PLANT GENE SYSTEMS AND THEIR BIOLOGY

Lee McIntosh and Joe Key, Organizers
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Poster/Discussion Sessions

Transcriptional Regulatory Elements (Keynote Address)

F 001 **REGULATION OF EUKARYOTIC TRANSCRIPTION BY THE INTERPLAY OF PROMOTER AND ENHANCER BINDING PROTEINS IN MAMMALIAN CELLS AND DROSOPHILA.** R. Tjian, J. Kadonaga, M. Briggs, P. Mitchell, D. Bohmann, U. Heberlein, B. England, M. Biggin, Dept. of Biochemistry, Univ. of Calif., Berkeley, CA 94720. 94720.

We have developed *in vitro* transcription systems to study the mechanism of promoter utilization by RNA polymerase II in eukaryotic cells. Reconstituted transcription reactions with partially purified components and DNA binding studies of purified proteins have revealed that transcriptional selectivity is mediated by complex control regions composed of interdigitated cis regulatory elements recognized by specific DNA binding proteins. Here, we report the identification and purification of several trans-activating factors that recognize and interact with control elements in mammalian and insect genes.

We previously identified a cellular transcription factor, Sp1, that binds to GC-box elements in the SV40 promoter as well as other viral and cellular genes. We have subsequently purified Sp1 to homogeneity, identified polypeptides of 105/95 kD as active species, raised monoclonal and polyclonal antibodies against Sp1 and recently cloned cDNA's encoding the Sp1 gene. DNA binding analysis has also uncovered several additional cellular activator proteins, AP1, AP2, and AP3, that interact selectively with different domains of the SV40 72 bp enhancer repeats. Binding studies suggest that some of these enhancer binding proteins may influence the activity of Sp1 and that T antigen, the virally encoded repressor of early transcription, can antagonize the action of these activator proteins. The relationship of these different trans-activators and repressors to mediate positive and negative regulation of SV40 will be discussed.

We have also developed cell-free extracts to study transcriptional regulation in Drosophila. In particular, we have made staged embryo extracts in an attempt to recapitulate the temporal program of alcohol dehydrogenase (Adh) and homeotic genes, *Ultrabithorax (Ubx)*, and *Antennapedia (Antp)* transcription *in vitro*. We find that the distal promoter of Adh is transcribed by staged extracts in a manner that follows the pattern observed *in vivo*. Analysis of promoter mutants, together with DNase footprint experiments, suggests that the regulation *in vitro* is at least partly due to the levels of transcription factor, Adf-1. This factor, which binds to and activates the distal promoter of Adh has been purified to homogeneity using specific DNA affinity chromatography. Surprisingly, the purified Adf-1 factor also binds strongly to the *Antp* P1 promoter. Its role in *Antp* transcription is currently being investigated.

We have also analyzed the signals involved in regulating transcription from the *Ubx* promoter in embryo and Kc cell extracts. Analysis of deletion and clustered point mutants indicate that sequences located both 5' and 3' to the *Ubx* start site affect the efficiency of initiation *in vitro*. Footprint analysis revealed the presence of several DNA binding proteins, including embryo-specific factors that selectively recognize these cis-regulatory elements. We are in the process of characterizing these factors in order to study their roles in regulating transcription during Drosophila embyogenesis.

Reproductive Biology - I. Flowering

F 002

Isolation and Characterization of Developmentally Regulated Sequences From Tomato Flowers. **Charles S. Gasser, Alan G. Smith, Kim B. Sachs, Sheila McCormick, Maud A. W. Hinchee, Dilip M. Shah, and Robert T. Fraley. Plant Molecular Biology, Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198.**

We have constructed cDNA libraries from the anthers and pistils of tomato flowers at several stages of development. A differential screening method has been used to isolate clones whose expression is differentially regulated during flower development. The temporal and organ specificity of these cDNA clones has been examined by Northern blot hybridization. Several of the clones appear to be expressed in a stage specific manner in either anthers or pistils. Additional clones have been found to be expressed in both anthers and pistils. A subset of the clones have been further characterized by *in situ* hybridization to sectioned floral organs. In these studies we demonstrate that the sequences we have isolated are expressed only in specific tissues or cell types in a given floral organ. All of these clones appear to come from single copy genes. Genomic clones have been isolated from a library of tomato leaf DNA for a number of the cDNA clones. The patterns of expression of these genes will be discussed in relation to the the process of floral development in tomatos.

F 003 DEVELOPMENTAL PATTERNS OF FLORAL INDUCTION IN TOBACCO. Carl N. McDaniel, Susan R. Singer, and Joan S. Gebhardt, Biology Department, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.

Floral induction has been extensively studied in photoperiodic plants but not in dayneutral plants. We have characterized floral induction in dayneutral Nicotiana tabacum cv. Wisconsin 38 by measuring the result of the induction process, floral determination (1,2). That is, an inductive signal acts on competent cells to elicit a new developmental fate which can be expressed in situ as well as in different environments (e.g. in isolation or in another place on the organism). In dayneutral tobacco both shoot apical meristems and internode cells become stably programmed to form flowers prior to the morphological appearance of flowers. The terminal meristem becomes determined for floral development about 4 nodes before it produces the terminal flower (3). At approximatley this same time some of the internode tissues in the middle of the plant gain the capacity to form floral shoots in culture (i.e. become florally determined). As the plant grows, tissues in more apical internodes gain the capacity to form floral shoots in culture. These data indicate a systemic signal(s) induces competent internode tissues and apical meristems to become committed to a program of floral development. Studies of N. tabacum cv. Maryland Mammoth, a short-day plant which differs from dayneutral tobacco by a single gene, indicate that the terminal meristem becomes florally determined just before or at the time of the initiation of the terminal flower. The control of the significant amount of vegetative growth which precedes floral induction and floral determination in both dayneutral and short-day tobaccos appears to involve the roots as well as the leaves. Rooting experiments indicate that in short-day tobacco the upper leaves can dominate over the root influence while in dayneutral tobacco the upper leaves are similar to the lower leaves in their interaction with the roots. Supported by grants from USDA (84-CRCR-1-1490) and NSF (DCB 84-09708).

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2. McDaniel, CN (1984) in Pattern Formation: A Primer in Developmental Biology, Malacinski, G, Ed., MacMillan, New York, pp 393-412.
3. Singer, SR and McDaniel, CN (1986) Dev. Biol., In Press.

F 004 THE INDUCTION AND INITIATION OF FLOWERS: GENERAL PERSPECTIVES, Daphne Vince-Prue, Department of Botany, University of Reading, Reading RG6 2AS, U.K.

The formation and growth of flowers involves a complex developmental sequence, with a number of component processes operating to produce a range of differentiated structures - the floral parts - which differ considerably in form between species. Although the initiation of flowers takes place at the shoot apical meristems, essential processes also occur in the leaves (induction) and, in some cases, controlling events can occur very early in the life of the plant (seed vernalization). The switch to flowering can be determined endogenously (autonomous induction) or by environmental signals, the most important of which are daylength and temperature. Flowering can also be modified by the application of a wide variety of chemical substances. The relationship between the induction process in autonomously-induced and daylength-induced flowering is reviewed briefly, before considering in more detail the events that take place during photoperiodic induction. The review concludes with a consideration of the nature of the cellular processes associated with vernalization, with induction in the leaf, and with the initiation of floral primordia at the apex.

Reproductive Biology - II. Fertilization

F 005 MOLECULAR ASPECTS OF SELF-INCOMPATIBILITY, M.A. Anderson, E.C. Cornish, S-L. Mau, A. Bacic, P.J. Harris and A.E. Clarke, Plant Cell Biology Research Centre, School of Botany, the University of Melbourne, Parkville, Victoria 3052, AUSTRALIA.

The interacting partners during fertilization in higher plants are pollen grains and the female pistil. If mating is compatible, pollen produces a tube which grows through the pistil to the embryo sac. In many plant families, inbreeding is prevented by rejection of the pollen tube after it has grown some distance down the style. Rejection is controlled by the product of a single gene, the S-gene, which has multiple alleles, S_1, S_2, S_3, S_4. We are investigating several aspects of self-incompatibility. (A) Nature of the S-gene: We have approached this by isolating cDNA encoding a M_r 32,000 style glycoprotein which segregates with the S_2-allele function (1). This S_2-cDNA has been used as a probe in hybridization histochemistry to show that the gene is expressed in the transmitting tract of the mature style. The S_2-cDNA shows homology in Northern blot hybridization experiments with mRNAs from mature styles of different S genotypes of Nicotiana alata and Lycopersicon peruvianum. (B) Nature of other style components: Arabinogalactan-proteins are major components of the extracellular mucilage of the female sexual tissues. These proteoglycans are developmentally regulated and are secreted in increased amounts in the stigma and ovary in response to pollination. We have isolated and partially characterized one group of these proteoglycans. (C) Structure of pollen tube walls: Saccharide analysis of isolated walls from in vitro grown pollen tubes show that, compared with vegetative cell walls, the walls of pollen tubes have a relatively simple composition: glucose and arabinose are the major components; the linkage analyses are consistent with the major wall polymers being a (1→3)-β-glucan (callose) and a (1→5)-α-linked arabinan with some branches through C(O)2. The arabinan is present in the outer layer of the wall and callose is present in the inner layer. Callose is absent at the tip of the pollen tube and is also deposited as plugs at regular intervals along the length of compatible pollen tubes. Information on pollen wall structure is essential to our eventual understanding of how the interaction of products of identical S-alleles in pollen and styles leads to arrest of pollen tube biosynthesis.

