmers and Alan B. Bennett, Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616

cDNA and genomic clones of a putative $Ca^{+2}$-ATPase in tomato have been identified. A tomato root cDNA library was screened with an oligonucleotide corresponding to the highly conserved ATP-binding domain of P-type ATPases. Two families of cDNA clones were identified (pLCA and pLHA). Sequence analysis of pLCA indicated it encodes a P-type ATPase with regions which are highly similar to the rabbit sarcoplasmic reticulum $Ca^{+2}$-ATPase, but not to other P-type ATPases. Screening of a tomato genomic library with pLCA yielded 12 positives. Based on restriction analysis all appear to be overlapping fragments of a single 20 kb segment. Genomic southern analysis indicated a single gene corresponding to pLCA. In northern analysis pLCA hybridized to mRNAs of 3.0, 4.5 and 6.0 kb in root tissue and to a single much less abundant 4.5 kb mRNA in leaf tissue indicating either differential transcription of the single gene, or differential processing of a single transcript.

**A 365** A PUTATIVE PROLINE RICH CELL WALL PROTEIN GENE IS EXPRESSED DIFFERENTIALLY IN JUVENILE AND MATURE ENGLISH IVY, HEDERA HELIX L., Ho-Hyung Woo, Wesley P. Hackett and Anath Das*, Departments of Horticulture and Biochemistry*, University of Minnesota, St. Paul, MN 55108

In English ivy, Hedera helix L., changes in phenotypic characters during development, referred to as phase change or maturation, occurs.

A putative proline rich cell wall protein (PRP) gene was isolated by differential screening of a cDNA library constructed from mRNA isolated from in vitro petiole culture. After 3 days of in vitro culuture, expression of this PRP gene was 5 to 10 times higher in mature petioles than in juvenile petioles. In the intact plant, this gene was expressed in wounded petioles of mature plants but not in wounded petioles of juvenile plants. It was not expressed in wounded lamina of mature plants. Thus, wound inducibility was organ and phase specific to petioles of mature plants.

In vitro, glutathione represses expression of the PRP gene in juvenile, young mature and mature petioles. In contrast, Wingate, V.P.M. et.al. (Plant physiology, 1988, 87:206-210) found that glutathione induces expression of defense genes chalcone synthase, phenylalanine ammonia lyase and HRGP in phaseolus. These conflicting results suggest that there are different mechanisms of regulation for different stress related genes.

**A 366** ***DE NOVO*** **INDOLE-3-ACETIC ACID BIOSYNTHESIS IN THE MAIZE MUTANT** ***ORANGE PERICARP*****, A TRYPTOPHAN AUXOTROPH.** Allen D. Wright, Michael B. Sampson, and M. Gerald Neuffer, Department of Agronomy, University of Missouri, Columbia, MO 65211 and Lech Michalczuk, Janet P. Slovin and Jerry D. Cohen, USDA-ARS Plant Hormone Laboratory, BARC, Beltsville, MD 20705 and Botany Department, University of Maryland, College Park, MD 20742.

The maize (*Zea mays* L.) mutant *orange pericarp* is a seedling lethal which lacks tryptophan synthase B subunit activity. This phenotype is caused by the mutation of two unlinked loci, orp1 and orp2. The mutant accumulates indole and can be rescued by treatment with tryptophan. The orange kernel color is due to indolic products which accumulate in the pericarp. We have used this mutant to test the hypothesis that tryptophan is the precursor to the plant hormone indole-3-acetic acid (IAA). IAA levels in aseptically grown mutant seedlings (10 d) were determined by quantitative mass spectrometry to be approximately 50 times that of the normal seedlings. Mutant and normal embryos were grown on media containing stable isotope labeled precursors, or 30% $D_2O$. All embryos grown with 30% $D_2O$ contained aromatic ring-deuterated IAA, indicating *de novo* synthesis from early precursors prior to entry into the shikimic acid pathway. The level of deuterium incorporation into tryptophan indicated no *de novo* synthesis of this amino acid in either the mutant or normal during the labeling period. The mutant and normal both incorporated $^{15}N$ from anthranilate into IAA but showed little or no incorporation of anthranilate into tryptophan. These results establish that indole-3-acetic acid can be produced *de novo* without tryptophan as an intermediate. *This work was supported, in part, by U.S. National Science Foundation grants DMB 8811027 (MGN) and DCB 8917378 (JPS, JDC).*

**A 367** **CELLULAR DISTRIBUTION OF SOYBEAN PROLINE-RICH PROTEIN RNAS**

R.Wyatt, R. Nagao and J. Key, Dept. of Botany, Univ. of Georgia, Athens, GA. 30602.

We have examined the in situ RNA expression patterns of a gene family which encodes proline-rich proteins in soybean (SbPRPs). Hong et al. (Plant Cell 1, 937-943, 1989) previously reported the developmental and organ-specific expression of the SbPRPs. Here, using in situ hybridization experiments, we report the spatial and temporal patterns of expression for the three genes, SbPRP1, 2 and 3 at the cellular level. **SbPRP1** RNAs were localized in several cell types of maturing hypocotyls, including small groups of cells within the phloem and cortical parenchyma. The expression diminished within the vascular tissue near the crown of the seedling; in this region cells within the pith primarily expressed SbPRP1. This pattern of expression persisted basipetally into the mature region of the root. **SbPRP2** RNAs were present in the vascular tissue of the hypocotyl, the parenchymous tissue of leaves and in developing buds, both apical and axillary. This gene was also expressed in the inner integuments of developing seed coats. **SbPRP3** RNAs, in the elongating region of the hypocotyl, were specifically localized to the endodermoid layer of cells surrounding the stele. SbPRP3 RNAs were also detected in the epidermal cells of leaves of light-grown cotyledons. The epidermis of etiolated cotyledons did not show SbPRP3 expression. Together, the members of this gene family exhibited examples of both cell-specific and developmentally sequential RNA expression.

*Control of Gene Expression*

**A 400** EXPRESSION OF A CHIMERIC GENE ENCODING A BRAZIL NUT METHIONINE-RICH SEED PROTEIN IN TRANSGENIC *BRASSICA NAPUS*, Susan B. Altenbach, Chiung-Chi Kuo, Lisa C. Staraci, Karen W. Pearson, Connie Wainwright and Jeffrey Townsend, The Plant Cell Research Institute, Inc., 6560 Trinity Court, Dublin, CA 94568

As part of an effort to enhance the methionine levels of seed proteins for use in animal feeds, we have transferred a chimeric gene encoding a methionine-rich seed protein to a commercial variety of canola (*Brassica napus* cv Cascade) using *Agrobacterium*-mediated transformation. The chimeric gene contains the regulatory regions from the phaseolin gene from French bean attached to cDNA sequences encoding the 17 kDa precursor form of a 2S albumin from Brazil nut. The Brazil nut 2S albumin contains 18.8% methionine and consists of two subunits of 9 kDa and 3 kDa which are derived from a 17 kDa precursor through a number of proteolytic processing steps. Seeds from transgenic *Brassica* plants accumulate significant quantities of the Brazil nut methionine-rich protein (about 2-4% of the total seed protein). The methionine-rich protein is processed correctly in the transgenic seeds and the subunits are able to associate into 2S protein species. The effect of the accumulated methionine-rich protein on the levels of methionine in the seed proteins of canola will be discussed.

**A 401 TRANSLATIONAL CONTROL OF HEAT SHOCK GENE EXPRESSION IN CARROT SOMATIC EMBRYOS**, Nestor R. Apuya and J. Lynn Zimmerman, Department of Biological Sciences, University of Maryland (Baltimore County Campus), Baltimore, MD 21228

It is generally accepted that the production of heat shock proteins (HSPs) during the heat shock response is primarily regulated at the transcriptional level and that the high level of HSP synthesis is driven by the high level of HS mRNA which is transcribed. We found that this is not the case in carrot cells and somatic embryos. Specifically, as the carrot cells enter the process of embryogenesis, their HS genes are not transcriptionally induced by the heat and the steady-state level of HS mRNA is low. In spite of this, globular embryos synthesize the full complement of all HSPs.

In order to know how globular embryos could produce equivalent HSPs from substantially fewer transcripts, we analyzed the polysome profiles of callus cells and globular embryos. We found that the distribution of HS17.7 mRNA in polysomes of callus and globular embryos are essentially the same, but that globular embryos have a higher level of the transcripts in the polysomes. These data indicate that the recruitment of HS transcripts to the polysomes is apparently enhanced in the globular embryos.

Northern analysis of total RNA revealed that callus cells accumulate ~ 12X HS mRNA than globular embryos, but this is not reflected in the polysome analysis. It is possible that the HS messages are not efficiently transported out of the nucleus of the callus cell. Alternatively, it could be possible that some of the HS transcripts in callus are localized in a "specialized organelle" (HS granules?). The net result of each of these possibilities suggests the selective sequestration of HS mRNA, and could dramatically influence the expression of the HS gene set.

**A 402** β-CONGLYCININ GENE SEQUENCES DIFFERENTIALLY PROGRAM TOBACCO ENDOSPERM AND EMBRYO EXPRESSION PATTERNS, Susan J. Barker and Robert B. Goldberg, Department of Biology, U.C.L.A., Los Angeles, CA 90024

We transformed tobacco plants with soybean β-conglycinin genes that encode respectively a 1.7kb β-subunit mRNA and a 2.5kb α'-subunit mRNA and compared the expression of these genes during tobacco seed development. We show that both mRNAs are restricted to storage parenchyma cells in the tobacco seed, as they are in soybean. The 2.5kb mRNA accumulates throughout the embryo and endosperm, whereas the 1.7kb mRNA is only detectable in the embryo, where it is localized to the cotyledons and upper axis. In soybean, the 2.5kb mRNA is located throughout the embryo, whereas the 1.7kb mRNA is restricted to the cotyledons. In addition, soybean endosperm does not detectably accumulate β-conglycinin mRNAs. In the tobacco embryo, the developmental accumulation of both mRNAs occurs progressively from the outside of the cotyledons and upper axis, to the inside, which is analogous to their accumulation in soybean cotyledons. In contrast, 2.5kb mRNA accumulation in tobacco endosperm occurs simultaneously throughout the structure and is detectable earlier than accumulation of either mRNA in the embryo. Consequently, 2.5kb and 1.7kb mRNA accumulation in tobacco differs temporally as well as spatially. The expression of chimeric β-conglycinin genes in transgenic tobacco indicates that 5' untranscribed sequences are responsible for the differential expression patterns of the two β-conglycinin genes.

**A 403** *Abstract Withdrawn*

**A 404** QUANTITATIVE PCR ASSESSMENT OF EXPRESSION OF TWO ACETOLACTATE GENES IN BRASSICA NAPUS. Faouzi Bekkaoui, Ivan Babic, Janet A. Condie, Maurice M. Moloney and William L. Crosby. Plant Biotechnology Institute, Saskatoon, S7N-OW9, Canada and Department of Biological Sciences, University of Calgary, Calgary, T2N-1N4, Canada

Acetolactate synthase (ALS) is the first enzyme in the pathway of branch-chain amino acids biosynthesis and is the inhibitory target of three classes of herbicides. In plants, all ALS genes thus far characterized have conserved sequence domains in the core region but differ in the 5' region. We have used PCR to quantitate the levels of the transcripts corresponding to ALS1 and ALS2 in different tissues of Brassica. Specific primers were designed in the 5' and 3' regions in order to differentiate between the two genes. First strand cDNA was made from RNA extracted from several different tissues. The transcripts corresponding to ALS2 were found in all tissues analysed, with a lower level in roots compared to leaves and cotyledons harvested at 3 weeks; also a higher level in floral buds compared to leaves harvested at 3 months. ALS1 transcripts were not detected in any of the specific tissue analyzed using either the 5' or the 3' primers. When the ALS1 gene was reintroduced into Brassica, using a heterologous promoter, the corresponding transcripts were present at a high level.

**A 405** IDENTIFICATION OF cDNA CLONES CORRESPONDING TO A PUTATIVE MAIZE SEED GLOBULIN PROCESSOR BY USING ANTI-IDIOTYPE ANTIBODIES, F.C. Belanger and A.L. Kriz, Department of Agronomy, University of Illinois, Urbana, IL 61801

A major protein present in maize embryos is a vicilin-like globulin of *Mr* 63,000 encoded by the *Glb1* gene. This protein, designated GLB1, is synthesized as a prepro-proprotein which undergoes extensive post-translational processing. The final processing step, controlled by the unlinked gene *Mep*, is a proteolytic cleavage near the amino terminus of proGLB1. In an attempt to identify the *Mep* gene product, antibodies to a synthetic peptide designed to span the *Mep*-catalyzed cleavage site were raised in rabbits. These antibodies were in turn used to raise anti-idiotype antibodies which should recognize proteins which interact with the original synthetic peptide sequence. A screen of an embryo-specific cDNA library with the anti-idiotype antibodies resulted in the identification of five immunoreactive clones; of these, three were found to to cross-hybridize and possess essentially identical restriction maps. The longest of these three clones was designated pcPOG1 and selected for further analysis. Northern blot analysis indicates that transcripts corresponding to pcPOG1 are present in developing embryos and in leaves of 7 day-old seedlings, but not in mature embryos, 3 day-old coleoptiles, or developing endosperm. A search of the current GenBank database with the pcPOG1 nucleotide sequence was unsuccessful in identifying related gene sequences. Experiments are in progress to determine if pcPOG1 corresponds to the *Mep* gene.

**A 406** TEMPORAL AND SPATIAL REGULATION OF GENE EXPRESSION DURING EMBRYOGENESIS IN BARLEY. Dianna J. Bowles, Laura Smith, Li Yi, Jane Handley, Helen Martin, Anthony Clark, Linda Donovan,

Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT. UK. Rob Lyne, Shell Research Ltd. Sittingbourne, Kent.

Embryogenesis can arise through development of a fertilized zygote *in planta*, or through a switch in developmental programmes such that an immature male gamete can differentiate directly into an embryo in culture. The existence of these two routes, leading to a near-identical morphological structure capable of germination and growth into a plant, offer a useful means of investigating the essential requirements for the construction of an embryo.

Screening of a cDNA library has led to the identification of a set of novel genes, characterised by a common spatial pattern of expression in barley embryos developing *in planta* or in culture. The genes are expressed in the scutellum, excluding the single cell layer of the scutellar epithelium. This similarity in pattern and the absence of expression from the root and shoot meristems suggests the genes encode products related to the function of the scutellar tissue: such as uptake, metabolism and transfer of nutrients to the embryonic axis. In contrast to the commonality of cell-type expression, the genes are differentially regulated with respect to the timing of their expression and their response to the growth regulators ABA and GA.

Expression patterns of these genes have also been analysed throughout the vegetative and reproductive tissues of the barley plant. Specific cell-types, often restricted to very limited populations of cells within a tissue and ill-defined by other criteria, can be shown to express certain of the genes identified in the embryo cDNA library.

**A 407** TWO POSITIVE AND ONE NEGATIVE REGULATORY ELEMENTS ARE INVOLVED IN DEVELOPMENTAL CONTROL OF β-PHASEOLIN EXPRESSION IN TRANSGENIC TOBACCO. Mark D. Burow, Partha Sen, Caryl A. Chlan, and Norimoto Murai. Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge LA 70803.

To elucidate 5'-elements in the β-phaseolin sequence that are responsible for developmental control, six progressive deletions in the 783 base-pair upstream region were made; the phaseolin coding and 1.0kbp downstream regions were preserved intact. These six mutants and the wild-type construct were transformed into tobacco leaf disks and mature plants were regenerated. ELISA analysis of protein extracts of mature seeds indicated the presence of a positive element between -296 and -228 relative to the transcription initiation site. Deletion of this region reduced phaseolin accumulation four-fold. A second positive element was found between -65 and -14, which contains the TATA region. Deletion of this region reduced expression over 150-fold further, to the lower limit of detection. Elimination of the fragment (-107 to -65) containing the CAT sequence did not alter the phaseolin concentration. Dot blot hybridization analysis of seed mRNA to labeled β-phaseolin genomic DNA confirmed the presence of these two regulatory elements. In addition, a negative regulatory element appears to exist in the region between -422 and -296. The level of hybridizing mRNA increased three-fold when this element was deleted; however, the increase in seed phaseolin was not statistically significant. No hybridizing mRNA was detected when the region containing the TATA element was eliminated. To determine the temporal control of phaseolin expression, immature seeds were collected weekly over a five-week period. Dot blot hybridization analysis indicated that the maximum steady-state phaseolin mRNA level occurred three weeks after pollination and that temporal regulation was lost when the region -65 to -14 was deleted. Experiments are in progress to determine the tissue-specificity of expression in the different mutants.

**A 408** POSITIVE AND NEGATIVE *cis*-REGULATORY DOMAINS OF THE PHASEOLIN 5'-FLANKING REGION CONTROL TEMPORAL AND SPATIAL REGULATION OF GENE EXPRESSION IN TOBACCO, Mauricio M. Bustos, Fatima A. Kalkan, Dilara Begum and Timothy C. Hall, Dept. of Biology, Texas A&M University, College Station, TX 77843-3258.

We have previously demonstrated the correct temporal and spatial regulation of a reporter β-glucuronidase (GUS) gene by the promoter and upstream region of a bean (*P. vulgaris*) β-phaseolin gene in tobacco[1]. Here we have used fluorometric and histochemical assays to show that this region has a modular internal structure, with at least five *cis*-acting DNA domains. A proximal positive domain, UAS-1 (-295/-109), is sufficient for high level seed-specific expression and it is most active in the embryonic cotyledons. Deletion of the second positive domain, UAS-2 (-468/-395), decreases expression in the embryonic axis. Two negative regulatory domains, NRS-1 and NRS-2 (-418/-295 and -518/-418) are required for correct temporal regulation; together they form part of a "time delay" mechanism that prevents transcription from the phaseolin promoter before the mid-maturation phase of development. The third positive domain functions as a quantitative element and contains an A/T-rich motif previously shown to activate gene expression in seeds and vegetative organs of tobacco[1]. The promoter region (-109/+20) can interact with the CaMV-35S enhancer to yield GUS expression primarily in vascular tissues of embryonic and vegetative cotyledons and hypocotyls.

[1]Bustos, M.M., et al. (1989) The Plant Cell 1, 839-853.

**A 409** THE INTEGRITY OF THE HORDEIN MULTIGENE FAMILY IS MAINTAINED IN THE FACE OF EVOLUTIONARY PRESSURE. Verena Cameron-Mills, Joycelyn Entwistle, Martin Müller and Mikael B.Sørensen. Department of Physiology, Carlsberg Laboratory, Gl. Carlsberg Vej 10. DK-2500 Valby. Denmark.
Hordein, the major storage protein synthesized in the endosperm of the developing barley seed, is composed of 4 classes of polypeptides, namely B, C, D and γ-hordein, encoded by multigene families on chromosome 5. The 6-8 C hordein genes located at the *Hor* 1 locus have been mapped to a 140 kbp fragment by PFGE. The hordein polypeptides have a high proline and glutamine content and the C hordein sequence consists of an octapeptide tandem repeat (consensus sequence - - PQQPFPQQ) which extends to all but the terminal 10-12 amino acids of the polypeptide. A single C to U mutation in the Q codons CAA/CAG can generate a stop codon, and the isolation of zein genes with in-frame stop codons has confirmed the frequency of these mutations. It has become a subject of increasing speculation as to how the integrity of a gene family is maintained where gene inactivation is a frequent event, without resorting to cycles of gene amplification. The homogeneity of the hordein genes within each *Hor* locus suggests that gene conversion could maintain a pool of active genes. The C hordein genomic clone λhor 1-14 has a 1044 bp open reading frame encoding 327 amino acids interrupted by an amber codon at base 481. In the first 25 amino acids of the $NH_2$-terminus, the deduced C hordein sequence of λhor 1-14 differs from the conserved actual amino acid sequence by a 6 amino acid insertion and 2 substitutions. We have set out to determine what functional activities this gene has retained and thus whether this mutated gene might remain within the pool of active genes. The C hordein coding region of λhor 1-14, cloned in a pDS expression vector, has been in vitro transcribed and translated in the wheat germ system. In addition to a 17 kd truncated polypeptide, a full length protein of 40 kd was synthesized, indicating read-through of the amber codon by a suppressor tRNA. The identity of this wheat germ tRNA species is being investigated. The 5' upstream region (421 bp) of the C hordein gene was fused in front of the GUS gene in a plasmid construct. The C hordein promoter was functional, since it could direct tissue specific expression of the GUS gene when introduced into developing barley endosperm cells by particle gun transformation.

**A 410** CHARACTERIZATION OF THE MAJOR NODULE-SPECIFIC GENE FAMILY IN Phaseolus vulgaris L. Francisco Campos, Mario Rocha-Sosa, Carolina Carsolio, and Federico Sánchez. Plant Molecular Biology and Biotechnology Unit. Universidad Nacional Autónoma de México, Cuernavaca, Morelos México.
The interaction between Rhizobium and the roots of leguminous plants results in the development of a novel organ: the nitrogen-fixing nodule. During the formation of the nodule, a set of nodule-specific genes or nodulins are differentially expressed. In P. vulgaris we have cloned and characterized one the most abundant nodule-specific transcripts, denominated nodulin-30. The nucleotide sequences of a cDNA and the corresponding genomic clones has been completed. Nodulin-30 presents two sequences resembling "Zn finger" motives, the aminoterminal domain contains a putative signal sequence for membrane translocation and a stretch rich in prolines at the carboxyl terminus. This nodulin has a great degree of homology with the major nodulin gene family and specifically with the nodulin-27 of soybean. We have cloned the most conserved "Zn finger" motif between soybean and common bean to establish the number of members of a possible "Zn finger" gene family in P. vulgaris and other legumes.

**A 411** SOLANUM NIGRUM : A MODEL PLANT FOR MOLECULAR GENETICS AND TRANSFORMATION STUDY, R.K. Chaudhuri, N. Bhattacharyya, A. Banik, D.K. Mukhopadhyay, P. Mallik and I. Chaudhuri, Molecular Biology Lab., Botany Deptt., Calcutta University, India.

The annual weed Solanum nigrum Linn. is a small herb which flowers in 8 weeks. Each plant bears 20 to 30 many-seeded berries. In the greenhouse the lifecycle can be shortened. It's response to cell and tissue culture is excellent. Complete plantlet regeneration, even from protoplast culture, is possible in test tubes. Amongst its cytotypes, diploid (2n=24), tetraploid (2n=48) and hexaploid (2n=72), gradual elimination of genetic material is noticed. Haploid genome of S. nigrum is 0.31 picogram which is about four times of that of Arabidopsis thaliana, and is reported as the penultimate genome size in reported angiosperms. Presence of low amount of repetitive DNA (13 percent at 50 mole x second per liter) is another advantage to work with that genome. Due to smallness of it's chromosome (25 megabase), isolation of chromosomal DNA by pulse-field electrophoresis and making a complete genome library are possible. Gene expression for glycoalkaloid (solasodine) biosynthesis in S. nigrum cells, a marker character, can be manipulated by external factors. This character as well as tissue culture response may be exploited for genetic transformation experiments. Results will be discussed.

**A 412** AN EMBRYO-SPECIFIC PROTEIN OF BARLEY *(HORDEUM VULGARE)*
Anthony J. Clark, Patricia Higgins, Helen Martin and Dianna J. Bowles. Department of Biochemistry and Molecular Biology, University of Leeds, LEEDS, LS2 9JT, U.K.

An immunological approach has been used to identify embryo-specific products that can be used as molecular markers of embryogenesis. Immunoadsorption of antisera to remove antigens common to embryos, meristamatic cells and callus, revealed one major embryo-specific antigen, a polypeptide of $M_r$ 17 kD. The antigen appeared about 18 days after the onset of embryo formation and remained at similar levels up to 6 days post-germination. The polypeptide could not be detected by protein staining suggesting it is a non-abundant product. Appearance of the antigen could be induced by culture of zygotic embryos *in vitro* on ABA ($10^{-6}$M) or mannitol (9% w/v). Cross-reactive products of near-identical molecular mass were observed in embryos of wheat, rye and oats but not in distantly related cereals, nor in embryos of dicot species. The timing of appearance of the antigen was different in embryos formed from microspores during anther culture *in vitro*. In the cultured material, the polypeptide of $M_r$ 17 kD preceded the appearance of morphologically distinct embryonic structures.

**A 413** NOVEL GENOME REARRANGEMENTS IN MAIZE, O. Prem Das and Joachim Messing, Waksman Institute, Rutgers University, Piscataway, N. J. 08855.

Our studies on the 27 kD zein locus have led to the identification of a somatic gene rearrangement which can contribute to genetic diversity in maize (PNAS, in press) and have provided evidence for gene conversion events in the evolution of this locus (MS submitted). Particular stocks of the maize inbred line A188 displaying somatic rearrangement at the 27 kD locus have been tested by genetic and molecular analysis for possible trans-activity on other loci. These include several RFLP loci on chromosome 7, where the 27 kD gene is located, and the classical P, B and R color loci located on other chromosomes. Our results indicate that these loci respond by the generation of alleles different from either parent, probably by intragenic crossover, and by increased levels of somatic mutations, whose products may be inherited. Cytological aberrations in meiosis are also seen in these stocks, though their progeny are viable. The activity(ies) responsible for these phenomena appear to be heritable through several generations. Our results suggest recombinational processes in these stocks which may be involved in generating allelic diversity through the maize genome.

**A 414** SINGLE CHAIN ANTIBODY (SCAB) ENCODING GENES: ONE-STEP CONSTRUCTION BY PCR AND EXPRESSION IN EUKARYOTIC CELLS George T. Davis*, William D. Bedzyk§, Edward W. Voss§ and Thomas W. Jacobs, *Department of Plant Biology* and Microbiology§, University of Illinois, Urbana, IL

Single chain antibodies (SCAbs) consist of the antigen-binding (VL and VH) domains of immunoglobulin light and heavy chains tethered by a short peptide linker. These reagents have a variety of potential uses in pharmacology and studies of cellular physiology. We are interested in applying SCAb technology to objectives in plant cell biology and hereby report a rapid method for the construction of single chain antibody encoding genes in addition to their expression in bacterial and eukaryotic cells. A structural gene encoding a SCAb directed against the aromatic dye fluorescein was synthesized by means of the simultaneous use of four PCR primers and templates of both light and heavy chain immunoglobulin genes consisting of cDNA from either plasmid clones or reverse transcribed hybridoma RNA. Two of the primers were partially complementary to one another and encoded the polypeptide linker which joins the immunoglobulin light and heavy chain variable domains of the SCAb polypeptide. A functional, hapten-binding product was synthesized from the gene thus constructed in both *E. coli* and *S. pombe*. Our results demonstrate that gene constructs encoding single chain antigen binding proteins can be synthesized very rapidly with only limited sequence information about the pertinent light and heavy chain immunoglobulin genes; and, that neither murine codon usage bias, *Thermus aquaticus* DNA polymerase infidelity, nor the eukaryotic cellular environment offer preclusive impediments to the synthesis of functional single chain antigen binding proteins in non-lymphatic, non-murine cells.

**A 415** MULTIPLE DNA-BINDING PROTEINS INTERACT WITH THE ARE OF *ARABIDOPSIS Adh*, Alice J. DeLisle[1] and Robert J. Ferl[2], [1]Department of Plant Science, University of Nevada, Reno, NV 89557 and [2]Department of Vegetable Crops, University of Florida, Gainesville, FL 32611

*Alcohol dehydrogenase* (*Adh*) is developmentally regulated and induced by hypoxic conditions in both *Arabidopsis* and maize. A region that shows sequence similarity to the Anaerobic Response Element of the maize *Adh* promoter is found upstream of the coding region of the *Arabidopsis Adh* gene. Following fractionation of an extract from an *Arabidopsis* suspension culture that constitutively expresses *Adh*, proteins that bind the ARE region were analyzed by gel mobility shift assays, southwesterns, and DNase I footprints. Multiple DNA-binding activities eluted from an anion exchange column across a salt gradient as determined by gel mobility shift assays. However, two distinct fractions showed positive bands by southwestern analysis, one containing a doublet around 150kD, and the other containing a 50kD band. In addition, the fraction containing the 50kD polypeptide also showed a DNase I footprint over the putative ARE. Possible implications of these results will be discussed.

**A 416** **THE TRANSCRIPTION FACTOR ASF-1 BINDS TO THE PEA LECTIN PROMOTER.** **B.S. de Pater[1], F. Katagiri[2], B.J.J. Lugtenberg[1], N.-H. Chua[2] and J.W. Kijne[1].** [1] Center for Phytotechnology, Botanical Laboratory, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands. [2] Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021, USA.

The pea genome contains one functional lectin gene, which is highly expressed in seed and poorly in leaf, stem and root. This expression pattern may result from regulation by different transcription factors. Analysis of the lectin promoter revealed that it contains several sequences resembling the as-1 binding site, to which the plant transcription factor ASF-1 binds. It has been demonstrated that an as-1 site coupled to a reporter gene causes high expression in root in contrast to leaf[1], probably because of the higher ASF-1 steady-state mRNA level in root[2]. Fusion of five as-1 sites to a reporter gene resulted in high expression in leaf also[3]. By using Electrophoretic Mobility Shift Assays (EMSA) and DNaseI footprinting, we found that one of the as-1-like sequences in the lectin promoter weakly binds to ASF-1 protein, encoded by a tobacco cDNA clone, and purified from E.coli. An immunologically related protein with similar binding properties appeared to be present in shoot, root as well as in seed from pea. This protein also binds weakly to the as-1-like sequence in the lectin promoter. Thus, low expression of the lectin gene in leaf, stem and root may result from the action of transcription factor ASF-1, whereas another yet unknown regulatory mechanism is responsible for high seed expression.

1 Lam E. et al. (1989) Proc. Natl. Acad. Sci. USA **86**, 7890-7894.
2 Katagiri F. et al. (1989) Nature **340**, 727-730.
3 Lam E. and Chua N.-H. (1990) Science **248**, 471-474.

**A 417** THE TOMATO HMGR GENE FAMILY IS DIFFERENTIALLY EXPRESSED IN DEVELOPING FRUIT AND SEEDLINGS, Susan M. Dean, Yoshihiro Mano, Jonathon Narita and Wilhelm Gruissem, Department of Plant Biology, University of California, Berkeley, CA 94720

HMG-CoA reductase catalyzes the first committed step in the isoprenoid pathway. This pathway is responsible for a variety of important plant compounds, including membrane sterols and carotenoids. Our laboratory has cloned and characterized three genes for HMGR from tomato that encode distinct isoforms of the enzyme. The members of the HMGR multigene family are differentially expressed during fruit development and seedling growth. The expression patterns of two of the members (HMG1 and HMG2) have been analyzed on Northern blots. These studies reveal that HMG1 mRNA is abundant during early fruit development when cells are undergoing division and expansion, but decreases as the green fruit reach their mature size. HMG2 mRNA is not detectable during early fruit development, but accumulates throughout the ripening process when carotenoids are synthesized. Western blot analysis of protein extracts from sequential stages of developing tomato fruit shows that the protein detected with an anti-HMGR antibody coincides with the expression pattern of HMG1 mRNA. In tomato seedlings preliminary results show that HMGR mRNA transiently decreases when dark-grown seedlings are transferred to continuous light. Northern blots done with gene-specific probes will enable us to determine which members of the HMGR gene family are giving rise to this pattern of expression in seedlings.

**A 418** SEQUENCES REGULATING CELL-SPECIFIC AND WOUND-INDUCIBLE EXPRESSION OF A GENE ENCODING 4-COUMARATE : CoA LIGASE. Carl J. Douglas, Karl D. Hauffe, and Mary Ellard, Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 2B1

A 600bp promoter fragment from the parsley 4CL-1 gene confers a complex pattern of developmental regulation upon the ß-glucuronidase (GUS) gene in transgenic tobacco. This expression is temporally controlled and is cell-type specific in both vegetative and floral organs, as judged by histochemical staining for GUS activity. The same promoter fragment confers wound-inducibility upon GUS in transgenic tobacco leaves. Analysis of the expression of a series of 5' deletions of the 600 bp promoter showed that a 210 bp fragment specifies full developmental expression of the reporter gene. Deletion of the 210 bp fragment by 36 or more bp resulted in loss of all histochemically-detectable expression. The 210 bp fragment contains several elements which are conserved in other genes encoding phenylpropanoid enzymes. A number of internally-deleted and mutagenized derivatives of the promoter have been constructed to test the role of these elements in regulating the developmental expression of 4CL-1-GUS fusions in tobacco. As well, potential regulatory elements are being tested for their ability to confer developmental regulation upon a heterologous minimal promoter. The location of elements within the 600 bp promoter which are required for wound inducibility is also being investigated.

**A 419** DIFFERENTIAL GENE EXPRESSION DURING IMBIBITION OF DORMANT AND NONDORMANT *AVENA FATUA* EMBRYOS, William E. Dyer, Plant and Soil Science Department, Montana State University, Bozeman, MT 59717

Seed dormancy is a unique form of plant developmental arrest that allows germination to be distributed temporally and spatially. The physiology of seed dormancy has been well characterized in *Avena fatua* but the molecular basis for dormancy maintenance and release is not understood in this or other species. Soluble embryonic proteins were isolated from dormant and nondormant (after-ripened) *A. fatua* 'AN265' caryopses from the same seed lot after 1, 3, 6, and 12 hours of imbibition. Two-dimensional silver-stained gels showed that by 6 hours, dormant embryos contained two distinct polypeptides not visible in nondormant embryos. By 12 hours, germination-specific proteins were visible in nondormant embryos. Poly($A^+$) mRNA was isolated from embryos imbibed under similar conditions and translated *in vitro* in a rabbit reticulocyte system. Two-dimensional gels showed that dormant embryos contained low levels of a message encoding a 38 kD protein absent in nondormant embryos. As judged by protein levels, the mRNA was present by 3 hours after the start of imbibition, and gradually declined over the next 9 hours. The results support the idea that seed dormancy is maintained by differential gene expression during early imbibition.

**A 420** **THE RADISH rRNA GENES: FROM STRUCTURE TO TRANSCRIPTION.** Manuel Echeverria, Dominique Tremousaygue, Françoise Grellet and Michel Delseny, Laboratoire de Physiologie Végétale, Université de Perpignan, 66025 Perpignan Cedex, France.

Plant nuclear rRNA genes are organised as thousand copies of a single transcriptional unit containing the 25S, 18S and 5.8S rRNA coding sequences separated by the external spacer (ES), a non coding region containing regulatory signals for the initiation and termination of transcription. In order to study cis and trans-acting elements that control rDNA transcription in dicotyledonous plants we sequenced the ES of radish. Main structural feature is the presence of seven 100 bp repeats separated by short dA stretches 300 bp upstream to a putative initiation site of transcription. This putative initiation site, mapped as a major signal of 5'end rRNA precursor by S1nuclease analysis, shows significant sequence homology with transcription initiation sites of animals. So we have begun to search for protein factors in radish crude nuclear extracts that bind to the ES. Using gel retardation assays and competition analysis two proteins binding to three different regions of the ES have been detected. One protein, factor A, binds to probe pRE 337 (nucleotides -1077 to -739 relative to putative transcription initiation site) containing three of the repeated ES sequences and the corresponding dA stretches. Factor A also binds to probe pRE 224 (-206 to +21). DNAse I foot-printing on the pRE 337 probe shows two protected region: the dA stretches and a short sequence, both present in probe pRE224 upstream to the putative initiation site. Another protein, factor B, specifically binds to probe pRE 259 (+150 to +410), downstream to the transcription initiation site. Experiments are going on to localise the specific sequence recognized by this factor. In summary, we have detected two radish nuclear proteins binding to the rDNA regulatory region. Whether factors A and B represent specific transcription factors for RNA polymerase I or play a more general role in genome expression remains to be elucidate.

**A 421** THE IDENTIFICATION AND CHARACTERIZATION OF PROTEINS INVOLVED IN THE TRANSCRIPTION OF AN EMBRYO SPECIFIC GENE ENCODING THE STORAGE PROTEIN CRUCIFERIN FROM BRASSICA NAPUS (OILSEED RAPE).

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**Department of Biological Sciences, University of Durham, Durham, DH1 3LE, U.K.**

The gene encoding an embryo storage protein, cruciferin, has recently been isolated and sequenced by this laboratory (Ryan, *et al.*, 1989). The 5' promoter sequence has been linked to the Gus gene, transformed into Tobacco and shown to be expressed in a tissue specific manor. We can therefore assume that all the 5' sequence needed for expression of cruciferin is present within this construct. Our aim is to identify both positive and negative *cis* acting regulatory proteins specific for the cruciferin gene. We have linked the promoter sequence to a liquid chromatography matrix and have isolated proteins from both embryo and leaf extracts that bind to the matrix in the presence of a large excess of free poly (dIdC).(dIdC). The 2.2 kb promoter sequence has been specifically fragmented into ~75 bp lengths to use as probes in several types of binding assays in order to identify the sequence specificity of the binding proteins.

Many eukaryotic transcription regulatory proteins have been shown to be modified by O-glycosylation (Jackson and Tjian, 1988). Such proteins have been shown to bind specifically to wheat germ agglutinin including the mammalian transcription factor Sp1 (Jackson and Tjian, 1989). We have partially purified plant proteins from rape embryo's that specifically bind to wheat germ agglutinin-agarose beads. We are investigating both the affect of the modified proteins upon the cruciferin gene in an *in vitro* transcription system (Yamazaki, *et al.*, 1990) and the similarity with O-glycosylated mammalian transcription factors.