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F 006 APOMIXIS IN PLANT IMPROVEMENT, Wayne W. Hanna, USDA/ARS, Coastal Plain Experiment Station, Tifton, GA 31793.

Apomixis is a genetically controlled method of reproduction in plants where an embryo is formed without union of an egg and a sperm. There are three basic types of apomictic reproduction: 1) apospory - embryo develops from a chromosomally unreduced egg in an embryo sac derived from the nucellus, 2) diplospory - embryo develops from an unreduced egg in an embryo sac derived from the megaspore mother cell, and 3) adventitious embryony - embryo develops directly from a somatic cell. In most forms of apomixis, pseudogamy or fertilization of the polar nuclei to produce endosperm is necessary for seed viability. These types of apomixis have economic potential because they can cause any genotype regardless of how heterozygous to breed-true. In addition to fixing hybrid vigor, apomixis can make possible commercial hybrid production in crops where efficient male sterility or fertility restoration systems for producing hybrids are not known or developed. Apomicts are usually highly heterozygous because of their asexual reproduction and release large amounts of genetic variability when crossed as males with sexual plants. The apomictic mechanism can be 1) obligate - reproduce only by apomixis, or 2) facultative - a plant can reproduce by apomixis and/or sexually. Obligate apomixis is the most desirable in commercially economical crops especially if cross-compatible sexual genotypes, needed to produce hybrids are available in the species. Although facultative apomixis has been reported in cereals such as sorghum and pearl millet, genes controlling obligate apomixis are mainly found in distantly related wild species. Therefore, novel and unique procedures are needed to transfer gene(s) controlling obligate apomixis to cultivated species. Apomixis has been reported to be mainly controlled by one or a few dominant or recessive gene(s) in most species which improves the possibility of gene transfer from the wild to cultivated species. More success can be expected when working with apomixis if the species or genotype with the greatest number of ideal characteristics is selected. These include: simple, dominant inheritance; strong expression; environmentally stable; effects good seed set; obligate reproduction and some male fertility. The transfer, development, and use of gene(s) controlling apomixis in important world food crops provides a unique opportunity to researchers for producing superior plants in the future. The challenge can be met by combining plant breeding, cytogenetic, cell culture, and molecular techniques.

Bashaw, E. C. 1980. Apomixis and its application in crop improvement. In: W. R. Fehr and H. H. Hadley (Editors), Hybridization of Crop Plants, American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin, pp. 45-63.

F 007 MITOCHONDRIAL GENES AFFECTING POLLEN FERTILITY IN MAIZE, C. S. Levings III and Ralph Dewey, North Carolina State University, Raleigh, NC 27695.
We have identified a unique mitochondrial gene sequence in the T (Texas) type of male-sterile maize (cms-T) that appears to be responsible for the cytoplasmic male sterility (cms) trait. The gene, designated URF 13, has arisen by intra molecular rearrangements involving other mitochondrial gene sequences. The URF 13 gene contains sequences common to the flanking or coding regions of atp6 and the 26S rRNA genes. Immediately, downstream of URF 13 is an open reading frame, termed URF 25, that is found in the mtDNA of other maize cytoplasms and in bean, pea, tobacco, rice and wheat. The function of URF 25 is unknown.
URF 13 is uniquely and abundantly (steady state) transcribed in cms-T. Western blot analysis indicates that URF 13 encodes a 13,000 molecular weight protein that is located in the membrane fraction of the mitochondria. Two nuclear genes, Rf_1 and Rf_2, restore cms-T plants to male fertility. In the presence of restorer genes, the transcriptional pattern of URF 13 is altered and the amount of the 13 kd protein is substantially reduced. Thus, the nuclear restorer genes are able to modify the expression of the mitochondrial gene, URF 13.

Summation

F 008 PERSPECTIVES IN FLOWERING. Anton Lang, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.
The first part of this paper will be a survey of certain physiological factors involved in the regulation of the transition from vegetative growth to flower formation, with emphasis on graft-transmissible materials, presumably hormones, that can promote or inhibit that transition. The promotive material or "florigen" has been demonstrated in eight different plant families, while clearcut evidence for the inhibitory material or "antiflorigen" so far exists in one family (Solanaceae) only. Florigen and antflorigen share a number of distinctive characteristics: They are formed in the leaves; they are freely interchangeable between different species of a genus and between species of different genera; and they are also freely interchangeable between different physiological plant types, namely long-day, short-day and day-neutral plants. They thus seem to be identical in different plants, and non-specific in a taxonomic and a physiological sense.
The second part of the paper will consist of a review of molecular approaches to flower formation, pointing out precautions that need to be observed in the light of the physiological information discussed, and emphasizing the need of controls necessary to be certain of the specificity of the results obtained.
In the third and last part of the paper, the existence and role of physical factors in flower formation will be considered. These factors seem to be inherent in the organism as a whole and to become operative after the genes required for flower formation have completed their action.

Genes Regulated by External Stimuli

F 009 THE LACTATE DEHYDROGENASE SYSTEM OF BARLEY: INDUCTION AND FUNCTION DURING OXYGEN DEFICIT, Andrew D. Hanson, Neil E. Hoffman, David Hondred (MSU-DOE Plant Research Lab, Michigan State University, East Lansing, MI 48824), A.H.D. Brown (CSIRO Division of Plant Industry, Canberra, Australia) and Danny C. Alexander (ARCO Plant Cell Research Inst, Dublin CA 94568).

Plant roots and seed tissues have a capacity for lactate glycolysis, as well as for ethanol glycolysis, when deprived of O_2. Lactate glycolysis is associated with cytoplasmic acidification; acidification may act as a signal to activate ethanol glycolysis (1) although, when excessive, it can hasten cell death (2). Precise controls over the amount and activity of lactate dehydrogenase (LDH) are therefore expected. LDH activity is induced up to 20-fold in roots and seed tissues of barley during O_2 deprivation; the induction of enzyme activity corresponds to an increase in the synthesis of LDH protein and in the level of LDH mRNA. In barley LDH, as in other plants and in animal LDH systems, the holoenzyme is a tetramer with subunits of ca. 40 kD. Genetic analysis of barley LDH isozyme variants indicates that there are two *Ldh* genes, very closely linked on chromosome 4, whose products associate randomly to generate a set of five LDH isozymes. The *Ldh* genes appear to be coordinately controlled during O_2 deficit. However, at least in hypoxic seed tissues, the regulation of the LDH system may be independent of that of the alcohol dehydrogenase system (3).

1. Roberts JKM et al (1984) Proc Natl Acad Sci USA 81:3379
2. Roberts JKM et al (1984) Proc Natl Acad Sci USA 81:6029
3. Hanson AD, Jacobsen JV (1984) Plant Physiol 75:566

F 010 HEAT SHOCK GENE FAMILIES OF SOYBEAN AND THE REGULATION OF THEIR EXPRESSION, Joe L. Key and Ronald T. Nagao, Department of Botany, University of Georgia, Athens, GA 30602.

When an organism is shifted from its normal growing temperature to some higher temperature (usually an increase of about 10 $\pm$ 4°C), most cells/tissues of the organism undergo a stress response referred to as heat shock (HS). The physiological and biochemical responses elicited by HS are numerous. Fundamental to this response, however, are changes in gene expression; the transcription of most genes expressed at the normal growing temperature and the translation of most of the related mRNAs slow dramatically or cease. The expression of several families of genes (HS genes) is rapidly turned on which were either not expressed at a detectable level before HS or which were enhanced several-fold in their expression. These HS mRNAs accumulate to high levels and are preferentially translated. With continuous HS (for example, at 40°C with soybean) transcription of the HS genes slows or ceases after 1 to 2 hr and HS protein accumulation slows after several hours. The slowing or shut-down of synthesis of HS mRNAs and proteins occurs as a result of mechanisms which may be referred to as autoregulation, apparently as a result of the accumulation of functional HS proteins. The expression of HS genes and the production of HS proteins at a permissive HS temperature correlates with the acquisition of thermotolerance, i.e., tolerance to an otherwise lethal temperature. The HS response may be elicited 1) by a rapid shift from normal to the optimum permissive HS temperature with maintenance at that temperature, 2) by a gradual increase in temperature (e.g. 3° per hr), 3) by a brief (3 to 10 min) exposure to a lethal (if given for 1 to 2 hr) HS temperature, and 4) by some chemical agents (e.g. arsenite, cadmium).

Soybean seedlings produce a large number (about 40) of HS proteins which range in MW from about 15 kD to about 110 kD. We have isolated cDNA and genomic clones for most of the families of HS genes of soybean. The sequence analysis of several of these genes will be presented along with some properties of the proteins they encode. Some aspects of the regulation of expression of the HS genes will be presented.

F 011 WATER STRESS, ABSCISIC ACID AND GENE EXPRESSION, John E. Mullet[1], Robert Bensen[1], Hugh Mason[1,2], Connie Bozarth[2], and John S. Boyer[1], Department of Biochemistry and Biophysics[1], Department of Soil and Crops Sciences[2], Texas A&M University, College Station, Texas 77843.

Soybean seedlings were grown in darkness for 48 hrs then transferred to water saturated vermiculite (1X) or partially desiccated vermiculite (0.13X). Transfer of seedlings to 0.13X vermiculite caused a reversible decrease in shoot growth rate. Plants in 0.13X vermiculite took 72 hours to reach the same height as plants grown for 24 hours in 1X vermiculite. During the first 12 hours after transfer of plants to 0.13X vermiculite shoot growth appeared to be inhibited by the lack of a water potential gradient required to supply water to the growing region. However, at later times additional factors limited shoot growth. Abscisic acid levels increased 30 fold in the region of shoot elongation of 0.13X plants to 19 ng/g dry wt. Abscisic acid levels peaked when shoot elongation of 0.13X plants was completely inhibited (24 hours post-transfer) and declined as growth recovered. Application of exogenous abscisic acid to well watered plants inhibited shoot growth suggesting that abscisic acid could limit shoot growth in plants whose water requirements are satisfied.

We next characterized proteins, polysomes and polyA$^+$RNA in hypocotyl tissue of 1X and 0.13X seedlings. Cell wall proteins were extracted and two proteins, a glycine-rich 28 kDa protein and an isoleucine and tyrosine-rich 70kDa protein, were characterized (see Bozarth, Mullet and Boyer, these abstracts). The 28 kDa protein was relatively abundant in cell walls of dividing cells; in contrast, the 70 kDa protein was found primarily in cell walls of the mature region of the shoot. Transfer of plants from 1X to 0.13X resulted in an increase of the 28 KDa protein in the dividing region of the shoot with relatively little change in the level of the 70 KDa protein.

Polysome and RNA populations were also assayed in 1X and 0.13X plants. The percent of ribosomes in polysomes declined in the shoots of 0.13X plants which had reduced growth rates. However, total polyA$^+$RNA or polysomal mRNA populations in 1X and 0.13X plants were found to be strikingly similar when analyzed by in vitro translation and two-dimensional gel electrophoresis. Only four in vitro translation products increased in relative abundance in the 0.13X plants. These data suggest that limiting water supplies can cause dramatic changes in growth rate without causing major changes in the polyA$^+$RNA population of the soybean hypocotyl.

Gene Regulation by External Stimuli (Biological)

F 012 Expression of TMV Sequences in Transgenic Plants and Their Effects on Disease Development. Roger N. Beachy, Richard S. Nelson, Patricia Powell Abel, Carl M. Deom, Melvin J. Oliver, and Robert T. Fraley*. Washington University, St. Louis, MO 63130; *Monsanto Company, St. Louis, MO 63107. Chimeric genes containing the promoter of the 35S RNA transcript of cauliflower mosaic virus were linked to cloned cDNAs that encode the 30 kDa or the coat protein (CP) coding sequences of the U_1 strain of tobacco mosaic virus (TMV). Chimeric genes were introduced into tobacco cells on a disarmed Ti-plasmid in Agrobacterium tumefaciens. Insertion and expression of the introduced genes were assayed in plants regenerated from transformed cells. Seedling progeny of the parental transformants were subsequently inoculated with a variety of TMV strains and the susceptibility of seedlings were assayed by noting disease development and replication of virus. Transgenic seedlings that expressed the TMV CP coding sequence have a reduced number of sites of infection. Virus replication in the inoculated leaves was likewise reduced compared to replication in control leaves. Virus spread to upper leaves was also reduced in transgenic plants compared to the controls. These features are apparently responsible for the reduction in disease symptom development in plants that express the CP coding sequence in a manner similar to that in cross-protection. Plants that express the 30 kDa protein, which is proposed to potentiate the cell-to-cell movement of TMV, were inoculated with TMV strains L and LS1. LS1 is a variant of L that does not move in a systemic fashion in plants held at 32°, a non-permissive temperature because of a mutation in the 30 kDa gene of this virus. Transgenic plants that express the 30 kDa gene of the U_1 strain complement the mutation of LS1 by potentiating the movement of LS1 at the non-permissive temperatures. The implications of these results for studies of disease resistance and susceptibility will be discussed.

F 013 REGULATION OF ETHYLENE BIOSYNTHESIS IN TOMATO FRUIT: PROPERTIES OF ACC-SYNTHASE, Anthony B. Bleecker, Gail Robinson and Hans Kende, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824.

The enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase catalyzes the conversion of S-adenosylmethionine to ACC, the immediate precursor to ethylene in higher plants. Elevated levels of ACC synthase are often responsible for the increased rates of ethylene biosynthesis which occur during specific developmental stages and as a result of a variety of stresses. We are currently using monoclonal antibodies against ACC synthase to characterize the enzyme and to investigate the mechanisms by which its activity is regulated in tomato fruit tissue. Immunopurification of ACC synthase from tomato fruit indicates that the activity is associated with a 50 kDa polypeptide. An enzyme linked immunosorbent assay (ELISA) capable of detecting 0.1 ng of antigen was developed. Wound-induced increases in ACC-synthase activity in tomato fruit tissue were correlated with changes in ELISA-detectable protein. *In vivo* labeling of wounded tissue with [^{35}S]methionine followed by immunoaffinity purification of ACC synthase yielded a single radioactive protein band at 50 kDa. Experiments involving pulse labeling with [^{35}S]methionine at various times after wounding showed that wound-induced increases in ACC synthase activity involve *de novo* synthesis of a rapidly turning over 50 kDa protein. (Supported by the Department of Energy under Contract No. DE-AC02-76ER01338 and the National Science Foundation through Grant No. DCB 8416492).

F 014 VECTORS FOR CLONING AND MANIPULATION OF FUNGAL PATHOGENICITY GENES, O. C. Yoder, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Two genes, selectable in wild type cells, are generally useful for transformation of plant pathogenic fungi: *amdS* from *Aspergillus nidulans* enclodes acetamidase, which allows growth on acetamide as the sole nitrogen source; *hygB* from *E. coli* encodes hygromycin B phosphotransferase and when fused to a fungal promoter is expressed in fungal cells, which permits growth in the presence of hygromycin B. Vectors carrying either gene integrate stably into chromosomal DNA, almost always at a single locus. If the vector has no homology with the fungal genome, integration occurs at apparently random locations; a single copy of the plasmid may integrate or there may be multiple copies arranged tandemly, head-to-tail. With a short region of homology (1 kb) between the plasmid and the genome, 50-70% of integration events occur by homologus recombination. With a longer region of homology (6 kb), virtually all integration events are at the homologous site; a single copy of the plasmid integrates by a single cross-over event which results in a nontandem duplication of the homologous region with the plasmid sequence situated between the two repeats. The latter observation suggests that it will be possible to mutate a specific gene in the genome by first disrupting a cloned copy of it *in vitro* and then replacing the wild type chromosomal gene with the mutant copy using either a one-step (double cross-over) or two-step (single cross-over followed by excision of the wild type gene) procedure. Co-transformation, using a mixture of two different plasmids, occurs at high frequency and results in integration of both plasmids at the same chromosomal locus. The available vectors transform at least five plant pathogenic fungal species, including members of the genera *Cochliobolus*, *Glomerella*, *Nectria*, and *Leptosphaeria*. Several fungal promoters have been isolated by selection from a library of random *Cochliobolus* genomic DNA fragments constructed at the 5' end of a promoterless *hygB* gene. Two promoters have been sequenced; both are translational fusions to the *hygB* coding region which add 60-80 amino acids to the N-terminus of the *hygB* polypeptide. A cosmid carrying a *hygB*-promoter fusion has been constructed. It accepts 40 kb inserts, which allows a genomic library to be screened using only 2000 transformants; there is a probability of 0.98 that any particular gene will be found. Thus, it is now feasible to clone pathogenicity genes by complementing a strain carrying a recessive allele with a library of DNA from a strain carrying the corresponding dominant allele.

Enzyme/Gene Systems in Metabolic Regulation

F 015 GLUTAMINE SYNTHETASE: ORGAN-SPECIFIC EXPRESSION OF GENES ENCODING DISTINCT POLYPEPTIDES IN A NITROGEN-FIXING LEGUME, Gloria M. Coruzzi, Scott V. Tingey*, Elsbeth L. Walker, and Janice W. Edwards, The Rockefeller University, New York, NY 10021-6399. *present address: E.I DuPont de Nemours Co., Wilmington, DE 19898.