**A 422** **Characterization and localization of fusions proteins comprising the α-subunit of the soybean seed storage protein β-conglycinin and β-glucuronidase in seeds of *Nicotiana benthamiana*.**

Leigh B. Farrell, Federico L. Sebastiani, Jacqueline J. DePaulo. Pamela Robeff and Roger N. Beachy, Department of Biology, **Washington University**, St. Louis, MO 63130

We are developing a system for high level expression and vacuolar targeting of heterologous proteins in seeds. As a first step, fusion proteins comprising a series of C-terminally deleted versions of the α-subunit of β-conglycinin fused to a glycosylation resistant version of the reporter gene β-glucuronidase (GUS), under the control the high level and seed specific promoter from the α-subunit of β-conglycinin have been transformed via *Agrobacterium tumefaciens* into *Nicotiana benthamiana* We have determined that fusion proteins comprising between 22 and 605 (full length) amino acids of the α-subunit fused to GUS express relatively high levels of GUS enzymatic activity in transgenic seed and, via Western analysis, that their migration in SDS-polyacrylamide gels corresponds well to predicted sizes. At present the subcellular location of each of these fusion proteins is being determined by fractionation of seed extracts on potassium iodide glycerol gradients and by immunogold labeling using electron microscopy.

**A 423** PROTEIN-DNA INTERACTIONS INVOLVED IN THE REGULATION OF MAIZE STORAGE PROTEIN GENES, G. Feix, K. Grasser, E. Griess, M. Haass, G. Hauptmann, W. Hetz, T. Quayle. Institut for Biology III, University of Freiburg, D-7800 Freiburg, Germany

Towards a better understanding of the transcriptional regulation of zein genes, an analysis of the specific interaction of their 5' flanking regions with nuclear proteins was perfomed. By working with a variety of in vitro techniques, we found that the core promoter region interacts with several nuclear proteins including HMG proteins and that several cis elements as far as 1.5 kb upstream of the coding regions may be important for the tissue and stage specific zein gene expression. The tissue specificity of some cis elements (i.e. the -300 box) could additionally be shown with transient transformation assays in protoplasts derived from an endosperm cell culture. Work is now in progress to isolate some of the genes encoding transacting nuclear factors involved in the transcriptional regulation of zein genes.

**A 424** GENE EXPRESSION STUDY IN CASSAVA USING HIGH VELOCITY MICROPROJECTILES

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Cassava (Manihot esculenta), which is cultivated for its starchy tuberized roots, is the fourth most important source of calories produced within the tropics. Cassava has not been subject to extensive breeding and there is considerable scope for improvement of cassava characteristics. The goal of our project is to genetically engineer cassava for virus resistance using the coat protein mediated protection strategy.

Since heterologous gene expression in the Euphorbiaceae has not been documented, we have developed a transient gene expression system to quantify the activity of different promoters and 3' terminators in cassava. The "GUS" gene was used as a reporter gene system and chimaeric plasmids were delivered by "bombarding" young cassava leaves with DNA coated tungsten microparticles. The level of expression mediated by some of our promoters was about three times stronger than that obtained using the CaMV 35S promoter. The biolistic process appears valuable for studying gene expression in cassava for which an efficient stable transformation procedure does not yet exist.

**A 425** CHARACTERIZATION AND EXPRESSION OF A CUCUMBER CLASS III CHITINASE GENE. Leslie Friedrich, James Beck, Mary Moyer and John Ryals. CIBA-GEIGY Biotechnology Research, P.O. Box 12257, Research Triangle Park, NC 27709-2257

A genomic clone encoding a class III chitinase protein (chitinase/lysozyme) from Cucumis sativis was isolated and sequenced. A 12kb EcoRI fragment was found to contain three distinct coding regions, apparently arising from a triplication event. These coding regions are highly homologous, but the 5′ and 3′ flanking regions have diverged. Based on cDNA cloning evidence, only the middle coding sequence is expressed. Three promoter deletions of the active gene were constructed and transformed into tobacco; the levels of foreign protein in the transgenic plants were determined by ELISA. In addition, transgenic tobacco plants containing a cDNA for this gene were found to process and secrete at high levels a fully active chitinase protein.

**A 426** SEED SPECIFIC REPRESSION OF GUS ACTIVITY IN TOBACCO PLANT USING ANTISENSE RNA, Toru Fujiwara, Philip A. Lessard and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130.
Manipulation of gene expression pattern is important for both basic research and application. β–conglycinin,7S storage protein of soybean, is expressed only in seed and its regulation is transcriptional. We used promoter of α' subunit of β-conglycinin for expression of antisense β-glucuronidase(GUS) transcript to repress GUS activity driven by CaMV35S promoter in seed specific manner. A T1 generation transgenic tobacco plant harboring pCaMV35S:GUS:α'3'end(homozygous in terms of T-DNA insertion,nopaline positive) was retransformed with pα':anti GUS:α'3'end using the binary plant transformation vector pMON850. Double transformants were regenerated and transformation confirmed by Southern hybridization. Although more than 70% of double transformants were nopaline negative, seed specific repression of GUS activity was observed and was correlated to the copy number of the GUS antisense insertion.

**A 427** Construction of a Regulated Promoter that Responds to Low Levels of Tetracycline, Christiane Gatz, Astrid Kaiser and Regina Wendenburg, Institut für Genbiologische Forschung, GmbH, Ihnestr. 63. 1 Berlin 33, FRG.

We have established the use of the Tn*10* -encoded Tet repressor-operator system to regulate the expression of a suitably engineered Cauliflower Mosaic Virus (CaMV) 35S promoter in transgenic tobacco plants. First, transgenic plants were generated which constitutively synthesize the Tet repressor protein. In a second transformation step, the *ß-glucuronidase* (*gus*) gene under the control of a modified CaMV 35S promoter, containing two *tet* operators, was stably integrated into the plant genome of a *tetR*$^+$ plant. Expression of the *gus* gene is repressed down to undetectable levels. Maximal induction is achieved already after 3h upon application of only 0.5 mg/l Tctetracycline (Tc) throughout the plant. This indicates that Tet repressor-operator complexes can form on plant chromosomes and block transcription. The fast and efficient induction makes the system extremely useful for specifically inducing expression of transferred genes at different stages of plant development without affecting other endogenous functions.

**A 428** DNASE I FOOTPRINT ANALYSIS OF TWO AUXIN-RESPONSIVE GENES OF SOYBEAN
V. Goekjian, R. Nagao and J. Key, Botany Dept., Univ. of Georgia., Athens, GA 30602
Aux 28 and Aux 22 are two auxin-responsive, tissue specific genes found in soybean. To better understand the regulation of these genes DNAase I footprinting and gel retardation analyses have been used to identify protein-binding DNA domains in the 5' flanking sequences. Multiple binding sites in the -827 to +51 region of the Aux 28 gene were found using either strand of a particular DNA fragment. The region between -10 and -100 shows a complicated pattern of protected regions and hypersensitive bands. These regions showed competition by specific and non-specific competitors including unlabeled fragments from the upstream region of the Aux 22 gene. Protein interactions at a sequence between -246 and -267 is specific as shown by additional competition experiments. Other DNA/protein domains were localized between -620 and -635 and between -770 and -800. Competition studies showed these interactions to be specific; also there is a relationship between the -770 to -800 sequence and an upstream region of the Aux 22 gene. There were no differences in the footprint patterns obtained with extract prepared from unincubated, auxin-depleted, and auxin-treated excised elongating soybean tissue. When extract was prepared from plumules or the apical, elongating, and mature regions of hypocotyls, the footprint patterns remained the same but the concentration of binding protein varied. This correlates with expression studies that showed Aux 22 and Aux 28 mRNA levels to be highest in elongating tissue. Preliminary footprint analysis of the Aux 22 gene showed two regions of protection between -300 and -550. Currently, further analyses in transgenic plants is in progress using a minimal promoter construct and the identified potential regulatory sequences of both genes.

**A 429**FUNCTIONAL ASSAY OF A RICE α-AMYLASE GENE PROMOTER SHOWING TISSUE SPECIFIC AND GIBBERELLIN INDUCIBLE EXPRESSION, Stanley Goldman, Yogesh R. Mawal, Barnabas L. Jenes and Ray Wu, Section of Biochemistry, Molecular and Cell Biology, Cornell University Ithaca,. N.Y. 14853
It is known that the α-amylase gene is induced to produce high levels of mRNA by the plant hormone gibberellin (GA). The tissue and temporal specific expression of a rice α-amylase gene (Osamy-B) was first investigated using RNA blots (Northern) and tissue specific transient assays of deletion mutants of the promoter sequence. The Northern blots of different rice tissues show that the α-amylase genes (approx 10 copies) are expressed in developing mature seeds from the day of imbibition through day 8. The gene expression is also seen in callus tissue derived from rice embryos. There is, however, no expression in root and little expression in leaf tissue. Biolistic bombardment of rice and oat aleurone tissues with α-amylase promoter:β-glucuronidase gene fusions shows that the reporter gene is expressed in rice and oat aleurone tissue using the X-Gluc (5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid) staining procedure. Quantitative assays were done by fluorometric determination of GUS enzyme product (4 Methyl- umbelliferone) revealing a GA responsive element 240 bp upstream of the transcription start site. DNA-protein gel retardation assays were used to determine the presence of protein factor(s) which specifically bind to the 180 bp promoter fragment 5' of the TATA box.

**A 430** A MOLECULAR MARKER FOR EMBRYONIC INDUCTION, P. Goupil, P. Hatzopoulos, R-L. You, M-S. Wang, F. Hempel, and Sung Z.R., Department of Plant Biology, University of California, Berkeley, CA 94720.

We have isolated a set of embryo specific genes that are expressed in somatic and zygotic embryos but not in adult organs i.e. leaves or roots. One of this genes, DC 8, has been well characterized. DC 8 belongs to the class of LEA ( Late Embryogeny Abundant ) genes; it encodes a hydrophilic protein located in the cytoplasm of the embryo and endosperm cells. The RNA and protein accumulation patterns in the somatic embryo are dependent on the presence of ABA. However, ABA cannot induce DC 8 expression in leaves.

Using transgenic carrots containing chimeric gene consisted of DC 8 promoter:GUS construct, we found that DC 8 is activated in early embryogenesis. In zygote embryos, DC 8 is first activated in the ovule. In somatic embryos, DC 8 is activated before callus induction (You et al, in preparation). In order to understand the molecular mechanism of DC 8 activation in maternaly and zygotic tissues, we are characterizing the trans-acting factors which interact with the cis-elements in the promoter region. The mechanism and hormonal regulation of DC 8 promoter is studied by deletion analysis in carrot protoplasts (transient assay) and transgenic carrots (Goupil et al,in preparation)

**A 431** HOMOLOGOUS RECOMBINATION IN *ARABIDOPSIS THALIANA*, Ursula Halfter and Lothar Willmitzer, IGF Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, Germany

Targeting an exogenous sequence to its homologous endogenous counterpart may be used to modify the coding sequence of endogenous genes to elucidate gene function by an altered phenotype. In higher eukaryotes the main difficulty of this approach is the very low ratio of homologous to illegitimate recombination. To establish such a system we started with a chimeric hygromycin phosphotransferase (HPT) gene which had been inactivated by a small deletion in the coding region. Transgenic plants harbouring this construct were transformed with a promoter-less intact coding region of the HPT gene using direct gene transfer. Gene targeting events result in hygromycin resistant calli. To distinguish between homologous, and non-homologous recombinations in which the HPT coding region gives rise to hygromycin resistance as a result of transcriptional or translational fusions to endogenous promoters, PCR technology was used. From a total of 3.8 x $10^8$ protoplasts having been transformed with the described targeting DNA 150 resistant calli have been selected, and 3 recombinants have been shown to give a correct PCR-signal and have resistant $F_1$ progeny which also by Southern blotting were shown to be due to homologous recombination. In comparison to the number of transformants due to illegitimate recombination we could enrich 400 fold for the gene targeting event and obtained a relative recombination frequency of 2 x $10^{-4}$ for the homologous recombination event in these experiments.

**A 432** DIFFERENTIAL EXPRESSION OF THE PLANT HOMOLOG OF THE YEAST CDC2/CDC28 GENE IN ALFALFA. Erwin Heberle-Bors[*], Heribert Hirt[*], Anikó Páy[*], János Györgyey[§], László Bakó[§], Kinga Németh[§], László Bögre[§], Rudolf J. Schweyen[*], and Dénes Dudits[§], [*]Institute of Microbiology and Genetics, University of Vienna, Althanstr. 14, 1090 Vienna, Austria, and [§]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, 6701 Szeged, POB 521, Hungary

The cdc2 protein kinase plays a central regulatory role in the control of the cell cycle of animals and yeasts. We have isolated a cDNA clone for the plant homolog of the yeast cdc2/CDC28 genes from alfalfa (Medicago sativa), cdc2Ms. The encoded protein reveals 64 % identity to the yeast and mammalian counterparts showing all the typical structural features known from these organisms. Antibodies raised against the 16 amino acid long synthetic PSTAIR peptide recognize a 34 kd protein in extracts of alfalfa cells. When transferred in fission yeast, the plant cdc2 homolog can complement a temperature-sensitive cdc2 mutant indicating that at least some of the components of cell cycle control identified in the yeasts are present also in plants. Unlike other organisms, alfalfa revealed an organ-specific transcription pattern of the cdc2Ms gene. Transcript levels were higher in shoots than in roots. In shoots, three transcripts were present in equal amounts. In roots and in suspension culture cells, predominantly one transcript was found. Suspension cultures that were induced to form somatic embryos by 2,4-D showed fluctuations in the transcription pattern of cdc2Ms during induction period and embryogenesis.

**A 433** HAPLOID TRANSFORMATION IN BRASSICA, *B.Huang, *S.Bird, *L.MacLellan, *L.Erickson, *E.Swanson, *R.Kemble,. **D.Simmonds, **B.Miki, and **W.Keller. *Allelix Crop Technologies, 6850 Goreway Dr. Mississauge, Ont L4V 1P1, Canada. **PRC, Agriculture Canada, Ottawa, Ont K1A OC6. Canada.

Haploid embryos can be produced readily in a number of Brassica species, particularly B.napus, by culturing microspores or anthers in vitro. Microspores, microspore-derived embryos and plants have been used as transformation targets. Microinjection has been used to deliver DNA into uninucleate microspores and dozens of fertile plants were regenerated from injected microspores. None of these plants was stably transformed. Experiments are being carried out to monitor DNA (by multiple PCR reactions) during and after the microinjection process and results will be presented.

Co-cultivation of microspore-derived embryos/explants with Agrobacterium tumefaciens yielded transgenic winter and spring canola(B.napus). Up to 3% of the embryos co-cultivated with A.tumefaciens produced transgenic plants, which were homozygous diploid after colchicine treatments. Results on optimizing conditions for haploid transformation will be discussed.

**A 434** OPTIMIZING PLANT EXPRESSION VECTORS, G.A. Huffman, L.R. Beach, J.M. Martich, M.M. Fall, L.E. Sims, M. Burrus, W.A. Marsh, S.E. Maddock and R.E. Bauer, Department of Biotechnology Research, Pioneer Hi-Bred International, Inc., 7300 N.W. 62nd Avenue, Johnston, Iowa 50131

Transient NPTII activity was optimized in maize and sunflower protoplasts by co-electroporation of various derivatives of the commonly available plasmid pCaMVNEO and a standard uidA (GUS) construct. The ratio of NPTII activity to GUS activity was increased by an order of magnitude in maize and by two orders of magnitude in sunflower. In the initial constructs, kan or uidA was fused to the CaMV 35S promoter and nos 3' region. All constructs tested in maize included the first intron from adh1 of maize upstream of the start of translation. Inserting a transcriptional leader from TMV increased transient expression of kan eightfold in sunflower, but only 50% in maize. Changing the translational start context of kan to match the eucaryotic Kozak consensus enhanced activity nearly threefold in sunflower, and removing 180 bp of extraneous Tn5-derived DNA following the stop codon of kan further boosted expression by more than threefold. The combined effect of these changes in maize expression vectors was over threefold. Replacing the nos 3' region with the 3' region from a proteinase inhibitor gene (pin II) from potato doubled NPTII activity in both maize and sunflower. A duplication of the upstream enhancing elements of the CaMV 35S promoter had no effect on transient NPTII activity.

**A 435** REGULATION OF LOW MOLECULAR WEIGHT HEAT SHOCK GENES AND FUNCTION OF THEIR PROTEINS IN CARROT, Cheol Ho Hwang and J. Lynn Zimmerman, Department of Biological Sciences, University of Maryland, Baltimore, MD 21228

Analysis of low molecular weight(LMW) heat shock gene expression during early development of Carrot shows a unique discontinuity between the level of transcript and the level of translated product. Specifically, the globular embryos produce low levels of LMW hs transcripts but synthesize an enhanced level of the corresponding hs proteins compared to those of the callus cell. The fact that the globular embryos enhance the synthesis of LMW hs proteins with a low level of corresponding messages lead us to consider that some *enhanced translation* mechanism is directing the expression of LMW hs genes. Although the details of the mechanism remain under investigation these observations open an important path for the functional elucidation of LMW hs proteins during embryogenesis. The low level of LMW hs transcripts in globular embryos in response to heat shock could represent a good target for blocking HS gene expression by using *antisense* message. Moreover,the enhanced synthesis of LMW hs proteins by globular embryos may imply an essential role for the LMW hs proteins, such that the interruption of their synthesis may lead to significant phenotypic alterations. For that purpose, a transgenic cell line containing an *antisense* LMW hs gene has been prepared and is being analyzed.

**A 436** OXIDATIVE STRESS IN PLANTS: EXPRESSION OF CATALASE GENES IN PEA, Sibel H. Isin and Randy D. Allen, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409
Catalase is a tetrameric heme-containing enzyme which catalyzes the conversion of hydrogen peroxide into oxygen and water. Catalase is found in all aerobic organisms and is one of the key enzymes in the active oxygen scavenging system. We have isolated full length catalase cDNAs from a pea λgt10 library using a cotton catalase cDNA as probe. The pea catalase cDNA is 1750 bp in length and its derived amino acid sequence shares significant homology with cotton and maize catalases. We have investigated the expression of pea catalase mRNA in germinated seeds and mature plants. The induction pattern of catalase mRNA in Paraquat treated pea plants have also been determined and compared to expression patterns of other oxidative stress response enzymes such as superoxide dismutase. Transgenic tobacco plants that express pea catalase constitutively at a high level are being developed. These plants will enable us to further investigate the role of catalase in the oxidative stress response.

**A 437** HIGH LEVEL GENE EXPRESSION IN PLANTS USING TOMATO GOLDEN MOSAIC VIRUS VECTORS CONTAINING CAULIMOVIRAL 35S PROMOTERS
**Stephen A. Jobling and Stephen G. Rogers**, Plant Molecular Biology, Monsanto Corporate Research, Monsanto Co., AA2G, 700 Chesterfield Village Parkway, St. Louis, MO 63198

Tomato golden mosaic virus (TGMV) is a DNA plant virus with a genome consisting of two single-strand circles of approximately 2.6 Kb each. The A component can replicate autonomously and when the coat protein gene is replaced by other coding sequences. TGMV A vectors have been constructed in which the coat protein promoter is replaced with that of either the cauliflower mosaic virus enhanced 35S or 35S promoter of carnation etched ring virus. These vectors (with the promoter in either orientation) replicate as well as wild type virus in agroinoculated *Nicotiana benthemiana* leaf discs. Expression of a mammalian gene (lipoprotein-associated coagulation inhibitor, LACI) from these vectors led to a 5-10 fold increase in RNA levels compared to the wild type promoter. When GUS was expressed from the same vectors only a slight increase in expression was observed, although an improved vector in which all coat protein sequences were removed gave significantly higher expression. Expression levels were very high in all cases; RNA levels of the gene replacing the coat protein can reach up to 20% of an infected cells poly(A)+ RNA and GUS was expressed at a level of at least 4% of the total cell protein. Other examples of the use of the TGMV vector system will be presented.

**A 438** CO-SUPPRESSION OF HOMOLOGOUS CHALCONE SYNTHASE GENES: MECHANISM AND DEVELOPMENTAL CONTROL, Richard Jorgensen, Timothy Robbins, and Carolyn Napoli, DNA Plant Technology Corp., 6710 San Pablo Ave., Oakland, CA 94608
The presence of an ectopic chalcone synthase (CHS) transgene in the petunia genome can result in the co-suppression of the expression of the endogenous, homologous CHS gene, as well as the transgene itself. It is probable that this is a general phenomenon to which most, if not all, genes are susceptible in plants. Data will be presented that indicate that co-suppression (1) is more efficient if the the sense strand of the transgene is expressed and (2) occurs whether the transgene is homozygous or heterozygous. Although the co-suppression state of a pair of homologous genes can be stable and heritable, it is also reversible. It appears that gene pairs can be reprogrammed in the development of axillary branches and in the formation of gametes or early in seedling development. In addition, somatic changes in the programming of co-suppressible CHS gene pairs can occur in a cooperative manner among cells of adjacent cell layers. Similarly, non-clonal, highly ordered pigmentation patterns in flowers also demonstate that cell-to-cell communication can determine the co-suppression state. Thus, co-suppression can be subject to stringent developmental control.

**A 439** CHARACTERIZATION OF THE EXPRESSION OF α-AMYLASE ISOZYMES AND (1-3;1-4)-β-GLUCANASE IN GERMINATING RICE AND BARLEY SEEDS, Erik E. Karrer, James C. Litts, and Raymond L. Rodriguez, Dept. of Genetics, Univ. of Calif. at Davis, Davis, CA, 95616

Steady-state levels of mRNAs encoding separate α-amylase isozymes and a (1-3;1-4)-β-glucanase were measured during germination of rice (*Oryza sativa*) and two varieties of barley (*Hordeum vulgare* L. cv. Himalaya and cv. Klages). Relative mRNA levels of each isozyme were quantified in both the embryo and the aleurone tissues of intact seeds germinated without exogenously applied growth regulators. We have found that α-amylase isozymes and (1-3;1-4)-β-glucanase were differentially expressed in each tissue of rice and barley. Two of the three subfamilies of the rice α-amylase multigene family were expressed and three genes of the RAmy3 subfamily (*RAmy3B*, *RAmy3C*, and *RAmy3E*) were preferentially expressed in aleurone tissues. Messenger RNAs encoding both low and high pI α-amylases and (1-3;1-4)-β-glucanase could be detected in germinated barley seeds. In each tissue of both barley varieties, the peak levels of mRNA encoding high pI α-amylases and (1–3;1–4)-β-glucanase always preceded those encoding low pI α-amylases. Peak levels of mRNA encoding high pI α-amylases and (1-3;1-4)-β-glucanase occurred two days earlier in the embryos of Klages barley than in embryos of Himalaya barley. A correlation between high levels of α-amylase mRNA and rapid germination rate was observed: Klages barley produced three times as much mRNA encoding high pI α-amylases and nearly four times as much mRNA encoding low pI α-amylases than the slower-germinating Himalaya variety while levels of (1-3;1-4)-β-glucanase mRNA remained the same.

**A 440 STRUCTURE-FUNCTION ANALYSIS OF A TOBACCO TRANSCRIPTION ACTIVATOR, TGA1A, Fumiaki Katagiri[1], Katja Seipel[1], Gunther Neuhaus[2], Gaby Neuhaus[2] and Nam-Hai Chua[1], [1]Lab. of Plant Mol. Biol., The Rockefeller Univ., New York, NY, USA and [2]Inst. for Plant Sci., ETH Zentrum, Zurich, Switzerland.**

TGA1a (373 amino acid residues), whose cDNA clone was isolated by "Southwestern" screening, is a tobacco transcription activator that binds specifically to the as-1 element (-83 to -63) of the CaMV 35S promoter. Based on their binding characteristics to a number of different DNA sequences, TGA1a appears to correspond to the tobacco nuclear factor ASF-1. Because of the abundance of TGA1a in roots, TGA1a is likely to be responsible for the in vivo function of as-1, which preferentially confers root expression on a promoter. We studied functional domains on the TGA1a molecule by deletion analysis. TGA1a derivatives obtained by in vitro translation were analyzed by gel retardation assays. A 65-amino acid region (aa#80-144) including a "bZIP" motif is sufficient and necessary for the specific DNA-binding, although there is a region in the C-terminal that affects the apparent binding affinity. The transactivation activity was analyzed by microinjection of purified TGA1a derivatives into cotyledon cells of transgenic tobacco carrying the -90 35S promoter (-90 to +8; containing as-1)-GUS fusion. The appearance of GUS activity in the injected cells was monitored by histochemical staining. An N-terminal region (aa#1-79) containing the "acidic domain" is important for the transactivation. This work is supported by Monsanto Co.

**A 441** ANALYSIS OF *cis*-ACTING ELEMENTS FROM GENES ENCODING β-CONGLYCININS, Philip A. Lessard, Toru Fujiwara, Randy D. Allen and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130

The soybean seed storage protein, β-conglycinin, is composed of three subunits, α', α and β. Genes that encode these subunits are expressed solely in seed tissues during mid- to late stages of seed development. In culture, expression of the β subunit gene can be modulated by altering the concentrations of available abscisic acid (ABA) or methionine. Response of the β subunit gene to ABA is likely to be transcriptional, though it appears to involve a mechanism distinct from the ABA response described by other groups (e.g. Marcotte et al., 1989, Plant Cell 1:969-976). Expression of the α' subunit gene is unaffected by changes in ABA or Met availability. A *cis*-acting seed-specific enhancer has been identified upstream from the promoter of the α' subunit gene. Nuclear factors have been identified which interact specifically with sequences within this enhancer; one of these factors does not bind to sequences 5' of the β subunit gene. Separation of the binding sites for each of these factors has helped correlate binding activity with function of the seed-specific enhancer. 5' terminal deletions have revealed the location of *cis*-acting elements within the β subunit gene. These terminal deletions will be used in defining the ABA and Met responsive elements in the β subunit gene.

**A 442** *IN VIVO* ANALYSIS OF PLANT RNA PROCESSING, Hua Lou, Andrew McCullough and Mary A. Schuler, Department of Plant Biology, University of Illinois, Urbana, IL 61801

A number of published *in vivo* and *in vitro* studies have demonstrated that the intron splicing machineries are not interchangeable between plant and animal systems or between dicot and monocot plant systems. The overriding goal of our research is to define the critical differences between monocot and dicot introns that prevent efficient *in vivo* expression in transgenic plants and to elucidate the mechanism for intron excision in plant nuclei. To this end, we have initiated a series of experiments in which monocot and dicot intron constructs were introduced into dicot nuclei on a replicating expression vector. The initial experiments have demonstrated that dicot introns are accurately and efficiently spliced from precursor transcripts generated by this expression system. The first intron in the pea rbcS 3A gene is accurately excised at 80-100% efficiency. The intron present in the wheat rbcS 4.3 gene is accurately excised from precursor transcripts, but much less efficiently than the pea rbcS3A-1 intron. To determine the basis of this difference in splicing efficiency, we have analyzed constructs in which the pea and wheat rbcS intron and exon sequences have been swapped. In addition, specific 5′ splice site mutations in the pea rbcS 3A-1 intron have been evaluated. These experiments have allowed us to define the cis-acting sequences required for accurate and efficient splicing of this dicot intron in dicot nuclei.

**A 443** SEQUENCE-SPECIFIC PROTECTION BY NUCLEAR PROTEINS IN THE PROMOTER REGIONS OF THE FIVE TOMATO rbcS GENES DIFFERS AMONG ORGANS, Thianda Manzara, Pedro Carrasco, Leslie Wanner and Wilhelm Gruissem, Department of Plant Biology, University of California, Berkeley, CA 94720

The five genes encoding ribulose-1,5-bisphosphate carboxylase (rbcS) from tomato are differentially expressed and show organ-specific, developmental and light-regulated control of steady-state mRNA levels. Although differential mRNA stabilities have been demonstrated, regulation has been shown to occur primarily at the level of transcription. Conserved DNA sequences have been identified in the 5' upstream regions of the five genes, and, in some cases, presence or absence of conserved sequences correlates with the observed patterns of expression. In this study, DNAse I footprinting assays were undertaken to determine the functional significance of the conserved sequences for all five rbcS genes. No differences were observed in the in vitro footprints generated by nuclear extracts derived from dark grown cotyledons versus cotyledons exposed to light, although only a subset of the rbcS genes is expressed in dark-grown cotyledons, and all five genes are light-regulated in cotyledons. In contrast, differences in expression among organs of tomato may be directly reflected by differences in the footprint patterns, as the DNAse I protection patterns generated by nuclear extracts from cotyledons, leaves, roots, and immature and mature fruit differed from one another at some, but not all sites. The relationships between binding sites identified in other plant genes and those defined in this study are examined.

**A 444** ISOLATION AND FUNCTIONAL ANALYSIS OF A PCNA HOMOLOGUE FROM *BRASSICA NAPUS* (CV. WESTAR). **Nancy A. Markley And Maurice M. Moloney. Department Of Biological Sciences, University Of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada. T2N 1N4.**

The Proliferating Cell Nuclear Antigen (PCNA) is an essential replication factor in eukaryotic cells. Together with another accessory protein, RF-C, PCNA functions as a part of a molecular mechanism to coordinate the sequential initiation of lagging and leading strand synthesis by Polymerase-α and -δ, respectively. Furthermore, PCNA specifically increases the processivity of Polymerase-δ, allowing for efficient replication by the leading strand polymerase. A heterologous probe specific to rice PCNA was synthesized by the polymerase chain reaction (PCR), and used to screen a *B. napus* (cv. Westar) apical cDNA library. A 1.0 Kb cDNA clone isolated by hybridization at low stringency (48°C) showed strong binding to the rice probe. The cDNA clone has been confirmed to be a PCNA homologue using the rice specific PCNA primers for PCR. Amplification of fragments of the predicted sizes (based on sequences published for mammalian and rice PCNAs) indicates that the structure of the gene has been conserved in *B. napus* . The complete sequence of the *B. napus* PCNA cDNA will be presented. Northern blot analysis of dividing and non-dividing tissue, as well as, transient expression of antisense PCNA RNA in protoplasts, is being used to test if a causal relationship exists between PCNA expression and cell cycling. These experiments represent one of the first attempts to study at the molecular level, the function of a cell cycle specific protein in higher plants. The structural and functional conservation of PCNA, and other cell cycle specific proteins, in a variety of diverse eukaryotes suggest that there may be a common mechanism(s) of cell cycle control. Analysis of cell cycle specific proteins in plants may provide an alternative approach for the elucidation of regulatory mechanisms of the eukaryotic cell cycle, and furthermore, may help to distinguish processes that are unique to the plant kingdom.

**A 445** REGULATION OF SOYBEAN *vspB* PROMOTER ACTIVITY BY JASMONIC ACID, Hugh S. Mason, Daryll B. DeWald and John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843
Soybean vegetative storage protein genes (*vspA* and *vspB*) are highly regulated in response to vegetative and reproductive development and environmental conditions. They are expressed abundantly in young growing shoot tissues, but can be induced in mature tissues by wounding or treatment with jasmonic acid, a prostaglandin-like plant hormone. In order to delineate regulatory sequences which respond to jasmonic acid, we have studied the activities of *vspB* promoter constructs in transfected tobacco protoplasts and transgenic tobacco plants. A 1.5 Kb fragment of the *vspB* gene containing promoter and enhancer-like sequences was used for making deletions and rearrangements which were then fused to the $\beta$-glucuronidase (GUS) coding sequence. Initial results indicate that treatment of transfected tobacco protoplasts with jasmonic acid-methyl ester enhances the expression of GUS constructs which contain the full 1.5 Kb fragment or a 5'-end shortened 0.9 Kb fragment of the *vspB* promoter. Expression of *vspB* promoter-GUS fusions in transgenic tobacco will be described.

**A 446** IMMUNO-GOLD LOCALISATION OF AN HSP70 IN PEA LEAF MITOCHONDRIA. Anthony L. Moore and Felicity Z. Watts. Department of Biochemistry, University of Sussex, Falmer, Brighton BN1 9QG, U.K.
We have recently cloned a mitochondrial HSP70 gene, PHSP1 (see Abstract - Watts and Moore). The PHSP1 protein has been shown to be associated with pea leaf mitochondria by Western blotting. To localise the protein to a specific region within mitochondria, we have carried out immuno-gold electron microscopy using antibodies raised against a peptide representing a typically mtHSP70 sequence. Preliminary results indicate that the protein is localised at the inner surface of the inner membrane. Results of a study to investigate the presence of ISP42 (Vestweber *et al.*, 1989) in pea leaf mitochondria will also be presented. The implications of these results with respect to their roles in protein translocation will be discussed.
Vestweber, D., Brunner, J., Baker, A. and Schatz, G. (1989) Nature 341, 205-209.

**A 447** DIFFERENTIAL EXPRESSION OF SPECIFIC MEMBERS OF THE TOMATO HMG CoA REDUCTASE GENE FAMILY IS CORRELATED WITH DEVELOPMENTAL AND ORGAN-SPECIFIC FUNCTIONS, Jonathon O. Narita, Xianju Cui, Grace S. Kim, and Wilhelm Gruissem, Department of Plant Biology, University of California, Berkeley, CA 94720
The enzyme HMG CoA reductase (HMGR) catalyzes an essential step in the synthesis of phytosterols, carotenoids, and other isoprenoid containing compounds. The tomato multigene family is composed of at least four genes., three of which have been analyzed. There is significant sequence identity between the carboxy-terminal "active-site" domains of these genes and HMGR genes isolated from Arabidopsis and yeast. Conversely, the amino-terminal membrane spanning domains show limited sequence homology in inter- and intra-species gene comparisons. During tomato fruit development a single gene, HMG-1 is expressed when cellular division and expansion occur, whereas mRNA for HMG-2 (and possibly other) genes accumulate only during fruit ripening (1). Previously, we have shown that inhibition of HMGR with the drug mevinolin will disrupt tomato early fruit development (2). Here we show that application of high levels of mevinolin to ripening fruit disks leads to loss of both photosynthetic carotenoids and de novo lycopene synthesis. These results are consistent with a direct role for HMGR in lycopene accumulation during the chloroplast to chromoplast conversion.
(1) Dean, S., Mano, Y., Narita, J., and W. Gruissem (1991) This abstract booklet.
(2) Narita, J. and W. Gruissem (1989) The Plant Cell, 1, 181-190.

**A 448** EXPRESSION OF A MITOCHONDRIAL PROTEIN ASSOCIATED WITH CYTOPLASMIC MALE STERILITY IN PETUNIA, Helen T. Nivison and Maureen R. Hanson, Section of Genetics and Development, Cornell University, Ithaca, NY 14853
An abnormal mitochondrial gene, *pcf*, is present in cytoplasmic male sterile (CMS) *Petunia* lines and CMS somatic hybrid plants, but absent from fertile *Petunia* lines and fertile somatic hybrids. *Pcf* is comprised of three regions: a segment of an *atp9* gene, portions of a *coxII* gene, and an unidentified reading frame termed *urfS*[1]. To determine whether the *pcf* gene is expressed at the protein level, antibodies were produced to four synthetic peptides which were specified by *pcf*. Each of the three regions of *pcf* was represented by at least one peptide. Anti-*urfS* peptide antibodies recognized a 25-kD protein which is present in sterile lines, absent from fertile lines, and greatly reduced in fertile plants carrying the sterile cytoplasm along with a single dominant nuclear fertility restorer gene[2]. This 25-kD protein is synthesized by isolated mitochondria and fractionates into both soluble and membrane portions of disrupted mitochondria. Further experiments with the peptide antibodies, pulse-chase labeled mitochondria, and a bacterial system which expresses *pcf* indicate that the 25-kD protein is a processed product of the full-length PCF protein.

[1] Young, E.G. and M.R. Hanson. 1987. *Cell* 50: 41-49.

[2] Nivison, H.T. and M.R. Hanson. 1989. *The Plant Cell* 1: 1121-1130.

**A 449** NEGATIVE REGULATION OF TRANSCRIPTION BY PHYTOCHROME IN LEMNA GIBBA Patricia Okubara and Elaine Tobin, Biology Department, University of California, Los Angeles, CA 90024.

The developmental photoreceptor phytochrome mediates changes in the transcription of a number of genes. Understanding phytochrome action on transcription entails the study of negative, as well as positive, regulation. Our research objective has been to examine the molecular mechanism(s) by which phytochrome decreases transcription. Three genes negatively regulated by phytochrome action from the aquatic monocot Lemna gibba have been isolated and characterized. mRNA levels for all three genes increases within 6 h in the dark and all three genes show reduced levels of transcription in response to a single illumination of red light. Detectable increases in nuclear transcripts for all three genes occurs within 2 h in darkness for both green and etiolated Lemna, but the reduction of transcription by red light is routinely observed only in etiolated plants. Southern blot analyses of Lemna genomic DNA indicate that there are 1 to 2 genes in each family. Chimeric constructions consisting of promoters ligated to reporter genes have been made for introduction into Lemna plants by microprojectile bombardment.

**A 450** EXPRESSION OF NODULE PHOSPHOENOLPYRUVATE CARBOXYLASE AND GLUTAMINE SYNTHETASE ISOFORMS IN RESPONSE TO HIGH $CO_2$ IN Phaseolus vulgaris, José Luis Ortega, Lourdes Blanco and Miguel Lara, Unidad de Biología Molecular y Biotecnología Vegetal, Universidad Nacional Autónoma de México, Apartado Postal 2-246 Cuernavaca, Mor. México.

One of the limiting factors for the nitrogen fixation in the Rhizobium-legume symbiosis is the photosynthetic capability of the plant. To search how the nodule metabolism is affected by the photosynthetic capacity, bean plants infected with Rhizobium were grown in high (1000ppm) or low (200ppm) $CO_2$. High $CO_2$ results in an increase in the nitrogenase activity. This effect is not associated with an increase in the glutamine synthetase (GS) or the phosphoenolpyruvate carboxylase (PEPc) activities. Analyses of the GS and the PEPc isoforms show that the nodule-specific isoforms of these enzymes decrease considerably in high $CO_2$.