Glutamine synthetase (GS), the enzyme responsible for ammonia assimilation in higher plants, occurs as distinct isoforms that are differentially localized within subcellular compartments as well as among various plant organs. We have characterized the distinct polypeptides, primary translation products, and mRNAs encoding GS which are present in the leaves, roots, and nodules of pea. Leaves contain two distinct GS subunit polypeptides (44 and 38 kd), which are localized to the chloroplast and cytosol respectively. The predominant GS polypeptides present in nitrogen-fixing root nodules of pea are a 38 kd polypeptide which is also found in roots, and three 37 kd polypeptides which are expressed most specifically, but not exclusively in nodules. Three different GS cDNA clones have been isolated from pea leaf, root and nodule cDNA libraries. GS mRNA hybrid-selected from poly(A)RNA of each organ translates in vitro into three distinct GS polypeptides (49, 38 & 37 kd). Two cDNA clones (pGS341 and pGS134) correspond to GS mRNAs which encode the 38 and 37 kd GS primary translation products. The levels of these GS mRNAs are induced 10-20 fold in nodules compared to leaves or uninoculated roots. A third GS cDNA (pGS185) corresponds to a larger GS mRNA species which is specific to leaf poly(A)RNA. This GS mRNA encodes a 49 kd GS polypeptide which has been shown by in vitro chloroplast uptake to be a precursor to the 44 kd chloroplast stromal GS polypeptide. Northern blot analysis has shown that light affects the steady state levels of the mRNA encoding chloroplastic GS, while the levels of mRNA for cytosolic GS are unaffected by light. Southern blot analysis of nuclear DNA and analysis of GS genomic clones has shown that the distinct GS mRNAs for chloroplastic and cytosolic GS are encoded by homologous but distinct nuclear genes. Future studies are directed at identifying the cis-acting DNA elements of the GS gene promoters which are involved in the differential expression of the distinct members of this gene family.

F 016 STRUCTURAL AND REGULATORY FACTORS THAT AFFECT THE ACTIVITY OF RUBISCO, Steven Gutteridge, Central Research and Development Department, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

The factors that determine the activity of Rubisco are becoming better defined and indicate that turnover can be modulated at a number of levels. Structural determinants, for example, have been deduced by chemical and enzymological methods which, in conjunction with crystallography, have pinpointed essential regions of the protein that co-operate in the reaction mechanism. This information is being used to direct in vitro manipulation of the enzyme to define the function of individual residues at the active site. With respect to the regulation of Rubisco a wide range of naturally occurring phosphate esters that resemble the substrate have been demonstrated to mediate in activation and inhibit catalysis in vitro. In many plants, however, there is a specific and unique inhibitor, 2-carboxyarabinitol-1-phosphate (2CA1P) that is synthesized in the dark and accumulates in the chloroplast stroma. This molecule resembles the structure of the transition state of the carboxylase reaction with higher affinity for the carbamylated ternary complex of Rubisco. Thus, in reduced light, (2CA1P) binds to the enzyme inhibiting turnover, but preserving the activated state.

F 017 MOLECULAR IDENTIFICATION OF THE ALTERNATIVE OXIDASE OF HIGHER PLANTS. Lee McIntosh and Thomas E. Elthon. MSU-DOE Plant Research Laboratory and Biochemistry Department, Michigan State University, E. Lansing, MI, 48824 U.S.A.
Plant mitochondria contain two types oxidases in their mitochondria: the "normal" cytochrome oxidase and an "alternative" CN-resistant oxidase. The expression of this alternative oxidase is developmentally regulated and tissue-specific. However, the exact function for this oxidase is unknown except in certain aroids. It has been suggested that it acts as a "spillover" mechanism for excess electrons from the cytochrome system. Until now, very little has been known about the regulation of the cytochrome and alternative oxidases in relation to each other. We have found a close temporal regulation of each pathway during the flowering response of the aroid Sauromatum guttatum. Not only is the cytochrome pathway shut down during the single day of flowering (with a concurrent rise in alternative oxidase function), but the cytochrome pathway is reactivated during senescence. Also, during this senescent phase the alternative pathway disappears.

Recently, the alternative oxidase of Sauromatum guttatum appendix mitochondria has been highly purified in our laboratory using a combination of cation exchange and hydrophobic interaction chromatography (Proceedings of the Second International Meeting of Plant Mitochondrial, 1986, in press; Elthon and McIntosh, 1986, Plant Physiology, 82, pp. 1-6). The partially purified alternative oxidase was found to require lipids for maximal activity. Polyclonal and monoclonal antibodies have been raised to this preparation. The polyclonal antibodies are proficient in immunoprecipitating the proteins responsible for alternative oxidase activity. Results from the purification steps and immunoprecipitation indicate that two polypeptides near 33 kilodaltons and 35 kilodaltons make up the alternative oxidase.

F 018 PLANT ACYL CARRIER PROTEINS. John B. Ohlrogge, Daniel J. Guerra, Phillip D. Beremand, David J. Hannapel, Salah Elhussein, and Jan A. Miernyk, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604

Plant cells require constitutive, housekeeping expression of fatty acid synthesis genes in all growing tissues. During seed formation, oil storing plants also express fatty acid synthesis genes in a tissue-specific developmental fashion. We are examining the nature of this dual regulation through studies of acyl carrier protein (ACP), the central cofactor for plant fatty acid synthesis. ACP is a small (9 kDa), plastid-localized, nuclear-encoded protein that is required in at least 10 reactions of fatty acid metabolism. In vitro mRNA translation and immunoprecipitation demonstrate that an ACP precursor is synthesized that contains a 5-6kDa transit peptide. Before uptake into plastids, the pantetheine prosthetic group of ACP is attached by holo-ACP synthase which we have localized to the cytoplasm. We have purified two isoforms of ACP from spinach leaves that appear to be coded by different genes. Western blot analysis detects only one major isoform in seed and root tissue. The two leaf isoforms have different activities in acyl transferase and acyl hydrolase activities, suggesting that their expression may regulate the distribution of acyl chains within the plant cell. A synthetic gene for spinach ACP-I has been constructed, cloned, and expressed in E. coli. In addition to producing ACP for biochemical studies, we are using this synthetic gene to probe the expression of ACP genes in plants.

F 019 BIOCHEMISTRY AND MOLECULAR BIOLOGY OF STARCH SYNTHESIS. Jack Preiss, Matthew Morell, Mark Bloom, William C. Plaxton, Ray Larsen*, and Thomas W. Okita.* Department of Biochemistry, Michigan State University, E. Lansing, Michigan, 48824 and *Institute of Biological Chemistry, Washington State University, Pullman, Washington, 99164. Regulation of starch synthesis in chlorophyllous and non-chlorophyllous tissues of plants, is exerted at the level of ADPglucose pyrophosphorylase, the enzyme catalyzing synthesis of ADPglucose, the glucosyl donor of starch synthase. 3-P-glycerate (3PGA) activates and orthophosphate (P_i) inhibits ADPglucose synthesis. 3PGA at high concentrations reverses or overcomes the inhibition caused by P_i. Regulation of ADPglucose synthesis by 3PGA and P_i is observed in all leaf cell types in C_3, C_4, and CAM plants and in guard cells. Pyridoxal-P (PLP) has been shown to activate spinach leaf ADPglucose pyrophosphorylase activity about 5- to 6-fold. PLP also activates the enzyme from maize endosperm and Arabidopsis thaliana leaves. It is not as effective as 3PGA since maximal stimulation by 3PGA is 20-fold. PLP can also inhibit the activation caused by 3PGA and similar to 3PGA it can overcome the inhibition caused by P_i. These results suggest that PLP binds at or close to the activator site. Reduction of the PLP onto the spinach leaf ADPglucose pyrophosphorylase with NBH_4 gives an enzyme with 5 to 6 higher activity in the absence of activator than the untreated enzyme when about 0.5 mole of [^{3}H]-PLP is bound per mole of enzyme. This covalently modified enzyme is highly resistant to phosphate inhibition in contrast to the untreated enzyme which is quite sensitive to P_i inhibition (K_i = 42 μM). Covalent binding of PLP to the ADPG pyrophosphorylase will aid in determining the amino acid sequence at the allosteric activator site. Using antibody prepared against spinach leaf ADPG pyrophosphorylase and λGT11, a cDNA clone of rice endosperm ADPglucose pyrophosphorylase has been isolated. It is an Eco RI fragment of 1480 Kb giving an open reading frame of 354 amino acids. This amino acid sequence corresponds to about 23% homology to the Escherichia coli ADPglucose pyrophosphorylase. Of interest is that there is homology in 5 of the 6 amino acids at a site reputed to be part of the binding site for ADPglucose in the E. coli enzyme. Recent studies also show that a calcium dependent but calmodulin independent protein kinase from soybean phosphorylates both the maize endosperm and spinach leaf ADPG pyrophosphorylase. The effects of phosphorylation on the regulatory kinetics of the enzyme are presently unknown.

Promoters/Enhancers/Transgenic Expression - I

F 020 ANALYSIS OF PHOTOSYNTHETIC PROMOTERS IN TRANSGENIC PLANTS

Pamela Dunsmuir, Caroline Dean, Jonathan Jones, David Gidoni, John Bedbrook. Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608.

We have used promoter fragments derived from the petunia chlorophyll a/b protein (Cab) genes, and the ribulose bisphosphate carboxylase small subunit (SSU) genes, to express foreign genes in plants. The Cab promoter/foreign gene fusions are expressed in tobacco at levels which approach the level of the endogenous gene in petunia. The SSU promoter/foreign gene fusions are expressed at levels which are less than 20% of the endogenous SSU gene levels. The difference between these two promoter systems for high level expression of foreign genes in plants may be due to the presence of an enhancer element within the intron of the SSU gene.

F 021 NODULE SPECIFIC EXPRESSION OF A CHIMERIC SOYBEAN LEGHEMOGLOBIN GENE IN TRANSGENIC LOTUC CORNICULATUS. Marcker KA, Stougaard J, Jensen EØ, Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Århus C, Denmark.

Recently we have constructed chimeric genes with the composition leghemoglobin (Lb) 5' flanking region - coding sequence of the E.coli chloramphenicol acetyl transferase Lb 3' flanking region. This chimeric gene has been transferred to yeast and it was shown that the expression of the gene is under control of heme at the translational level. Using an Agrobacterium rhizogenes vector the chimeric gene was introduced into the genome of another legume species, Lotus corniculatus. Nodule specific expression of the chimeric gene was found in root nodules formed on fully regenerated plants inoculated with the lotus microsymbiont Rhizobium loti. Expression under the control of the 5' upstream region of the soybean gene was regulated at the level of RNA and followed the correct developmental timing. Sections of the 5' Lb region have been deleted from the chimeric gene and the resulting genes have been transferred to Lotus corniculatus. At least two distinct regions of the Lb flanking sequence are apparently important for the nodule specific expression of the chimeric genes. The nature of these two regions will be discussed. The entire Lbc_3 gene has also been transferred into Lotus by a similar method. It was shown that the gene is transcribed and the mRNA is translated into a stable protein product.

F 022 CIS- AND TRANS-ACTING ELEMENTS FOR LIGHT-INDUCED AND TISSUE-SPECIFIC EXPRESSION OF PLANT GENES, Ferenc Nagy, Cris Kuhlemeier, Pamela J. Green, Robert Fluhr, Steve A. Kay, Marc Boutry, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 20021-6399.

Nuclear genes encoding the chlorophyll a/b binding protein and the small subunit of ribulose 1,5-bisphosphate carboxylase are expressed in a tissue-specific, light-inducible manner. The light-regulated expression of these genes is mediated by phytochrome and manifested at the transcriptional level. In vitro DNA sequence manipulation coupled with expression in transgenic plants revealed different cis-acting elements for regulated expression of these genes. One of these elements, an upstream ca.260 bp enhancer-like sequence of the Cab-1 or of the rbcS-3A gene confers phytochrome regulated, tissue-specific expression on constitutive promoters. Detailed analysis of these two sequences allowed us to identify multiple regulatory elements within this region. The position and sequence of these negative and positive regulatory elements as well as their contribution to regulated gene expression (i.e. phytochrome mediated light-induction, tissue-specificity) will be discussed. In addition to cis-acting regulatory elements, our studies on trans-acting factors have led to the identification of DNA binding proteins specific for the rbcS-3A upstream region. The interaction of these proteins with known light regulatory sequences will also be discussed.

Promoters/Enhancers/Transgenic Expression - II

F 023 CIS-REGULATORY ELEMENTS IN HEAT SHOCK AND T-DNA PROMOTERS, William B. Gurley, Eva Czarnecka and Wesley B. Bruce, Department of Microbiology and Cell Science, 1059 McCarty Hall, University of Florida, Gainesville, Florida 32611.

Functional domains related to transcriptional activity have been identified in the 5'-flanking regions of two genes that function in plants: the "780" gene of T-DNA (T-right) and the heat shock gene hsp 17.5E from soybean. The 5'-flanking regions of these genes were mutated by 5'- and internal deletions, and also by the introduction of small duplications. The effects of these alterations on promoter activity were monitored by measuring steady state RNA levels in sunflower tumors using a T-DNA-based vector system. Precise quantification of transcript levels by S1 nuclease was made possible by incorporation of a homologous reference gene into the vector.

The heat shock gene hsp 17.5E contains several regions of high homology to the consensus sequence established for heat inducibility of animal genes. At least two of these heat shock elements (HSE's) are located TATA-proximal (positions -49 to -100) and appear to be essential for transcriptional activity. Several upstream clusters of HSE's are located between positions -359 and -582 bp. Removal of sequences upstream of position -95 bp reduces heat inducible transcription.

Deletion analysis of the 780 gene promoter suggests that this apparently nonspecialized T-DNA promoter has a structural complexity similar to plant genes regulated by developmental and environmental factors. The 780 promoter is comprised of three functional domains: the TATA, the upstream element, and the activator. The activator is located between -229 and ca. -440 bp and may contain multiple subelements. This element shows enhancer-like properties in its capacity for bidirectional action and in its TATA-distal position. The "780" gene activator appears to be able to completely replace the upstream element when positioned immediately upstream to TATA.

DNase I footprinting studies indicate specific interactions of nuclear factors in the promoter regions of both the heat shock and T-DNA genes.

F 024 TARGETING OF PROTEINS INTO AND ACROSS CHLOROPLAST MEMBRANES, Kenneth Keegstra*, Cynthia Bauerle*, Karen Cornwell*, Alan Friedman*, Thomas Lubben*, Thomas Moore*, Sjef Smeekens+, and Peter Weisbeek+, University of Wisconsin, Madison, WI 53706* and University of Utrecht, Utrecht, The Netherlands+.

Most chloroplast proteins are encoded in the nucleus and synthesized in the cytoplasm. These proteins must cross either one, two or three membranes to reach their proper location. Chloroplast membrane proteins contain additional information that direct their insertion into the proper membrane. The first step during import into chloroplasts is thought to be an interaction of precursor proteins with receptors on the outer envelope membrane. We have employed several different approaches to study this interaction. Chemical cross-linking experiments have provided evidence that a 66kD envelope protein interacts with the precursor to Rubisco SS. Experiments using chimeric precursors have provided evidence that a transit peptide is necessary and sufficient to direct binding to the chloroplast surface and to cause import into chloroplasts. Targeting of imported proteins to non-stromal locations or insertion into chloroplast membranes requires additional steps. Studies with plastocyanin, an imported protein located in the thylakoid lumen, have lead to the conclusion that targeting to the lumen occurs in two steps. The first is transfer across the envelope membranes and the second is transfer across the thylakoid membrane. The plastocyanin transit peptide has separate domains that direct each step. Proteins directed to the envelope membranes are currently under investigation in an effort to determine the steps involved in their localization.

F 025 NEW DEVELOPMENTS IN THE ENGINEERING OF INSECT RESISTANT PLANTS Mark Vaeck, Arlette Reynaerts, Herman Höfte, Marc Van Montagu and Jan Leemans, Plant Genetic Systems N.V., J. Plateaustraat 22, B-9000 Gent, Belgium.

The bt2 gene from Bacillus thuringiensis berliner 1715 encodes a 130 KDa protein (Bt2) that is highly toxic to lepidopteran larvae. The active toxin is a 60 KDa polypeptide, derived from the NH2-terminal half of the molecule (Höfte et al., 1986, Eur. J. Biochem.). Modified bt2 genes have been constructed: truncated genes encoding the active toxin and gene fusions containing NH2-terminal fragments of bt2 fused to the neomycin phosphotransferase gene from Tn5. The latter encode stable fusion proteins which are equally toxic to insects as the original Bt2 and which exhibit specific NPTII enzyme activity, comparable to intact NPTII protein.
Transgenic plants expressing chimeric genes consisting of strong constitutive plant promotors and modified bt2 genes were constructed using Agrobacterium vectors. Plants expressing elevated levels of fusion proteins could be isolated by direct selection of shoots growing on high concentrations of kanamycin.
Both truncated and fused proteins are produced in plants at varying levels and were immunologically quantified. In greenhouse tests, expression levels between 50-200 ng/g fresh weight conferred resistance to feeding damage by the tobacco hornworm. The insect resistance trait was stably inherited in subsequent generations and segregated as a dominant Mendelian allele.

F 026
UBIQUITIN AND SELECTIVE PROTEIN DEGRADATION, Richard D. Vierstra, Dept. of Horticulture, Univ. of Wisconsin-Madison, Madison, WI 53706.