**A 451** REGULATION AND EVOLUTION OF THE CLASS-I PATATIN GENES OF POTATO

William Park, Greg May, Jie Du, and Hongyong Fu, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

Patatin is a family of lipid acyl hydrolases that accounts for 30-40% of the soluble protein in potato tubers. Class-II patatin genes are an ancestral form expressed at low levels in tubers and also expressed in roots of a wide range of solanaceous species. Class-I patatin genes are normally expressed only in tubers, but they can be induced to express at high levels in leaves and stems by sucrose. The trans-acting factors required for the sucrose induction of patatin expression are present in a wide range of solanaceous plants. While the cis-acting elements involved also appear to be highly conserved, the way they are arranged and the way the endogenous patatin genes respond to environmental signals differs in nontuberizing species and in some evolutionarily divergent tuberizing species. The data suggest that the Class-I patatin genes may have evolved from components found on two different chromosomes of nontuberizing species.

**A 452** TRANSGENE EXPRESSION VARIABILITY (POSITION EFFECT) OF CAT AND GUS REPORTER GENES DRIVEN BY LINKED DIVERGENT T-DNA PROMOTERS, Cindy Peach and Jeff Velten, Molecular Biology Program, Plant Genetic Engineering Laboratory and Chemistry Department, New Mexico State University, Las Cruces, NM 88003-0001, USA

Forty-five individually transformed clonal tobacco callus lines were simultaneously assayed for both chloramphenicol acetyltransferase (CAT) and ß-glucuronidase (GUS) activity resulting from introduced reporter genes driven by the adjacent and divergent agropine promoters. Excluding lines in which one or both of the enzyme activities was essentially zero, the activities of the reporter genes varied by as much as a factor of 136 (CAT) and 179 (GUS) between individual transformants. Superimposed upon the high degree of inter-clonal expression variability was an intra-clonal variability of 3-4 fold. The observed degree of intra-clonal reporter gene activity may be more extreme because of the regulatory characteristics of the agropine promoters, but must still be addressed when considering the limitations of reporter gene-based analysis of transgene function and structure. There was no consistent correlation between the expression levels of the CAT and GUS genes since the ratio of GUS to CAT activities (nmol/min/mg) within individual lines varied between 0.05 and 49 (nearly 1000 fold). Even divergent transcription from two directly adjacent promoter regions (both contained within a 479 bp TR-DNA fragment) is insufficient to guarantee concurrent expression of two linked transgenes. Our quantitative data was compared to published data of transgene expression variability to examine the overall distribution of expression levels in individual transformants. The resulting frequency distribution indicates that most transformants express introduced transgenes at relatively low levels, suggesting that an unknown, though potentially large, number of *Agrobacteria*-mediated transformation events may result in silent transgenes.

**A 453** BIOCHEMICAL COMPARATIVE ANALYSIS OF ACTIN ISOFORMS IN ROOT TISSUE OF Phaseolus vulgaris, Glicine max and Zea maiz . Héctor F. Pérez and Federico Sánchez. Plant Molecular Biology and Biotechnology Unit. UNAM. Cuernavaca, Mor. México.

We have developed a simple procedure for the enrichment of plant actin from root tissues. Using low ionic strenght extraction, DEAE batch fractionation and ammonium sulfate concentration it is possible to enrich plant polypeptides tha are recognized by monoclonal anti-actin antibodies. These polypeptides comigrate with animal actin in two dimensional polyacrilamide gels.

Using the above procedures we have determined plant actin isoform expressión in root tissues of Phaseolus vulgaris (black bean), Glicine max (soybean) and Zea maiz (corn).

We discussed the above results and compare them with the kappa, lambda and mu actin classes described by genetic methods.

**A 455 CHARACTERIZATION OF A GENE ENCODING A CRUCIFERIN SUBUNIT: COORDINATE EXPRESSION OF CRUCIFERIN GENES DURING EMBRYOGENESIS.**
Joakim Rödin, Staffan Sjödahl, Lars-Göran Josefsson and Lars Rask, Department of Cell Research, Swedish University of Agricultural Sciences, BMC, Box 596, S-751 24 Uppsala, Sweden.

A gene (cru1) encoding for a subunit of cruciferin, the 12S storage globulin in rapeseed *(Brassica napus)*, has been cloned and sequenced. It consists of about 2200 bp including three short intervening sequences. The positions of the exon-intron boundaries are the same as in 11-12S storage globulin genes in legumes and other dicots. The gene cru1 encodes a cruciferin polypeptide of 509 amino acids including an amino terminal signal sequence of 23 amino acids and an α polypeptide of 296 amino acids and a β polypeptide of 190 amino acids. This gene belongs to one of three different families encoding cruciferin subunits. By use of gene specific probes for this gene and for genes encoding the two other cruciferin subunits we have estimated the number of genes in the three different cruciferin subfamilies to 3-4 member each per haploid genome by Southern analysis. The probes have also been used to study the expression and accumulation of these three types of cruciferin transcripts during the embryogenesis of rapeseed. Data from these experiments indicate regulation on both transcriptional as posttranscriptional level.

**A 456** ISOLATION AND ANALYSIS OF A GENE EXPRESSED DURING EARLY SEED DEVELOPMENT IN BARLEY, Ru Liu[1], Odd.A.Olsen[2], John Davies[3] and Nigel Halford[1], 1. Dept. of Biochemistry and physiology, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, U.K. 2. Dept. of Biology, Division of General Genetics, Oslo University, Norway. 3. Dept. of Biological Sciences, Durham University
We are investigating the control of earley seed development in barley and are attempting to identify genes specifically during this stage. A cDNA library was constructed from mRNA extracted from barley (Hordeum vulgare L. cv. Bomi) seed 18 days after anthesis. Following differential screening for seed specific, a 940 bp cDNA clone was isolated. The cDNA clone has been sequenced and mapped to the short arm of chromosome 4. Southern blot analysis shows the gene has low copy number, and Northern blot analysis indicates that it is expressed at low level. This result was confirmed in preliminary in situ experiments. The insert of the cDNA clone was used to screen a barley genomic library and a single hybridising clone has been isolated and sequenced. Various length of promoter fragments are being fused to the GUS repoter gene and their expression are going to be tested in two systems-transient expression assay and stable intergration into tabacco using Agrobacterium tumefaciens.

**A 457** GENOMIC ORGANIZATION AND EXPRESSION OF A ZEIN GENE CLUSTER IN MAIZE, Irwin Rubenstein and Changnong Liu, Department of Plant Biology and Plant Molecular Genetics Institute, University of Minnesota, St. Paul, MN 55108
Five zein subfamily 4 (SF4) genes are clustered in a tandem array within a 56 kb region of the maize inbred W22 genome. There are two types of zein SF4 genes. One type (T1) contains no early in-frame stop codons. The other (T2) contains one or two early in-frame stop codons. T1 genes have 5 to 20 fold higher steady-state levels of mRNA transcripts than T2 genes. cDNA clones with nucleotide sequences identical to the sequences of genes representing both types of SF4 genes have been isolated. Of the 6 zein SF4 genes sequenced to date 5 are T2 genes. The 5' and 3' non-coding nucleotide sequences for the two types of genes are similar. The amino acid sequences in the regions downstream of the early in-frame stop codons for the T2 genes are similar to the amino acid sequences found in the T1 gene. These results suggest that the SF4 zein T2 genes are biologically functional.

**A 458** ISOLATION OF TWO *ARABIDOPSIS THALIANA* cDNA CLONES CONTAINING HOMEODOMAIN-RELATED SEQUENCES. Ida Ruberti[1], Giovanna Sessa[1], Sabrina Lucchetti[2] and Giorgio Morelli[2]. [1]Centro di studio per gli Acidi Nucleici c/o Dipartimento di Genetica e Biologia Molecolare Università di Roma La Sapienza, P.le Aldo Moro 5 00185 Roma Italy [2]Unità di Nutrizione Sperimentale, Istituto Nazionale della Nutrizione, via Ardeatina 546 00178 Roma Italy.
Several reports at the 32nd Maize Genetics Meeting have revealed that a substantial fraction of plant regulatory genes share homology with animal genes like *c-myc*, *myb-b*, *fos* and *jun*. Moreover, E. Vollbrecht (USDA Plant Gene Expression Center) has reported that the maize *knotted* locus, a dominant leaf morphology mutant previously cloned by tagging with DS, contains a homeodomain-related motif, homologous to the *MAT* locus of yeast and to the *gooseberry* gene of *Drosophila*.
To isolate plant genes containing homeodomain-related sequences, we have taken a different approach. Using as a probe a degenerate oligonucleotide derived from the most conserved region of the homeodomain (Burglin T.R., Finney M., Coulson A. and Ruvkun G. 1989 Nature 341, 239-2437), we have screened a cDNA library from *Arabidopsis thaliana*. In this way we were able to isolate several clones out of 60.000. DNA sequencing confirmed that two out of four selected clones contain sequences corresponding to the conserved helix-3 region of the homeodomain.

**A 459** TEMPORAL ORDER OF RNA EDITING AND PROCESSING OF THE COX2 TRANSCRIPT IN PETUNIA MITOCHONDRIA . Claudia A. Sutton, Kim Dixon Pruitt* and Maureen Hanson, Section of Genetics and Development, Cornell University, Ithaca, NY 14853 , *Boyce Thomson Institute, Ithaca, NY 14853.
The *cox2* transcript of *Petunia hybrida* contains a group II intron of ca 1.3 kb. The mature transcript is edited (C to U changes) at distinct sites. We undertook experiments to determine if editing occurred pre- or post-splicing. Two oligonucleotides were synthesized that were complementary to a 25 nt segment of the second exon of *cox2* and differed only in that one was complementary to the genomic sequence and one to the edited RNA sequence. These were then used to probe blots of mitochondrial RNA. We developed specific hybridization conditions to discriminate between edited and unedited RNAs. RNA editing of the second exon of *cox2* appears to occur very rapidly and to precede splicing of the intron. cDNA clones representing unspliced transcripts have been sequenced to determine whether all of the RNA edits found in the mature transcript occur before intron removal.

**A 460** THE UPSTREAM REGION OF CAULIFLOWER MOSAIC VIRUS (CaMV) 35S PROMOTER WORKS AS A NEGATIVE REGULATORY ELEMENT IN HeLa CELLS.
Makoto Takano, Kumiko Takeda, Kazuhiko Sugimoto and Joh-E Ikeda, Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, JAPAN
CaMV 35S promoter originally drives the transcription of the 35S RNA of plant virus (CaMV) and also works in animal cells (HeLa cell). This means that the CaMV 35S promoter has cis acting elements which function in both plant and animal cell. So far, we found two distinctive regions in the 35S promoter. One enhances the promoter activity and the other represses the enhancement in HeLa cells. In the present work we have examined the negative control element.
A series of deletion mutants of the CaMV 35S promoter sequences were constructed and linked to the CAT genes. These constructs were transfected into HeLa cells mediated by Lipofectin. By measuring CAT activities, we found that the sequence between -163 and -130 bp relative to the mRNA initiation site exerts a negative effect on the promoter activity. Furthermore, the DNA sequence between -347 and -125 bp represses the expression from SV40 early promoter (in pSV2cat) when it was inserted to the upstream of the promoter in either orientation. But this sequence no longer acts as a negative regulator when it is located on 3' flanking of the CAT gene of pSV2cat or when it connects to the RSV LTR promoter. Therefore, we concluded that the cis acting elements including in this region may not fully satisfy the difiniton of silencer but repress the promoter activity in an enhancer-specific manner.

**A 461** **THE SV-40 NUCLEAR LOCALIZATION SIGNAL AND THE DNA BINDING DOMAIN OF PLANT B-ZIP PROTEINS DIRECT EFFICIENT IMPORT INTO PLANT NUCLEI.**

A.R. VAN DER KROL AND NAM-HAI CHUA, Plant Molecular Laboratory, Rockefeller University New York fax 212-570-8327

The import of large proteins (>60 kD) into the nucleus depends on the presence of a nuclear localization signal (NLS) which has been identified in numerous mammalian, amphibian and yeast proteins. Insights into nuclear targeting processes obtained from non-plant systems can be of value in the plant field where this process has not been studied so far. To test the degree of conservation of the nuclear import mechanism in mammals and plants we examined whether the mammalian SV40 large T-antigen NLS will function in plants cells. The nucleotide sequence encoding the SV40 NLS was fused to the GUS coding sequence and the chimeric GUS gene (SV40-GUS), under control of the CaMV 35-S promoter, was introduced into tobacco plants. Subcellular localization of the GUS in transgenic plants was scored using histochemical assay. The results show that the SV40-GUS is efficiently imported into the plant nucleus.

We also analyzed several putative NLS's from different plant DNA-binding proteins. Preliminary results from these experiments show an overlap of a NLS and the DNA binding domain of the B-Zip proteins TGA-1A and TGA-1B. This functional overlap can impose a considerable constraint on the evolution of B-Zip DNA binding proteins.

**A 462** CHARACTERIZATION OF ALPHA- AND BETA- TUBULIN GENES IN MAIZE (*Zea mays*). Richard Villemur[1], Nandini Mendu[1], Russell H. Goddard[2], Nancy A. Haas[1], Wang Duo[1], Susan M. Wick[2], D. Peter Snustad[1,3], and Carolyn D. Silflow[1,2,3]. Dept. of Genetics/Cell Biology (1), Dept. of Plant Biology (2), and Plant Molecular Genetics Institute (3), Univ. of Minnesota, 1445 Gortner Ave., St-Paul MN 55108.

Microtubules are filamentous polymers, composed of alpha- and beta-tubulins, that are intimately involved in two key developmental processes in plants: cell division and cell expansion. Tubulins are encoded by multi-gene families in higher eukaryotes. Maize is an attractive system for studying tubulin isotype expression in higher plants because growth and development in this plant are well characterized. We have isolated many maize tubulin clones from pollen, endosperm and shoot seedling cDNA libraries. Complete and partial DNA sequences derived from these clones revealed that 12 different tubulin cDNAs were isolated: six alpha and six beta. Based on their sequence similarities, the 6 alpha-tubulin genes can be classified into three sub-families. Genes in one of these sub-families are highly expressed in pollen because they represented more than two-thirds of the pollen alpha-tubulin cDNA clones isolated. We subcloned the divergent 3' non-coding sequence of all 12 tubulin cDNAs to prepare gene-specific hybridization probes (GSP). Southern blot analysis with the GSPs showed that most, if not all, of the alpha-tubulin genes have been cloned and probably half of the beta genes. We also derived specific isotype antibodies, in chicken, against each of the alpha-tubulin sub-families and to two of the beta-tubulins using synthetic oligopeptides derived from the carboxyl terminal sequence as antigens. This part of the protein has been shown to be highly divergent between different tubulin isoforms, whereas most of the rest of the protein is highly conserved. We will discuss the expression of each tubulin isotype in different maize tissues and during different development stages of specific maize tissues using the GSPs and the specific antibodies as tools.

**A 463** TARGETING A FOREIGN PROTEIN TO LEAF PEROXISOME OF TRANSGENIC TOBACCO PLANTS, Micha Volokita and Pazia Gonen, Department of Biochemistry, The Weizmann institute of Science, Rehovot 76100, ISRAEL

Glycolate oxidase (GLO), a key enzyme of the glycolate metabolism pathway, is located in the leaf peroxisome. To identify the peroxisomal sorting sequences of GLO, several chimeric genes were constructed by fusion in-frame the GLO gene or portion of the gene to a truncated β-Glucuronidase (GUS) gene. The last six residues of the carboxy-terminal portion of GLO are sufficient in order to target GUS to the leaf peroxisome of transformed tobacco plants.

**A 464** CLONING AND CHARACTERIZATION OF THE *R-r* ALLELE OF ZEA MAYS,
Elsbeth L. Walker, Timothy P. Robbins, Jerry L. Kermicle and Stephen L. Dellaporta, Yale University, New Haven, CT 06511

The *R* locus of maize encodes a *myc*-like DNA binding protein which controls anthocyanin production. The *R-r* allele conditions pigmentation of the aleurone layer of seeds and of plant parts including the anthers, coleoptile and seedling leaf tip. Derivative alleles of *R-r* which lack the ability to pigment either plant (P) or seed (S) occur at a high frequency ($8 \times 10^4$ and $4 \times 10^4$, respectively) at meiosis. The independent loss of (P) and (S) components of *R-r* implies a duplicate structure in which displaced synapses between (S) and (P) followed by crossing-over lead to the loss of one of the components.
Through a molecular analysis we have established that *R-r* comprises a triplication of DNA. Genomic clones spanning >120 kbp of DNA of *R-r* have been used to determine the order and orientation of the three *R-r* components. Sequence analysis of the breakpoints of the *R-r* triplication has shown that the potential for pairing exists for some but not all members. Microprojectile bombardment experiments will confirm the identity of each member of the *R-r* triplication. These data have led to a new model which explains the behavior of *R-r* and its cross-over derivatives.

**A 465 CHEMICAL INDUCTION OF GENE EXPRESSION IN PLANTS**
Eric Ward, Shericca Williams, Sandra Dincher, Leslie Friedrich, Jean-Pierre Métraux* and John Ryals, Agricultural Biotechnology Research Unit, CIBA-GEIGY Corp., Research Triangle Park, NC and *CIBA-GEIGY AG, Basle, Switzerland

We will describe the effects of a novel "immunization" chemical that induces a broad range of systemic resistance to pathogens by triggering an inherent "immune" reaction in target plants. The molecular basis for this response has been investigated. We will present data concerning some of the genes involved and their induction patterns as a result of chemical treatment. One of the immunity-associated genes in tobacco encodes the pathogenesis-related protein PR-1. By using PR-1/GUS fusions, we have defined the cell types that respond to the chemical. We have also developed a tightly regulated chemically-inducible expression system for heterologous genes in plants.

**A 466** CHARACTERISATION OF A MITOCHONDRIAL HSP70 GENE FROM *P. SATIVUM*.
Felicity Z. Watts and Anthony L. Moore, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, U.K.

Using an *S. pombe* gene SSP1, (Powell and Watts, Gene in Press) as probe, we have isolated a full-length cDNA from *P. sativum* encoding a member of the HSP70 gene family. DNA sequence analysis indicates that this gene (PHSP1) is more homologous to mitochondrial HSP70 genes and *E. coli* Dnak, than to cytoplasmic HSP70 genes from pea or other organisms. This is the first identification of a plant mitochondrial HSP70 gene. Comparison of the PHSP1 protein sequence with that of the cytoplasmic HSP70 for which the 3D-structure has recently been determined, indicates that the PHSP1 protein contains all three of the amino acids proposed by Flaherty *et al.* (1990) to be involved in binding ATP. Regions of the protein that are highly conserved or which are typical of mitochondrial HSP70s have also been identified. The potential significance of these regions will be discussed. Western blotting of cytoplasm and purified mitochondria using antibodies aganist a peptide corresponding to a sequence not observed in cytoplasmic HSP70s, confirms that the protein is predominantly associated with the mitochondria. Isolation of mitochondria from pea leaves incubated at different temperatures for 30 minutes prior to harvesting, indicates that expression of the protein is not induced by either cold or heat shock, implying that PHSP1 encodes a heat shock cognate protein. Studies are currently underway to determine the role of this protein, and to further localise it within the mitochondria and the results are presented separately (Moore and Watts).
Flaherty, K.M. *et al.*, Nature (1990) 346, 623-628.

**A 467** FACTORS AFFECTING THE REGULATION OF EMBRYO-SPECIFIC GENES IN MICROSPORE EMBRYOS OF *BRASSICA NAPUS*. **Ronald W Wilen, Gijs JH van Rooijen, Larry A Holbrook* and Maurice M Moloney. Department of Biological Sciences, University of Calgary, Calgary Alta, Canada, T2N 1N4; * Plant Biotechnology Institute, NRC, Saskatoon Sask, Canada, S7N. 0W9**

*Brassica napus* seeds accumulate three major proteins, napin, cruciferin, and oleosin, during embryogenesis, accounting for 90% of total seed protein. We have utilized the microspore-derived embryo system of *B. napus* to investigate hormonal and environmental regulation of these storage protein genes. Microspore embryos express all three genes, although the level of storage protein mRNA detected was minimal until the embryos were at a late cotyledonary stage. The accumulation of mRNA encoding these three proteins was dramatically increased with exogenous abscisic acid (ABA, 1 - 50 μM) if applied at heart stage or later. An ABA-like response in transcript accumulation was also noted when the embryos were cultured in the presence of an osmoticum (osmotic pressure = 25.5 bars). Time course studies of napin and cruciferin mRNA accumulation indicated the ABA treatment elicited a much faster inductive response than observed with the osmotic stress treatment. Endogenous ABA was measured (by GC-MS) during osmotic treatment, and was found to increase four to sixfold within 2 h of plating on the osmoticum. These findings initially suggested osmotically induced storage protein gene expression was mediated by ABA. When the time course northern blots were reprobed with an oleosin clone, a different expression pattern was noted. The induction of oleosin mRNA transcripts was Jasmonic acid (JA) was found to be a natural product in microspore embyros. When JA was applied to the microspore embryos, induction of these mRNAs was detected at levels comparable to those observed after ABA treatment. We are investigating the interrelationships between ABA, JA and environment through the use of antagonists to ABA receptor binding.

**A 468** CHARACTERIZATION OF A NUCLEAR PROTEIN FROM CAULIFLOWER WHICH BINDS A SEQUENCE WHICH IS SUFFICIENT TO CONFER SHOOT APICAL MERISTEM SPECIFIC EXPRESSION TO A MINIMAL PROMOTER, Mary E. Williams and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021

We have identified a 5′ deletion of the pea stem specific gene (S2) promoter to -75 which confers expression specifically in the shoot apical meristem. Within this 75 base promoter, there are two copies of the palindrome ANCACGTGNT. When four copies of this palindrome are fused to a heterologous TATA box, CaMV 35S, expression is again detected in the shoot apical meristem. A weak binding activity to this sequence is detected in tobacco leaf nuclear extracts. Cauliflower nuclear extracts contain a much higher specific activity. Footprint analysis of the promoter region using cauliflower extracts demonstrate binding across both copies of this sequence. Specific sequence preferences of this DNA binding protein will also be presented.

**A 469** SEARCH FOR THE FUNCTION OF AN ARABIDOPSIS GENE WHICH SHARES SIMILARITY TO YEAST GENES CDC10 AND SWI6, Hong Zhang, Yie-Hwa Chang, Susan Hanley, and Howard Goodman, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

An *Arabidopsis* cDNA clone (designated AB5-13) with similarity to yeast genes CDC10 and SWI6 was isolated. The similarity occurs at the 33-amino acid motifs of the yeast genes, and this motif is also shared by neurogenic gene Notch of *Drosophila*, and homeotic genes lin-12 and glp-1 of *C.elegans*. The fact that this motif is conserved in a set of evolutionarily distant organisms suggests that it plays an important role in the cell. Notch, lin-12 and glp-1 appear to encode a membrane-bound receptor-like molecule, and SWI6 was shown to be a *trans*-acting factor controlling HO gene expression in yeast. We would like to study the function and regulation of this gene (AB5-13) in higher plants.

The partial clone AB5-13 is 1.3 kb long, and the 33-amino acid motif is repeated 4 times in this clone. The corresponding genomic sequence shows that this portion of the gene is split by 6 introns. The genomic southern indicates that there is only one copy of this gene in *Arabidopsis* genome, and northern data shows a relatively abundant message in leaf tissue with a mobility of 2.4 to 3 kb. AB5-13 was mapped to chromosome five, 7.4 cM away from RFLP marker λbAt555. We also expressed AB5-13 in yeast mutant swi4 (a homolog of SWI6), and it weakly boosted the expression of lac Z under the control of the HO gene promoter. Currently we are testing the activity of AB5-13 in the swi6 mutant. Meanwhile, we are looking for a full length cDNA clones and characterizing the genomic sequences. Antisense RNA experiments are also being pursued.

*Genetic Dissection of Hormone-and Light-Regulated Gene Action*

**A 500** DEVELOPMENT OF A TRANSPOSON-MUTAGENESIS-SYSTEM FOR *ARABIDOPSIS THALIANA*, Thomas Altmann, Alison Jessop, Peter C. Morris, Renate Schmidt and Lothar Willmitzer, IGF Berlin GmbH, Ihnestr. 63, 1000 Berlin 33, Germany

Using both the intact, autonomous maize transposable element *Activator* (*Ac*) as well as non-autonomous derivatives ("*Ds*"-elements), we are trying to set up an efficient insertion mutagenesis system for *Arabidopsis thaliana*. *Ac* has been shown to exhibit a germinal excision frequency of about 0.2 to 0.5 % in transgenic *Arabidopsis*. As a monitor we are therefore using the bar gene that is activated during excision of *Ac* by its fusion to the TR1´ promotor. It confers resistance to the herbicide Basta. This marker gene allows selection of very large seedling populations by spraying the herbicide as well as testing single plants by using a non destructive leaf painting assay. Currently about 100 independently transformed, diploid lines are screened for the germinal excision frequency of their integrated *Ac* copy. Two "*Ds*" -elements have been constructed: $Ds_A$ is a pure deletion derivative of *Ac* lacking 2.8 kb of internal sequences. $Ds_B$ contains a bacterial gene (supF) to facilitate the recloning of the element and a chimaeric bar gene useful for genetic analysis. For both "Ds"-elements the NPT II gene conferring resistance to Kanamycin / G418 is used as excision marker. As non-mobile source of trans-activating transposase an Ac clipped wing ($Ac_{cl}$) was generated by precise deletion of one of the terminal 11 bp inverted repeats of *Ac*. Transposition of $Ds_B$ promoted by $Ac_{cl}$ could be detected by G418 selection of regenerating leaf explants from plants carrying $Ds_B$ and $Ac_{cl}$. DNA analysis and the recloning of both the empty $Ds_B$ donor site and the 5´ region of $Ds_B$ together with new flanking sequences confirmed its excision and reinsertion. Data derived from crosses of 5 $Ac_{cl}$ lines with 7 $Ds_A$ and 3 $Ds_B$ lines indicate a much higher competence of $Ds_A$ to be transactivated than $Ds_B$. Up to about 25% Kanamycin resistant F2 plants were obtained in the progeny of crosses between $Ac_{cl}$ and $Ds_A$. As an alternative transposase expressing construct, the cDNA of *Ac* under the control of the CaMV 35S promotor has been introduced into *Arabidopsis* and is currently being tested for its ability to activate $Ds_A$ and $Ds_B$.

**A 501** MAPPING OF AC ACCEPTOR SITES IN THE TOMATO GENOME: IMPLICATIONS FOR TRANSPOSON TAGGING STRATEGIES, B. Baker, C. Corr, R. Hehl, J. Prince*, S. Tanksley, S.*, McCormick, , B. Osborne, Plant Gene Expression Center, USDA, University of California, Berkeley, Albany, CA, 94710.*Department of Plant Breeding, Cornell University,Ithaca, NY,14853-1902

One of the objectives of our research is to develop and utilize the maize transposons *Activator* (*Ac*) and *Dissociation* (*Ds*) as versatile genetic tools for insertional mutagenesis and isolation of genes critical to host disease resistance in tomato. Knowledge of how a transposable element moves and information about the behavior of the transposon in a given species can substantially enhance recovery of transposable element mutations in a gene of interest. Our specific objectives include determination of the (1) frequency of transposition, (2) the nature of transposon target sites, and (3) the distance of *Ac* transposition. The transposition behavior of *Ac* and *Ds* has been favorably assessed in 30 available tomato transgenic lines over at least two plant generations. It has been demonstrated that *Ac* transposes germinally and somatically at a high frequency (10-80%) for 3 generations; integrates in hypomethylated chromosome regions; retains structure and sequence upon transposition; induces an 8 bp duplication of target sequences and often transposes to unique and low copy genomic sequence. *Ac* displays no negative dosage effect in tomato and *Ac* acceptor sites are both linked and unlinked to donor sites. We have also demonstrated that $Ds^{neo}$ (*Ds* carrying a neo gene) transposition is efficiently induced in *Ac* and *Ds* crosses; transposed $Ds^{neo}$ is transmitted to F2 progeny plants and some $Ds^{neo}$ acceptor sites are unlinked to donor sites. We are locating *Ac* and *Ds* transposition acceptor sites on the tomato genetic map. The results of this work indicate that *Ac* transposes to chromosomes on which it originated as well as to several other chromosomes and displays, as in maize, a tendency to make short or clustered hops. These results have important implications for developing and implementing transposon strategies.

**A 502** REDUCED ETHYLENE BIOSYNTHESIS IN TRANSGENIC TOBACCO PLANTS EXPRESSING S-ADENOSYLMETHIONINE HYDROLASE, Richard K. Bestwick[1], Wendy Wagoner[1], Jill A. Kellogg[1], William Pengelly[2], Lyle Brown[3] and Adolph Ferro[1], [1]Agritope, Inc., 15425 SW Koll Parkway, Beaverton, OR 97006, [2]Oregon Graduate Center, Beaverton, OR, [3]Oregon State University, Corvallis, OR.

Control of ethylene biosynthesis in plants will lead to new varieties with improved pre- and post-harvest physiology. We have utilized the bacteriophage T3-encoded enzyme S-adenosylmethionine hydrolase (AdoMetase or SAMase) to generate transgenic tobacco plants with reduced capacity to synthesize ethylene. SAM is the metabolic precursor of 1-aminocyclopropane carboxylic acid (ACC), the proximal precursor to ethylene. SAMase catalyzes the conversion of SAM to methylthioadenosine (MTA) and homoserine. The 470 base pair SAMase gene was substituted for the GUS gene in the Agrobacterium vector pBI121 which placed SAMase under the influence of the constitutive CaMV 35S promoter. This construct was used to transform tobacco using Agrobacterium-mediated gene transfer. Kanamycin-resistant regenerated plants were analyzed for SAMase expression and ethylene biosynthesis. Southern blot analysis showed integration of the SAMase gene was successful and the gene was in the expected configuration. Northern blots using leaf mRNA showed active transcription of a 0.8kb SAMase-specific mRNA species. Enzymatic assays of leaf extracts demonstrated that SAMase activity was present. Leaf disk assays of transgenic plants showed ethylene evolution to be less than 50% of control plants either with or without napthalene acetic acid, an inducer of ethylene biosynthesis. Furthermore when ACC and ethylene levels were measured and their values combined, leaf disks from SAMase transgenic plants had less than 40% of control plants. Current efforts are aimed at controlling SAMase expression with nonconstitutive promoters to obtain tissue and stage specific expression of SAMase.

**A 503** GENETIC TRANSFORMATION, PROMOTER TAGGING AND DISEASE RESISTANCE IN SUGARCANE, Robert G. Birch, Tricia Franks, Carl S. O. Rathus and W. V. Shiromi Basnayake, Department of Botany, The University of Queensland, Australia, 4072

Conditions have been established for reliable analysis of foreign promoters using transient expression assays following electroporation of sugarcane protoplasts. Stably transformed callus can be regenerated under selection for kanamycin resistance, and cotransformation with non-selected markers has been demonstrated, but plants are not readily regenerated from protoplasts. Microprojectile bombardment of embryogenic sugarcane callus has allowed regeneration of G418 resistant sugarcane plantlets which appear to be transgenic. Non-toxic *in situ* luciferase assays are being developed to detect chimeric transformants, and for real-time analysis of promoter activity in intact plants.

Bacterial genes for resistance to albicidin phytotoxins have been cloned. Resistance mechanisms include toxin binding, toxin inactivation and a toxin resistant target. Albicidin phytotoxins, produced by *Xanthomonas albilineans*, block DNA replication in bacteria and proplastids. Bacterial resistance genes will be transferred into sugarcane with appropriate promoters to evaluate effects on phytotoxin and disease resistance.

**A 504** PHYTOCHROME OVEREXPRESSION IN TRANSGENIC ARABIDOPSIS.
Margaret Boylan and Peter H. Quail, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

Monocot phytochrome has previously been shown to be biologically active when expressed in transgenic dicot tissues. We are interested in using Arabidopsis as a model dicot system for analysing the effects of elevated phytochrome content on plant growth and development. For this purpose, we have generated transgenic plants that overexpress oat phytochrome. In etiolated R1 seedlings, obtained by selfing the primary transformants, phytochrome spectral activity is three-fold higher than wild-type levels, indicating that the introduced oat protein is photoreversible. When grown in the light, these seedlings segregate for hypocotyl length, with a ratio of 3 short: 1 normal length hypocotyl. The short hypocotyl phenotype is easily distinguishable from wild-type and correlates with the amount of increased phytochrome found in the transformants. Further biochemical and physiological analysis of phytochrome overexpression in these plants will be presented.

**A 505** MONITORING TAM3 TRANSPOSITION IN TOBACCO AND *ARABIDOPSIS* USING AN SPT ASSAY.
R.A.Burton, S.R.Scofield, J.D.G.Jones and C.R.Martin

John Innes Institute and the Sainsbury Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.

The *Antirrhinum* transposon, Tam3, and the maize transposon, Ac, show homology both in the sequence of their inverted repeats and in the derived animo acid sequence of Tam3 cDNAs and the Ac transposase (Sommer *et al.*, 1988, in Plant Transposable Elements, pp. 227-235, ed. Nelson, Plenum Press). In comparison to Ac, Tam3 has some special features, including higher transposition activity at lower temperatures.

To study Tam3 activity further we have transferred it to tobacco. We found an appreciable level of excision in primary transformants but failed to detect transposition in subsequent generations. An associated specific methylation of the element was detected (Martin *et al.*, 1988, EMBO J. 8:997-1004). We have improved our ability to investigate Tam3 activity by establishing a phenotypic assay, based on streptomycin resistance, that has successfully been used to monitor Ac activity (Jones *et al.*, 1989, Science 244:204-207). In both tobacco and *Arabidopsis* we have detected variable Tam3 activity in the selfed progeny of both primary transformants and of the F1 generation. Most plant lines show activity in only a small (<10 %) of the self progeny. In addition, a number of plants which contain Tam3 in one particular orientation with respect to the antibiotic resistance gene show evidence of "leakiness", as manifested by an intermediate resistance phenotype. Studies at the RNA level have been initiated to investigate this further. We have found that Tam3 is specifically methylated in *Arabidopsis* as well as tobacco and are investigating Tam3 methylation in those few plants that show high activity. Finally, we have found that the element shows unaltered transposition frequencies at a lower temperature.

**A 506** TRANSCRIPTIONAL REGULATION OF A FERREDOXIN GENE BY PHYTOCHROME IN *ARABIDOPSIS*, Timothy Caspar, David Somers, and Peter Quail, Dept. of Plant Biology, Univ. of Calif., Berkeley, CA 94720 and USDA Plant Gene Expression Center, Albany, CA 94710

Ferredoxin is an electron transfer protein which functions in both green and non-green plastids. The abundance of ferredoxin mRNA has been shown to be regulated by phytochrome in pea (Dobres, et al. Plant Mol Biol 8:53). In order to study the phytochrome regulatory system in *Arabidopsis*, we have cloned an *Arabidopsis* ferredoxin gene. Northern blots using RNA from etiolated and red- or white-light irradiated seedlings show a rapid 3- to 10-fold light-induced increase in ferredoxin mRNA. The red light response is partially reversed by a subsequent far red treatment, indicating that it is phytochrome-mediated. In order to determine the mechanism of regulation, we fused the ferredoxin promoter to reporter genes and expressed these in *Arabidopsis* both stably, using *Agrobacterium*-mediated transformation, and transiently, using particle gun gene transfer. In both types of transformants the reporter genes were light-regulated, demonstrating that the ferredoxin promoter is sufficient to mediate light-regulation. Furthermore, the reporter gene was expressed at several hundred-fold lower levels in roots than in cotyledons indicating that the promoter mediates tissue-specific expression as well. These results contrast with those obtained with the pea ferredoxin gene, studied in transgenic tobacco, where the data suggested that the light-regulatory elements are located largely within the transcribed region (Elliot, et al. Plant Cell 1:691). The different results may derive from differences in the experimental systems used or, alternatively, to inherent differences in the regulatory mechanisms controlling the pea and *Arabidopsis* ferredoxin genes.

**A 507** **FUNCTIONAL ANALYSIS OF OAT PHYTOCHROME BY DELETION MUTAGENESIS**

**Joel R. Cherry[1], David Hondred[1], Howard Hershey[2], & Richard Vierstra[1]**

**[1] Dept. of Horticulture, Univ. of Wisconsin, Madison, WI 53706 [2]Agricultural Products Dept., E.I. DuPont deNemours & Co., Wilmington, DE 19880.**

**We are currently using a transgenic phytochrome expression system to identify domains within the photoreceptor required for biological activity. Plants expressing the full-length oat protein exhibit a striking "light exaggerated" phenotype characterized by a decreased apical dominance, increased chlorophyll content, and dwarfism. Processive deletions of the amino and carboxy-terminal ends of the protein were expressed in tobacco and assessed for biological activity using plant phenotype as an assay. In addition, truncated proteins were characterized biochemically with respect to chromophore attachment, photoreversibility, rate of dark reversion, dimerization, and form-dependent ubiquitination and degradation. Preliminary results indicate that the biological activity of oat phytochrome is extremely sensitive to minor deletions in both the N- and C-terminus. In general, C-terminal deletions are unable to attach chromophore and/or dimerize. Presumably because of these deficiencies, these C-terminal truncations are not biologically active. In contrast, "Large" phytochrome (lacking residues 7-69) is capable of chromophore attachment, dimerization and is photoreversible like the native chromoprotein, but is altered in its spectral properties. Interestingly, tobacco plants expressing "Large" phytochrome are phenotypically indistinguishable from untransformed controls, indicating that these phytochromes are biologically inactive in tobacco. This N-terminal deletion thus identifies a domain within the phytochrome molecule which is not required for assembly or photoreversibility, but which is essential for biological activity.**

**A 508** SITE DIRECTED MUTAGENESIS OF THE GENOME OF HIGHER PLANT CELLS VIA ELECTROPORATION.

Marcel J.A. de Groot[1], Remko Offringa, Mirjam P. Does, Jorgen de Haan, Tom J.W. Stokkermans, Paul J.J. Hooykaas[2] and Peter J.M. van den Elzen[1]. 1)MOGEN International nv, Einsteinweg 97, 2333 CB Leiden, The Netherlands. 2)Clusius Laboratories, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

A study was initiated to establish whether homologous recombination of DNA occurs in plant cells after introduction of the DNA via electroporation. For this purpose we constructed defective derivatives of the NPTII gene that contain a deletion or a frameshift mutation either in the 5' or the 3' part of the gene. In cotransformation experiments a 5' deletion and either a 3' deletion or a 3'FS mutant NPTII gene, linearized at different sites within the gene, were simultaneously introduced into protoplasts of *Nicotiana tabacum* SR1. Depending on the combination of the mutant genes and the linearization site restoration of Km' was found with a frequency of 1.1 up to 29.5% of the transformed calli. Transformation of each of the mutant NPTII genes separately did not give rise to any Km' calli. The presence of an intact NPTII gene formed via homologous recombination was confirmed through molecular and genetic analysis of plants regenerated from the recombinant calli. In addition to these experiments gene targeting experiments were performed. Protoplasts containing a 3' deleted NPTII gene as a chromosomal target, were transformed with a 5' deleted NPTII gene. Experiments are in progress and results will be discussed.