Intracellular protein degradation is an important process in the regulation of plant development and metabolism and in the ultimate expression of many foreign genes in transgenic plants. To date both the mechanisms responsible for selectivity and the ultimate routes of catabolism for plant proteins remain obscure. In an attempt to understand these processes, we have begun to characterize at the biochemical and molecular level a recently-identified proteolytic pathway in plants involving the small (76 amino acids) protein, ubiquitin. In this pathway first described in reticulocytes, ubiquitin is covalently ligated to proteins predestined for catabolism and appears to serve as a reusable recognition signal for selective proteolysis. The presence of ubiquitin in plants has been confirmed and immunological, enzymatic and structural studies of the molecule isolated from oats (Avena sativa) indicate that it is remarkably homologous to the animal form. The plant and animal proteins differ by only three residues out of the 76 amino acid sequence and X-ray crystallographic analyses show near identical tertiary conformations. In addition to ubiquitin, plants also contain a multitude of diverse ubiquitin conjugates in vivo. Enzymatic activities involved in conjugate formation and degradation have been identified in vitro. Conjugation requires ATP, will use both endogenous and exogenous proteins as substrates, and is detectable in extracts from all plant species and tissues examined. One function of the ubiquitin system has been identified recently during the study of the selective degradation of the plant photoreceptor pigment, phytochrome. We find that photoconversion of the stable Pr form (t/2>100h) to the rapidly-degraded Pfr form (t/2=2h) induces the rapid formation of phytochrome-ubiquitin conjugates in vivo. Kinetic analyses suggest that such conjugates represent intermediates of phytochrome breakdown. Studies on the molecular organization and regulation of the ubiquitin proteolytic pathway have been initiated recently in Arabidopsis with the sucessful cloning and characterization of ubiquitin gene(s).

Molecular and Genetic Modification of the Photosynthetic Apparatus

IN VITRO MUTAGENESIS OF psbA GENES IN SYNECHOCYSTIS 6803

F 027 Christer Jansson, Richard Debus, Heinz Osiewacz, Michael Gurevitz and Lee McIntosh, Plant Research Laboratory, Michigan State University, East Lansing Mi 48824.

Cyanobacteria are phototrophic organisms with oxygenic photosynthesis much like that in chloroplasts of higher plants and algae. The prokaryotic nature of cyanobacteria makes them attractive for molecular biological studies on the mechanism and regulation of photosynthesis. As a model system we have chosen the cyanobacterium Synechocystis 6803, mainly because of two major advantages with this organism; i)it can be transformed by exogeneous DNA which is integrated into the chromosome by homologous recombination, and ii)it can grow heterotrophically. This allows us to inactivate photosynthesis genes by in vitro mutagenesis and subsequently shuttle the genes back into the cells to replace the native genes. We can then grow the cells heterotrophically and map the inhibitory sites by various functional analyses, such as partial electron transport reactions. Furthermore, we can investigate what portion of a gene that is essential for a certain function and also identify regulatory sequences. psbA is a gene that encodes the 34-30 kDa Q_B-binding protein in Photosystem II. In higher plants and algae the psbA gene is found in the chloroplast genome and codes for a precursor protein with a C-terminal extension. In those systems the protein has been found to have a very high turnover, possibly as a consequence of harboring the secondary quinone acceptor Q_B. In Synechocystis 6803 we have found that the psbA gene belongs to a small multi-gene family with three copies. These different copies are differentially expressed with one copy, psbA-1, beeing almost silent under our growing conditions. Consequently, when we mutagenized psbA-1 no effect was found on O_2 evolution, fluorescence kinetics or Western blot analyses. Inactivation of psbA-1 and psbA-2 seems to induce a de novo or increased expression of psbA-3. We have recently cloned psbA-3 and inactivated also this copy. We will now test if we can force expression of psbA-1 by inactivating psbA-2 and psbA-3, and also study photosynthesis and assembly in the tripple transformant, devoid of active psbA genes.

F 028 LIMITING FACTORS IN PHOTOSYNTHESIS - AN OVERVIEW, Donald R. Ort, USDA/ARS and Department of Plant Biology, University of Illinois, Urbana IL 61801.
Most often factors limiting photosynthesis, whether internal or external, are evaluated from the stand point of maximum rates at light saturation. That is, the approach has been to ask, with abundant light available what are the specific reactions in the chloroplast and physical factors within the leaf that prevent the plant from utilizing more of the excess light. There can be no question that a wide range of important insights about photosynthetic limitations have resulted from this approach. However, it should be recognized that, even on a cloudless day, only a small portion of the total photosynthesis occurring within a dense agricultural crop canopy takes place at saturating light intensities. From this viewpoint it is not surprising that evolution has provided for numerous mechanisms and chloroplast features apparently dedicated solely to low light operation. Indeed, most of these are circumvented, redundant or, in some cases, possibly even hazardous at light intensities that saturate total photosynthesis. The provisions for low light operation to be discussed represent a very significant investment by the plant in synthesis and maintenance further emphasizing the importance of the photosynthesis carried out at below saturation irradiance levels.

Selected examples of certain environmental factors that may have their most severe effects on growth and yield through impairment of low light performance of photosynthesis will be discussed. Consideration will be given as to how this view of limiting factors in photosynthesis could be included in development of crop improvement strategies.

F 029 **MOLECULAR GENETICS OF PLANT CAROTENOID MUTANTS** P. Scolnik, G. Giuliano, D. Pollock, E. Wurtzel and P. Hinton. Cold Spring Harbor Laboratory, P.O. BOX 100, New York 11724. Our lab is conducting studies on the biosynthesis of carotenoids in plants and on the effects that the lack of colored carotenoids has on chloroplast differentiation and nuclear gene expression. The first model chosen was the tomato *ghost* plant, in which a single nuclear mutation (*gh*) blocks an early step on the carotenoid biosynthetic pathway. As in other plants mutated in early steps of carotenoid biosynthesis viability is severely affected due to photooxidation of the chloroplast membranes. In the case of *ghost*, however, the mutation is somatically unstable and, as a consequence, green sectors form during the development of the plant. We used tissue culture techniques to selectively propagate green *ghost* tissue. Tissue culture-propagated *ghost* plants are almost as efficient as their wild type counterparts in growth rates, flowering and fruit setting. In contrast, *ghost* plants started from seed are only rarely viable. We now use tissue culture to routinely produce homozygous recessive *ghost* plants for molecular and biochemical studies. Biochemical characterization of the pigments accumulated in the different *ghost* tissues shows that colored carotenoids and chlorophyll accumulate in the green sectors but no chlorophyll is detected in the white sectors, which contain high levels of the colorless carotenoid phytoene. We analyzed the ultrastructure of chloroplasts and chromoplasts. In white leaves the plastids formed show an underdeveloped membrane system and, in general, they have irregular shapes. In green *ghost* leaves, however, normal chloroplasts develop. Fruits of *ghost* plants tend to rapidly lose chlorophyll during ripening. Analysis of their chromoplasts indicates that the formation of crystaloids, membrane structures that are the site of accumulation of carotenoids, is affected in *ghost* fruits. Analysis of the steady-state mRNA levels for SSU and CAB shows that in white leaves they are sharply reduced but they are similar to wild type in green leaves. *In vitro* transcription experiments show that the rates of transcription of SSU and CAB genes are very low in the white sectors but they are normal in the green areas. *In vitro* transport experiments indicate that the synthesis of a set on nuclear-encoded chloroplast proteins is affected in *ghost* plants. In conclusion, *ghost* is a somatically unstable mutant in carotenoid biosynthesis that shows two phenotypes during development: white and green. In white leaves carotenoid biosynthesis is blocked at the level of dehydrogenation of phytoene, chlorophyll does not accumulate, plastids do not develop into mature chloroplasts and transcription of a set of nuclear genes coding for chloroplast proteins is affected. In contrast, green *ghost* leaves accumulate chlorophyll and colored carotenoids, chloroplasts differentiate normally and transcription of nuclear genes is not affected. We are also studying the process of biosynthesis of carotenoids in the photosynthetic bacterium *Rhodobacter capsulatus*. Genes were mapped and our attention is now being focussed on *crtI*, the gene that may code for the enzyme phytoene dehydrogenase.

Finding New Genes

F 030 TRANSPOSON TAGGING OF MAIZE REGULATORY AND RESISTANCE GENES USING MUTATOR, Jeffrey L. Bennetzen, April D. Cresse, Willis E. Brown, R. Paul Fracasso, Cathleen Carter-Peek, and David Horvath, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Many of the most interesting loci in higher plants, including the genes that regulate development, tissue-specific gene expression, or pest and pathogen resistance, have not yet been associated with any gene product. Therefore, these genes are not accessible to molecular cloning by the standard cDNA, oligonucleotide, or heterologous expression approaches. The technique of "transposon tagging" has been used extensively in Drosophila and bacteria, and recently in plants, to provide access to single, dominant genes which may lack an identified product but which generate an unambiguous phenotypic change upon insertional inactivation by a transposable element. The Mutator transposable element system is particularly well suited to the transposon tagging approach due to its high mutagenic activity (inactivation rates of 10^{-3} to 10^{-5} per generation for any specific gene), its ability to induce mutations at most or all maize loci, the distinctive somatic instability of many Mutator-induced mutations, the structural similarity of the insertions in Mutator-derived mutations, the absence or low copy number of Mutator transposable elements in most maize lines, and the ease by which a Mutator-induced mutant may be stabilized by crossing to an inactive Mutator-derived line. In order to fine tune the Mutator system for use in transposon tagging, we have investigated the relationship between Mutator mutagenic activity, Mutator-derived bronze mutability, and Mu element copy number, transposition, and modification. Our data indicate that any maize line with a high copy number of unmodified Mu elements which also shows Mutator-derived bronze mutability will have high mutagenic activity. Maintenance of high mutagenic activity can be drastically affected by the crossing program followed, in particular, the frequency of crosses between Mutator plants and the choice of genetic backgrounds Mutator is crossed into.