**A 509** ANALYSIS OF MUTATIONS IN GENES WHICH CONTROL THE PHOTOMORPHOGENESIS OF *Arabidopsis thaliana*, **Xing-Wang Deng* and Peter H. Quail, UC-Berkeley/USDA Plant Gene Expression Center, Albany, CA 94710**

Mutations of *Arabidopsis thaliana* showing ***constitutive photomorphogenic*** (***cop***) phenotypes regardless of the presence of light have been isolated. So far, mutants isolated are all recessive and belong to six different loci. The phenotypes observed include opening and enlargement of cotyledons, absence of apical hook, and short hypocotyl. Mutants in one of the six loci, ***cop***1, have seedling morphology when dark-germinated that is indistinguishable from continuous white-light-germinated seedlings, except the cotyledons of the dark-grown mutants are slightly purple instead of green. Mutants in the ***cop***1 locus have been most extensively characterized thus far. All four alleles of ***cop***1 result in plants that are much smaller and have mucg lower fertility when grown in the green house, even after multiple rounds of backcrossing. However, there are appreciable differences among different alleles. Genetic complementation studies show that ***cop***1 is a different gene to the ***det***1 mutant isolated by Chory et al. (Cell, 58, 99 999, 1989). At the cellular level, the cell differentiation, tissue organization and plastid morphology of cotyledons of dark-germinated ***cop***1 mutants are similar to those of light-germinated seedlings. At the molecular level, mRNA levels for genes normally induced or repressed by light are already elevated or repressed in dark-germinated ***cop***1 mutant seedlings. Furthermore, adult plants having ***cop***1 mutation do not display the changes in mRNA levels normally observed for most of light regulated genes upon dark adaptation. Studies with transgenic plants having various promoter-Gus fusion constructs indicate that changes in the mRNA levels for these genes are primarily due to modulation of promoter activities. Analyses of double mutants of ***cop***1 and ***hy***1 (the later being deficient in active phytochrome) show that ***cop***1 is epistatic to ***hy***1. However, as for wild type plants, germination of ***cop***1 mutant seeds is still inhibited by far-red pulse treatments, and the diurnal fluctuation of cab mRNA levels is unaltered.

*X.-W.D is a Du Pont Fellow of The Life Sciences Research Foundation.

**A 510** MOLECULAR CHARACTERIZATION OF A LEAKY GL1 MUTANT OF ARABIDOPSIS, Jeff Esch and M. David Marks, School of Biological Sciences, University of Nebraska, Lincoln, NE 68588-0118.

Several mutations are know to effect trichome formation on Arabidopsis. Mutations at the GL1 locus have been reported to result in a complete loss of trichomes on the leaf surface and stem. However, we have recently received a gl1 mutant (designated gl1-2) from George Redei (University of Missouri) that has a reduced number of trichomes. Molecular characterization of the GL1 gene has revealed that it encodes a DNA binding protein with myb homology. A Gl1 probe was used to screen a gl1-2 genomic library under stringent hybridization conditions. Restriction enzyme analysis of the clones revealed that two distinct clones were isolated. Preliminary DNA sequence data indicates that the two clones are nearly identical to each other as well as to GL1. The relationship of the apparent gene duplication to the gl1-2 mutant phenotype is under investigation.

**A 511** EXPRESSION OF HEAT SHOCK 90 GENES IN PHARBITIS NIL, Roderick Felsheim and Anath Das, Department of Biochemistry and the Plant Molecular Genetics Institute, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108

We have cloned members of the hsp90 gene family from Pharbitis nil (Japanese Morning Glory). One member has been completely sequenced and the derived protein sequence exhibits about 80 % sequence conservation with known hsp90's if conservative substitutions are allowed. Low stringency Southern analysis and cloning experiences suggest 6-7 members of the family in P. nil. and 4-5 members in Arabidopsis thaliana. RNase protection studies show that at least two members are light regulated, with hsp90A message levels peaking two hours after a dark period of at least 6 hours. Currently gus fusions are being used to localize expression of the hsp90 genes in Nicotiana.

**A 512** **TISSUE-SPECIFIC AND HORMONAL REGULATION OF A GENE ENCODING BARLEY (1→3,1→4)-ß-GLUCANASE,** Geoffrey B. Fincher, Tapas K. Ghose and David C. Baulcombe, Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH, U.K.

Two (1→3,1→4)-ß-glucan 4-glucanohydrolase (EC 3.2.1.73) isoenzymes are expressed in the scutellum and aleurone of germinating barley grain, where they are secreted into the starchy endosperm and function to depolymerize the (1→3,1→4)-ß-glucans that constitute up to 70% of the cell walls. Levels of both (1→3,1→4)-ß-glucanase mRNA and secreted enzyme in isolated barley aleurone layers are enhanced by gibberellic acid. The enzymes are the products of two, single copy genes which probably evolved by duplication of a common ancestral gene. A gene for isoenzyme EI has been screened from a barley genomic library and sequenced. The primary structures of the gene and the corresponding protein are typical of plant genes and proteins with respect to the sequence, size and position of motifs for transcription, RNA processing, translation and protein processing. Specific oligonucleotides have been prepared to define the expression sites of the two genes, using Northern analyses and by amplifying specific cDNAs through the polymerase chain reaction. In the tissues examined, transcription of (1→3,1→4)-ß-glucanase isoenzyme EII is restricted to the aleurone of germinated grain. In contrast, the gene for isoenzyme EI is transcribed in a broader range of tissues, including the scutellum and aleurone of germinated grain but also at relatively high levels in young leaves. The promoters of the (1→3,1→4)-ß-glucanase isoenzyme EI gene have been spliced onto the GUS reporter gene for investigating cis-acting elements that may be responsible for the tissue-specific and hormonal regulation of the gene during cell wall metabolism in barley development. The regulation of expression can be compared to other cereal genes that have been introduced into cereal protoplasts or directly into a range of tissues with the microprojectile gun.

**A 513** MULTIPLE DNA-PROTEIN INTERACTIONS UPSTREAM OF PLANT LIGHT- REGULATED GENES, Pari Tavladoraki, Elena Ceccarelli and Giovanni Giuliano, ENEA-Biotechnology, PO Box 2400, Rome 00100 AD, Italy

Plant promoters of the light-regulated ribulose biophosphate carboxylase/oxygenase gene family contain several evolutionarily conserved regions, among which the closely clustered L, I and G boxes (Giuliano et al.. Proc. Natl. Acad. Sci. USA 85. 7089-7093 (1988)). The consensus sequences for the three boxes are ATTAACCA (L box), GATAAG (I box) and (A/C)CACGTGG (G box). The I box shows homology to the GATA elements present in chlorophyll a/b binding protein (CAB) gene promoters (Gidoni et al., Mol. Gen. Genet. 215, 337-344 (1989)).

Using oligonucleotides containing the tomato RbcS-3A L, I and G boxes, we detect three distinct DNA binding activities. One (GBF) shows a high specificity for the G box. The other two ($IBF_1$ and $IBF_2$) show distinct DNA affinity spectra and are able to bind, with varying affinities, the tomato I box, the tomato L box and the tomato GATA element. At least two of the activitico, GBF and $IBF_1$, are evolutionarily conserved between tomato and *Arabidopsis*. Tests for cooperativity of binding between different factors and for light-regulation of factor abundance are under way.

**A 514** MOLECULAR CLONING AND CHARACTERISATION OF DELILA, A GENE REGULATING THE ANTHOCYANIN BIOSYNTHESIS PATHWAY IN ANTIRRHINUM MAJUS, W. Justin Goodrich, Jorge Almeida, Timothy P. Robbins, Rosemary Carpenter and Enrico S. Coen, Department of Genetics, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK.

Antirrhinum majus plants carrying mutations at the delila locus have flowers in which anthocyanin pigment is absent in the tube but present in the lobes. The wild type delila product is necessary in trans for normal expression levels of the pigment biosynthesis genes nivea, pallida and incolorata in the tubes of flowers. A region of the pallida promotor important for regulation by delila has been defined through a series of overlapping deletions. An unstable delila allele del-602 was isolated from a large scale transposon mutagenesis programme, and shown to carry a copy of the transposon Tam 2. Using Tam 2 sequences as a probe, part of the delila locus was isolated and used to obtain full length cDNA clones. The sequence of the delila cDNA shows strong homology to the Lc locus, a member of the R gene family which regulate pigment production in diverse organs of Zea mays plants. Delila and Lc also share homology with the myc gene family in the helix-loop-helix domain, a region important for protein dimerisation and DNA binding. The expression patterns of members of the delila gene family should help understand the development and evolution of pigmentation patterns.

**A 515** DE NOVO SYNTHESIS OF AN INTRON BY THE MAIZE TRANSPOSABLE ELEMENT, DS., L. Curtis Hannah, Lynwood Ingham, Michael Giroux, Donald McCarty, and John Baier, Department of Vegetable Crops, University of Florida, Gainesville, Florida, 32611
Since the discovery of intervening sequences or introns within genes, much effort has been directed towards identifying their origin(s) and their role(s) in the host organism. Recent insight into a possible origin of introns has come from the discovery that transposable elements can sometimes alter RNA processing. Newly-created transcripts are produced by use of a splice junction within the element itself or a site created upon insertion into the gene. Here, we report two novel splice reactions involving the well-characterized maize element, dissociation or Ds. In one transcript, *Ds* located within an exon of the shrunken-2 (Sh2) gene alters splicing of an adjacent, upstream intron such that splicing now involves the use of a 3' acceptor site normally silent in the wild type gene. This results in the inclusion in the mature RNA of sequences usually spliced from the processed transcript. Coupled to its ability to act from a distance to alter splicing, this *Ds* element with its associated duplication of host sequences also can behave like a conventional intron. A 5' donor site arising from one copy of the duplicated host sequence is sometimes spliced to the downstream 3' acceptor site. This removes the *Ds* element plus one copy of the host duplication and results in a transcript of wild type sequence at this junction. This latter situation then represents a direct demonstration of the creation of an intron within a modern eukaryotic gene.

**A 516** **CHARACTERIZATION OF AUXIN-BINDING PROTEINS WHICH EXHIBIT ALTERED ACTIVITY IN THE *diageotropica* MUTANT OF TOMATO.** G.R. Hicks and T.L. Lomax. Department of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331-2902.

We have recently identified auxin-binding proteins (ABPs) of 40 and 42 kDa in plant plasma membranes using photoaffinity labeling with azido-IAA ($^3$H-5N$_3$-IAA). These polypeptides are present in lower quantities or display greatly reduced photoaffinity labeling in hypocotyl membranes of an auxin-insensitive, agravitropic mutant of tomato (*diageotropica*, *dgt*) compared to the isogenic parent line (VFN8). We are currently developing a purification protocol for the azido-labeled proteins. Initial enrichment involves a temperature-induced phase separation in the nonionic detergent Triton X-114. Under these conditions, the 40-42 kDa ABPs partition into the aqueous (hydrophilic) rather than the detergent (hydrophobic) phase. This behavior is similar to that of certain integral proteins that aggregate to form water-filled channels through membranes such as the acetylcholine receptor and ion transporters. By gel filtration under native conditions, azido-labeled protein can be detected as a radioactive peak at ca. 80 kDa suggesting that the 40-42 kDa ABPs aggregate to form a dimer. We are subjecting Triton X-114 extracted material to HPLC which should provide sufficient enrichment for two-dimensional electrophoresis and microsequencing of polypeptides immobilized on PVDF (Immobilon) membranes. Microsequence will be useful for designing molecular probes to study the role of the 40-42 kDa ABPs in gravitopism and for determining their relationship to other ABPs.

**A 517** ABSCISIC ACID AND DROUGHT INDUCED GENES IN BARLEY
Tuan-hua David Ho, Scott Uknes, Bimei Hong and Rivka Barg, Department of Biology, Washington University, St. Louis, MO 63130

As part of our effort to investigate the action of abscisic acid (ABA) we have isolated 18 independent cDNA clones encoding ABA regulated mRNA in barley aleurone layers. In this paper we describe the unique features of two of these clones, pHv A1 and pHv A22. The protein encoded by pHv A1 is 23 kD in size and it contains nine imperfect repeats with a sequence of T-E-A-A-K-Q-K-A-A-E-T, which are also found in some LEA (late embryogenesis abundant) proteins. While this protein is quite abundant in mature aleurone layers, its basal level in seedlings is very low. However, this protein can be induced by ABA or drought stress to accumulate in all the tissues in three-day-old seedlings. In contrast, the Hv A1 gene is only expressed in roots of older plants. The mRNA encoded by pHv A22 is induced by ABA within 30 min of treatment, reaches a peak of abundance after 4 hr and then returns to background levels after 12 hr of continuous ABA treatment. Protein synthesis inhibitors in the presence of ABA can superinduce this mRNA by more than 20 fold, indicating that this mRNA is normally under strong negative regulation conferred by a protein(s). Hv A22 mRNA is normally expressed at a very low level late during seed development in aleurone tissue and during drought stress in roots. Hv A22 encodes a protein of 14.5 kD which contains several unusual structural features such as a phosphorylation site by serine kinase, a leucine-rich region, a highly positively charged region and four properly spaced histidines which may be able to chelate divalent cations. The potential role of these ABA-induced proteins will be discussed.

**A 518** ***Ac/Ds*** **TRANSPOSON TAGGING SYSTEM IN** ***Arabidopsis***

M. Honma, C. Waddell and B. Baker. Plant Gene Expression Center, USDA, Albany, CA, 94710

We are developing a transposon mutagenesis/tag system in *Arabidopsis thaliana* utilizing the maize transposon family *Activator/Dissociation (Ac/Ds)*. *Ac* has been shown to transpose in several heterologous plant systems, including *Arabidopsis*. We have designed a two element system consisting of a stable *trans*-activator ($Ac^{st}$) and a responsive element *(Ds)*. This system creates stable insertions that act as both genetic and physical markers. The *Ds* element will transpose to other sites when *trans*-activated by $Ac^{st}$, but these new *Ds* insertions become fixed when $Ac^{st}$ segregates away from *Ds*. Our system consists of three genetically marked components such that both the presence of $Ac^{st}$ and *Ds* -bearing *Agrobacterium* T-DNAs, as well as the *Ds* excision and reintegration events, can be monitored genetically:

1) ***Ds*** **elements** carrying herbicide resistance genes, either *Arabidopsis ALS* (encoding resistance to chlorsulforon) or *Streptopmyces hygroscopicus bar* (encoding resistance to phosphinothricin, PPT).

2) **Excision marker** bearing the *Ds* element; *Ds* excision results in kanamycin resistance. These Kan:: *Ds* constructs also carry a hygromycin resistance gene.

3) **Stable** $Ac^{st}$ **transactivator** in which the *Ac* promoter has been deleted and replaced with strong heterologous promoters to drive transposase expression. These $Ac^{st}$ *trans*-activator constructs have both selectable (DHFR, encoding resistance to methotrexate) and screenable (GUS) markers.

All constructs have been introduced into *Arabidopsis* utilizing hygromycin, kanamycin, chlorsulforon or methotrexate for selection in tissue culture. Segregation analysis of selfed progeny using these same selective agents, as well as PPT will be presented. We have also introduced the *Ds* elements into transgenic tobacco lines that carry *Ac* and have preliminary evidence that some of the *Ds* elements can excise in the presence of *Ac*.

**A 519** FRUIT-SPECIFIC PROMOTER OF THE TOMATO 2A11 GENE CONTAINS STRONG NEGATIVE AND POSITIVE REGULATORY ELEMENTS,

Catherine M. Houck and Mark J.J. Van Haaren, Calgene, Inc., 1920 Fifth Street, Davis, CA 95616

Fruit-specific expression of ß-glucuronidase (GUS) activity was produced in transgenic tomato plants when the GUS coding region was flanked by 5' and 3' sequences of the fruit-specific tomato gene, 2A11. Deletion studies on the 5'-region revealed a number of strong regulatory elements involved in the proper expression of the 2A11 gene. A 4.0 Kbp and a 1.3 Kbp 5'-region conferred high level, fruit-specific GUS expression, while a 1.8 Kbp 5'-region produced no GUS activity in leaf or fruit tissue indicating that a strong negative regulatory element (silencer) is present in the 5' region.

We divided the 5' region of the 2A11 gene into small fragments, ranging in size from 211 to 634 bp and used these short DNA fragments in *in vitro* protein binding studies. These studies revealed the presence of at least four fruit-specific and one general protein binding domains. Furthermore, each of these promoter fragments as well as overlapping fragments were tested for the ability to enhance ß-glucuronidase expression from a truncated 35S promoter in transgenic plants. This revealed the presence of four fruit-specific and three general or leaf-active enhancer elements. Comparison of the results obtained with these two approaches allowed us to draw a functional map of the 2A11 promoter.

**A 520** ANALYSIS OF T-DNA TAGGED MUTANTS WITH ABNORMAL FLOWER DEVELOPMENT,

Beatrice Howard and Alice Cheung, Department of Biology, Yale University, New Haven, CT, 06511

We have used T-DNA as an insertional mutagen to tag genes in *Arabidopsis thaliana* . Several putatively T-DNA tagged mutants have been isolated. Ongoing work on two of these mutants, BH18 and BH178, will be described. BH178 displays a late flowering phenotype while BH18 displays an abnormal transition to undifferentiated growth during development of the primary inflorescence. Both BH18 and BH 178 have single T-DNA inserts. Current results on gene isolation, genetic mapping of the induced mutations, genetic complementation by the recovered wild type genes as well as physiological and molecular analyses of these mutants will be presented.

**A 521** CYTOKININ AND AUXIN ACTIVATE GENE EXPRESSION THROUGH A PROTEIN KINASE C-LIKE PATHWAY IN *NICOTIANA PLUMBAGINIFOLIA*, Stephen H. Howell, Janice A. Dominov, John J. Schwarz, Laura Stenzler and Scott M. Leisner, Boyce Thompson Institute, Tower Road, Cornell University, Ithaca NY 14853

Cytokinins stimulate developmental events in *Nicotiana plumbaginifolia* cultures which culminate in shoot formation. We are studying how cytokinin (benzyladenine, BA) alters the pattern of gene expression in differentiating cultures, and we have focussed on a BA-induced gene, *cyr 16*. In other plants, the equivalent gene is activated by a variety of stimuli including auxin, heat shock and heavy metals. The addition of BA to *N. plumbaginifolia* cultures rapidly induces *cyr* 16 RNA accumulation within 15 min, and induction levels reach about 20-fold in 24 hrs. In *N. plumbaginifolia,* both cytokinin and auxin stimulate *cyr* 16 expression in a synergistic manner, but each with different kinetics. To determine the signal transduction pathway by which these two plant hormones activate *cyr* 16 gene expression, pharmacological agents were used to mimic or block *cyr* 16 gene expression. It was found that the calcium ionophore A23187 and the phorbal ester, TPA, stimulated *cyr* 16 expression. These two agents acted synergistically to give levels of expression comparable to the growth hormone. The action of BA was found to resemble more closely that of TPA, rather than the ionophore, because BA synergized the action of A23187, but not TPA. In animal systems, these results are characteristic of hormone induced gene expression via a protein kinase C-mediated pathway. However, in plants, phorbal ester-activated protein kinase C activities have not been reported.

**A 522** MOLECULAR EVOLUTION OF PHOSPHOENOLPYRUVATE CARBOXYLASE GENE INVOLVED IN C4 PHOTOSYNTHESIS IN MAIZE, Katsura Izui, Takao Kawamura, Satoru Okumura, Shuichi Yanagisawa, Hiroyuki Toh*, Department of Chemistry, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan and *Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka, 565 Japan.

Comparison was made for the sequence structures among three maize PEPCs. In addition to the cDNA clone for PEPC involved in C4 photosynthesis (C4-PEPC) (Yanagisawa et al. FEBS Lett. 229, 107-110 (1988)), we newly isolated cDNA clones for the so-called C3-type PEPC (C3) and the root-type PEPC(R). (1) The homologies(%) in amino acid and nucleotide sequences were as follows, respectively; C4/C3, 77, 71; C4/R, 80, 71; C3/R, 85, 77. (2) No exon shuffling seems to have occurred during evolution as judged from the amino acid sequence homologies (60-85%) in each exon between C4 and C3. (3) The G+C contents(%) of C4, C3 and R-forms were 62, 51 and 48, and the G/C preferences(%) in the third letter of codons were 84, 53 and 44, respectively, indicating that a remarkable G/C preference is associated with the evolution of the C4-PEPC gene. (4) From RFLP analysis the 3 PEPC genes were shown to be located on the different chromosomes. The C4-PEPC gene seems to have been incorporated in the GC-isochore region. (5) The 9 PEPC sequences hitherto reported were aligned and phylogenic tree was constructed. A simple interpretation of the tree is that the C4 and C3 PEPC had branched far earlier than the branching of dicots and monocots.

**A 523** CHARACTERIZATION OF A NEW CLASS OF AUXIN TRANSPORT INHIBITORS
Jill A. Jacobson, Gloria K. Muday, Sandra Brunn and Philip Haworth, Sandoz Crop Protection, 975 California Avenue, Palo Alto, CA 94304

Auxin (indole acetic acid) is a hormone which affects growth and development of plants. In a growing shoot, auxin is synthesized at the tip and transported in a polar fashion to the base, where it exerts its effects on growth. Several synthetic phytotropins, including naphthylphthalamic acid (NPA), have been described. These inhibit the process of polar auxin transport. We have characterized a new class of phytotropins. These molecules bind with high affinity to the NPA binding protein in plant plasmalemma and competitively inhibit binding of NPA. We have determined the molecular domain of the inhibitor responsible for binding to plasmalemma and have raised antisera to these molecules. In addition, we have obtained an azido derivative which may potentially be used for purification of the NPA binding protein. We would like to purify this protein so that we may develop tools which will aid us in cloning the gene.

**A 524** TRANSCRIPTIONAL CONTROL OF DORMANT EPIPHYLLOUS BUD REACTIVATION IN KALANCHOE MORTAGEI, Yogesh T. Jasrai, Prashant N. Bhatt and Atul R. Mehta,Botany Department, Maharaja Sayajirao University of Baroda, Baroda - 390 002, India.

There are dormant meristemoids located in the notches on crenate margin of Kalanchoe mortagei leaves. The detachment of leaf from plant causes the growth reactivation of these dormant buds with their visible appearance on 4th day. As a part of the project investigating the factors responsible for dormant bud outgrowth, we report here the effect of inhibitors of RNA and protein synthesis. Both actinomycin-D (AC) and cycloheximide (CH) inhibited the outgrowth of dormant buds. Time-course experiments, involving transfer of reactivated buds to inhibitors seperately, revealed that new proteins are transcribed and translated upto first 8 and 18 hours of leaf isolation respectively. This was further substantiated by the finding that (14C)serine incorporation in protein was maximum upto first 12 hours of leaf detachment. Extensive changes in the qualitative analysis of proteins by isoelectric focusing technique were noticed. The results clearly demonstrate that in Kalanchoe mortagei the growth reactivation of dormant epiphyllous buds is proceded by synthesis of new proteins.

**A 525** IDENTIFICATION OF A NEGATIVE REGULATORY SEQUENCE OF A HIGH a-AMYLASE GENE IN RICE, Ju-Kon Kim, Jun Cao and Ray Wu, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY14853.

The α-amylase genes are known to be highly stimulated by the plant hormone, gibberellin (GA), in cereal aleurone cells. We have cloned and sequenced a rice genomic clone, OS*amy-c*, and found that the gene has three introns and four exons which encode a very high pI α-amylase of approximately 45 kDa. During rice germination, the accumulation of the mRNA corresponding to this high pI subfamily was stimulated by 20-fold in the presence of exogenous $GA_3$. Gel retardation assay and foot-printing analysis identified seed-specific protein factors which bind to pyrimidine-rich 5' regulatory region of OS*amy-c*. Expression analysis of the α-amylase gene was carried out with a series of OS*amy-c* 5'-deletion constructs fused to the *Gus* reporter sequence. The constructs were introduced into rice aleurone cells by the microprojectile bombardment method, which led to the identification of two functionally distinct regulatory sequences. Deletion of the sequence located between -231 and -50 relative to the transcription start site resulted in large increase in GUS expression in embryoless seeds imbibed in water whereas GUS expression remains high for all the constructs in embryoless seeds imbibed in 5μM $GA_3$ indicating that the sequence between -231 and -50 contains a negative regulatory element (s). The sequence located between -50 and +25 was sufficient for constitutive expression regardless of the presence of $GA_3$. Thus, the GA-dependent expression of the OS*amy-c* gene appears to act by reversing the inhibitory effect produced by the negative regulatory sequence.

**A 526** A GENETIC APPROACH TO THE MECHANISM OF LIGHT-ACTIVATED GENE EXPRESSION IN *CHLAMYDOMONAS REINHARDTII*, Karen L. Kindle*, Jim Blankenship*, Sevastiani Petridou^ and Kenneth Foster^, *Plant Science Center, Cornell University, Ithaca, NY 14853 and ^Department of Physics, Syracuse University, Syracuse, NY 13244

The expression of many nuclear genes that encode chloroplast proteins is induced by light. We are interested in defining genetically the signal transduction chain between light reception and increased gene expression. We have recently developed technology for high efficiency transformation of the nuclear genome of *Chlamydomonas*. Initially, we are using this technology to define cis-acting sequences which mediate light-activated gene expression of a nuclear gene encoding one of the chlorophyll a/b binding proteins in *Chlamydomonas* (*cabII-1*). As a means of isolating mutations along the signal transduction pathway, we have made fusions of the *cabII-1* promoter to the structural gene for nitrate reductase (*nit-1*) and have introduced this chimeric gene into a *nit-1* mutant. Nit+ transformants have been recovered and are being tested for light dependent nit-1 gene expression. These transformants will be mutagenized; nit- mutants (selected by resistance to chlorate) should include those that are unable to express the chimeric gene in response to light. These mutations may be involved in photoreception, signal transduction, light-induced transcripiton factors, etc.

We have isolated a cDNA whose expression is induced rapidly by light and may be under the control of phytochrome. Low intensity action spectra have shown that the gene is specifically induced by red light, at intensities similar to those required for the low fluence phytochrome response in higher plants. Experiments to test for far-red reversibility are in progress and preliminary results are encouraging. If these preliminary results hold up, this will be the first physiological evidence for phytochrome in *Chlamydomonas*, and it will allow a genetic approach to signal transduction via this important higher plant photoreceptor.

**A 527** TRANSPOSON MUTAGENESIS IN MAIZE USING THE MOBILE ELEMENT ACTIVATOR (AC), Roger W. Krueger, Thomas Brutnell*, Dick Johnson, Catherine Mackey, Stephen L. Dellaporta*, DeKalb Plant Genetics, Groton, CT 06340 and *Department of Biology, Yale University, New Haven, CT 06511

An efficient transposon tagging strategy involving the mobile element Activator Ac was applied to a large population of maize. F1 plants were screened for dominant mutations, and F2 segregating populations were screened for recessive mutations. A diversity of phenotypic mutants was obtained including defective embryo (dek), dwarf, leaf disease mimic, leaf and kernel pigmentation, and male sterility. The technique yields important genetic alleles useful in both developmental and clonal analysis. The genes tagged by an Ac insertion can be isolated and studied by efficient cloning strategies and inverse polymerase chain reaction. Preliminary analysis of selected genes will be presented in this report.

**A 528** **ANALYSIS OF PROTEINS WHICH INTERACT WITH GIBBERELLIN REGULATED α-AMYLASE PROMOTERS IN BARLEY**, Michael B. Lanahan, T-H. David Ho, Biol Dept. Washington University., St. Louis, MO 63130

Gibberellic acid ($GA_3$) transcriptionally activates α-amylase genes in barley aleurone cells. Ascisic acid (ABA) suppresses this expression. We have assayed nuclear extracts from aleurone cells treated with or wiothout GA3 and ABA for proteins which bind to sequences in α-amylase promoters. Our results indicate numerous specific protein/DNA interactions. There does not appear to be major differences in the abundance of these factors from $GA_3$, ABA or untreated samples. There are however reproducible differences in the mobilities of the complexes on non-denaturing gels. To further define sequences responsible for protein/DNA interactions, complexes were footprinted with DNAse I. Oligos corresponding to these sequences are being used to further define the protein/DNA interactions. Based on our results we believe that GA-regulated transcription of barley α-amylase may be more complicated than in rice. In rice a single GA-dependent factor, which recognizes a single site on a rice promoter, has been described. We have been unable to identify an analogous factor.

**A 529** STRUCTURAL EVIDENCE FOR A POSSIBLE TRANSPOSABLE ELEMENT IN *G. MAX*, Howard M. Laten[1] and Roy. O. Morris[2], [1]Department of Biology, Loyola University of Chicago, Chicago, IL 60626 and [2]Department of Biochemistry, University of Missouri at Columbia, Columbia, MO 65211

Using the polymerase chain reaction, we have amplified a collection of related nuclear DNA sequences in *G. max* characterized by inverted repeats. The elements are not present in *A. thaliani* or *S. oleracea*. The total length of the amplified DNA is approximately 790 bp. The sequence of the repeat bears a moderate resemblance to the terminal inverted repeats found in Ds from *Z. mays*, TAM3 from *A. majus*, TPC1 from *P. crispum*, and TST1 from *S. tuberosum*, but not TGM1 from *G. max*. Restriction enzyme analysis suggests limited variabilty within these elements. Approximately half the elements are characterized by a single EcoRI site, half possess an MboI site, half have a HpaII site, and half have an MboI site. All of the elements appear to contain a conserved TaqI site. Members of the collection have been cloned into pUC18. The sequence of the element, its distribution in *G. max*, and its possible relationship to plant transposons will be presented.

**A 530** DETECTION AND cDNA CLONING OF TRANSCRIPTS FROM TOMATO FRUIT HAVING PARTIAL HOMOLOGY TO THE 22 kD AUXIN BINDING PROTEIN OF MAIZE, T.L. Lomax, C.J. Gaiser, G.R. Hicks, and D.L. Rayle. Department of Botany and Plant Pathology, Oregon State University, Corvallis OR, 97331-2902.

Using an azido auxin as a photoaffinity label, we have recently identified auxin binding proteins of 40 and 42 kDa ($ABP_{40-42}$) in the plasma membranes of divergent groups of plants. These polypeptides are present in lower amounts or bind azido auxin poorly in hypocotyl membranes of an auxin-insensitive, agravitropic mutant of tomato (*diageotropica*, *dgt*) as compared to the isogenic parent line (VFN8). This indicates that the mutation is in a developmentally-regulated gene which is involved in both auxin perception and the gravitropic response of plants. Circumstantial evidence indicates that the $ABP_{40-42}$ share some homology with a 22 kDa auxin binding protein ($ABP_{22}$) found in the ER of maize. Northern blots of mRNA from VFN8 and *dgt* were incubated with a 35-mer oligonucleotide probe corresponding to the published sequence of $ABP_{22}$ (Tillman et al., EMBO J. 8: 2463-2467).The probe hybridized strongly to a single maize mRNA of approx. 900 bases, corresponding to the known size of $ABP_{22}$ and also to a mRNA of approx. 2000 bases in both *dgt* and VFN8 mRNA. We speculate that this larger mRNA species may encode $ABP_{40-42}$ in dicots. The hybridization data suggest that the *dgt* mutation does not result in the alteration of $ABP_{40-42}$ gene expression at the transcriptional level. We have used the oligonucleotide probe to isolate several clones from a tomato fruit cDNA library. These clones will be useful for examining the relationship of the $ABP_{40-42}$ to $ABP_{20-22}$ and for elucidating the nature of the *dgt* lesion.

**A 531 CHARACTERIZATION OF CIS ACTING SEQUENCES INVOLVED IN THE TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF EXPRESSION OF THE Em GENE OF WHEAT,** William R. Marcotte, Jr. and R. S. Quatrano, Department of Biology, University of North Carolina, CB# 3280, Coker Hall, Chapel Hill, NC 27599-3280.

The ultimate level of gene expression associated with a given physiological response is dependent upon transcriptional, post-transcriptional, translational and other processes. We have described the preliminary identification of ABA-responsive sequences of the Em gene from wheat using a rice protoplast transient assay (Marcotte, et al. (1988), **Nature** 335:454-457; Marcotte, et al. (1989), **The Plant Cell** 1:969-976). These sequences were shown to reside in transcribed and non-transcribed regions, suggesting the involvement of transcriptional and post-transcriptional components. A detailed analysis of the sequences involved in the transcriptional component of the regulation has shown that a 76 bp sequence is necessary and sufficient for greater than 10-fold induction of expression in the presence of the phytohormone ABA. In addition, mutation of 2bp in a sequence motif conserved among other ABA-regulated genes, as well as genes responding to other stimuli, reduces or eliminates the ability of the 76 bp sequence to mediate ABA induction (Guiltinan, et al. (1990), **Science** in press). Further studies have reduced the sequences necessary and sufficient for ABA-mediated induction and will be presented. Preliminary analyses of the post-transcriptional component of Em regulation has identified sequences in the 5' untranslated region and the Em 3' region as being involved. Experiments are currently underway to delineate the role(s) of these sequences in the expression of chimeric genes. Their expression will be compared to chimeric genes containing comparable regions of another ABA-regulated gene which is not post-transcriptionally regulated. The results from these experiments will be presented. Supported by grants from the U.S. Department of Agriculture Competitive Grants Program (90-37262-5281 to W.R.M. and 89-37262-4456 to R.S.Q.).

**A 532 INSERT INDEPENDENT MUTATIONS INDUCED IN TRANSFORMED PLANT CELLS DURING *AGROBACTERIUM* COCULTIVATION.**

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Nitrate reductase(NR)-deficient mutants were selected from haploid and diploid heterozygotic (nia/wt or cnxA/wt) protoplasts and leaf disk cultures of *Nicotiana plumbaginifolia* after *Agrobacterium* cocultivation (pGA472).The chlorate resistant isolates were tested for the presence of the "T"-DNA (kanamycin resistance) only after the NR deficiency had been established.

A total of 57 independent NR deficient mutants from the kanamycin resistant group were further analysed by Southern blot hybridization Contrary to the fact that the NR deficiency preferentially occured in the transformed cell population,there was no indication of integrated "T"-DNA sequences in the mutated NR genes.The result suggests the existence of another type of mutagenesis related to the transformation process in the competent cells.The nature of these mutations is under investigation.

**A 533** LIGHT-REGULATED EXPRESSION OF Al-3, A CAROTENOID BIOSYNTHETIC GENE OF *NEUROSPORA CRASSA*. Giorgio Morelli*,Simona Baima*, Alessandra Carattoli• and Giuseppe Macino•.*Unità Nutrizione Sperimentale, Istituto Nazionale della Nutrizione, via Ardeatina 546 00178 Roma Italy, •Dipartimento di Biopatologia Umana, sezione Biologia Cellulare, Università La Sapienza 00185 Roma Italy.
We are studying the photoregulated expression of carotenoid biosynthesis in *N.crassa*. The al-3 gene, which has been previously cloned in our lab, codes for the geranyl-geranyl pyrophosphate synthetase, an enzyme which has been shown to be inducible by blue-light. For this reason we analyzed the expression of al-3 gene in different light regimes. We found that the al-3 gene is a very fast regulated gene; the level of its mRNA is already increased after 5' of illumination and reaches a maximum after about 15'. The al-3 photoinduced expression is transient and a dark incubation period is necessary to restore light sensitivity. In fact, the first induction is followed by a refractory period and maximum competence for a second light induction is restored only after about 2 h. The rate of decay of al-3 mRNA to the dark level is slower in mycelia maintained in continuous light than in mycelia exposed to a single pulse of light. We are now studying the effect of continuous light on the expression of the al-3 gene.

**A 534** INSERTIONAL MUTAGENESIS OF THE *P* LOCUS OF MAIZE USING *Ac*. Maria A. Moreno-Gomez, Jychian Chen, Irwin Greenblatt, and Stephen L. Dellaporta. Department of Biology, Yale University, P. O. Box 6666, New Haven, CT 06511.
The *P* locus of maize, located on the short arm of chromosome one, is a complex locus that appears to be involved in the regulation of flavonoid biosynthesis. To understand the genetic organization of this locus, a genetic and molecular analysis has been undertaken using a transposon-mediated insertional mutagenesis approach. The transposable element *Activator* (*Ac*) was used as the transpososn mutagen to generate over 200 mutations of *P-RR* gene. Each allele conditions variegation in the cob and pericarp tissue. These alleles have been characterized by genetic analysis, Southern hybridizations, and PCR sequencing to localize *Ac* within the *P* cistron. Our results show that *Ac* insertions are dispersed over a 20 kb chromosomal region with tight clustering in three regions. The variegation pattern conditioned by each allele appears to be a reflection of position rather than *Ac* dosage. Based on striping patterns several critical regions for *P* function have been identified. Also, the pattern of short range transpositions of *Ac* within this 20 kb region has been determined.

**A 535** DETERMINATION OF THE ROLE OF THE NPA BINDING PROTEIN IN THE POLAR TRANSPORT OF IAA, Gloria Kressin Muday, Jill A. Jacobson, Sanda A. Brunn, and Phillip Haworth, Sandoz Crop Protection, 975 California Ave, Palo Alto, CA 94304

Indole acetic acid (IAA) is transported from the tip of stems towards the base in a polar fashion. This polar transport can be inhibited by phytotropins such as naphthylphthalamic acid (NPA). NPA binds specifically and with high affinity to a single protein in plasmalemma. To determine the role of the NPA binding protein in the process of auxin transport, we are employing a variety of approaches. The kinetics of NPA binding have been carefully analyzed and we are currently determining the interactions between NPA and IAA as they bind to plasmalemma. The ability of other compounds to interact with the NPA binding protein is being explored to determine the natural ligand that interacts with this protein *in vivo*. The physiological effects of auxin transport inhibition by NPA and other inhibitors and reversal of this inhibition by auxins are being studied by examination of arabidopsis and tomato root growth in the presence of these compounds. Lastly, the uptake of IAA by right-side out vesicles is being used to estimate IAA uptake into plant cells under various conditions. NPA binding activity is being used to etimated IAA efflux under the same conditions to determine the relationship between IAA influx and efflux during plant growth and development.

**A 536** SPATIAL EXPRESSION PATTERNS OF AN AUXIN-INDUCIBLE PROMOTER
R.Nagao, M. Ainley, R.Wyatt and J. Key, Botany Dept., U. of Ga., Athens, GA. 30602
Two genes from Arabidopsis thaliana corresponding to the auxin-inducible genes Aux28 and Aux22 of soybean have been isolated (T. Conner **et al.** PMB in press). The regulatory properties of the promoter region of one of these genes, At-Aux 2-11, was analyzed in transgenic Arabidopsis plants. In an initial investigation of potential regions effecting spatial expression, deletions of the 5' flanking region were fused to the $\beta$-galactosidase reporter gene (lacZ) and assayed for control of tissue specific expression. A 400 bp fragment from the 5' region conferred an expression pattern similar to the longest 5' promoter region tested, a 3.0 Kb fragment. Histochemical localization of $\beta$-galactosidase activity in the transgenic plants revealed spatial and temporal expression patterns highly correlated with tissues and processes associated with auxin action. Expression of the Aux 2-11/lacZ construct was detected in the elongation zones of both roots and shoots. As these systems matured expression in these regions became restricted to developing metaxylem elements. The promoter was also very active in the root cap and expression increased in the stele of the root where branch root initials occured. In the hypocotyl of light-grown seedlings expression was limited to the elongation zone, whereas etiolated seedlings exhibited expression along the entire hypocotyl, within most cell types. The cotyledons exhibited expression primarily within the epidermis, especially in epidermal cells having trichomes and also in developing vessel elements.

**A 537** ORGAN-SPECIFIC EXPRESSION OF ACC SYNTHASE GENES IN POLLINATED ORCHID FLOWERS, Sharman D. O'Neill[1] and Abraham H. Halevy[2], [1]Department of Botany, University of California, Davis, CA 95616 and [2]The Hebrew University of Jerusalem, Rehovot, Israel

Flower senescence represents an example of plant organ senescence that is often highly regulated by pollination. The regulation by pollination involves the production of signals moving from the site of pollination (stigma) that lead to the induction of ethylene biosynthesis in the gynoecium and later to enhanced ethylene sensitivity and ethylene evolution in the perianth. Differential screening of *Phalaenopsis* orchid flower gynoecium and perianth cDNA libraries has resulted in the isolation of a number of cDNA clones, including one or more putative ACC synthase cDNAs. Targeted screening has led to the identification of a number of other ACC synthase cDNAs, and at least one cDNA for cx-cellulase. These results will be presented along with an analysis of the spatial and temporal pattern of expression of the ACC synthase genes during the time course of pollination-induced flower senescence and in relation to ethylene production and changing ethylene sensitivity.
This research was supported by a grant from the American Orchid Society.

**A 538** SITE DIRECTED MUTAGENESIS OF THE GENOME OF HIGHER PLANT CELLS VIA AGROBACTERIUM, Remko Offringa, Marcel J.A. de Groot, Mirjam P. Does, Henne H. Haagsman, Peter J.M. van den Elzen and Paul J.J. Hooykaas, MOGEN International nv, Einsteinweg 97, 2333 CB Leiden and Leiden University, Clusius Laboratories, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.
We determined whether *Agrobacterium* T-DNA is a suitable substrate for homologous recombination in the plant cell. A 5' and a 3' deleted version of a NPTII construct were used for the detection of such recombination events in protoplasts of *Nicotiana tabacum* SR1. Our results show that homologous recombination occurs at high frequencies (1 - 4%) between T-DNAs that have been co-introduced from separate bacteria into tobacco protoplasts. More importantly, we demonstrate that a target locus in the plant genome is altered after homologous recombination with a T-DNA that is introduced into the plant cell via *Agrobacterium tumefaciens* (EMBO J., in press). This gene targeting occurred at a low frequency ($10^{-5}$) and we are now optimizing our system to enrich for recombinants in a large pool of transformed cells. One approach will be to use the T-DNA aux-2 gene as a negative selectable marker.
Our finding opens the possibility to use the *Agrobacterium* transformation system for site directed mutagenesis (SDM) of the plant genome. The SDM-technique will have its implications for the use of reverse genetics in basic plant research as well as for the genetic engineering technology of crop plants.

**A 539** LIGHT-DEPENDENT EXPRESSION OF THE GLYCINE DECARBOXYLASE MULTIENZYME COMPLEX IN PEA LEAF MITOCHONDRIA, David J. Oliver, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843, USA.

Relatively few good developmental systems have been described for plant mitochondria. One of the most striking is the expression of the four component enzymes of the photorespiratory glycine decarboxylase multienzyme complex, called P, H, T, and L-proteins. Together with the enzyme serine hydroxymethyl transferase, these proteins catalyze the conversion of two moles of glycine to one each of serine, $NH_3$, and $CO_2$. The amount of the enzyme increases about 10 fold during the transition from etiolated to green leaves where it reaches a final concentration in the mitochondrial matrix of over 130 mg/ml. This is enough protein to increase the density of the organelles. The cDNAs have been cloned for the H and P-proteins. Run on transcription experiments show that both genes are under transcriptional control. The increase in the abundance of the mRNA for both genes closely parallels the kinetics for expression of the mRNA for the Rubisco small subunit and the chlorophyll A/B binding protein. This includes a common four hour time delay despite the fact that the only observed change in the mitochondria through the greening process is the accumulation of the photorespiratory proteins suggesting that expression is controlled by common mechanisms. Both P and H-protein are synthesized with N-terminal extensions that are removed following uptake into the mitochondria. Although this process can occur in vitro, the rates are slow.

**A 540** MECHANISMS OF TRANSPOSON DUPLICATION IN TRANSGENIC PLANTS, Peter W. Peterson, Yusen Tong, John I. Yoder, Department of Vegetable Crops, University of California, Davis, CA 95616

We have observed that amplification of the maize transposable element Ac can occur when introduced into the tomato genome. Amplification was observed in the R1 progeny of a primary R0 transformant which contained a single Ac element. The R2 progeny contained between one and sixteen copies of Ac; evidence that the duplicated copies were not segregating as a single genetic locus. These and other previous results indicated that amplification resulted from the transposition process. To further analyze amplification events, we are determining the genetic linkage of the amplified copies by examining the segregation of Ac elements in outcrosses. In the course of these studies, we observed amplification in the selfed progeny of a R2 plant which originally contained a single copy of Ac. This indicates that amplification is probably not a rare event and can occur at different generations after introduction into the genome. The results of these and other studies under way may allow us to investigate whether amplification occurs by replicative transposition or by a repair mechanism following conservative transposition as suggested for *Drosophila* P elements.

**A 541** MOLECULAR ANALYSIS OF DESICCATION TOLERANCE: ABA-AND DESICCATION INDUCED GENES IN THE RESURRECTION PLANT ***CRATEROSTIGMA PLANTAGINEUM***, Detlef Piatkowski, Katharina Schneider, Francesco Salamini and Dorothea Bartels, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln Carl-von-Linne Weg 10, Germany

Leaves of the resurrection plant ***Craterostigma plantagineum*** (Hochst.) can be desiccated up to 1 % relative water content and are still viable after rehydration. Several new proteins appear during the desiccation, they are also inducable in callus tissue by ABA-treatment. For six of these proteins we have isolated and characterized the corresponding cDNA-and genomic clones. Three clones show homologies to desiccation-related genes expressed in a number of other plants, whereas the other three appear to be specific for *Craterostigma*. Analysis of the regulatory regions of these genes will be presented. Promotor regions are investigated by deletion analysis and fusions to GUS-and Lux-reporter genes have been made.

**A 542 FURTHER GENETIC AND PHYSIOLOGICAL ANALYSES OF THE *aux1* MUTATION OF *Arabidopsis*** F. Bryan Pickett, Max O. Ruegger and Mark Estelle, Dept. of Biology, Indiana University, Bloomington IN 47405.
The analysis of hormone response in higher plants will benefit from a combined approach utilizing methodologies of physiology, genetics and molecular biology. These approaches are currently being applied to the study of hormone resistant mutations in *Arabidopsis thaliana*. Recessive mutations in the *AUX1* locus result in agravitropic root growth and a high level of resistance to the inhibition of root growth by auxin and ethylene. Other ethylene responses are normal in *aux1* plants. The elongation of the hypocotyl of etiolated *aux1* plants is inhibited by ethylene treatment. In addition, the leaves of mature plants lose chlorophyll and accumulate peroxidase activity after ethylene treatment. The root specific resistance to ethylene and auxin and the root agravitropism of the *aux1* plant lead to the conclusion that the effects of the *aux1* mutation are limited to the root. Recently, root growth experiments in which *aux1* plants were exposed to various levels of one hormone while being maintained at one concentration of the other hormone have demonstrated a specific enhancement of ethylene sensitivity by auxin. Ethylene does not have a reciprocal effect on auxin sensitivity. These observations suggest that multiple hormone resistance in mutant plants could be due to cross regulation between different hormone response pathways. The *AUX1* locus has been mapped to the second chromosome and ordered with respect to the markers *er* (erecta) and *py* (thiamine auxotrophy) using cis mapping techniques. We are currently using flanking visible markers to perform fine mapping of RFLPs near *AUX1*. The two closest flanking RFLPs will be used to initiate a chromosomal walk to *AUX1*.

**A 543** EXPRESSION OF TWO GENES MARKERS OF CELL DIVISION IN MAIZE EARLY EMBRYOGENESIS, P. Puigdomènech, L. Ruiz-Avila, R. Raz, L. Montoliu, M. José, M.D. Ludevid, J. Rigau and J.A. Martínez-Izquierdo. Dep. Genética Molecular. CID-CSIC. Jordi Girona, 18. 08034 Barcelona. Spain.
Two genes highly expressed in meristematic tissues of *Zea mays* have been characterized. One codes for a hydroxyproline-rich glycoprotein (HRGP) located in the cell wall, the other one codes for alpha-tubulin. The expression of these two genes in maize embryos has been studied. The HRGP gene is expressed in tissues rich in dividing cells and it is induced by wounding. This HRGP appears to be coded by a small number of genes (one or two). The sequence of genomic clones from different varieties of maize and related species has been obtained. The expression of this gene during embryogenesis has been studied. In maize embryos both HRGP mRNA and protein are located mainly in the axes while no evidence is found of the protein or mRNA in scutellum therefore raising the question of what type of protein is present in the corresponding cell wall.
Alpha-tubulins are coded in maize by at least two families of genes formed by two or three members each. One of these groups is a tandem repeat of two genes (gene I and II) preferentially expressed in the radicular organs of the plant. A member of another group (gene III) codes for a protein highly homologous to the two other genes but it shows a highest accumulation in immature embryos in a way that correlates with cell division.

**A 544** PATHWAYS OF ABA ACTION DURING MAIZE EMBRYOGENESIS: RESPONSES OF *VIVIPAROUS* MUTANTS TO ABA AND LIMITATION OF WATER UPTAKE
Carol J. Rivin, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330

Abscisic acid (ABA) is a global effector of plant embryo maturation. Different pathways of ABA action during maize embryogenesis were discerned by comparing the effects of culturing wildtype and *viviparous* mutant embryos on high levels of ABA or with high osmoticum. A large and diverse group of polypeptides characteristic of maturing wildtype embryos is absent in mutants that are deficient in embryo ABA synthesis (*vp5* mutant) or embryo ABA response (*vp1* mutant). This protein set accumulates in immature wildtype and *vp5* embryos treated with exogenous ABA, but not in *vp1* embryos. When wildtype immature embryos are cultured with high osmoticum, they express most of the ABA-regulated proteins, while a subset of proteins respond only to ABA specifically. Mutant *vp1* and *vp5* embryos cultured with high osmoticum also synthesize many ABA-set proteins, but they do not make all of those synthesized by similarly treated wildtype embryos, indicating that the missing proteins are modulated by *de novo* embryo ABA synthesis in response to limiting water uptake. We are investigating the distinction between these proteins and those that are induced only by exogenous ABA. For the group of maturation proteins that are appropriately accumulated in *vp1* and *vp5* mutant embryos cultured with high osmoticum, ABA synthesis and response cannot be direct requirements. We suggest that these proteins are regulated by a pathway for which limiting embryo water uptake (imposed environmentally or by ABA) is the more direct developmental cue.

**A 545** MOLECULAR ANALYSIS AND REGULATION OF GENES CODING FOR AUXIN-BINDING PROTEINS

Etienne Schwob, Soon-Yong Choi, Larry L. Ilag and Dieter Söll
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Gravity is among the most important determinants of plant growth. Auxin is the only phytohormone directly involved (together with calcium) in the initiation of the gravitropic growth response. There is ample evidence which implies specific receptors for this hormone. Such putative receptors have recently been purified (1), and some of these auxin-binding proteins (ABP) show tissue-specific and developmentally regulated expression.

In collaboration with Dr. Palme's group in Cologne (FRG) we have cloned from *Zea mays* the axr1 gene encoding the major auxin-binding protein from corn coleoptile membranes. The DNA sequence (7 kb) of the gene containing four introns and up to 2 kb of the promoter has been determined. Deletions and fusions of this promoter to the GUS reporter gene have been made in order to characterize its functional domains. The regulation of the expression of the axr1 gene was studied in a transient expression assay after transformation of the *Black Mexican Sweet* corn cell suspension culture by electroporation. The tissue-specific expression of these constructs is tested in transgenic plants, by Ti-mediated transformation of *Arabidopsis* roots.

Comparative experiments are currently being done with genes coding for other auxin-binding proteins from corn (axr4) and *Arabidopsis* (axr1, p60). The regulation of these different genes and the specific advantages of each system (e.g. purification of trans-acting factors from corn; gravitropic mutants from *Arabidopsis*) should allow us to begin to understand the molecular mechanism of auxin perception and action.

1. Hesse,T., Balshüsemann,D., Bauw,G., Vandekerckove,J., Puype,M., Löbler,M., Klämbt,D., Schell,J. and Palme, K. (1989). EMBO J. 8: 2453-2461.

**A 546** **Effects of *Ac* transposase gene fusions on somatic and germinal excision of *Ds*::SPT in tobacco,** **Steve Scofield, Kate Harrison, Steve Nurrish and Jonathan Jones, Sainsbury Laboratory, John Innes Centre for Plant Science, Norwich NR4 7UH, ENGLAND.**

We have constructed fusions between *Ac* transposase and the CaMV 35S promoter, the octopine synthase (*ocs*) promoter and the nopaline synthase (*nos*) promoter. The activities of these fusions were assayed by crossing plants containing the fusions to a line containing a *Ds* element inserted in streptomycin phosphotransferase, (*Ds*::SPT), which provides a visual marker for somatic and germinal excisions of *Ds*. These studies have relevance for optimizing gene tagging strategies, understanding the regulation of transposon activity, and monitoring the onset of promoter activity during development.

Our measurements indicate that the *nos* and *ocs* fusions give rise to very low rates of germinal excision. The CaMV 35S fusion was found to give germinal excision rates comparable or slightly higher than *Ac* control constructs, though significant pod to pod variation was observed. Southern blot analysis of germinal excision progeny resulting from the CaMV 35S fusion shows that *Ds* excision is associated with reinsertion, and that siblings sometimes carry the same transposition event.

The size and shape of somatic revertant sectors of *Ds*::SPT are a direct consequence of the timing of the excision event. The patterns of variegation resulting from the fusions indicates that each promoter triggers *Ds* excisions at different times in embryo development. The 35S promoter turns on before *ocs*, and *nos* becomes active last. These observations lead us to believe that fusions to transposase may be very useful for analyzing temporal and spatial aspects of promoter activity during development.

**A 547** A FAR UPSTREAM ELEMENT WITHIN THE ARABIDOPSIS THALIANA PLASTOCYANIN PROMOTER IS DEPENDENT ON FUCNTIONAL CHLOROPLASTS, Sjef Smeekens, Oscar Vorst, Alice Lever and Peter Weisbeek, Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

We have cloned the plastocyanin gene of Arabidopsis thaliana and have shown that the expression of this gene is light controlled at the steady state mRNA level. Analysis of transgenic tobacco plants harbouring the plastocyanin promoter fused to the ß-glucuronidase reporter gene showed that this light dependence is regulated at the transcriptional level. Within the plastocyanin promoter an upstream region has been identified which functions as a strong transcriptional enhancer. Deletion of this region reduces expression 350-fold. Interestingly, the enhancing capacity of this region is fully dependent on the presence of functional chloroplasts. Treatment of transgenic seedlings with a variety of chemicals which suppress chloroplast development results in loss of enhancing capacity of this promoter element. These treatments did not have an effect on the activity of the Arabidopsis thaliana ferredoxin promoter in transgenic tobacco seedlings.

**A 548** PLANT RNA DIRECTS ETHYLENE BIOSYNTHESIS IN *XENOPUS* OOCYTES, Pietro Spanu, Dieter Reinhardt and Thomas Boller, Botanisches Institut, University of Basel, Hebelstr. 1, CH-4056 Basel, Switzerland.

Ethylene biosynthesis is induced in tomato cell suspension cultures treated with elicitor. This is due to an increase in the activities of both ACC synthase and of the ethylene forming enzyme. RNA was extracted from these cells and injected into *Xenopus* oocytes. The oocytes did not form any ethylene when incubated without treatment or treated with RNA from uninduced tomato cells. However they started to produce ethylene upon treatment with RNA from elicitor-stimulated tomato cells; the ethylene forming enzyme expressed in oocytes had similar biochemical properties (apparent $K_m$, ability to distinguish stereoisomeric analogues of ACC, sensitivity to inhibitors) to that of tomato cell cultures. We conclude that mRNA for both ACC synthase and ethylene forming enzyme are translated in oocytes to form catalytically active enzymes; thus the complete pathway for ethylene biosynthesis is expressed. This finding is currently being used to investigate molecular mechanisms controlling ethylene biosynthesis and to characterize the ethylene forming enzyme.

**A 549** ABA REGULATION OF TWO DISTINCT PLANT EMBRYO GENES, Terry L. Thomas, Molly A. Bogue and Jeevalatha Vivekananda, Department of Biology, Texas A&M University, College Station, TX 77843-3258

We are interested in the *cis*- and *trans*-acting factors controlling the expression of two distinct plant embryo genes: a carrot gene called *Dc*3 and a sunflower gene encoding the seed protein helianthinin. *Dc*3 is a *lea*-class gene normally expressed during carrot embryogenesis; *Dc*3 is also expressed in embryonic and in non-embryonic tissues in response to exogenous abscisic acid (ABA). Conversely, the sunflower helianthinin genes, as other seed protein genes, are expressed exclusively in the developing seed. Discrete *cis* regulatory elements contained within the upstream regulatory ensembles (UREs) of the carrot *Dc*3 gene and the sunflower helianthinin genes were identified by analysis of transcriptional fusions with the $\beta$-glucuronidase (GUS) reporter gene in transgenic tobacco. We found that the *Dc*3 URE includes promoter proximal elements that respond to developmental cues and are insensitive to ABA; more distal elements are responsive to ABA and are apparently insensitive to embryo-specific signals. A 2.4 kb upstream region of a helianthinin gene confers rigorous developmental GUS expression in transgenic tobacco seeds with no detectable GUS activity in non-embryonic tissues. Discrete elements of the helianthinin URE confer novel regulatory patterns when analyzed outside the context of the complete sunflower regulatory complex; these include ABA-responsive elements. In the full-length helianthinin URE, these elements only respond to ABA in the developing seed suggesting that the helianthinin gene contains additional regulatory elements that ensure hierarchical control in the developing seed.

**A 550** CHARACTERIZATION OF NUCLEAR GENES ENCODING CHLOROPHYLL BIOSYNTHETIC ENZYMES FROM ANGIOSPERMS AND GYMNOSPERMS , Michael P. Timko, Anthony J. Spano, Katherine F. Boese, and Zhenghui He, Department of Biology, University of Virginia, Charlottesville, VA 22901

Chlorophylls and their derivatives play a fundamental role in the energy absorbing and transducing activities of all photosynthetic organisms. Light is known to regulate chlorophyll synthesis at two points in the biosynthetic pathway: first, at the the formation of ALA, the first committed precursor to all plant tetrapyrroles and second, at the level of protochlorophyllide reduction. This latter activity is mediated by the enzyme NADPH-protochlorophyllide oxidoreductase (Pchlide reductase). In angiosperm species, Pchlide reductase is a light-dependent enzyme requiring reduced pyridine nucleotides and stoichiometric amounts of light quanta for activity. In contrast, gymnosperms are able to synthesize chlorophyll in the dark as well as in the light by virtue of a light-independent Pchlide reductase activity. We have isolated and characterized cDNAs and nuclear genes from white pine (Pinus strobus) and pea (Pisum sativum) encoding the Pchlide reductase. The structure of these genes and the expression characteristics of their encoded products during light-induced development will be presented. In addition, our progress toward the characterization of gene families encoding earlier biosynthetic steps of the pathway will be discussed.

**A 551** EFFECTS OF THE INTRODUCTION OF AGROBACTERIUM RHIZOGENES ROL GENES ON PLANT AND FLOWER DEVELOPMENT IN TOMATO PLANTS. Van Altvorst, A.C., Bino, R.J., Lindhout, W.H. and J.J.M. Dons, Department of Developmental Biology, Centre for Plant Breeding Research, P.O.B. 16, 6700 AA Wageningen, The Netherlands

The individual *rol* genes of *Agrobacterium rhizogenes* Ri-plasmid were introduced into *Lycopersicon esculentum* (tomato) to study their effect on the growth and development of the plant. Punctured cotyledon transformations were carried out to obtain tomato plants transgenic for the T-DNA genes *rol A, rol B and rol C*, which are involved in the hairy root formation. Regenerated plants showed the same characteristic morphological phenotype, specific for the different *rol* genes. Transgenic *rol* A plants manifested a severe wrinkling of leaves, a reduced flower bud length, hypostyly and a decreased pollen viability. *Rol B* plants were characterized by a reduction of the internode length and a reduction of the apical dominance. The strong phenotypic expressions of *rol* A and *rol* B genes were not observed in *rol ABC* plants, suggesting an antagonistic effect of the different *rol* genes. Also *rol* B and *rol* ABC plants displayed a reduction in flower bud size and a loss of male fertility. The transformed plants showed a higher auxin sensitivity. Southern blot analysis proved the integration of the genes.

**A 552** A VISUAL ASSAY FOR DETERMINING FORWARD INSERTION RATES OF HETEROLOGOUS TRANSPOSABLE ELEMENTS IN TRANSGENIC TOMATOES, John I. Yoder, Department of Vegetable Crops, University of California, Davis, CA. 95616

We are interested in developing a transposon mutagenesis system for tomato for gene isolation, gene replacement, and gene disruption studies. We have determined that the maize transposable element Ac is active during the somatic development of tomato by hybridization criteria which measure both excision and insertion events. Because only a small fraction of transposition events are sexually transmitted, the examination of transposition in somatic tissue is more powerful than relying on events which are genetically inherited. We are using an endogenous tomato gene whose phenotye can be readily visualized in intact stems and leaves to determine the frequency with which a targeted gene is inactivated by a heterologous transposable element. Because gene inactivation can be recognized in intact plants, the assay also allows us to determine the developmental timing of insertion events. The results of using the insertion assay to determine the mutagenic potential of Ac in tomato will be presented.

*Genetic Dissection of Plant Development*

**A 600** REGULATION OF C4 GENE EXPRESSION IN DEVELOPING LEAVES AND COTYLEDONS OF GRAIN AMARANTH. James O. Berry, Jiangliang Wang, and Ping-Xu, Dept. of Biological Sciences, SUNY-Buffalo, Buffalo, NY, 14260.

Immunofluorescence microscopy and *in situ* hybridization were used to examine the cell type-specific expression of genes encoding ribulose 1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase), in the $C_4$ dicotyledonous grain plant *Amaranthus hypochondriacus*. In mature leaves the chloroplast-encoded large subunit (LSU) gene and nuclear-encoded small subunit (SSU) genes of RuBPCase are expressed only in leaf bundle sheath cells, while the nuclear-encoded PEPCase genes are expressed only in mesophyll cells. Similarly, cotyledons of six day and older seedlings show Kranz anatomy and cell type-specific expression of genes encoding these $C_4$ enzymes. In both leaves and cotyledons a unique developmental program occurs for the two subunits of RuPBCase. At very early stages of development of these tissues LSU and SSU polypeptides and mRNAs are found in both mesophyll and bundle sheath cells. As these tissues mature the RuBPCase subunits and mRNAs become specifically localized to the bundle sheath cells, indicating that the cell type-specific expression of these genes is determined in part by the developmental program of the tissue. In contrast, PEPCase gene expression is specifically localized to mesophyll cells throughout leaf and cotyledon development.

**A 601** DEVELOPMENT BIOLOGY OF MALE-FERTILITY IN *ARABIDOPSIS THALIANA*, Abdul M. Chaudhury, Stuart Craig, Ethan R. Signer and Elizabeth S. Dennis CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia, Massachusetts Institute of Technology, Cambridge, Massachusetts

In an attempt to understand the development of the male part of a bisexual flower (*Arabidopsis thaliana*) we have isolated nineteen sporophytic nuclear recessive mutants that confer male-sterility. They fall into several phenotypic categories including mutants devoid of released pollen, mutants with aberrant pollen, a mutant with apparently normal but infertile pollen, and a mutant whose male-sterility is due only to short filament length. Eight of these nineteen mutants define seven complementation groups. By sectioning anthers at different stages of development we have analyzed the microspore development of wild-type flowers and of seven mutants defining six complementation groups. Mutants belonging to different complementation groups demonstrated different developmental "end points". In the mutants *ms4, ms5,* and *ms15* microsporogenesis is arrested at a stage earlier than the formation of tetrads. In *ms2,* although normal tetrads are formed, the locules harboring the microspores collapse soon afterwards, and no further development of the microspore occurs. In the previously isolated mutant *ms1-1* and the allelic mutant *ms1-2,* tetrad formation is apparently normal but the released microspores are abnormal in appearance. In *ms10* pollen development proceeds further, leading to the release of the apparently normal self-infertile pollen.

These mutants not only define various developmental steps leading to male-fertility, but promise to lead to the genes that are important in coding for the structural and regulatory proteins important for the male-fertility in a hermaphrodite flower.

**A 602** ANALYSIS OF GENOMIC CLONES REPRESENTING TWO SELF-INCOMPATIBILITY ALLELES IN *PETUNIA INFLATA*, Craig E. Coleman and Teh-hui Kao, Department of Molecular and Cell Biology, Penn State University, University Park, PA 16802.

The solanaceous species, *Petunia inflata*, displays monofactacorial, gametophytic self-incompatibility (SI). cDNAs representing three alleles of an SI-associated, pistil-specific gene encoding a single 24-25 kDa glycoprotein (S-protein) was used as probe to isolate two clones representing the S1 and S3 alleles from a *P. inflata* genomic library. The coding region and much of the flanking regions have been sequenced from both clones revealing a surprising level of interallelic heterogeneity. The genomic clones have been inserted into binary vectors for transfer into diverse genetic backgrounds through *Agrobacterium* -mediated transformation. Additionally, nested deletions of the 5' flanking region of the S3 allele have been constructed for use in transient assays to identify elements which are important in transcriptional control. Finally, flanking fragments of these two genomic clones are being used to probe DNA from related self-incompatible species in order to identify other components of the self-incompatibility locus.

**A 603** INTRACELLULAR AND EXTRACELLULAR SORTING OF THE TOBACCO PR PROTEINS AND PR GENE EXPRESSION DURING FLORAL DEVELOPMENT.
John R. Cutt, David C. Dixon, François Côté*, Alain Asselin* and Daniel F. Klessig, Waksman Institute, Rutgers University, PO Box 759, Piscataway, NJ 08855 and *Département de Phytologie, Université Laval, Sainte-Foy, Québec, G1K 7P4, Canada.

**The pathogenesis-related PR-1 and PR-2 gene families of tobacco are regulated by both stress and developmental signals. In response to TMV infection these genes are coordinately induced, however, during floral development they can be coordinately or differentially activated in various organs. We have found that the PR-1 and PR-2 genes exhibit an identical pattern of expression in sepals. In contrast, only the PR-1 genes were expressed in the developing seeds. The expression of the PR genes in floral organs implies they perform a function in normal developmental processes.**

**In TMV-infected tobacco leaves, the acidic PR-1 proteins are synthesized and secreted into the apoplastic space. We have detected the accumulation of the PR-1 proteins in the vacuoles of crystal idioblast cells - a morphologically distinct cell type that contains crystals of calcium oxalate in their vacuoles. A second intracellular localization has also been shown for the PR-1 proteins within the cells of the developing ovule. The data suggests that the PR-1 proteins can be differentially targeted to multiple cellular locations and that this may be controlled by variations inherent in the specific cell type.**

**A 604** LECTIN-LIKE TRANSCRIPTS HIGHLY EXPRESSED IN DEVELOPING LEAF PRIMORDIA OF *Pisum sativum L.*
Michael S. Dobres, Jhang Ho. Pak, Laurel Belmont, Dipanwita Maiti and Albert List. Department of Bioscience, Drexel University, Philadelphia, PA 19104

The seeds of leguminous plants contain high levels of carbohydrate-binding proteins called lectins. These lectins are typically synthesized in cotyledons during the mid-maturation stage of seed development and are localized within protein bodies of storage vacuoles. In contrast , immunologically related non-seed legume lectins have been localized in root tips and shoots of several different legumes. Although, the molecular properties of many lectins have been well characterized, the biological roles of these proteins during plant development remain unknown.

We recently reported the characterization of a shoot-specific transcript corresponding to the cDNA pEA207 encoding a protein with significant sequence identity to several leguminous seed lectins. This transcript displays a striking degree of organ and stage specific expression: It accumulates in the actively growing bud of the pea plant and is either absent or present at very low levels in the expanded leaves below the bud (Dobres and Thompson, 1989, Plant Physiology **89**: 833-838). We have used pEA207 to isolate four different cDNA clones corresponding to four different pEA207 genes The coding region of each gene family member displays 99% amino-acid identity to each other. Curiously, the amino-acid differences between each gene serve to alter potential N-glycosylation sites within the predicted protein. The physiological significance of these glycosylation-site differences remains to be determined. *In situ* hybridization reveals that pEA207 is highly expressed in epidermal and procambial tissue subjacent to the apical dome but it is not highly expressed in the protodermal tissue of the apical dome. Our working hypothesis is that the pEA207 gene product may play some role in controlling the rate of cell growth in tissue immediately below the apical meristem.

**A 605** ISOLATION OF A DOMINANT CONDITIONAL LETHAL GENE WITH POTENTIAL USES IN PLANTS.
Stanton B. Dotson and Ganesh M. Kishore, Monsanto Company, St. Louis, MO 63198. A heterologous conditional lethal gene would be useful as a negative selectable marker in plant transformation and for cell ablation in developmental biology studies. A protoxin was identified which was inactive against plants and bacteria. Screening a collection of microbes identified a *Pseudomonas sp.* capable of utilizing the protoxin as a nutritional source. The first step in degradation was identified as the enzymatic conversion of the protoxin into the toxin. The enzyme was purified to homogeneity and the gene cloned and sequenced. The gene was engineered behind the RecA promoter for expression in *E. coli*. Expression of the gene had no observable effects on *E. coli* growth when plated on minimal medium except in the presence of the protoxin which completely inhibited growth confirming the original hypothesis for a conditional lethal phenotype. Potential applications for this gene as a negative selectable marker and for conditional cell ablation will be presented.

**A 606** EXPRESSION OF HEAT SHOCK PROTEIN HSP70 IN DEVELOPING TOMATO POLLEN, Nicholas B. Duck and W. R. Folk, Department of Biochemistry, University of Missouri, Columbia MO 65211.

The 70 kD heat shock proteins (hsp70's) were first characterized as being abundantly expressed following heat and heavy metal stress in most organisms. They are encoded by a multigene family whose members are differentially regulated. Some family members are expressed in the absence of stress and are called heat shock cognates (hsc70's). The purpose of this investigation was to examine the expression of hsp/hsc70's in developing pollen of tomato (Lycopersicon esculentum cv.VF36). *In situ* hybridizations using hsc70 riboprobes and antibodies directed against hsp/hsc70's were used to examine 70 kD heat shock protein expression during pollen development, both with and without heat stress.

**A 607** CHARACTERIZATION OF PROTEINS ASSOCIATED WITH THE TRANSITION FROM SHOOT REGENERATION COMPETENCE TO CALLUS DETERMINED GROWTH IN INTERNODAL STEM EXPLANTS OF *Populus deltoides*, Stephen G. Ernst and Virginia I. Miller, Department of Forestry, Fisheries and Wildlife, University of Nebraska, Lincoln, NE 68583-0814 USA

Previous experiments demonstrated that competence for shoot regeneration and callus determined growth of *Populus deltoides* internodal stem explants could be manipulated *in vitro*. Developmental state specific proteins were observed using one- and two-dimensional polyacrylamide gel electrophoresis. Spots from 2D gels were removed and concentrated on 1D gels for electroblotting onto PVDF or nitrocellulose membranes for amino-terminal sequencing. One set of proteins were associated with either shoot or callus determined growth: a set of three 32 kDa proteins (putative isoforms) with pI's of 6.5 to 7.5 that are present only in tissue that is determined for production of adventitious shoots; and a single 35 kDa protein with a pI of approximately 7.0 that is present in much higher relative concentration in tissues that are determined for callus growth. Both groups are hydrophobic membrane-associated proteins. The second set of proteins were associated with changes in competence for shoot regeneration. These include, among others, 77, 65, 51 and 26 kDa proteins which are associated only with those treatments for which shoot induction competence has been stimulated in *Populus deltoides* stem explants. Amino acid sequence data and sequence comparisons will be discussed.

**A 608** A cdc2 GENE, ITS 34-KDA PRODUCT, AND HISTONE H1 KINASE ACTIVITY IN PEA. Heidi Feiler, Betty Prewett and Thomas Jacobs, Department of Plant Biology, University of Illinois, Urbana, IL 61801.

Macroscopic form and growth dynamics in higher plants are directly influenced by cell division patterns in meristems. However, little is known about the molecular basis of mitotic regulation in plant cells. We have selected *Pisum sativum*, the garden pea, for an analysis of the ubiquitous *cdc2*-centered cell regulatory system, originally identified in fission and budding yeasts. A monoclonal antibody (MAb-J4) raised against *Schizosaccharomyces pombe* p34 recognizes a polymorphic signal at 34 kDa in pea protein extracts, comparable to the phosphorylated isoforms of mammalian p34. In other systems, p34 protein kinase activity is regulated by phosphorylation state and quaternary associations. Nondenatured protein extracts of meristematic pea tissues were fractionated by gel filtration, electrophoretically separated under denaturing conditions, and immunoblotted. p34 crossreactive material was apparent in both low and high molecular mass fractions, indicating that pea p34 occurs as both a monomer and as part of a high molecular mass complex. Histone H1 kinase activity was found predominantly in the higher molecular mass fractions. We also report the cloning of the pea *cdc2* homologue (*Pscdc2*) by polymerase chain reaction combined with a novel immunological screening approach. *Pscdc2* DNA sequence analysis reveals perfect conservation of the hallmark "PSTAIR" sequence motif found in all *cdc2* gene products analyzed to date. Experiments are in progress to test the ability of this gene to complement a fission yeast *cdc2* mutation. Northern blot analysis of *Pscdc2* expression in various pea tissues shows that the 1.35 kb transcript is enriched in mitotic tissues.

## A 609 *AM*GRP-1, A GLYCINE RICH, TAPETUM SPECIFIC GENE IS A PUTATIVE TARGET OF HOMEOTIC GENES IN *ANTIRRHINUM MAJUS*

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Homeotic genes in flowers, as analyzed so far, show homology to transcriptional activators. Their gene products presumably regulate flower morphogenesis by activating or repressing a specific set of target genes. We have used differential screening of a subtracted, 'flower specific' cDNA library with probes derived from inflorescences of either wildtype, or the homeotic mutant *deficiens*$^{globifera}$ to isolate several independent cDNA clones that are 'down-regulated' in the mutant.

The derived protein of one of these cDNA clones (*am*GRP-1) shows homology to structural proteins, 50% of its amino acids are glycines, and it carries an export signal. The corresponding message is specifically expressed in tapetum cells of developing anthers. The message is absent in *deficiens*$^{globifera}$ which shows homeotic transformation of anthers to carpels. Furthermore, the gene is virtually not expressed in the weak *deficiens* alleles which develop functional tapetum tissue. In analogy to known glycine rich proteins, we assume that *am*GRP-1 is cell wall located. We discuss the possibility of homeotic genes controlling structural cell wall proteins as one way of regulating morphogenesis.