F 031 INTRODUCTION OF TRANSPOSABLE ELEMENTS INTO ARABIDOPSIS, Jose M. Martinez-Zapater, Ruth Finkelstein and Chris R. Somerville. Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Arabidopsis thaliana has both a rapid life cycle and a small genome, characteristics which make it well suited for genetic analysis. In order to extend these studies to the molecular level, it would be helpful to have a transposable element to use as an insertional mutagen. Since no endogenous transposable elements have yet been found in this species, we are trying to introduce transposons from other eukaryotic species by Ti plasmid mediated transformation and, when possible, to modify them to be more convenient as transposon tags. The P element of Drosophila is one of the most thoroughly characterized eukaryotic transposable elements. The regions required for both transposase synthesis and transposition of the element have been identified making it amenable to manipulation and, in principle, useful as a transposable element system in plants. We have constructed several modified derivatives in which the P element transposase gene is under transcriptional control of the 70 Kd heat shock protein (hsp70) promoter, allowing us to induce transcription by exposure to a brief heat-shock. A functional transposase, if produced in response to heat shock, should mobilize, in trans, a non autonomous P-Element that could act as a mutagen. We will discuss the progress in analyzing the expression and activity of the P element in tobacco and Arabidopsis.

F 032 SPLICING OF MAIZE TRANSPOSABLE ELEMENTS FROM EXON SEQUENCES PERMITS THE SYNTHESIS OF ACTIVE PROTEINS, Susan R. Wessler, George Baran and Marguerite Varagona, Botany Department, University of Georgia, Athens, GA 30602.

One hallmark of the controlling element alleles of maize is their phenotypic diversity. No two alleles have the same phenotype. To understand the molecular basis for this diversity we are analyzing the Ac/Ds alleles of the waxy (wx) locus. We have cloned eight of these alleles and have quantified wx RNA and protein in several. Surprisingly, we have found very different molecular mechanisms responsible for the different patterns of expression displayed by each allele.

The most novel mechanism is displayed by the wx-m9 allele. Strains harboring this allele have significant wx expression in spite of a 4.3 kb insertion in exon sequences. Northern blot analysis and sequencing of cDNAs of wx-m9 transcripts indicate that the 4.3 kb element is efficiently spliced from wx RNA. We have discovered a 20 bp sequence in the Ds element that encodes 3 splice sites; one in each reading frame. In this way the element can be spliced out and the correct reading frame maintained without regard for the site of Ds insertion.

Analysis of other Ac and Ds alleles of waxy reveals that splicing is a common occurrence. Furthermore, preliminary analysis of other DNA insertions into the waxy gene (not Ac or Ds) indicates that these elements may also be spliced from wx RNA.

These data suggest that the maize elements have evolved mechanisms that permit gene function in spite of insertion into exon sequences.

Generating Mutants in Flowering and Hormone Response

F 100 MOLECULAR STUDIES IN THE OIL PALM (ELAEIS GUINEENSIS), Suan-Choo Cheah, Palm Oil Research Institute of Malaysia, Bangi, Selangor, Malaysia.

The oil palm is grown in Malaysia for its mesocarp and kernel oil. Improvement in the palm has proceeded in the direction of increasing oil yield and fatty acid unsaturation. Except for the effect of the sh locus affecting kernel shell thickness in improving oil yield, there is little information on other gene loci in the palm genome even though several single gene mutations affecting fruit and vegetative characters are known. Its long breeding cycle of a minimum of 7 to 9 years is a major impediment to genetic studies. We have therefore resorted to molecular techniques in an attempt to map mono-gene fruit characters. Random cDNA and genomic fragments are being cloned so that they may serve as RFLP markers. The progeny of existing breeding programmes will be analysed for the linkage of these characters to RFLP loci. It is envisaged that the assignment of the RFLP markers to linkage groups will be achieved by in situ hybridisation.

F 101 TRANSCRIPTION OF SEED STORAGE PROTEIN GENES IN BRASSICA NAPUS EMBRYOS, Alice J. DeLisle and Martha L. Crouch, Indiana University, Bloomington, IN 47405.

Two seed storage protein families, cruciferin and napin, accumulate during embryogeny in Brassica napus. Accumulation of these proteins is accompanied by high levels of their respective mRNAs. The proteins will also accumulate in a similar manner when early expansion phase embryos are cultured in the presence of luM abscisic acid (ABA), and this accumulation is accompanied by an increase in the levels of the corresponding mRNAs. We have determined whether cruciferin and napin transcription rates parallel the mRNA levels in embryos developing in the seed, as well as in embryos cultured with and without ABA.

The cruciferin and napin mRNAs for both seed storage proteins are high during the expansion phase of embryo development, representing as much as 11% and 8% respectively, of the total embryo mRNA. The transcription rates for both gene families relative to total transcription as measured in isolated nuclei is high during the same phase of embryo development. Cruciferin transcription rate increases 5-fold whereas cruciferin mRNA level increases 30-fold. In late expansion phase, cruciferin transcription rate declines while the mRNA level remains high, suggesting that there are transcriptional and posttranscriptional controls of cruciferin mRNA level. Napin transcription rate and mRNA level increase in parallel (3-4 fold) during expansion phase. However, the mRNA level declines before the transcription rate, indicating that a higher transcription rate is required to maintain the mRNA level.

When embryos are cultured in the presence of ABA, the levels of cruciferin and napin mRNAs are about 2-fold higher than in embryos cultured on basal medium. The transcription rate of the seed storage protein genes shows a similar 2-fold increase in the presence of ABA, suggesting that the transcription rate can account for the increase in the levels of the seed storage protein mRNAs. ABA has no significant effect on either actin or rDNA transcription, which shows that the effect is not a general effect on transcription.

F 102 MAJOR REGULATOR GENES, PHYTOHORMONE LEVELS AND SPECIFIC GENE EXPRESSION FOR REPRODUCTIVE ORGANOGENESIS IN **MERCURIALIS ANNUA L.** (2n = 16). Bernard Durand, Jean-Paul Louis, Saïd Hamdi, Elisabeth Cabré, Long Xi Yu, Brigitte Guérin, Gérard Teller, Université d'Orléans, 45067 Orléans Cedex 2, France

In plants as in animals, genes for sex determination are considered by the authors as major regulator genes. They control stamen or ovary development and normal sex expression. After stamen induction, sterility-determining genes as well as a cytoplasmic factor, control abnormal pollen formation in sterile plants. The constructed homozygous or heterozygous combinations of the 3 sex genes governing different physiological phenotypes (strong, weak males or females) and those governing the various degrees of fertility (total or semi-sterility or restored fertility) were used for auxin and cytokinin measurements. The analyses show that sex genes control the endogenous **auxin** contents: strong males are richer (175ng/100 g fresh weight) than intermediate (155ng) and weak males (90ng) or females (35ng). On the other hand, females contain **trans-zeatin** which has never been detected in male genotypes. The feminizing activity of cytokinins and the masculinizing effects of auxins are confirmed **in vivo** by the obtention of phenotypic flowers sexually opposite with respect to the genetic sex of the treated plant. The combinations for various sterile and restored fertile plants contain unexpected **Cis-Zeatin** beside increasing quantities of auxin: in spite of the female inducer (zeatin) a normal stamenogenesis results in these restored fertile strains. The endogenous hormones in turn control the synthesis of specific mRNAs expressed in each kind of sexual organ. The isolation and cloning of these specific mRNAs are now in progress. In **Mercurialis**, normal sexual organogenesis results from a hierarchy of regulator genes controlling the hormonal balances that switch on (or off) the sets of genes specifically expressed in each sexual differentiation.

F 103 TIMETABLE OF EARLY SEED AND FRUIT DEVELOPMENT IN SOYBEAN, C.D. Dybing, K.P. Sharma, S.G. Rugenstein, C. Lay, and C. Paech. USDA-ARS and SD State University, Brookings, SD 57007.