## A 610 Molecular Analysis of *deficiens*, a Homeotic Gene Controlling Flower Morphogenesis in *Antirrhinum majus*

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The homeotic gene *deficiens* participates in the genetic control of floral organogenesis in *Antirrhinum majus*. Its protein product belongs to a group of putative transcription factors ( SRF, MCM1, AG ) that share a common DNA binding domain. In order to get insight into structure and function of the *deficiens* locus we analyzed several morphoalleles, the three classical ones ( *def*$^{chl}$, *def*$^{nic}$, *def*$^{gli}$ ) and two new alleles recently obtained by transposon mutagenesis.

The structure of the deficiens gene has been established. Three different kinds of alterations were observed in the mutant alleles. The *def*$^{chl}$ allele displays a 4 basepair alteration in the promotor that leads to a reduced mRNA level. The *def*$^{nic}$ allele has nearly wildtype mRNA level but shows a point mutation within the putative DNA binding domain. The other three alleles, *def*$^{gli}$, *def 23*, *def 136b* carry transposon insertions that lead to a strong reduction of the mRNA. We discuss the molecular structure of these alleles with respect to their phenotypes.

## A 611 CELL-TYPE SPECIFIC EXPRESSION OF MAIZE STALK GENES:

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We are investigating the cell-type specific expression of maize stalk genes. We have isolated cDNAs from a 8 week old maize stalk cDNA library. Several of the genes isolated are differently expressed in pith versus rind cells. We are using in situ hybridization to identify the cell-type specific expression patterns in stalk and in other organs as well as studying their expression during stalk development.

**A 612** CLONING OF *er* LOCUS IN *ARABIDAPOSIS* BY CHROMOSOME WALKING, Inhwan Hwang, Takayuki Kohchi and Howard M. Goodman, Department of Molucular Biology, Massachusetts General Hospital, Boston, MA 02114.

In *Arabidopsis*, several morpholoical mutants (*erecta, asymmetric,* etc) have been isolated. As an initial step to study the regulation of plant morphogenesis, we are attempting to clone the *er* locus. The *er* mutation shows multiple effects on various parts of a plant: very compact inflorescences, short petiole and height, blunt fruit, and strong apical dominance. However, the biological activity of the locus is not known. To clone the *er* locus, we decided to do chromosome walking to the locus and complement the mutant with candidate DNA fragments. We have initiated walking from a neighboring RFLP marker. To locate the locus more precisely, we have used a fine mapping strategy using double mutants *er, cp2* and *er, hyl*. Each of these double mutants was crossed to the wild type of Columbia ecotype. The recombinants were isolated from the F2 generation. DNAs were prepared from pools of F3 plants and used to examine the segregation of RFLPs. We were able to construct a contig which contains the *er* locus and whose size is over 1 Mb using the yeast artifical chromosome libraries. Now we have located the *er* locus within a 20 kb region in a 120 kb YAC clone. Functional complementaion is currently being used to identify the *er* locus.

**A 613** GENETIC AND MOLECULAR ANALYSIS OF FLORAL HOMEOTIC GENES IN ARABIDOPSIS. Vivian Irish and Ian Sussex. Department of Biology, Yale University, New Haven, CT 06511.

The apetala-1 (ap1) mutation results in the loss of petals and the homeotic conversion of sepals to bracts. Based on the synergistic interactions of ap1 with two other homeotic floral mutations, apetala-2 (ap2) and agamous (ag), we have proposed that interactions between these three genes are required to establish the determinate floral pattern. We have tested this model by constructing an ap1, ap2, ag triple mutant combination, as well as constructing other double mutant combinations using several different ap1 and ap2 alleles. We will also present data on our progress towards molecularly defining the ap1 locus and other genes involved in floral development.

**A 614** EXPRESSION PATTERNS OF STRUCTURAL AND REGULATORY GENES IN *ANTIRRHINUM* FLOWERS. D.P.Jackson, F.Culianez-Macia, A.Prescott, K.Roberts, C.Martin. Depts. of Cell Biology and Genetics,John Innes Institute,John Innes Centre for Plant Science Research,Colney Lane,Norwich NR4 7UH,UK.

The spatial and tissue-specific regulation of gene expression is of fundamental importance in the differentiation of multicellular organisms. We have investigated the effects of genetic factors, both cis- and trans-acting, on the spatial expression of genes in the anthocyanin biosynthetic pathway. In particular we have shown that a single regulatory locus, *Delila*, controls the tissue-specific expression of *Nivea*, an early gene in the pathway, and the spatially-specific expression of *Pallida* and *Incolorata*, which act late in the pathway. In order to investigate these effects further we have attempted to isolate transcriptional regulators similar to *C1*, a regulator of anthocyanin biosynthesis from maize, which contains the DNA-binding domain of the *myb*-oncoproteins.

Six genes containing this domain have been isolated from a cDNA library prepared from flowers of *Antirrhinum majus*. Each gene also contains an acidic domain in the carboxy-terminal half of the deduced protein sequence suggesting that they could function as transcriptional activators. Analysis of the expression patterns of these genes has allowed general conclusions to be made about their function(s). In addition for one of the genes, which is expressed only in flowers, analysis of tissue-specific expression by *in-situ* hybridisation has allowed more detailed predictions, since its' expression is strongest in the nectary and a layer of cells in the transmitting tract of the style, both of which are involved in secretion. Phenotypes observed in transgenic *Antirrhinum* roots and tobacco have given further insights into the possible regulatory roles of these genes, and these will be discussed with regard to our general conclusions about the roles of myb-related genes in plants.

**A 615** FLOWER DEVELOPMENT IN A GIBBERELLIN-DEFICIENT (*gib-1*) MUTANT OF TOMATO, Steven E. Jacobsen and Neil E. Olszewski, Department of Plant Biology, University of Minnesota, St. Paul, MN 55108.

In an effort to develop a system to study changes in gibberellic acid mediated gene expression that occur during flower development, we have studied the biology of the *gib-1* mutant of tomato. This GA deficient mutant initiates flower primordia but flowers do not complete development unless supplied with exogenous GA. To determine when *gib-1* flower development stops and if development can be restored following cessation, wild type and *gib-1* flowers of different lengths were treated with GA and then the number of days to petal reflex was recorded. In wild type, a nearly linear negative correlation exists between bud length and the number of days to petal reflex for all flowers tested (lengths ranged from 0.2 to 13.2 mm at treatment). For *gib-1* flowers, this same negative correlation exists only for buds 0.2-2.5 mm long at treatment. Buds between 2.5 and 3.7 mm have a similar number of days to petal reflex and most buds longer than 3.7 mm do not develop normally. This suggests that mutant flowers reach a developmental block at a length of 2.5 mm and then remain developmentally arrested but still rescuable by GA until they reach a length of 3.7 mm. Further analysis indicates that anthers of arrested flowers (2.5-3.7 mm) contain pollen mother cells that are in G1 of premeiotic interphase. When arrested buds are treated with GA, premeiotic DNA synthesis and callose accumulation are evident by 48 h post treatment, and within 66 h the first visible stage of prophase I of meiosis occurs. Developmentally arrested anthers are currently being used in differential screening experiments to identify clones to mRNAs that are more abundant in anthers 8 h after GA treatment.

**A 616** ISOLATION AND CHARACTERIZATION OF ROOT-PREFERENTIAL CDNA CLONES FROM ZEA MAYS. Isaac John, Bruce M. Held, Eve S. Wurtele, and James T. Colbert. Department of Botany, Iowa State University, Ames IA, 50011.

Roots are the primary plant organ specialized for absorption of water and nutrients. To begin investigation of the molecular control of root development and function we have screened for genes that are preferentially expressed in roots. A λZAP II cDNA library was produced using poly(A)$^+$RNA isolated from the roots of nine-day-old corn seedlings. Growth of the seedlings on germination paper allowed harvest of complete root systems, including root hairs. The cDNA library contained about $5x10^5$ recombinants. Differential screening of about 100,000 plaques, using single-stranded cDNA produced from nine-day-old roots or nine-day-old shoots, allowed identification of three distinct cDNA clones that were preferentially expressed in roots. The cDNA clones were about 2.5 kbp (pZRP2), 0.6 kbp (pZRP3), and 1.2 kbp (pZRP4) in length. Northern blot analysis indicated that each of these cDNA clones was approximately full-length. The mRNAs corresponding to these clones were 25- to 80-fold more abundant in roots than in leaves. Genomic Southern blot analysis suggested that one of the cDNA clones is a member of a multigene family. Partial nucleotide sequences of these cDNA clones revealed no significant homology with other sequences currently in the GENEBANK repository.

**A 617** PURIFICATION AND CLONING OF *Brassica napus* STEAROYL-ACP DESATURASE, Martin Kater, Gregory Koningstein, H.John J.Nijkamp and Antoine Stuitje, Department of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

*Brassica napus* stearoyl-ACP desaturase was purified to near homogeneity from approximately 100 gr developing seeds using a purification protocol which is essentially as described by McKeon and Stumpf (J.Biol. Chem., 257: 12141-12147 (1982)). Following preparative SDS-PAGE the protein was transferred to a PVDF membrane and subjected to N-terminal sequence analysis. The remaining purified protein was transferred to nitrocellulose and used to raise a potent polyclonal antibody preparation in a Swiss-mouse. The antibody was able to immunoprecipitate rapeseed Δ9-desaturase activity and could detect single polypeptides of an apparent mass ranging between 33 (rapeseed) and 40 kD in all plant species analysed. Screening of a *B.napus* seed-specific λ-gt11 c-DNA library with the antibody preparation resulted in the purification of several c-DNA clones.Using these c-DNA clones an apparent full-length was isolated and sequenced revealing an open-reading frame for 385 aminoacids that includes a 73 aminoacids presequence preceding the N-terminal sequence found in the purified protein. Several PCR-mediated constructions were expressed in *E.coli* resulting in the synthesis of either full-length or truncated versions of the protein. Depending on growth conditions, western-blotting indicated processing of presequence in *E.coli* even with a truncated version of the protein. Results will be presented that suggest an aberrant folding of the protein in *E.coli* , which may explain the absense of stearoyl-ACP activity.

**A 618** ISOLATION AND CHARACTERIZATION OF SHOOT APEX SPECIFIC cDNA CLONES FROM TOBACCO, Alan J. Kelly, Michelle T. Zagotta, Regina A. White and D. Ry Meeks-Wagner, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The transition from vegetative to reproductive growth results in the commitment of the apical meristem to floral organ formation. Developmental studies have yielded valuable information regarding the developmental states of the tobacco shoot apex. In order to identify the molecular signals that initiate floral development we have begun a molecular analysis of the early steps in flowering by identifying genes transcribed in cells of the shoot apex of tobacco during the floral transition. Due to the difficulty in obtaining sufficient amounts of apex tissue for molecular cloning experiments we utilized PCR technology for the differential screening of an unamplified, subtracted cDNA library made from transition shoot apices. This approach enabled the isolation of apex-specific and floral apex-specific cDNA clones. We are currently investigating the expression patterns of the corresponding genes in day-neutral and photoperiodic cultivars of tobacco.

**A 619 DEVELOPMENTALLY REGULATED EXPRESSION OF A POLLEN-SPECIFIC GENE IN BRASSICA CAMPESTRIS**, Bruce Knox, Piyada Theerakulpisut, Mohan B. Singh, Huiling Xu, Sean Davies and John Pettitt*, School of Botany, University of Melbourne, Parkville Victoria 3052, and *Department of Pathology and Immunology, Monash Medical School, Prahran, Victoria 3181, Australia.

Poly(A)+ RNA was isolated from pollen collected at anthesis and used to construct a cDNA library in the vector lambda-gt 10. cDNAs representing genes that are preferentially expressed in pollen were isolated from the library by differential screening using cDNA probes made from leaf, flower (without anthers), fruit and seedling. Several cDNA clones that showed signals only with pollen under stringent hybridization conditions were selected for further characterization. cDNA clone Bcp1 was chosen as representing a class of mRNAs that is differentially expressed and very abundant in mature pollen. Bcp1 hybridized with mRNAs from pollen of other genera in the family Brassicaceae but not with pollen from other plant families. In situ hybridization showed that transcripts are detectable in the mature pollen grains, but are not expressed in the anther wall cells. Earlier in development, expression of this transcript was detected in the tapetal cells, but not in pollen until the bicellular stage. These results indicate that in pollen, haploid-specified gene expression is operative, while earlier in development, diploid-specified expression occurred in the neighbouring cells of the tapetum. The cDNA has been sequenced and the deduced amino acid sequence shows no homology with other known proteins. A genomic clone has been isolated from a lambda-library, and its promoter region is being identified by sequencing of the upstream region, and transformation experiments.

**A 620** GENETIC INTERACTIONS WITH THE *TUNICATE* LOCUS OF MAIZE. Jane A. Langdale, Erin E. Irish and Timothy Nelson, Dept. of Biology, Yale University, P.O. Box 6666, New Haven, Ct 06405 .

The *Tunicate* (*Tu*) mutation in maize is a co-dominant mutation that causes glumes (bracts) in both the male and female inflorescences to become enlarged. This mutation also affects sex determination, insofar as homozygous *Tu/Tu* plants develop pistils in the normally staminate tassel. As part of a comprehensive effort to understand floral morphogenesis in maize, we have examined interactions between the *Tu* locus and other mutants that affect vegetative and sexual differentiation. Synergistic interactions were seen in double mutant combinations with *tasselseed (ts)1*, *ts2*, *ts4*, *Ts5*, *Ts6*, *Teopod1*, *Teopod2* and *Corngrass*. A partially corrective phenotype was observed in combination with *Vestigial glume*. In light of these observations, we discuss possible roles for the *tu* gene product.

**A 621** EXPRESSION OF FLOWER-SPECIFIC GENES FROM *NICOTIANA TABACUM* AND ANALYSIS OF THEIR PROMOTERS, Bruce P. May, Qing Gu, M.-J. Morse, and Alice Y. Cheung. Department of Biology, Yale University, New Haven, CT 06511.

Several flower-specific genes have been isolated by differential screening of a cDNA library prepared from *Nicotiana tabacum* flower buds. Characterization of expression of the genes has revealed genes expressed throughout the flower, genes expressed in some but not all whorls, and genes expressed only in stamens or only in pistils. These genes also show temporally regulated expression during flower development. Genomic copies of the genes are being cloned and promoter elements that are responsible for the various specificities of expression are being analyzed. Continuing results from these efforts will be presented.

**A 622** THE LAM-1 GENE CONTROLS LATERAL EXPANSION IN DEVELOPING LEAF PRIMORDIA, N.A. McHale, Department of Biochemistry & Genetics, Conn. Agric. Expt. Station, P.O. Box 1106, New Haven, CT 06504.
Lateral expansion of the leaf lamina in tobacco begins with the establishment of meristematic initials located at the margins of emerging primordia. Cell divisions perpendicular to the main axis produce an elliptical lamina which enters a second phase of lateral expansion out from the midrib in regions associated with emerging lateral veins. We have isolated a nuclear mutation (lam-1) in N. sylvestris that blocks lateral expansion of the lamina. Emerging primordia grow normally in length, but remain narrow and cylindrical, suggesting an early defect in establishment of marginal initials. Lateral development of the vascular system is also restricted. Branch veins are initiated normally, but turn and grow parallel to the midrib. Mutant leaves reach their normal length (30 cm) but never expand beyond 2-3mm. Thin sections show normal differentiation of epidermis as well as palisade and spongy parenchyma. We are establishing a system for T-DNA mediated gene tagging in N. sylvestris. One of our objectives is to tag and clone the lam-1 gene to gain further information on mechanisms governing morphogenesis in the vegetative meristem

**A 623** CO-SUPPRESSION OF FLORAL PIGMENTATION GENES IN *PETUNIA*, Joseph Mol, Rik van Blokland, Nico van der Geest, and Jan Kooter, Department of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

We have recently shown that introduction of extra gene copies for the flavonoid-specific enzymes chalcone synthase (CHS) or dihydroflavonol 4-reductase (DFR) into *Petunia hybrida* can lead to a reduction in flower pigmentation (v.d. Krol et al., 1990, Plant Cell, 2, 291-299). Up to 25% of the transformants is affected whereas the remainder look normal. In white flowers transcripts of both the endogenous and the transgene(s) are undetectable whereas in fully pigmented flowers both transcripts co-exist.
We addressed the question whether initial co-expression of endogenous and transgene(s) leading to elevated enzyme and metabolite levels causes the co-suppression phenomenon. We therefore introduced half-sized chs cDNA fragments driven by the CaMV35S promoter into *Petunia*. Both halves appeared to be equally effective in co-suppression. This makes it unlikely that co-suppression is caused by elevated enzyme levels rather unlikely.
Our present research is focussing on the possible involvement of co-transcription in the establishment of co-suppression. Two strategies are adopted: first run-on experiments are carried out in isolated nuclei and second, gene fusions containing the chs or dfr structural sequence are introduced that are prevented from being transcribed.
The possible relationship beteween "sense" and "antisense" effects is discussed.

**A 624** MUTANT PHENOTYPES OF *ARABIDOPSIS THALIANA* DERIVED FROM T-DNA TRANSFORMANTS, Peter C. Morris, Thomas Altmann, Alison Jessop, Renate Schmidt and Lothar Willmitzer, Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1000 Berlin 33, Germany.
T-DNA insertion mutagenesis is an increasingly popular method of generating mutant plants for study at a molecular level and *Arabidopsis thaliana* is an ideal plant in which to carry out this mutagenesis because of its small genome size and other attendant advantages. We present here data based on the analysis of the progeny of some 600 independent T-DNA transformed *Arabidopsis* plants, which have yielded a variety of altered phenotypes. The incidence of readily observable different phenotypes is approximately 5%. Included in these are transformed lines with altered colour (pale green or albinos), size (dwarves), leaf morphology (angustifolia), flowering time (both early and late), silique shape and growth habit. Of especial interest are three independent mutant phenotypes which show diminished apical dominance; however reduced auxin sensitivity does not appear to be the cause of this. Also described are plants which show abscisic acid insensitivity; they germinate in the presence of 30 μM ABA. Our current work is geared toward a genetic and molecular analysis of selected mutants. Several of these phenotypes can be attributed to somaclonal variation rather than insertion mutagenesis. However data on the cosegregation of mutant phenotypes with the T-DNA insertion indicate that insertion mutation is the basis for many of the phenotypes.This should allow for the isolation and characterisation of those genes whose disruption underlies the phenotype. Our initial results towards this end are presented.

**A 625** GENETIC ENGINEERING OF REVERSIBLE STERILITY IN TREES: APPROACHES, PROBLEMS, AND PROGRESS, Lorraine S. Nyers and Steven H. Strauss, Department of Forest Science & Genetics Program, Oregon State University, Corvallis 97331-5705.

*TREES* present many opportunities and problems for genetic engineers. A number of problems can be ameliorated, and opportunities exploited, by deployment of sterile trees. (1) Because of their outcrossing habit and usual admixture with wild stands, release of introduced genes into natural tree populations is a severe concern. Genetic sterility would provide reliable and long lasting protection against uncontrolled entry into natural gene pools. (2) A number of studies indicate that sterile trees would have enhanced wood production due to removal of the drain of sexual reproduction. (3) Vegetative propagation is effective in many species, allowing sterile trees to be multiplied for commercial plantings. (4) Production of seed from specific crosses, including hybrids, could be enhanced via male sterility---which is largely unavailable for trees from conventional tree breeding. Finally, (5) production of pollen from shade trees is often a health nuisance, and shedding of reproductive parts a sanitary nuisance.

Production and use of sterile trees presents several challenges. Can promoters active in the early phases of reproductive development be found, thereby minimizing investment of energy in reproductive primordia? To what extent can promoters and constructs from agronomic species be used? Can a generic sterility construct be produced that would be effective in a broad array of tree species? This is important because a wide variety of phylogenetically distinct species are important in forestry and horticulture. What kinds of suicide genes would be most effective? Restoration of fertility under specific conditions would be desirable to allow continued sexual breeding. What are the options for producing reversible sterility? The poster reviews our research program and progress to date working with poplar, and discusses the pros and cons of various approaches.

**A 626** CHROMOSOME WALKING TO THE *GL2* LOCUS IN *ARABIDOPSIS*
D. Oppenheimer, J. Esch, K. Coleman, B. Hauge*, H. Goodman*, D. Marks.
School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588, and *Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Our lab is using trichome development in *Arabidopsis* as a model system in which to study the molecular basis of cellular differentiation in plants. The *gl2* mutation of *Arabidopsis* is a nuclear recessive mutation which maps to chromosome 1. This mutation results in a loss of trichomes from the first two leaves, and a reduced number of trichomes on the surface of the rest of the leaves of the plant. Correlation of the physical map with the RFLP map of *Arabidopsis* has allowed the isolation of two overlapping cosmid clones which should contain the entire *gl2* locus. These cosmids have been restriction site mapped, and overlapping restriction fragments covering both of these cosmids have been cloned into a binary Ti-plasmid vector. These clones have been used in *Agrobacterium* -mediated gene transfer experiments with *gl2/gl2* plant tissue to complement the *gl2* mutation The results of the genetic complementation experiments will be presented.

**A 627** MOLECULAR CHARACTERIZATION OF AN APOMICTIC BACKCROSS HYBRID DERIVED FROM Pennisetum glaucum AND P. squamulatum, Peggy Ozias-Akins and Wayne W. Hanna, Department of Horticulture and USDA/ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA 31793
P. squamulatum reproduces by obligate apomixis (apospory). In order to introduce this trait into cultivated P. glaucum (pearl millet), it has been necessary to manipulate the ploidy level of pearl millet and to include a bridging species, P. purpureum, in the cross. Only with this approach has sufficient male fertility been maintained to allow repeated backcrossing to pearl millet. Male fertility is essential since the apomictic plants can only be used as male parents. A backcross 3 ($BC_3$) plant has been obtained that has a single supernumerary chromosome and is apomictic. DNA from P. squamulatum can be detected in $BC_3$ by restriction fragment length polymorphisms revealed with several genomic probes. Further characterization is being carried out with chromosome banding and genomic blocking techniques, as well as polymerase chain reaction using random primers.

**A 628** CHARACTERIZATION OF A GENE FAMILY IN *ARABIDOPSIS* WHICH HAS SEQUENCE SIMILARITY TO THE S-LOCUS OF *BRASSICA OLERACEA*, Sara E. Ploense and Robert E. Pruitt, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108
Identification of different types of individuals in the self-incompatibility system of *Brassica oleracea* is genetically controlled by a single gene , the S-locus. This gene is involved in a recognition process which arrests self-pollinations at the stigma surface. The product of the gene is a glycoprotein which is abundantly expressed in the stigma of incompatible *Brassica* species but is not easily detected in the pollen or anthers. The gene has been cloned and shown to be a member of a large multigene family in *Brassica oleracea*. The possible functions of the other members of this gene family are unknown though one gene is abundantly expressed in the stigma, coincident with S-locus expression. We have recently shown that there are six genes found in the self-compatible weed *Arabidopsis thaliana* which cross-hybridize with members of the S-locus family. Sequence characterization of four of the *Arabidopsis* genes shows they are quite similar to some members of the *Brassica* family: within their coding regions members of both gene families share blocks of highly conserved sequence interspersed with blocks of variable sequence. One member of the *Arabidopsis* gene family is apparently a psuedogene. Unlike the *Brassica* genes characterized to date, expression of the *Arabidopsis* gene product is not readily detectable. Work is in progress to delineate the spatial and temporal patterns of expression of these genes via transformation of *Arabidopsis* with promoter/GUS fusions and reverse transcriptase PCR.

**A 629** GENETIC ANALYSIS OF CELLULAR INTERACTIONS DURING FERTILIZATION OF *ARABIDOPSIS THALIANA*, Robert E. Pruitt, Thomas F. Horejsi, Brenda K. Pierskalla and Sara E. Ploense, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108
Fertilization in the angiosperms is a process which is regulated by the interactions of the male and female reproductive tissues. These regulatory interactions occur at various steps during the fertilization process. We have isolated several classes of mutations which interrupt the fertilization process at different times, probably by interfering with the ability of the male reproductive tissues to interact properly with the female reproductive tissues. One class of male sterile mutations produces pollen grains which are capable of effecting fertilization except that they are not recognized by the stigmatic papillar cells and therefore cannot germinate. These pollen grains can be rescued by providing the missing recognition functions on other pollen grains. Another class of male sterile mutations produce pollen grains which germinate and grow normal pollen tubes, but the pollen tubes fail to locate the wild-type ovules within the ovary. We have also isolated different types of female sterile mutations: one class which blocks the penetration of the growing pollen tubes through the stigma and two types which arrest ovule development prior to maturity. These last classes are of interest because the ovules which arrest very early in development are incapable of attracting pollen tubes, while those which arrest later do attract pollen tubes even though the ovule is incapable of developing into a viable seed. Genetic and biochemical experiments are in progress to further characterize these mutations.

**A 630** A T-DNA INSERTION MUTANT OF *ARABIDOPSIS THALIANA* WITH ABNORMAL FLORAL MORPHOLOGY, Judith L. Roe, Kenneth A. Feldmann*, and Patricia Zambryski. Department of Plant Biology, University of California, Berkeley, CA 94720, and *E. I. DuPont DeNemours and Co., Inc., Wilmington, DE 19880-0402.

We are interested in the mechanisms involved during the development of floral organs in higher plants. To identify gene products involved in this complex process we are using the technique of transposon insertion mutagenesis. We have generated a T-DNA insertion mutant by transformation of Arabidopsis thaliana with Agrobacterium tumefaciens [carrying a chimeric nptII gene (which confers resistance to kanamycin to a transformed plant) within the T-DNA region of the Ti plasmid] using the seed transformation method. Kanamycin-resistant progeny were screened for visible mutations in the subsequent generation. A line carrying a T-DNA insert was observed which displayed abnormal floral mophology. The mutant flowers are sterile and have a reduced number of sepals, petals and stamens compared to wild-type. The carpels do contain ovules, but do not completely fuse. This line was characterized for segregation ratios for $kan^r$ and for the mutant floral phenotype. Plant sequences flanking the T-DNA inserts in the mutant have been cloned, and cosegregation analysis of the T-DNA inserts suggests that one of these T-DNA inserts cosegregates with the mutant phenotype. Backcrosses with wild-type plants are being analyzed to confirm that this cosegregating T-DNA insert is responsible for the mutant phenotype. To characterize the wild-type gene which has been disrupted in the mutant, transcripts from the region are being analyzed by Northern analysis in wild-type plants. Also, cDNA clones have been isolated and sequencing of these clones is in progress.

**A 631** ISOLATION AND CHARACTERIZATION OF A PROLINE-RICH cDNA AND THE CORRESPONDING GENOMIC CLONE, HIGHLY EXPRESSED IN YOUNG TOMATO FRUIT, Yehiam Salts[1], Ruth Vacs[1], Rivka Barg[1], Mohamad Abu-Abied[2], Daniel Zamir[2] and Wilhelm Gruissem[3], [1] Department of Plant Genetics, The Volcani Center, ARO, POB 6, Bet-Dagan 50250, Israel. [2] Faculty of Agriculture, The Hebrew University, Rehovot 76100, Israel. [3] Department of Botany, University of California Berkeley, CA 94720, USA

A cDNA library was constructed in λgt10, from poly $A^+$ RNA isolated from young tomato fruits (6-8 mm in diameter, about one week after anthesis). Differential screening with tomato seedlings cDNA was used to select clones that are differentially expressed in young fruit. One of these, a 1.3kbp cDNA clone 1-36-1 is expressed in young fruit at a level that is 2000, 1000, 60 and 30 times higher than in mature-green fruit, leaf, root and stem, respectively. It's level of expression is already high in ovules at anthesis. Another 1.4kbp cDNA clone - 1E1 - and a 12kbp genomic clone corresponding to 1-36-1 were isolated. Sequence analysis revealed the following facts: a. the existence of an intron in the 3' untranslated region of the gene; b. an identity between the shared regions of the 1E1 cDNA and the genomic clone; c. cDNA clones 1-36-1 and 1E1 are both truncated and lack the 5' terminal region, they differ at the 3' ends, both harbor a poly-A tail but 1-36-1 terminates at a position which is 75bp upstream of 1E1; d. the open reading frame would encode a new proline-rich protein composed of repeating amino acids blocks. Using RFLP analysis cDNA clone 1-36-1 was mapped as a single gene to the tomato chromosome 7, between markers TG143 and TG166.
This work is supported by a BSF grant No. 87-00397, and by grant No. I.1626.89 from BARD, The United States-Israel Binational Agricultural Research & Development Fund.

**A 632** SEASONAL EXPRESSION OF A VEGETATIVE STORAGE PROTEIN AND ENZYMES OF NITROGEN ASSIMILATION IN CICHORIUM INTYBUS ROOTS, Konrad A. Sechley, J.Derek Bewley, Department of Botany, University of Guelph, Guelph, Ontario, Canada. N1G 2W1.

Little is known of the biochemical and molecular regulation of proteins associated with overwintering success in roots of perennial species such as chicory. Seasonal partitioning of compounds between the root and shoot results in extensive modulation of protein, nitrogen, and carbohydrate status of the roots. We found, by Western analysis, that the 6-fold increase in protein during the winter includes an 18 kD vegetative storage protein (VSP), and several enzymes involved in nitrogen (N) assimilation (nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase, and glutamate dehydrogenase (GDH)). Seasonal alterations of in vitro activities of these enzymes parallels the amount of protein (except for GDH) and accounts for changes in the soluble N pool. We have purified and N-terminally sequenced the VSP, synthesized corresponding oligomers, and by anchored PCR prepared a VSP-cDNA probe. Coupled with available cDNA's for GS and NR, seasonal alterations in transcription of these seasonally dynamic proteins is being examined.

**A 633** FUNCTION AND EXPRESSION OF S-ALLELES IN SELF-INCOMPATIBLE AND PSEUDO-SELF-COMPATIBLE PETUNIA T.L.Sims, P.D.Collins, J.J. Okuley and K.R. Clark, Department of Molecular Genetics, Ohio State University, Columbus, OH 43210.
We are investigating the structure and expression of different alleles of the S-locus in self-incompatible (SI) Petunia and in pseudo-self-compatible (PSC) varieties in which the self-incompatibility response has broken down [Clark *et al*, The Plant Cell 2 815-826 (1990)]. Two cases of breakdown in the self-incompatibility response are of particular interest: (1) Selfing of an SI stock, homozygous for the $S_1$ allele, gave progeny segregating 3:1 for SI versus PSC. (2) MSU1093 is a PSC variety having S-locus sequences that are expressed at a level indistinguishable from that in a self-incompatible stock. Crossing MSU1093 to the PSC line 80-15-5, previously characterized as a pollen-part mutation by Flaschenreim and Ascher, [TAG 54 97-101 (1979)] gave high levels of seed set (PSC) when MSU1093 was used as the female parent, but gave no seed set (SI) when MSU1093 was used as the pollen parent. This result suggests that MSU1093 and 80-15-5 share the same S-allele, with one line (80-15-5) showing defects in the pollen response, and the other line (MSU1093) showing defects in the style response. We are presently characterizing the structure and expression of S-alleles in each of these varieties, in an attempt to determine the molecular basis for SI breakdown. To determine if transfer of a specific S-allele can result in an alterated ability to discriminate among different S-alleles, we have used A. tumefaciens transformation to insert the $S_2$ allele into the $S_{1,1}$ background. Transformed plants are being regenerated and will be characterized for the function and expression of the transferred S-allele.

**A 634** CHARACTERIZATION OF RIBONUCLEASE ACTIVITY OF S-ALLELE ASSOCIATED PROTEINS IN PETUNIA INFLATA, Anuradha Singh, Yunjun Ai and Teh-hui Kao, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802
We previously identified three S-allele associated proteins from Petunia inflata, a species with gametophytic self-incompatibility, and obtained cDNA sequences encoding these proteins. The S-proteins share regions of sequence homology with two fungal ribonucleases, T2 and Rh. The S-proteins were purified to homogeneity, and ribonuclease activity was found to co-migrate with the purified S-proteins on activity staining gels. Here we report on the biochemical characterization of this catalytic activity, and examine its relevance to the biological function of S-proteins. The activity has a pH optimum of 7.0 and a broad temperature optimum. The S-proteins have similar specific activities, but vary markedly in their ability to hydrolyze various polyhomoribonucleotides. The activity is inhibited by zinc or copper, and by 1mM diethylpyrocarbonate. The uptake of labelled proteins by pollen tubes, and their effect on pollen of various genotypes is now being examined, to test whether the nucleolytic function of S-proteins is exerted in an S-allele dependent manner in situ.

**A 635** **ISOLATION OF cDNAS ENCODING POLLEN-SPECIFIC PROTEINS OF RYE-GRASS: INTRACELLULAR TARGETTING TO AMYLOPLASTS**, Mohan B. Singh, Piyada Theerakulpisut, Philip Taylor, Penelope Smith, Asil Avjioglu, Sean Davies, Terryn Hough and Bruce Knox, School of Botany, University of Melbourne, Parkville Victoria 3052.

Pollen grains of rye-grass Lolium perenne contain two major polypeptides of $M_r$ 32K and 35K. Both these proteins act as elicitors of the allergic response. Immunoblotting with specific monoclonal antibodies shows that expression of these polypeptides is pollen-specific. We have isolated and sequenced cDNA clones corresponding to both of these proteins. The clone encoding the 32K polypeptide has a predicted protein sequence with a hydrophobic putative signal peptide sequence of 25 amino acids that has motifs similar to chloroplast transit peptides. Immunoelectron microscopy shows that the 35K polypeptide is located in the cytosol, while the 32K polypeptide occurs in the starch granules (see figure). There is no amino acid sequence homology of these two polypeptides with any other known protein. Approaches to understanding the natural function of these molecules are being pursued by following developmental expression of the genes.

**A 636** MUTATIONS AFFECTING INFLORESCENCE DEVELOPMENT IN *ARABIDOPSIS THALIANA*,
D. R. Smyth, John Alvarez, Yu Xiang-Hua and Catherine L. Guli, Department of Genetics & Developmental Biology, Monash University, Clayton, Melbourne, Vic. 3168, Australia
A large number of recessive mutants with modified inflorescence structure have been isolated following EMS mutagenesis of the Landsberg *erecta* ecotype. We have investigated three classes. The first have inflorescences with more branching than wild type and more cauline leaves. Individual flowers are also abnormal, with more sepals in irregular locations around an often abnormal gynoecium. Some mutants also have a few petals and stamens. At least three of these mutations are allelic with *leafy* (Haughn, G.W. & Somerville, C.R. 1988 Dev. Genet. **9**, 73-89). The *leafy* locus is closely linked to *yellow inflorescence* on chromosome 5.
A second class has several normal flowers on each flowering stem before development terminates in a compound flower. This flower often has two or three gynoecia surrounded by irregular numbers of sepals, petals and stamens. We have had many independent occurrences of this mutation which we call *triple flower*. Its locus lies near the top of chromosome 5.
The last class has many fewer flowers per stem than wild type. In this case, though, the inflorescene terminates in a short, undifferentiated outgrowth of the apical meristem. The flowers produced usually have fewer sepals and stamens but more petals than wild type, and a pin-shaped gynoecium with reduced or no ovaries. The standard mutation of this class, which we call *pinoid*, is not allelic with *pin-formed* (Goto, N. *et al.* 1987 Arab. Inf. Serv. **23**, 66-71). Its locus is close to *asymmetric leaves* on chromosome 2.
We anticipate that a molecular description of the action of these genes when in wild type form will help reveal the gene cascade involved in setting up decisions in early inflorescence development.

**A 637** CLONING AND CHARACTERIZATION OF THE $p34^{cdc2}$ HOMOLOGUE OF *ZEA MAYS*
V. Sundaresan, J. Colasanti, and M. Tyers, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
In animal and yeast cells, entry into mitosis or meiotic M phase is initiated by activation of the $p34^{cdc2}$ protein kinase. When activated, this kinase phosphorylates a number of substrates (Histone H1, nuclear lamins, $pp60^{src}$, etc.) resulting in the onset of M phase. As an initial step towards studying the regulation of cell-division during plant development, we have isolated cDNA clones encoding $p34^{cdc2}$ homologues from a higher plant, *Zea mays* (maize). A full length cDNA clone, *cdc2ZmA*, was isolated and sequenced. Comparison of the deduced amino acid sequence of the maize $p34^{cdc2}$ protein to that of human and *S. pombe* $p34^{cdc2}$ shows it to be 66% and 63% identical respectively. Southern blot analysis, as well as isolation of another cDNA clone, *cdc2ZmB*, that is 96% identical to *cdc2ZmA*, indicates that maize has multiple *cdc2* genes. Studies of expression of the maize *cdc2* gene(s) by Northern blot analysis indicate that there is a correlation between the abundance of *cdc2* mRNA and the proliferative state of the tissue, as well as the amount of *cdc2*- specific histone H1 kinase activity.

**A 638** DIFFERENTIAL GENE EXPRESSION DURING GERMINATION AND CYTOKININ-MEDIATED BUD INDUCTION IN *PICEA ABIES* (NORWAY SPRUCE).
Sundås A. and Engström P.
Department of Physiological Botany, Uppsala University, Box 540, S-751 21 Uppsala, Sweden.

A pulse-treatment of isolated seed embryos of *Picea abies* with cytokinin efficiently and reproducibly induces a synchronous de novo-formation of adventitious buds from subepidermal cells. This treatment also effects the organ development during germination of the embryo; e.g. chloroplast maturation is delayed and cell elongation is suppressed. To study the mechanisms by which these developmental processes are regulated we have attempted to isolate cDNA clones corresponding to genes that are differentially expressed during germination and early bud differentiation.

A cDNA library was constructed from RNA isolated from 5 days old cytokinin-treated embryos. Differential screening was carried out and we isolated approximately 40 clones which gave differential signals.

By Northern blot analysis two main categories of clones were identified; one which is only expressed in developing embryos but not in the seed or the mature seedling and a second category which is expressed both in the embryo and in the mature seedling. Classes that are either enhanced or suppressed in the cytokinin-treatred embryos were found in the embryo category and clones with suppressed expression in cytokinin treated embryos were found in the second category. Clones that are expressed at higher levels in the cytokinin-treated embryos than in the *in vitro*-germinating embryos are candidates for clones corresponding to genes which have functions in dividing cells and in developing meristems since these are the processes that are more active in the cytokinin-treated embryos. The characterization of these clones by DNA sequencing and *in situ* hybridizartion experiments will be presented.

**A 639** CHARACTERIZATION AND CLONING OF FLOWER-SPECIFIC AND DEVELOPMENTAL STAGE-SPECIFIC DNA BINDING PROTEIN OF THE EPSPS PROMOTER FROM PETUNIA

Hiroshi Takatsuji, Masaki Mori, Philip N. Benfey, Ling Ren and Nam-Hai Chua, Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021

5-enolpyruvylshikimate-3-phosphate synthase(EPSPS) catalyzes an essential step in the shikimate pathway leading to the biosynthesis of aromatic amino acids, flavonoids and anthocyanins. The expression of the EPSPS gene is flower specific and developmental-stage specific in the flower. The specific expression pattern is conferred by the -1752 to -823 region of the EPSPS gene in the transgenic plants.

We have prepared nuclear extracts from petunia petals and detected DNA-binding activity in this region. DNasesI footprinting experiments revealed four strong binding sites(EP1-EP4) and several weaker binding sites. Based on competution experiments, these sites appear to interact with the same factor. By screening a cDNA library constructed from petunia petal RNA, we obtained a full-length cDNA clone encoding protein(EPF1) that binds to the EP1 site. Sequence analysis showed that EPF1 contains two repeats of the C2/H2-type zinc finger motif. The expression pattern of the EPF1 gene parellels that of the EPSPS gene, suggesting that EPF1 is a positive transcription factor of the EPSPS gene. Supported by a grant from Monsanto Co.

**A 640** A *Brassica* S-GENE PROMOTER TARGETS CELL SPECIFIC DEATH IN TRANSGENIC *Arabidopsis*.

Mary K. Thorsness, M. K. Kandasamy, Mikhail E. Nasrallah and June B. Nasrallah. Section of Plant Biology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

In *Brassica* the S-locus controls the self-incompatibility response that blocks self-fertilization. Genes derived from the S-locus are expressed in a cell-type specific manner. In the pistil, S-gene expression is confined to the papillar cells at the surface of the stigma. S-gene expression in the anther is not well defined.

Promoter sequences from an S-locus gene were used to genetically direct cell death in the self-compatible crucifer *Arabidopsis*. A gene fusion was constructed that combined the promoter region of the *Brassica* SLG-13 gene and the diptheria toxin A (DT-A) gene. DT-A is a potent inhibitor of translation that can serve as a very sensitive indicator of gene expression. Flowers of *Arabidopsis* transformants that carried the DT-A gene fusion had distinct structural defects. The papillar cells at the stigma surface were stunted, and had striking ultrastructural abnormalities. Anther development was also affected in some flowers. These defects rendered transformants that carried the toxic gene fusion self-sterile.

**A 641** ANALYSIS OF POLLEN SPECIFIC GENE EXPRESSION IN TOBACCO,

Koen Weterings, Jan Schrauwen, Wim Reijnen & George Wullems, Department of Molecular Plant Physiology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

Molecular insight into the processes underlying pollen development and germination can be obtained by analyzing pollen specific gene expression. For this purpose we have constructed a cDNA library against mRNA from mature pollen. By differentially screening this library a pollen specific clone was isolated (p303;2100bp; full length). p303 is solely present in pollen; not in other generative or vegetative tissues. By in situ hybridization expression has been localized within the generative cell of the pollen grain. The cDNA clone is expressed in a wide range of plant species. After the microspore mitosis the first 303 transcripts appear and they accumulate until anthesis. This pattern is also found in in vitro ripened pollen. At least during the first two hours of germination de novo synthesis of 303mRNA takes place. p303 probably has a function during pollen tube growth or fertilization. Sequence analysis of the cDNA clone has not revealed similarity to other published sequences. A genomic clone has been isolated and will be used for isolating pollen specific promoter sequences.

Another cross hybridizing clone, p903 is 8000bp in length and hybridizes to a larger transcript on Northern blot. The regulation of p903 expression and its relation to p303 is presently under study.

**A 642 CLONED cDNA AND GENOMIC DNA SEQUENCES ENCODING CALMODULIN AND CALMODULIN-LIKE PROTEINS AND THEIR EXPRESSION IN TRANSGENIC PLANTS**, Raymond E. Zielinski, Vincent Ling, and Imara Perera, Department of Plant Biology, University of Illinois, Urbana, IL 61801

Calmodulin (CaM) is a $Ca^{2+}$-binding protein involved in the transduction of external signals to intracellular response elements. We have cloned cDNA sequences encoding CaM from *Arabidopsis*, barley, and carrot. The *Arabidopsis* genome encodes at least two CaM isoforms (ACaM-1 and ACaM-2) that differ in four of 148 amino acid residues. Northern blot and PCR experiments indicate that ACaM-1 and ACaM-2 mRNAs are expressed to equal extents in all vegetative and flowering tissues. It is not clear, however, whether there are significant biochemical differences between ACaM-1 and ACaM-2 proteins. A barley CaM cDNA was introduced into tobacco *via Agrobacterium*-mediated transformation. A CaMV 35S promoter was used to drive sense and anti-sense CaM mRNA expression with the aim of testing the effects of over- and under-production of CaM. In both cases, the steady state levels of CaM mRNA could be altered by 10- to 30-fold. However, CaM protein levels never varied by more than two-fold. Our results suggest that CaM protein accumulation is strongly regulated post-transcriptionally. We have also cloned cDNAs encoding a structurally related, 21.6-kDa $Ca^{2+}$-binding polypeptide from *Arabidopsis* (p21). The derived amino acid sequence encoding p21 shares 65% similarity with the CaM sequences, but only three of the predicted sequence changes alter proposed $Ca^{2+}$-binding ligands.

*Late Abstracts*

**Paramutation of the Maize *R* Gene**, Mary Alleman and Jerry Kermicle, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706

*R* is a regulatory gene of the anthocyanin pathway in maize. During paramutation, the *R* alleles in certain heterozygotes interact; one allele induces a change in the other allele in a highly-specific, directed manner. The altered (paramutant) allele, designated *R'*, confers a reduced level of anthocyanin pigmentation on the kernel. The phenotype is inherited in the absence of the inducing (paramutagenic) allele. Both paramutant and progenitor *R* alleles show imprinting effects in the endosperm. *R* and *R'* specify a fully pigmented phenotype when inherited from the female parent but darkly (*R*) and lightly (*R'*) mottled phenotypes when inherited from the male parent.

The molecular consequences of paramutation include the hypermethylation of cytosine residues in *R* locus DNA. Paramutant *R'* is hypermethylated relative to its progenitor based on gel blot analysis using restriction enzyme isoschizomers. Identical methylation patterns were produced using the DNA from tassel, immature ear, leaf, root and seedling. Analysis of endosperm DNA from reciprocal crosses involving *R* and *R'* with an *R* locus deletion showed that both alleles are more methylated following male transmission than following female transmission or in the sporophytic tissues tested. Treatment of dry seed with the alkylating agent diethyl sulfate reduced the extent of paramutation in *R'* and the strength of the paramutagenic allele by 20-50%. Phenotypic variants are recovered in at least 80% of the progeny of the treated material. According to the rules governing the tassel and ear progenitor cells in the dry seed, these frequencies suggest that the DES induced changes occur in most cells of the dry seed. Methylation patterns of DNA obtained from $M_0$ plants and their progeny indicate that *R* DNA was demethylated during the chemical treatment.

ISOLATION AND CHARACTERIZATION OF cDNA AND GENOMIC DNA ENCODING WHEAT 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE (HMGR), Kazuko Aoyagi, Anne Beyou, Larry Fang, David Korman, Keith Moon, and Tom H. Ulrich, Sogetal, Inc., 3876 Bay Center Place, Hayward, CA 94545

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is a key enzyme in the isoprenoid biosynthetic pathway which gives rise to membrane sterols, carotenoids, sesquiterpenes, diterpenes, and a variety of other isoprenoid containing compounds. In order to directly isolate expressed wheat HMGR gene fragments, degenerate oligonucleotide primers were designed to regions of amino acid sequence homology reported to be highly conserved among several diverse organisms. Using these primers and the polymerase chain reaction (PCR), partial cDNA fragments encoding wheat (*Triticum aestivum*) HMGR were synthesized from first-strand cDNA templates made from total wheat RNA. Comparison of sequence data obtained from several of these cloned wheat HMGR cDNAs shows that they represent a small number of discrete sets of expressed HMGR genes which share a high degree of HMGR sequence homology among themselves as well as among other organisms including tomato, *Arabidopsis*, hamster, human, *Drosophila* and yeast. At present, several genomic clones have been isolated from a wheat λ EMBL3 genomic library. The cloning strategy and characterization of these cDNA and genomic clones will be discussed.

A NEW BIOACTIVE XYLOGLUCAN UNDECASACCHARIDE AND CONFIRMATION BY CHEMICAL SYNTHESIS OF THE STRUCTURE OF A PREVIOUSLY CHARACTERIZED BIOACTIVE NONASACCHARIDE. Christopher Augur, Lu Yu, Alan Darvill and Peter Albersheim, Complex Carbohydrate Research Center and the Dept. of Biochemistry, University of Georgia, 220 Riverbend Rd. Athens GA 30602

Xyloglucan, a hemicellulose isolated from soluble extracellular polysaccharides of suspension-cultured sycamore (*Acer pseudoplatanus*) cells, was digested with a purified fungal $\beta$-1,4-endoglucanase. A nonasaccharide (XG9) from this digest, inhibited 2,4-dichlorophenoxyacetic acid (2,4-D)-stimulated elongation of etiolated pea stem segments. The inhibitory activity of XG9 exhibited a concentration optimum around $10^{-8}$ M. The fucosyl-galactose side chain of XG9 is essential but not sufficient for biological activity. It was therefore of interest to determine the biological activity of related xyloglucan oligosaccharides with the characteristic disaccharide side chain. An undecasaccharide containing two fucosyl-galactose side chains consistently exhibited greater inhibition of 2,4-D stimulated growth than did XG9. XG8, which lacks the terminal fucose of XG9, is inactive. We are studying the role of a fucosidase present in cell walls that removes the essential fucose residue. The inhibitory properties of chemically synthesized XG9[1] is indistinguishable from that of XG9 isolated from xyloglucan.

Acknowledgements: This work is supported in part by U.S. Department of Energy grant DE-FG09-85ER13425, and by U.S. Department of Energy grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers Program.

[1]*Tetrahedron Letters*, 1990, *31*:3035-3038.

REGULATION OF ANTISENSE SEQUENCES IN PLANTS UTILIZING tRNA PROMOTERS, June E. Bourque and William R. Folk, Department of Biochemistry, University of Missouri, Columbia, Missouri, 65211.

The major function of tRNA is its involvement in the translation of mRNA into protein of the ribosome. While the functions of translation are mediated by the ribosome, the fidelity of protein synthesis is dependent on the esterification of the correct amino acid to the cognate tRNA, as well as on the specificity of codon-anticodon interactions between mRNA and tRNA. The tRNA structure must contain all information necessary for specific interactions with components in the translation apparatus that interact with all tRNAs. The $tRNA^{met}_i$ gene encodes the initiator tRNA utilized for virtually all cytoplasmic protein synthesis in plants and as such is functional in every plant tissue making it ideal for use as a constitutively expressed promoter. We have isolated, cloned and sequenced a plant $tRNA^{met}_i$ gene from soybean and *Arabidopsis*. We have placed several regions from the chloramphenicol acetyltransferase (CAT) gene immediately downstream of the coding region. *In vitro* transcription assays, using human 293 whole cell extracts, indicated that the $tRNA^{met}_i$ gene correctly initiated transcription, reading through the antisense sequence, and terminated at a predicted stretch of 4-5 thymidine residues. We have achieved suppression of the target gene, with differential expression from the sequences of the 3' and 5' portions of the CAT gene, by electroporating DNA and RNA(transcribed using the T7 polymerase) into carrot plant cells. These results as well as the fate of the RNA within the cell will be discussed.

SPECIFIC REGULATION OF A TOBACCO GLYCINE-RICH PROTEIN GENE BY A FUNGAL GLUCAN PREPARATION, K. Brady, A.G. Darvill, and P. Albersheim, Department of Biochemistry, and Complex Carbohydrate Research Center, 220 Riverbend Road, The University of Georgia, Athens, GA 30602

A glucan preparation from the mycelial cell walls of the fungus *Phytophthora megasperma* f.sp. *glycinea* protects *Nicotianae* species against viral infection when applied before, during, or up to 8 hours after virus innoculation (Kopp *et al.*, (1989) *Plant Physiol. 90*:208). This glucan treatment causes no significant increase in the levels of any of several early and late phenylpropanoid pathway enzymes, a variety of pathogenesis-related proteins, hydroxyproline-rich glycoproteins, lignin or lignin-like substances, or callose (Unpublished results of Rouster, Kopp, and Fritig). Nucleic acid probes have been used to assay for increased mRNA accumulation in plants treated with the fungal glucan. The glucan treatment does not appreciably alter the mRNA accumulation levels of the genes for CHS, PAL, HMG-CoA, HRGP, PR-1a, PR-1b, PR-E, PR-B, chitinase, and $\beta$-1,3-glucanase. However, mRNA levels corresponding to a glycine-rich protein (GRP) gene are significantly increased within 15 minutes by the glucan treatment. Cellular localization studies of the responding GRP gene are currently being done and these results will be presented.

Acknowledgements: This work is supported in part by U.S. Department of Energy grant DE-FG09-85ER13425, and by U.S. Department of Energy grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers Program.

PROMOTER ANALYSIS OF CYTOSOLIC GLUTAMINE SYNTHETASE FROM PEA

Timothy Brears and Gloria M Coruzzi
Laboratory of Plant Molecular Biology, Rockefeller University, 1230 York Avenue, New York, NY 10021

The promoter from the pea gene for cytosolic GS3A has previously been shown to confer phloem-specific expression to the GUS reporter gene in leaves, stems, roots, and cotyledons of transgenic tobacco (Edwards et al 1990; PNAS 87:3459). This phloem-specific expression pattern correlates with the role of cytosolic GS3A in the synthesis of glutamine for nitrogen transport. Here, we show that the pea GS3A promoter also confers phloem-specific gene expression when introduced into an amide transporting legume (alfalfa), in leaves, roots, and cotyledons. Studies are underway to assess GS3A expression in nodules of the transgenic alfalfa. Promoter deletion analysis has been undertaken to define the minimal region of GS3A required for phloem-specific expression in tobacco and alfalfa. The 5' deletions define a critical region of the GS3A promoter to be contained within -174 relative to the start of transcription, while the 3' deletions which encompass the GS3A mRNA leader also seem to contain regulatory elements. In parallel, gel-shift analysis has been utilized to assess the protein binding activity of the *cis*-regulatory sequences which we have identified to be important for expression *in vivo*. The region of the GS3A promoter from -174 to the start of transcription binds numerous proteins present in nuclear and whole cell extracts of pea and tobacco, among which is the factor GT1 which has been implicated in regulating other plant genes (e.g. rbcS). We are attempting to clone these various DNA binding proteins by southwestern screening of cDNA expression libraries.

MOLECULAR CLONING OF A DISEASE RESISTANCE GENE FROM MAIZE, Steven P. Briggs and Gurmukh S. Johal, Pioneer Hi-Bred Int'l, Johnston, IA 50131

The nuclear Hm1 gene confers dominant resistance to the ear mold and leaf blight pathogen, Cochliobolus (Helminthosporium) carbonum, race 1. A collection of Hm1 mutants was tested for cosegregation of the mutant hm1 alleles with transposable element-containing restriction fragments. The hm1-656 and hm1-1369 alleles cosegregated with a single Mu1 and Mu3 fragment, respectively. These fragments were cloned and restriction-mapped, revealing that the element insertions were within 1 kb of each other. Aberrant transmission frequencies indicated that the hm1-1790 allele is associated with a deletion. Hybridization with flanking RFLP markers and probes from the hm1-656 and hm1-1369 alleles showed that the deletion spanned the region of the Mu1 and Mu3 insertions but extended no farther than 5 cM on either side of the Hm1 locus. The hm1-656 and hm1-1369 probes also detect polymorphisms between the hm1-1040 allele and it's progenitor. Thus, in each of four cases, a forward mutation at Hm1 occurred concomitantly with a rearrangement of the cloned region.

SEASONAL EXPRESSION OF A LIGNIN SPECIFIC O-METHYLTRANSFERASE CLONED FROM ASPEN DEVELOPING SECONDARY XYLEM, Wilbur H. Campbell, Robert C. Bugos, Vincent L.C. Chiang, Phytotechnology Research Group, Department of Biological Sciences and School of Forestry, Michigan Technological University, Houghton, MI 49931

In hardwoods such as aspen (*Populus tremuloides*), O-methyltransferase (OMT) catalyzes *meta*-specific methylation of precursors of coniferyl and sinapyl alcohols, the monomers which undergo peroxidase-catalyzed free radical polymerization to form guaiacyl and syringyl lignins. We purified aspen bispecific OMT from developing secondary xylem and prepared a monospecific antibody. Antibody screening of a lambda gt11 cDNA library made from poly(A)$^+$ RNA of aspen developing xylem, gave a 1.5 kb clone for OMT, called Ptomt1. The deduced amino acid sequence of Ptomt1 matched internal peptides sequenced from OMT and expression of Ptomt1 in *E. coli* yielded lysates with bispecific OMT activity. Northern blots of RNA from aspen developing xylem showed the 3' untranslated region of Ptomt1 hybridized specifically to a 1.7 kb transcript. Aspen developing xylem was collected from field trees over the growing season of 1990 and analyzed for OMT mRNA, OMT and coniferyl alcohol dehydrogenase (CAD) activities. Two peaks of activity for both enzymes and OMT mRNA were found, with highest levels in late July and early August. The high late season OMT and CAD activities and OMT mRNA levels correlate with the formation of "latewood" in aspen, which is a more fibrous type of wood with fewer vessels. Differentiation of xylem depends on auxin transported from apical shoots, but control of the seasonal changes in xylem may involve other phytohormones or regulatory factors as modulators of expression of the genes for lignin precursor biosynthetic enzymes.

TISSUE-SPECIFIC AND PATHOGEN-INDUCED REGULATION OF ß(1,3)-GLUCANASES IN *NICOTIANA PLUMBAGINIFOLIA* PLANTS, Carmen Castresana, Fernanda de Carvalho, Godelieve Gheysen, Dirk Inzé and Marc Van Montagu
Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium)

To understand the regulatory mechanisms controlling the expression of plant defense-related genes, the ß(1,3)-glucanase *gn1* gene isolated from *N. plumbaginifolia* has been examined in detail. Expression of the endogenous gene in *N. plumbaginifolia* and a reporter ß-glucuronidase gene under the control of the *gn1* promoter in transgenic tobacco plants has been examined. Expression has been determined in healthy plants as well as after several stress treatments. A detailed histological analysis has been performed to identify the specific cells where the expression mediated by the *gn1* promoter takes place. In addition, the expression characteristics of a second ß(1,3)-glucanase gene (*gn2*) will be described. The results obtained suggest that the characterized ß(1,3)-glucanases play an as yet undefined role both in plant development and in the defense response against pathogen infection.

THE USE OF YEAST AS A TOOL TO STUDY AN *Aspergillus nidulans* TRANSCRIPTIONAL REGULATOR, Yun C. Chang and William Timberlake, Department of Genetics, University of Georgia, Athens, GA30602

Conidiophore formation during the asexual life cycle of *A. nidulans* requires the sequential transcriptional activation of many genes. One of these genes, *brlA*, plays a pivotal role in regulating this developmental process. Several lines of evidence indicate that *brlA* is a transcription regulator. To elucidate how *brlA* regulates transcription, we have used a heterologous gene expression system to identify genes under the direct control of *brlA*. A library was first constructed by inserting *A. nidulans* DNA upstream of *Cyc1-lacZ* fusion gene in a pLGΔ178-derived vector (Guarente et al. 1984). This library was transformed into a yeast strain containing a *brlA* gene which was under the *Gal1-10* promoter control. We induced *brlA* expression by using galactose as the sole carbon source and screened the library for response to *brlA* induction by plating the library on X-gal containing medium. Several clones were identified which showed a response to *brlA* activity. We sequenced these *A. nidulans* DNA containing clones and further showed that they did not respond to induction of *brlA* genes possessing missense mutations in the zinc finger regions. This heterologous gene expression system may provide a general way to identify the targets of transcriptional regulators.

Guarente, L., Lalonde, B., Gifford, P. and Alani, E. Cell 36: 503-511, 1984

ANALYSIS OF A PUTATIVE REVERTANT OF RESPIRATORY FUNCTION FOR THE PETUNIA CMS-ASSOCIATED LOCUS, M.B. Connett*, S. Izhar**, and M.R. Hanson*, *Department of Genetics and Development, Cornell University, Ithaca, NY, 14850, USA and **Volcani Center, ARO Institute of Field and Garden Crops, Bet Dagan, 50-250, Israel.

Cytoplasmic male sterile (CMS) lines of Petunia had previously been found to exhibit differences in respiratory electron transport compared to isonuclear male fertile lines and a fertility-restored line. We have analyzed somatic hybrids of a Petunia hybrida male fertile line and a Petunia parodii CMS line for partitioning of respiratory electron transport between the cytochrome oxidase and alternative oxidase pathways. The data are consistent with the hypothesis that the Petunia CMS-associated mitochondrial locus pcf is also associated with altered electron transport. Furthermore, a somatic hybrid line that has given rise to revertants to male fertility shows anomalous respiratory partitioning. This line is being examined for an altered pcf locus.

SEQUENCE ANALYSIS OF THE RICE TUNGRO BACILLIFORM VIRUS (RTBV) - A double strand DNA virus infecting monocots, Alexandre de KOCHKO[1], Rongda QU[1], Gary S.LACO[1], Maitrayee BHATTACHARYYA[1], B.L. Subba RAO[1], Maria KANIEWSKA[1], J. Scott ELMER[2], Dean E. ROCHESTER[1], Christine SMITH[2] & Roger N. BEACHY[1], 1: Department. of Biology, Washington University, St. Louis, MO 63130. 2: Monsanto Company, St. Louis, MO 63198

The Rice Tungro Bacilliform Virus (RTBV) is known to produce very severe disease in rice plants in Southeast Asia when the plants are coinfected with the Rice Tungro Spherical Virus (RTSV). RTBV has a double strand DNA genome of 7969 bp with five open reading frames on one of the two strands. ORF3 is a very large open reading frame whose predicted translational product is a polyprotein of 1665 amino acids. We identified on ORF3 the sequence encoding the 33 kD coat protein (CP), and confirmed its identity by expressing the CP sequence in *E.coli* . The amino acid sequence of ORF3 predicts consensus sequences for the proteinase activity and for a reverse transcriptase activity. These proteins, in association with the identification of a sequence complementary to met tRNA and its predicted role in priming of reverse transcriptase, suggests a relationship of RTBV with caulimoviruses, and predicts that RTBV is a pararetrovirus. A DNA fragment that contains a transcriptional promoter has been found in the intergenic region which starts at the end of ORF5. To date, the products encoded by ORFs 1-2-4-5 are unkown.

CLONING OF THE TEMPERATURE SENSITIVE FLORAL REGULATORY GENE *APETALA2-1* FROM *ARABIDOPSIS THALIANA*. Bart den Boer[1], Marc van Montagu[1], K. Diana Jofuku[2], and Jack K. Okamuro[2], [1]Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium and [2]Department of Biology, University of California, Santa Cruz, CA 95064.

Little is known about the molecular mechanisms that govern pattern formation in higher plants. Genetic studies in *Arabidopsis thaliana* have identified several regulatory loci that play a crucial role in the formation of the plant flower (1,2). Recently we cloned one of these genes by insertional mutagenesis using T-DNA from *Agrobacterium tumefaciens* and the transposable element *Tam3* from snapdragon. *Flower-1 (fl-1)* is a T-DNA/*Tam3* induced homeotic floral mutation. Allelism tests indicate that *fl-1* is a mutant allele of *Apetala2*, one of the best characterized floral regulatory loci in Arabidopsis (3). Using *fl-1* plant DNA sequences that flank the T-DNA insertion as a probe, we have cloned the corresponding gene region from the temperature sensitive mutant *apetala2-1 (ap2-1)* (4). By comparing the nucleotide sequences of *ap2-1* and its wildtype counterpart, we hope to identify sequences necessary for *Apetala2* activity. Results of our progress towards this goal as well as of gene expression studies using wildtype and mutant flowers will be presented.

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IMPORT OF ACYL CARRIER PROTEIN INTO CHLOROPLASTS: CoA-DEPENDENT MODIFICATION BY A CHLOROPLAST HOLO-ACP SYNTHASE, Michael D. Fernandez, and Gayle K. Lamppa, Department of Molecular Genetics and Cell Biology, The University of Chicago, 920 E. 58th St., Chicago, IL 60637.

Recently, we determined that import of a spinach acyl carrier protein (ACP) into chloroplasts does not require addition of the phosphopantetheine prosthetic group, and subsequently identified an enzymatic activity within the chloroplast that transferred the phosphopantetheine from CoA to ACP after its translocation (Fernandez and Lamppa, *Plant Cell* **2**, 1990). We have investigated the substrate specificity of this chloroplast holo-ACP synthase in order to ascertain both its role in the pathway of ACP maturation and its function within the cell. We have shown, using the products of an *in vitro* import reaction as the substrates, that the mature form of ACP (apo-ACP), after entry into the chloroplast and removal of the transit peptide, is modified by the holo-ACP synthase. Modification occurs optimally at 37 °C, and is inhibited by 5 mM 3',5'-ADP and 2 mM EDTA. An ACP construct (matACP) lacking the transit peptide was also converted to the holo-ACP form in an organelle-free assay, independent of precursor cleavage. The matACP construct was used to monitor the chromatographic separation of the holo-ACP synthase from the transit peptidase. Using fractions enriched for the holo-ACP synthase, it was demonstrated that the precursor of ACP is also modified in the presence of CoA, and subsequently can be proteolytically processed directly to holo-ACP. Superose-12 gel filtration analysis indicates that the holo-ACP synthase has an apparent $M_r$ of ~50,000. Taken together, our results provide evidence that the chloroplast contains the essential enzymatic machinery to produce holo-ACP from either the precursor immediately upon its import, or after its proteolytic maturation. Thus, we have individually reconstituted, *in vitro*, each step of a complete pathway for the synthesis and maturation of ACP.

THE DISCOVERY OF AN ANTIFREEZE-LIKE PEPTIDE IN SNOW MOLD WITH EPITOPIC HOMOLOGY TO THE WINTER FLOUNDER AFP, Fawzy Georges, W. Jay Newsted, Mohammed Saleem, Adrian J. Cutler, Bob Papish and Sandra Polvi, Plant Biotechnology Institute, National Research Council Canada, Saskatoon, Saskatchewan, Canada S7K 7R9

Snow molds are low-temperature-tolerant fungal pathogens of Canadian winter cereals. Their ability to develop and grow at temperatures approaching 0°C suggests that they may possess substances relating to low temperature tolerance similar to those found in the serum of the Atlantic winter flounder or the hemolymph of overwintering insects. We examined the protein extracts from four snow mold species, *M. borealis*, *C. psychromorbidus*, *T. idahoensis* and *T. incarnata*, by Western analysis using a polyclonal antibody raised against the flounder AFP. We observed, in only one species, *C. psychromorbidus*, a strong cross-reactivity with a low molecular mass peptide (~3200 Da) closely resembling that of the flounder AFP (3700 Da). The peptide appears to be expressed abundantly in response to low temperature treatment. At 10°C the peptide level is minimal and located mainly in the insoluble protein fraction, while at 5°C it accumulated copiously in both soluble and insoluble protein fractions. Thus, the peptide seems to be membrane-targetted. Preliminary Southern analyses of genomic DNA from *C. psychromorbidus* suggests that a degree of homology with the flounder AFP DNA may exist. Purification and further characterization of this peptide, and its regulatory mechanisms, is in progress with a view to extending its use to higher crop plants. We designate this peptide psychromorbidin.

IDENTIFICATION OF FUNCTIONAL DOMAINS IN THE MAIZE TRANSCRIPTIONAL ACTIVATORS *C1* AND *B*. Stephen A. Goff, Karen C. Cone, Michael E. Fromm, Vicki L. Chandler. Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan St., Albany, CA 94710; Division of Biological Sciences, University of Missouri, Columbia, MO 65211; Department of Biology, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The maize anthocyanin pigmentation regulatory proteins encoded by the *C1* and *B* genes were analyzed for functional domains by fusion with well characterized transcriptional activators. The putative DNA-binding region of **C1** fused to the transcriptional activation domain of **GAL4** activated transcription of anthocyanin structural gene promoters in *c1* aleurones, *c1 Rscm2* embryos, and *c1 r* embryogenic callus. Cells receiving these constructs accumulated purple anthocyanin pigments. The **C1** acidic region fused to the **GAL4** DNA-binding domain activated transcription of a **GAL4**-regulated promoter. The basic-helix-loop-helix domain of the *B*-encoded protein was also analyzed by deletion and substitution with heterologous helix-loop-helix and basic-helix-loop-helix domains. Results from these studies demonstrate the importance of these functional domains.

REGULATION OF INTRACELLULAR CALCIUM LEVEL AND MEMBRANE POTENTIAL BY ABSCISIC ACID IN BARLEY ALEURONE PROTOPLASTS, Sjoukje Heimovaara-Dijkstra, Mei Wang and Freek Heidekamp, Center for Phytotechnology RUL-TNO, Department of Molecular Plant Biotechnology, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The plant hormone abscisic acid (ABA) has been considered to play an important role in seed dormancy. Intracellular calcium is regarded as a major secondary messenger in hormone signal transduction. In order to understand the working mechanism of ABA in the regulation of seed dormancy, the effects of ABA on intracellular calcium concentration and on membrane potential have been investigated in barley aleurone protoplasts. Cytosolic calcium concentrations of these protoplasts were measured after stimulation with ABA by using the calcium-sensitive fluorescence dye Indo-1. Stimulation of protoplasts with ABA results in a rapid decrease of the cytosolic calcium concentration. The membrane potential of these protoplasts was monitored upon ABA stimulation by measuring the distribution of the lipophilic cation tetraphenylphosphonium. Upon stimulation with ABA a membrane potential hyperpolarization was observed which is possibly due to activation of plasma membrane $H^+$-ATPases. The possible effects of ABA-induced changes in intracellular calcium and membrane potential are discussed.

**ISOLATION AND CHARACTERIZATION OF THE SUCROSE PHOSPHATE SYNTHASE GENE FROM *Arabidopsis thaliana*.** Luis Herrera- Estrella, Araceli Oropeza, Beatriz Jimenez, Graciela Salerno, Horacio Pontis, Eduardo Zabaleta. Centro de Investigacion y Estudios Avanzados, Apartado Postal 629, 36500 Irapuato, Gto, Mexico.

The partitioning of assimilates from their site of synthesis to the sink regions of the plant is clearly of considerable agronomic importance. Sucrose is a major factor with regard to the source-sink relationship, since in most plants the solar energy converted to chemical energy is stored and translocated in the form of sucrose. It is then transported to the sink organs (tubers, roots or seed), where it is transformed into the final reserve substances. Sucrose phosphate synthase is the enzyme responsible for sucrose biosynthesis in plants. To understand the molecular events that regulate sucrose synthesis in plants we undertook the task of isolating the genes encoding SPS. Using a cDNA clone that we have previously isolated from wheat leave RNA, full length cDNA and genomic clones of SPS have been isolated for *Arabidopsis thaliana*. The molecular characterization of the gene and initial studies of its regulation will be presented.

A FAMILY OF PUTATIVE SOLUTE TRANSPORT PROTEINS IN PLANT VACUOLES, H.Höfte, K.D. Johnson, L. Hubbard and M.J. Chrispeels. Dept. Biology, Univ. of California, San Diego, La Jolla, CA 92093 and Dept. Biology, San Diego State Univ., San Diego, Ca 92182 (KDJ). A cDNA encoding an abundant, seed-specific, 27 kDa tonoplast intrinsic protein (TIP) from plant storage vacuoles was isolated from bean seeds. The deduced amino-acid sequence predicts a protein with 6 transmembrane domains. TIP is related to a growing family of intrinsic membrane proteins from various sources among which MIP (major intrinsic polypeptide), a channel-forming protein in mammalian lens fiber cells and GlpF, a protein mediating the uptake of glycerol across the inner membrane of *E.coli*. These data suggest that TIP is also involved in the transport of solutes across the tonoplast. The bean cDNA was used to isolate 3 TIP-like genes from an *Arabidopsis thaliana* genomic library. The temporal and tissue distribution of the transcripts of two of these genes was investigated. One messenger is specifically expressed in seeds and appears in developing siliques starting from 9 days after pollination. Interestingly, the second gene is expressed in all tissues investigated except seeds. Using a specific antiserum generated against a fragment of the protein encoded by this gene we could show that this protein is also tonoplast specific. These data suggest that different TIP isoforms have complementary functions in different tissues. We are currently investigating the cell-specific expression of a beta-glucuronidase reporter gene under control of the promotors of these genes in transgenic *Arabidopsis* plants. (Supported by grants from NSF and the USDA and an EMBO fellowship to HH).

DIFFERENTIATION OF PLASTIDS IN DEVELOPING PLANT TISSUE, Yasuko Kaneko*†, Hisashi Matsushima† and Kenneth Keegstra*, *Department of Botany, University of Wisconsin, Madison, WI 53706, †Department of Regulation Biology, Saitama University, Urawa, 338, Japan

During plant development, plastids exhibit very dramatic structural changes during their differentiation into specialized plastids, such as chloroplasts. Although the functions of the various plastids in developing tissues is not well defined, ultrastructural observation suggests some specific changes during each step of plant development. In order to characterize the plastids in developing plant tissues, ultrastructural differentiation of the plastids was examined by electron microscopy and correlated with expression of some of nuclear-encoded plastid proteins using molecular and immunocytochemical techniques. Two different developmental systems are being investigated. First is plastid differentiation in young developing pea leaves following germination, in which, plastids were observed in amoeboid stage, starch accumulating stage, and prolamellar body stage successively before they fully differentiate into chloroplasts. Differentiation of plastids was also examined during flower bud formation on tobacco pedicel segments cultured in vitro. Plastids with few lamellar structures containing electron dense material were observed in actively dividing meristematic tissue which eventually developed into flower primordia, while large starch accumulating plastids were observed in the sustaining cell layers. Results from molecular and immunocytochemical analysis will also be presented.

ANALYSIS OF THE ACTIVITY OF THE MAIZE TRANSPOSABLE ELEMENT, *Ac*, IN *ARABIDOPSIS* Janis Keller, Eda Lim, Doug James, and Hugo Dooner, DNA Plant Technology Corp., Oakland, CA 94608

*Ac* activity has been studied in 78 independent transformants of *Arabidopsis*, ecotype WS, using the *Ac*::SPT assay of Jones et al. (Science 1989). In this assay, when seedlings are germinated on streptomycin, green variegation against a white background indicates *Ac* activity. Fully green seedlings arise as a consequence of germ-line excision events. 74 of the 78 transformants have single *Ac* inserts by Southern blot and genetic segregation analysis and have very low *Ac* activity. In general, less than 10% of the seedlings are variegated and very few green progeny are obtained. Plants with multiple inserts show greater *Ac* activity and give more green progeny. This finding suggests a positive dosage effect for *Ac* in *Arabidopsis*, as has been observed in tobacco. The frequency of germinal excision events in the transformants with multiple loci is variable ranging from 0 to 41 %. Evidence will be presented that multiple green progeny from one individual may arise from premeiotic events, that reinsertion of the element occurs about 60% of the time, and that the variability of numbers of germinal events is due to the stochastic nature of *Ac* activity in *Arabidopsis*, i.e. transposition can occur at any point in the life cycle of the plant.

Attempts have been made to map the T-DNA insertion sites relative to the *Arabidopsis* RFLP map using IPCR generated T-DNA border fragment probes. However, despite a survey of a large number of restriction enzymes and 6 ecotypes of *Arabidopsis*, no RFLP was found with any of the IPCR probes analyzed. This finding which indicates a lower level of polymorphisms than anticipated in *Arabidopsis*, will be discussed in more detail.

PURIFICATION AND PARTIAL CHARACTERIZATION OF ENZYMES INVOLVED IN THE BIOSYNTHESIS OF POLY(γ-GLUTAMYLCYSTEINYL)GLYCINES, [(γ-EC)$_n$G's], GLUTATHIONE AND THEIR PRECURSORS IN *DATURA INNOXIA*. Cheryl R. Kuske, Pamela J. Anderson and Paul J. Jackson. Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

Metal-tolerant cell suspension cultures of *Datura innoxia* synthesize large amounts of poly(γ-glutamylcysteinyl)glycines [(γ-EC)$_n$G's], polypeptides that specifically bind certain toxic metal ions. In order to characterize the regulation of (γ-EC)$_n$G production, we are purifying enzymes involved in the synthesis of (γ-EC)$_n$G, glutathione, and their precursors. Purified proteins are being partially sequenced, and this information will be used to generate DNA primers for the amplification and isolation of genes encoding these enzymes. *O*-acetylserine sulfhydrylase, which catalyzes the formation of cysteine from *o*-acetylserine, has been purified. Physical and kinetic characteristics of this enzyme will be presented. An assay for γ-glutamylcysteine dipeptidyl transpeptidase has also been developed and this enzyme has been partially purified.

EMBRYO ABLATION IN *BRASSICA NAPUS* USING MODIFIED *PSUEDOMONAS* EXOTOXIN, Michael Lassner and Ann Koning, Calgene Inc., 1920 Fifth St., Davis, CA 95616

Cell autonomous cytotoxins could be powerful tools to study the spatial and temporal specificity of plant promoters. We have utilized a modified form of exotoxin A from *Psuedomonas aeroginosa* for such studies. The exotoxin was modified to act intracellularly and to exhibit low toxicity to mammalian cells. When fused to a constitutive promoter, the modified cytotoxin inhibited cell function in electroporated tobacco protoplasts. The exotoxin was fused to napin regulatory sequences and transformed into *B. napus*. The transgenic *Brassica* plants were phenotypically normal except for having shrivelled seeds--the shrivelled seed phenotype segregated as a mendelian trait linked to the exotoxin containing T-DNA. Examination of embryo development in the shrivelled seeds revealed that embryo development halted concomitant with the onset of napin expression.

PHENYLALANINE AMMONIA-LYASE GENE EXPRESSION IN TOBACCO IS NOT INDUCED BY TOBACCO MOSAIC VIRUS INFECTION, Huub J.M. Linthorst, Frans Th. Brederode and Joh. F. Bol, Department of Biochemistry, Leiden University, Einsteinweg 5, 2333 CC Leiden, The Netherlands

Infection of Samsun NN tobacco with tobacco mosaic virus (TMV) results in a hypersensitive response accompanied with the induced synthesis of a large number of proteins, among which are cell wall proteins and pathogenesis-related (PR) proteins. By using a cDNA probe corresponding to phenylalanine ammonia lyase (PAL) we have studied the expression of PAL upon wounding, ethephon treatment and TMV infection. Tobacco PAL is 82% similar to bean PAL and is encoded by approximately four genes in the tobacco genome. In non-stressed tobacco leaves, PAL mRNA was found to be present at very low levels, which became higher 24 to 48 h after wounding, in agreement with results obtained with other plant species. However, neither TMV infection nor ethephon treatment did induce PAL expression in the inoculated or the systemic leaves. In contrast, expression of PR-1 was induced to high levels by these treatments. These results are in contrast to those of Legrand *et al.* (Phytochem. 15, 1353-1359, 1976) who found a rapid increase in PAL activity upon TMV infection.

THE β-GLUCANASE GENE FAMILIES OF BARLEY AND RICE, James C. Litts, Carl R. Simmons, Amin Nouirey, Ning Huang, Erik E. Karrer, Steve J. Reinl, and Raymond L.Rodriguez, Department of Genetics, University of California, Davis CA 95616

Several diverse β-glucanases are involved in various processes during the life cycle of the plant. During germination, particularly in barley but also in a variety of other cereals, (1-3;1-4)-β-glucanases help make the stored reserves of the endosperm available to other hydrolases. A (1-3)-β-glucanase may play a role in protecting the germinating barley seed from fungal attack. Other β-glucanases are involved in coleoptile growth. (1-3)-β-glucanases have been well characterized as pathogenesis-related proteins in dicots and are hormone-inducible in some cases. We are interested in characterizing some of the members of this family of related proteins in cereals. Southern blots of barley and rice DNA probed with different β-glucanase probes consistently show several bands at low stringency. We have obtained clones in bacteriophage λ of several of these cross-hybridizing fragments. Comparison of the protein sequences deduced from the nucleotide sequences of these clones with other protein sequences reported in the literature revealed a great diversity of sequences. Several of our sequences fall into groups whose function, either catalytically or physiologically, is not yet known. Pairwise comparisons were used to group the genes according to their sequence similarity. The homology between different groups of β-glucanases is generally around 45%. B-glucanase genes obtained from both monocots and dicots share a common structure. The signal peptide coding region is interrupted by a large intron, while the entire mature peptide is encoded by a continuous open reading frame. The sequence of the (1-3;1-4)-β-glucanase isozyme I gene has been reported. Here we present the sequence of one of the unknown β-glucanase genes (designated λHV34) we have isolated from barley. The accumulation of mRNA encoding the (1-3;1-4)-β-glucanase of barley is enhanced in barley aleurones by GA. We have not found λHV34 transcripts in the germinating seed.

RECONSTITUTION OF A FUNCTIONAL RED CLOVER NECROTIC MOSAIC VIRUS BY RECOMBINATIONAL RESCUE OF THE CELL-TO-CELL MOVEMENT GENE EXPRESSED IN A TRANSGENIC PLANT, Steven A. Lommel and Zhonnguo Xiong, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616

An infectious transcription system has been developed for both red clover necrotic mosaic virus (RCNMV) RNA-1 and RNA-2. The 1.5 kb RNA-2 encodes a single 35 kDa polypeptide which facilitates cell-to-cell movement. Inoculation of RNA-1 infectious transcripts to transgenic *Nicotiana benthamiana* plants constitutively expressing the 35 kDa RCNMV cell-to-cell movement protein resulted in systemic infection. Virus from the non-inoculated, systemically infected leaves contained both genomic RNA-1 and RNA-2. Sequence analysis of the RNA-2 indicated that the 5' terminus of RNA-1 recombined with the 35 kDa cell-to-cell movement protein mRNA generating a chimeric RNA-2 molecule which could be replicated and packaged into virions. The reconstituted progeny virus composed of wild type RNA-1 and chimeric RNA-2 was able to form a systemic infection with wild type symptoms on normal *N. benthamiana* plants.

PHOTOAFFINITY LABELLING AS A TOOL TO STUDY AUXIN-BINDING PROTEINS IN WILD TYPE AND AUXIN MUTANTS, Heather Macdonald, Anne Blonstein and Patrick King, Friedrich Miescher-Institut, Postfach 2543, 4002 Basel, Switzerland

The photoaffinity label 5-azido-indole-3-acetic acid (azido IAA) was used to search for membrane-associated and soluble proteins that interact with auxin. Three soluble proteins from Hyoscyamus muticus which bind strongly with the label have been studied. In one case, the binding is apparently specific to auxins, auxin transport inhibitors and auxin analogues. The other two proteins seem to show indole-specific binding. A collection of auxin-resistant mutants of Nicotiana plumbaginifolia is being studied using azido IAA, to try to identify differences between the mutants and the wild type.

α-1,4-D-OLIGOGALACTURONIDES STIMULATE THE FORMATION OF FLOWERS AND INHIBIT THE FORMATION OF ROOTS IN TOBACCO EXPLANTS, V. Marfà, S. Eberhard, D. Mohnen, A. Darvill and P. Albersheim, Dept. of Botany and Biochemistry, and Complex Carbohydrate Research Center, 220 Riverbend Road, The University of Georgia, Athens, GA 30602; F. Cervone and G. de Lorenzo, Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza", Roma, ITALY 00100.

We modified a tobacco thin cell layer morphogenesis bioassay for testing morphogenesis-regulating activity of plant cell wall fragments. Pectic fragments were released from suspension cultured sycamore (*Acer pseudoplatanus*) cell walls by treatment with a purified endopolygalacturonase (EPG) from *Aspergillus niger*. Addition of pectic fragments to a culture medium containing 1.5 μM IBA and 0.9 μM kinetin induced the formation of flowers on the explants. The EPG-released pectic fragments are known to be primarily composed of the polysaccharides rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II), and α-1,4-linked-oligogalacturonides (OGs). Pectic components were purified and tested for flower-inducing activity. The larger OGs induced flowers to form on the explants. RG-I, RG-II and small OGs did not induce flowers to form. Highly purified OGs obtained by partial acid hydrolysis of citrus pectin were also able to induce flowers to form. OGs with a degree of polymerization (DP) of 12-14 were the most active at inducing flowers exhibiting half maximum activity at ≈ 0.4 μM. OGs with DPs<10 showed little or no activity at 4 μM. OGs with DPs>10 inhibit the formation of roots on the explants incubated in a medium containing 15 μM IBA and 0.5 μM kinetin. OGs also inhibit the formation of roots on tobacco leaf disc explants. The ability of oligogalacturonides to regulate morphogenesis is another biological activity of this pleiotropic oligosaccharin. Acknowledgements: This work is supported in part by U.S. Department of Energy grant DE-FG09-85ER13425, and by U.S. Department of Energy grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers program.

**LOW TEMPERATURE INTERRUPTS CIRCADIAN REGULATION OF TRANSCRIPTION OF NUCLEAR GENES ENCODING CHLOROPLAST PROTEINS IN CHILLING-SENSITIVE PLANTS.** Susan J. Martino-Catt[1] and Donald R. Ort[1,2]. Department of Plant Biology[1], University of Illinois and USDA/ARS[2], Urbana, IL 61801.

Under normal growth chamber conditions tomato (*Lycopersicon esculentum*) plants show marked changes in protein synthesis in response to light/dark cycles. In addition to these diurnal oscillations, the synthesis of certain proteins continues to oscillate in the absence of any external cues indicating the presence of an endogenous circadian rhythm in gene expression. Our studies have focused on three nuclear encoded chloroplast proteins: the major light harvesting chlorophyll a/b binding protein of Photosystem II (Cab), ribulose bisphosphate carboxylase/oxygenase (rubisco) activase and the small subunit (SSU) of rubisco. The net synthesis of both Cab and rubisco activase demonstrates a circadian rhythm in conditions of extended dark and light. In contrast, SSU is synthesized abundantly at all times of the cycle. Using various lengths of low temperature exposure given at different times during the day, we were able to demonstrate that chilling interrupts the normal circadian pattern of expression for Cab and rubisco activase. When plants are returned to room temperature, expression of these genes proceeds from the point in the rhythm they were prior to low temperature treatment. Analysis of steady state mRNA populations for Cab and rubisco activase at low temperature indicates the normal oscillation of mRNA has also been arrested. Transcription assays with isolated nuclei indicate gene transcription to be undetectable at low temperature. These data suggest the steady state levels are being maintained by message stabilization at low temperature rather than by altering rates of gene transcription. Further studies are being done to examine possible factors which might be involved in stabilizing these messages at low temperature.

Molecular Analysis of Genes That Direct Conidiophore Morphology in *Aspergillus nidulans*, Bruce L. Miller, Karen Y. Miller, Jiangou Wu, Tina M. Toennis and Angela R. Edwards, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843

Conidiation in *A. nidulans* is characterized by the orderly differentiation and spatial organization of well-defined cell types into a reproductive apparatus, the conidiophore. Chains of haploid conidia are produced upon this structure. Potential loci that control conidiophore morphology and conidia formation fall into two major genetic regulatory networks, one (Bristle) directing the linear progression from vegetative hyphal elements to conidia, the second (Stunted) directing conidiophore morphology. Lesions in either the Stunted (*stuA*) or Medusa (*medA*) genes result in aberrant patterns of cell differentiation and highly modified conidiophores. Products of these two loci are believed to act by modifying functions of the Bristle network. The gene product of at least one member of the Bristle network, *abaA*, is incorrectly localized in the conidiphore of the *stuA*1 type-mutant. The *stuA* gene has a complex organization that includes alternate patterns of RNA processing and two sets of transcriptional initiation sites that are differentially regulated. Several small ORFs are found in the long untranslated leaders of the *stuA* mRNAs suggesting additional, post-transcriptional regulation. A *stuA*(p)::*lacZ* fusion gene has been used to study translational regulation of the 590 amino acid *stuA* gene product and its *in situ* localization during conidiophore development. In a *medA*26 mutant, the expression of the abacus, *abaA*, transcript is negligible, suggesting the *medA* gene product is necessary for enhancement of *abaA* gene expression and differentiation of the conidiogenic phialide cell.

**REVERSIBLE INACTIVATION OF A TRANSGENE IN *ARABIDOPSIS***

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Several *Arabidopsis thaliana* plants carrying multiple copies of the hygromycin phosphotransferase gene do not transmit their resistant phenotype to the progeny in a Mendelian manner although the complete gene was meiotically inherited. The difference between genotype and phenotype was caused by transcriptional inactivation of the resistance gene. Tissue from sensitive transformants could regain antibiotic resistance under callus-inducing conditions. Rare, spontaneous reactivation was in addition observed after outcrossing with wildtype plants or different sensitive transformants, but gene expression was often again lost in the next generation. A reduction of the copy number was not a prerequesite for spontaneous reactivation. We shall discuss a possible interaction between homologous transgenic sequences.

**Analysis of regulation of transacting factors *in vivo*.** Gunther Neuhaus[1], Gabriele Neuhaus-Url[1], Ingo Potrykus[1], Fumiaki Katagiri[2], Katja Seipel[2], Nam-Hai Chua[2], [1]Institute for Plant Sciences, ETH - Zürich, CH-8092 Zürich, Switzerland, [2]Lab. of Plant Mol. Biol., The Rockefeller University, New York, USA.

So far the microinjection technique was only used for transformation experiments. In our laboratory we have extended the range of applications by using the unique advantages of this system: 1. Injection can be performed into defined single cells, even in multicellular structures. 2. Targeting of the injected substance to different cell compartments (nucleus, cytoplasm). 3. Injection of a defined quantity (titration of molecules). 4. Injection of different kinds of molecules (DNA, RNA, proteins, chemicals etc.) and all combinations. Using these possibilities we analysed the regulation of the transcription factor TGA1a, a tobacco transcription activator that binds specifically to the as-1 element (-83 to -63) of the CaMV 35S promoter *in vivo* (see also abstract Katagiri et al.: Structure-function analysis of a tobacco transcription activator.).

TRANSFER OF THE METHOMYL-SENSITIVITY TRAIT OF cmsT MAIZE TO TRANSGENIC TOBACCO PLANTS, Alan M. Myers, Carla M. Koehler, Jintai Huang, Ethan Hack and Robert W. Thornburg, Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011

The mitochondrial gene T-*urf13* from cmsT maize previously has been transferred to *E. coli* or yeast, and shown to cause sensitivity to HmT toxin, the causative agent of southern corn leaf blight. In this study T-*urf13* was introduced into the nucleus of transgenic tobacco plants. To target the T-URF13 protein to mitochondria, the T-*urf13* coding region was fused to that of the mitochondrial targeting sequence from $F_o$ ATPase subunit 9 of *N. crassa*. This gene fusion, prN9/T-*urf13*, was expressed using an enhanced 35S promoter from cauliflower mosaic virus. Several transgenic plants containing from one to three copies of prN9/T-*urf13* were examined for susceptibility to methomyl, a functional analog of HmT toxin, and for the ability to produce functional pollen. All tested plants containing prN9/T-*urf13* were killed when exposed to methomyl at a concentration of 9 mM, while control transformants were resistant to this concentration of the drug. Several prN9/T-*urf13* transformed plants were grown to maturity. Anthers from the first flowers of three different plants shed pollen, however, approximatley 90% of the pollen grains failed to stain with $I_2$/KI, suggesting they might not be functional. Furthermore, germination of pollen from the prN9/T-*urf13* plants was markedly abnormal compared to pollen from control plants. Additional analyses, including crosses between the prN9/T-*urf13* transformants and control plants, are in progress to further characterize pollen function in the methomyl-sensitive plants.

SITE-DIRECTED MUTAGENESIS OF THE D1 POLYPEPTIDE OF THE PHOTOSYSTEM TWO REACTION CENTER COMPLEX, Peter J. Nixon and Bruce A. Diner, E. I. du Pont de Nemours & Co., Experimental Station, CR&D Department, P.O. Box 80173, Wilmington, DE 19880-0173

In oxygenic photosynthesis, the light-induced oxidation of water to oxygen is catalysed by the membrane-bound protein-chlorophyll complex designated Photosystem Two. At the heart of this complex are two polypeptides called D1 and D2, which by analogy to reaction centers from purple photosynthetic bacteria are each thought to span the thylakoid membrane five times and bind all the redox-active components necessary for light-induced charge separation. The location of the manganese cluster that oxidises water is unknown, but the D1 polypeptide appears a strong candidate since the redox-active species $Y_Z$ which oxidises the cluster has been identified as tyrosine-161 of the D1 polypeptide. We describe the construction and analysis of mutants of the cyanobacterium Synechocystis PCC 6803 in which specific carboxylate, histidine, tyrosine and tryptophan residues of D1 have been altered. Of the 24 amino acids examined, we conclude that aspartate-170 is a ligand to the manganese cluster and that aspartate-342 and histidine-332 are excellent candidates for such a role.

ISOLATION OF AUXIN-INDUCED GENES FROM *ARABIDOPSIS THALIANA*,
Jennifer Normanly and Gerald R. Fink, The Whitehead Institute, Nine Cambridge Center, Cambridge, MA 02142

We have isolated three auxin-induced genes from *Arabidopsis thaliana* by probing an *A. thaliana* cDNA library with a pea cDNA clone which encodes an auxin-induced polypeptide of unknown function (1). The *A. thaliana* cDNA clones which hybridized to the pea sequence were then used as probes in Northern analysis of *A. thaliana* tissue (either leaf, hypocotyl, or stem) which had been incubated in the presence of the auxin, indole acetic acid (IAA). One of the cDNA clones (pIAA6-1) shows a five-fold induction in all three types of tissue upon the addition of IAA. The maximal induction in hypocotyl tissue occurs one hour after addition of the hormone, while maximal induction in leaf is reached after 6 hours. The second cDNA clone (pIAA6-23) shows an IAA response after one hour in both hypocotyl and leaf tissues, and the third cDNA clone (pIAA6 16) shows an IAA response in hypocotyl tissue only, with maximal induction reached after one hour. The DNA sequence of pIAA6-23 is 64% and 75% identical to the soybean auxin-induced genes Aux22 and Aux28 (2), respectively. We will fuse the promoter regions from the genomic clones which correspond to these cDNAs to the reporter gene encoding beta-glucuronidase (GUS). Transgenic plants containing these chimeric genes will be used to determine when and where these auxin-induced genes are expressed, and will enable us to screen for mutants which are unresponsive to auxin.

(1) Theologis, et al., J. Mol. Biol. (1985) 183:53-68.
(2) Ainley, et al., J. Biol. Chem. (1988) 263:10658-66.

**A DNA SEQUENCE INDUCING MUSHROOM DEVELOPMENT IN SCHIZOPHYLLUM,**
Carlene A. Raper and J. Stephen Horton, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405.

Mushroom production (fruiting) is normally under the control of the multiallelic mating-type genes in the Basidiomycete, *Schizophyllum commune*. We have isolated a cloned sequence, called *FRT1*, which upon transformation into the *S. commune* genome induces the *de novo* development of mushrooms in unmated isolates. Thus, *FRT1* overides the normal requirement of a mating interaction for fruiting. It also enhances normal mushroom development during the sexual cycle of this fungus that occurs after mating. We have shown, by DNA-DNA hybridization analyses, that this sequence has more than one location in the normal genome. It also shares some sequence similarity with one of the *Sc* genes (previously cloned by Mulder and Wessels, 1986. Exp. Mycol. 10: 214) that is transcriptionally regulated during mushroom development in this fungus. *FRT1* appears to be a regulatory member of a family of genes involved in the development and differentiation of the sexually reproducing organ in this simple eukaryote. We are presently characterizing this gene on the molecular level in order to determine its mode of action.

HEAT STABLE ABSCISIC ACID RESPONSIVE PROTEINS: POSSIBLE ROLE IN CONFERRING RESISTANCE TO ENVIRONMENTAL STRESS,
Robertson, Albert J., Masaya Ishikawa and Lawrence V. Gusta, Crop Development Centre, University of Saskatchewan, Saskatoon, Sask. Canada S7N 0W0.

Exogenous (±)ABA (75 µM) increased freezing tolerance to <-40°C compared to -7°C for control bromegrass suspension cultures grown at 25°C for 7 days. ABA treated cells also showed increased resistance to heat, salt and osmotic stresses. Electrophoretic analyses identified proteins (25 to 30, 42 to 46, and 200 kD) which increased in intensity during both ABA, low temperature and salt and osmotic stresses. High resolution comparative 2D SDS-PAGE of total cell protein isolated from control cultures and those treated with 75 µM(±) ABA identified 33 ABA responsive proteins. Proteins increased by low temperature, salt and osmotic stress formed a subset of the ABA responsive proteins. Approximately 25 of the ABA responsive polypeptides were resistant to coagulation after 30 min. at 90°C, including the abundant 42 to 46 kD family of proteins induced by (±) ABA, salt and osmotic stresses. In vitro translation of poly A+ RNA isolated from ABA treated cell cultures identified a series of abundant translates in the 42-46 kD region and some minor translates in the 25 to 30 and 70 to 75 kD region which also showed thermostability. To test the hypothesis that these thermostable proteins play a role in conferring tolerance to thermosensitive proteins, in vitro heat denaturation studies were performed. Control protein fractions coagulated after 8 min. at 50°C, whereas protein fractions containing heat stable ABA induced proteins showed no evidence of heat induced coagulation after 20 mins. The role played by ABA induced heat stable proteins in conferring tolerance to environmental stresses will be discussed.

## TRANSFORMATION OF THE MOSS *PHYSCOMITRELLA PATENS*

Didier G. SCHAEFER and J:-P. ZRYD, Laboratory of Cellular Phytogenetics, Collège propédeutique, University of Lausanne, CH-1015 LAUSANNE, SWITZERLAND.

Protoplasts of the moss *Physcomitrella patens* were transformed using a PEG procedure and expression vectors coding for kanamycin and hygromycin resistance. Two classes of transformants were recovered. The first one, which occurred at a relative transformation frequency (RTF) of about $10^{-5}$, was characterised by a normal pattern of growth, stability of the new phenotype in the absence of the selection pressure, meiotic transmission and mendelian segregation of the resistance. These clones were therefore considered to be true transgenic mosses. The second class, which occured at a RTF around 20%, was characterised by alteration in growth and development and loss of the new character in the absence of the antibiotic. The new phenotype was mitotically unstable and was transmitted through meiosis at very low frequency. Segregation analysis and molecular data will be presented. We shall also discuss the nature of the second class of transformants.

PROTEIN SECRETION FROM BARLEY ALEURONE TISSUE, Maarten J. Schans, Bob Bakhuizen, Martien P.M. Caspers, Freek Heidekamp, Betty E. Valk, Joyce S. Velterop, Mariska A. De Vries, Mieke J. Van Zeijl and Karin M.C. Sinjorgo, Center for Phytotechnology RUL-TNO, Department of Molecular Plant Biotechnology, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The barley aleurone tissue provides an interesting system for an investigation of the mechanism of secretion in plant cells. During germination of barley seeds, a secretory apparatus (endoplasmic reticulum, golgi apparatus etc.) develops in the aleurone cells and hydrolytic enzymes are synthesized and secreted. These processes appear to be dependent on the hormone gibberellic acid (GA), which is thought to originate from the embryo. It has been demonstrated that isolated aleurone layers as well as protoplasts derived from this tissue show a GA-dependent secretion of hydrolytic enzymes.
Therefore, one of our approaches for studying the mechanism of protein secretion in barley aleurone tissue is based on transient gene expression in aleurone protoplasts. We have demonstrated that the cml and pat genes encoding the cytoplasmic proteins chloramphenicol-acetyltransferase and phosphinothricin-acetyltransferase, respectively are expressed in this system. Experiments including the analysis of secretion of proteins encoded by these genes fused to barley α-amylase signal sequences are in progress. Secondly, using isolated aleurone layers, studies are being performed in which the synthesis and secretion of hydrolytic enzymes from this tissue is monitored under various experimental conditions. The morphology of the cells is followed and compared to morphological changes occuring in barley aleurone of germinating seeds.

CHITINASES AND β-GLUCANSES IN RICE CELL SUSPENSION CULTURES OF LYSINE MUTANTS AND WILD TYPE CELLS, G.W. Schaeffer and P. Ueng, USDA, ARS, Plant Molecular Biology Laboratory, Beltsville, MD 20705

The biochemistry associated with inhibitor mutant selections in higher plants may contribute to crop improvements. The rice cells insensitive to inhibitory levels of lys+thr produced plants with improved lysine in protein as well as increased protein in seeds. The biochemical alterations responsible for the mutants are being examined. We report here the relationships between enzyme assays, stages in cell culture, protein levels and specific enzyme activity expressed on the basis of weight, mg protein and unit absorbance at 280nm. The results show that the overall growth characteristics of the mutant and controls are similar. The incorporation of 3H-lysine is different at 3, 5 and 10 days post dilution and large molecular weight proteins appear less regulated during early stages of cell growth than the controls. The protein content of the mutant is higher during the early stages of cell cycle in mutant than in the control. Chitinase is exported into the medium and strongly expressed at 5 days after the reinoculation. Methodologies for the purification of chitinases are given. Two clones of rice the chitinase gene, RC-13 and RC-16, have been isolated and identified from the rice EMBL3 genomic library with a bean chitinase probe.

THE EXPRESSION OF THE CAULIFLOWER MOSAIC VIRUS (CaMV) 62 KDA INCLUSION BODY PROTEIN IN TRANSGENIC NICOTIANA BIGELOVII ALTERS THE HOST RESPONSE TO CaMV INFECTION. J.E. Schoelz, *K.-B. Goldberg, and *J.M. Kiernan. Dept. of Plant Pathology, University of Missouri, Columbia, MO, 65211 and *Dept. of Plant Pathology, University of Kentucky, Lexington, KY, 40546.

The gene VI coding sequence of CaMV strain D4 was transferred into the genome of N. bigelovii through the use of the Agrobacterium vector pGA472. Virus D4 was chosen as the source of gene VI because previous work demonstrated that the gene VI product of this virus determined systemic infection of N. bigelovii. In order to demonstrate complementation, transgenic plants that expressed the CaMV 62 kD gene VI product and non-transformed controls were inoculated with recombinant virus H31, a virus composed of W260 and CM1841 sequences which could not systemically infect non-transformed N. bigelovii. Transgenic N. bigelovii inoculated with H31 developed systemic symptoms 3-4 weeks after inoculation while the non-transformed N. bigelovii controls remained symptomless. In order to show that no changes had occurred within the H31 genome that would affect host specificity, the H31 virus was isolated from systemically infected leaves of the transgenic plants and re-inoculated to both non-transformed and transgenic N. bigelovii. Again, the transgenic N. bigelovii developed systemic symptoms while the non-transformed controls remained healthy. We are now testing other strains of CaMV to determine if the transgenic N. bigelovii are susceptible to all CaMV strains.

STRUCTURE AND REGULATION OF A DEFENSE-RELATED BETA-GLUCANASE GENE IN RICE (*ORYZA SATIVA*), Carl R. Simmons, James C. Litts, Ning Huang, and Raymond L Rodriguez, Department of Genetics, University of California, Davis 95616

A rice beta-glucanase gene has been characterized and its regulation analyzed at the level of mRNA expression. The gene is expressed at relatively low levels in many tissues, such as shoots, callus, and developing panicles, but it is expressed at relatively high levels in the roots. Shoots treated with fungal elicitors, ethylene, salicylic acid, and heat shock all express the gene at higher levels (up to a 30-fold induction). Expression in the shoots is not enhanced by auxin, gibberellic acid, or abscisic acid. Expression in the roots is not affected by any of the above treatments and appears to be constitutive. PCR-amplified cDNA clones which span the intron have been sequenced to confirm the gene expression and the location of the intron. The beta-glucanase has 85% amino acid homology to the barley germination 1,3;1,4-beta-D-glucanases, and it is by hybridization homology the closest beta-glucanase in the rice genome to the barley germination beta-glucanases. The gene is, however, barely expressed if at all in germinating rice seeds. The molecular weight of the mature peptide is calculated to be 32 kD and the pI is 6.2. The gene has a very large intron of 3.1 kb in the codon of the 25th amino acid of the signal peptide. The gene exhibits a very strong codon bias of 99% GC in the third position of the codon in the region coding for the mature peptide, but only 58% in the signal peptide region.

MOLECULAR ANALYSIS AND REGULATION OF GENES CODING FOR AUXIN-BINDING PROTEINS
Etienne Schwob, Soon-Yong Choi, Larry L. Ilag and Dieter Söll
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Gravity is among the most important determinants of plant growth. Auxin is the only phytohormone directly involved (together with calcium) in the initiation of the gravitropic growth response. There is ample evidence which implies specific receptors for this hormone. Such putative receptors have recently been purified (1), and some of these auxin-binding proteins (ABP) show tissue-specific and developmentally regulated expression.

In collaboration with Dr. Palme's group in Cologne (FRG) we have cloned from *Zea mays* the axr1 gene encoding the major auxin-binding protein from corn coleoptile membranes. The DNA sequence (7 kb) of the gene containing four introns and up to 2 kb of the promoter has been determined. Deletions and fusions of this promoter to the GUS reporter gene have been made in order to characterize its functional domains. The regulation of the expression of the axr1 gene was studied in a transient expression assay after transformation of the *Black Mexican Sweet* corn cell suspension culture by electroporation. The tissue-specific expression of these constructs is tested in transgenic plants, by Ti-mediated transformation of *Arabidopsis* roots.

Comparative experiments are currently being done with genes coding for other auxin-binding proteins from corn (axr4) and *Arabidopsis* (axr1, p60). The regulation of these different genes and the specific advantages of each system (e.g. purification of trans-acting factors from corn; gravitropic mutants from *Arabidopsis*) should allow us to begin to understand the molecular mechanism of auxin perception and action.

1. Hesse,T., Balshüsemann,D., Bauw,G., Vandekerckove,J., Puype,M., Löbler,M., Klämbt,D., Schell,J. and Palme, K. (1989). EMBO J. 8: 2453-2461.

**THE SIGNAL FOR A LEAKY UAG IN SEVERAL PLANT RNA VIRUSES INCLUDES THE TWO DOWNSTREAM CODONS.** Jim Skuzeski, Lindy Nichols, Ray Gesteland and John Atkins. HHMI, University of Utah, Salt Lake City, UT 84112.
The ability of ribosomes to bypass certain stop codons is commonly utilized by plant RNA viruses to express internal protein coding regions. To analyze the sequence requirements for stop codon bypass, we constructed β-glucuronidase (GUS) expression vectors in which the $NH_2$-terminal coding region of GUS was interrupted by short sequences corresponding to the three known classes of leaky termination sites in plant viral RNAs, rendering synthesis of GUS subject to readthrough of stop codons. Analysis of GUS expression directed by the constructs in transfected tobacco protoplasts indicated that the sequence, -CAA-UAG-CAA-UYA-[1], which is found in tobacco mosaic virus, beet necrotic yellow vein virus and turnip yellow mosaic virus, supported readthrough at an efficiency of up to 5%. Mutational analysis showed that readthrough of the UAG stop is drastically affected by certain mutations in the 3′ flanking CAA-UYA sequence. In general, 3′ flanking sequences of the form CAR-YYA[1] conferred leakiness to a stop codon. Readthrough of the other two classes of leaky termination sites was not detected in the transient expression assay, suggesting that more distant sequences are involved. [1]Y=pyrimidine, R=purine.

ARE MODIFICATIONS IN FLOWER DEVELOPMENT ASSOCIATED WITH CHANGES IN MITOCHONDRIAL GENOME ORGANIZATION AND EXPRESSION UPON SOMATIC HYBRIDIZATION IN CYTOPLASMIC MALE STERILE LINES OF *NICOTIANA*?
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Somatic hybrid/cybrid plants were produced by one-to one electrofusion of defined selected protoplast-protoplast and protoplast-cytoplast pairs (microfusion) of male fertile *N. tabacum* and different alloplasmic cms mutant lines (with cytoplasms of *N. bigelovii* and *N. debneyi*) followed by microculture of the fusion products obtained. Analysis of the transmission genetics of organelles was performed using chloroplast (Str $^{r/s}$) and mitochondrial (mtDNA RFLPs) markers. In addition, variation in flower morphology, developmental histology and SEM studies on floral bud formation were carried out for cybrids from cms + cms' and cms + mf fusion combinations. Changes in mitochondrial genome organization as well as mitochondrial gene expression studied at the level of *in organello* translation products and mt transcripts were detected. Correlative evidences supporting an association of the observed changes with the homeotic floral phenotypes (petalody and feminization of stamina) will be discussed.

**TOBACCO NUCLEAR FACTOR CG-1: DNA-PROTEIN CROSSLINKING STUDIES AND COPURIFICATION BY DIFFERENTIAL SEQUENCE-SPECIFIC AFFINITY CHROMATOGRAPHY WITH A 21 KD POLYPEPTIDE** **Dorothee Staiger[1], Jeff Schell and Klaus Palme,** Max Planck-Institut für Züchtungsforschung, D-5000 Cologne 30, FRG
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The nuclear factor CG-1, identified in a variety of plant species including tobacco and snapdragon, binds specifically to the palindromic sequence motif CACGTG residing within the UV light responsive sequence of the Antirrhinum majus chalcone synthase promoter. Photoactivated DNA-protein crosslinking revealed three polypeptides with apparent molecular masses of approximately 21, 32 and 42 kD, respectively, binding to the CACGTG motif. Proteins interacting with the CACGTG motif were purified from tobacco seedlings using differential sequence-specific affinity chromatography. Denaturing polyacrylamide gel electrophoresis revealed a major polypeptide of 21 kD present in the affinity purified fractions.

Developmental regulation of Ri plasmid rolC gene in transgenic plant system. H. Uchimiya[1], Y.Oono[1], A.Suzuki[1],A.Kato[1],N.Fujii[2], R.Matsuki[2],T.Satomi[2] K.Kanaya[2]. 1)Botany Dept. Hokkaido Univ., Sapporo, Japan,and on leave from 2) Inst. Biol.Sci.,Tsukuba Univ.,Ibaraki, Japan.

We obtained carrot cells possessing the 5'-noncoding sequence of the rolC - GUS gene. When such cells were cultured in medium containing 2,4-D, substantial reduction in GUS activity was observed. Upon transferring the cells from a 2,4-D-containing medium to one devoid of 2,4-D, enhanced expression of GUS in somatic embryo development was recorded. Activation and suppression of GUS activities, in concord with embryogenesis, by $GA_3$ and ABA, respectively were also noted. The specific binding of a nuclear protein isolated from different tissues including roots, leaves and embryos to the 5'-noncoding region of the rolC detected by gel-shift suggested organ related binding factors. Footprinting with DNase I revealed the protection of a region where a common sequence identical to the AT-1 box (AATATTTTTATT) of the promoter of light-inducible genes was found.

PURIFICATION AND CHARACTERIZATION OF LIPOXYGENASE FROM GERMINATING BARLEY, Betty E. Valk, Anneke C. Douma, Ingrid Kokkelink, Simone van der Veen and Albert Doderer, Center for Phytotechnology RUL-TNO, Department of Molecular Plant Biotechnology TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Up to 5 % of the dry weight of barley seed consists of lipids, which are mainly stored in the embryo and in the aleurone layer. Upon germination, a significant decrease in the lipid content of the seed is observed. One of the metabolic pathways involved in this process of lipid degradation, is the lipoxygenase route. In the present study the key enzyme of this pathway, lipoxygenase, has been purified and characterized from embryo's of germinating barley seed. The purification procedure yielded pure preparations of two isoenzymes of lipoxygenase. Both isoenzymes have a molecular weight of 90 kD as determined by SDS-PAGE and crossreact with antibodies raised against pea lipoxygenase on Western blots. In addition, both lipoxygenases have an apparent $K_m$ of 2.0 μM for linoleic acid and a pH-optimum of 6.5. They differ however in several other properties. One of the lipoxygenase isoenzymes is active in a narrow pH-range, its isoelectric point is 5.2 and it exclusively forms the 9-hydroperoxide of linoleic acid as a product. The other isoenzyme is active in a broad pH-range, has an isoelectric point of 6.5 and it solely forms 13-hydroperoxide as a reaction product. Sequence data of one of the purified proteins indicate some homology with known sequences of lipoxygenases of other plants. Further characterization of the enzymes as well as the cloning of their corresponding genes are in progress.

POTATO VIRUS X REPLICATION IS ALTERED IN CO-INFECTIONS WITH POTATO VIRUS Y, Vicki Bowman Vance, Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208.

Potato virus X (PVX) and potato virus Y (PVY) co-infect tobacco to cause a classic synergistic disease. In the acute stage the disease is characterized by a dramatic increase in the accumulation of infectious PVX particles, with no corresponding change in the accumulation of PVY particles. We have examined the accumulation of PVX genomic RNA and coat protein in doubly versus singly infected tobacco leaves. Our experiments indicate that the steady state levels of both viral components increase in doubly infected plants to about the same extent (3-7 fold) as the level of infectious PVX particles. The level of PVX subgenomic coat protein mRNA found associated with polyribosomes of synergistically infected plants is also increased to a similar extent. Pulse labelling experiments suggest that the increase in PVX coat protein is due to an increased rate of synthesis. Interestingly, the level of PVX (-) strand RNA template increases disproportionately in doubly infected tissue, to a level three times higher than that of the virion or its component parts. This result suggests that PVX/PVY synergism involves an alteration in the normal regulation of the relative levels of PVX (+) and (-) strand RNAs during viral replication.

ANTHOCYANIN REGULATORY GENES FROM MAIZE (*B-PERU* AND *C1*) ACTIVATE THE ANTHOCYANIN PATHWAY IN WHEAT, BARLEY, AND OAT CELLS.

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Expression of the anthocyanin pathway in maize requires an active B or R gene and an active C1 or Pl gene.[1] Using particle bombardment, we have introduced *B-Peru* and *C1* cDNA clones, each controlled by a 35S promoter-intron array, into wheat (*Triticum aestivum, T. durum*), barley (*Hordeum vulgare*) and oat (*Avena sativa*) tissues. Purple-pigmented cells appeared 24-48 hours later in each case. Introduction of the genes individually did not result in anthocyanin production. Additional data on transactivation of maize reporter gene constructs, and tissue, genotype, and species specificity will be presented.

The significance of these results lies in 1) the inter-specific conservation of regulatory gene function for inducing this multi-step biosynthetic pathway, and 2) the potential for employing these genes to create pigmentation as a transformation marker in these species, as has been reported for maize.[2]

[1]Goff, S.A. *et al.* 1990. EMBO Journal 9:2517-2522.
[2]Ludwig, S.R. *et al.* 1990. Science 247:449-450.