Many soybean flowers are shed without developing into seed-bearing fruits. Decisions involved in this process include ovule abortion and ovary abscission. For ovaries with 3 ovules, the basal ovule is more likely to abort than the other 2, but controlling factors are mostly unknown. Ovary abscission, though usually high in genotypes with long racemes, is a product of genetic, hormonal, and environmental factors. Gene action controlling flower abscission is additive with relatively few genes involved. Selection for low abscission increased 3-seeded pods, seed per raceme, seed weight, and seed yield. Flower abscission and raceme length were more stable genotypic characteristics across years than number of flowers per raceme. Cytokinin (1 mM benzyladenine) applied to the raceme increased set of both pre- and post anthesis ovaries and shortened the rest period that the zygote normally experiences for a few days after anthesis. Earliest detected effect from cytokinin application was an increase in thickness of peduncle and pedicel tissues after 3 days. The increased sink demand resulting from increased fruit number caused little change in chlorophyll, dry weight per square cm, or RuBP carboxylase activity in the subtending leaf. Effectiveness of cytokinin application on preanthesis ovaries was diminished in the field as compared to the greenhouse.

F 104 AUXIN RESISTANT MUTANTS OF ARABIDOPSIS WITH AN ALTERED MORPHOLOGY
Mark A. Estelle and C.R. Somerville. MSU-DOE Plant Research Lab, Michigan State University, East Lansing, MI. 48824

For a number of reasons the conventional biochemical analysis of IAA metabolism and IAA action has been extremely difficult. As an alternative we are attempting to identify some of the functions involved in IAA metabolism and action by isolating mutants of Arabidopsis which are resistant to exogenous auxin. We have isolated a total of 28 mutants which are significantly resistant to indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) by screening large numbers of mutagenized seed on agar plates containing auxin. These mutants fall into at least 3 complementation groups. In addition to auxin resistance, mutations in each complementation group confer a distinctive morphological phenotype. We have named the locus defined by one of these complementation groups Axr-1 for auxin resistance and have characterized these mutants in some detail. The axr-1 mutations all behave as single gene recessive traits and all confer a number of developmental abnormalities including an apparent reduction in apical dominance, a reduction in root branching, loss of normal geotropic response, and a failure to self-fertilize due to a reduction in stamen elongation. In addition the mutants exhibit reduced 2,4-D induced ethylene biosynthesis from mature leaves and form callus poorly under standard conditions. We believe that both resistance to auxin and the developmental aberrations are due to a change in a protein involved in some aspect of IAA metabolism or IAA action. We plan to continue our analysis of this interesting locus by examining IAA transport and by identifying and characterizing auxin binding proteins in both wildtype and axr-1 plants.

F 105 FLOWER DEVELOPMENTAL MUTANTS IN PETUNIA HYBRIDA. Anton G.M. Gerats, Dept. of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam.

A number of mutants are known in Petunia hybrida that influence floral development: gp (green petals) causes the development of a second ring of sepals instead of petals; bl (blind) prevents the development of a corolla; ch 1-4 (choripetalous) prevent the normal fusion of the petals. alf (aberrant leaf and flower) causes especially flower development to be very aberrant; px (phoenix) disturbes normal tube development and sometimes causes the occurrence of new flowers growing through the tube of old ones.

The mutations are either spontaneous or induced by mutagenic treatment. The alf and px mutants are probably insertion mutants.

The alf mutation was characterized on the biochemical level in cooperation with Russel Malmberg (Athens, GA) and appears to cause a two-three fold increase in putrescine content in old leaves as compared to the wildtype. Enzyme activity (arginine decarboxylase) and mRNA levels were enhanced in a similar way.

The unstable systems of Petunia hybrida appear to give rise to developmental mutants in a high frequency.

F 106 FLAVONOID BIOSYNTHESIS IN PETUNIA HYBRIDA. Anton G.M. Gerats, Dept. of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam.

Over thirty genes are known that influence flavonoid synthesis either quantitatively or qualitatively in Petunia hybrida. Interactions between a number of genes are described, indicating that specifically the flavonols kaempferol and quercetin and the anthocyanidin delphinidin are favorite endproducts in the pathway; other products, like myricetin and cyanidin are only produced in high amounts when the other routes are shut off completely. Phenotypic data will be presented that show: 1) tissue-specific expression of genes within the flower and 2) that duplicate(d) genes differ in strength and tissue specificity of expression.

The gene Hf1 for example (responsible for 3' and 5' hydroxylation of the β-ring) prevents the synthesis of cyanidins completely and is expressed in both corolla and tube of the flower. In contrast, the gene Hf2 (which is functionally duplicate) blocks cyanidin synthesis only partly and is expressed only in the corolla of the flower.

F 107 MOLECULAR CHARACTERIZATION OF TOMATO POLYGALACTURONASE PG2A PROTEIN AND cDNAs, William R. Hiatt[1], Judith Pearson[2], Colin J. Brady[2] and Raymond E. Sheehy[1], [1]Calgene, Inc., Davis, CA 95616. [2]Plant Physiology Unit, Division of Food Research, CSIRO, and School of Biological Sciences, Macquarie University, North Ryde NSW, Australia 2113.

Tomato cDNA clones of the polygalacturonase gene have been isolated and sequenced. One clone is nearly full-length and contains a single open reading frame. The amino acid sequence of polygalacturonase (PG2A) has been determined and used to confirm the identity of cDNA clones. Polygalacturonase 2A appears to be synthesized as a precursor of 457 amino acids (Mr = 50,075) which is subsequently processed to 386 amino acids (Mr = 41,833). The 71 amino acid pre-sequence has characteristics of signal peptides common to secretory proteins. The expression of polygalacturonase mRNA is restricted to ripening fruit and is not detectable in homozygous fruit containing the rin (ripening inhibitor) mutation. The relationship of PG2A to PG2B, a slightly higher molecular weight form, will be discussed.

F 108 THE ISOLATION AND CHARACTERISATION OF AUXIN MUTANTS IN VITRO Patrick J. King, Anne D. Blonstein, Yvan Fracheboud, Jurg Oetiker, Martin Suter, Ludwig Waelder, Ewald Weber. Friedrich Miescher-Institut, 4002 Basel, Switzerland

Two approaches to the isolation of mutants altered in biosynthesis of, or response to, auxins have been taken using haploid protoplast cultures: 1) the cloning of conditional lethal mutants showing a requirement for auxin at high temperature; 2) the selection of clones with increased resistance to auxins or related compounds. Temperature-sensitive auxin auxotrophs have been isolated from protoplast cultures of Hyoscyamus muticus and Nicotiana plumbaginifolia and are being characterised at both the plant and the cell level. In addition to basic biochemical and genetic analyses, we have been able to repair the temperature-sensitive phenotype of an H. muticus clone by transformation with the auxin genes of the Ti plasmid. We are working towards the use of transformation systems to isolate the plant genes affected in these auxotrophic mutants. Lines with elevated resistance to auxins are being examined at the plant level.

F 109 STUDIES OF GENE EXPRESSION IN THE TOMATO FRUIT RIPENING MUTANT nor, Matthew G. Kramer, John N. Bell, Greg Holtz, and Leona Fitzmaurice, SIBIA, Inc., La Jolla CA 92037.

In contrast to normal cultivars, the fruit ripening mutant, nor, produces a non-climacteric fruit in which ripening occurs very slowly and ripening changes such as carotenoid accumulation and polygalacturonase activity are virtually inhibited. This mutation has been mapped to a single locus on chromosome 10, but the mechanism by which the nor mutation affects diverse ripening processes is not understood.

Comparative two-dimensional gel electrophoretic studies of in vitro translation products derived from nor fruit tissue RNAs at different developmental stages indicate that gene expression in nor does not evidence the same pattern of tissue-specific, developmentally-regulated expression observed in normal fruit tissue. A set of genes expressed only during early stages of fruit development in normal tissue continue to be expressed during late development in nor. Translation products of another set of genes, expressed late during fruit development in normal tissue, are not observed in nor.

Northern blot studies using several fruit-specific, developmentally-regulated cloned sequences indicate that the nor mutant exhibits an effect on mRNA expression and/or stability.

F 110 MOLECULAR GENETIC ANALYSIS OF FLORAL DIFFERENTIATION, D.R. Meeks-Wagner, E.S. Dennis, and W.J. Peacock. Division of Plant Industry, CSIRO, G.P.O. Box 1600, Canberra City, A.C.T. 2601, Australia.

We have used the tobacco 'Thin Cell Layer' (TCL) explant system of Dr. K. Tran Thanh Van (1) to isolate cDNA clones specific to hormonally induced floral tissue. TCL explants were made by stripping tissue from the floral branches of Nicotiana tabacum L. cv. samsum. This tissue, which contains epidermal, sub-epidermal, and parenchyma cells, has the ability to give rise to fully differentiated organs in less than 20 days without intermediate callus formation; the 5 to 10 vegetative or floral buds formed on each explant show synchronous development. Culture conditions for the formation of vegetative and floral buds were identical except that vegetative cultures used zeatin as a cytokinin, while floral cultures used kinetin.

A cDNA library made from 'day 7' floral explants was screened with radioactive cDNA from 'day 7' floral and 'day 7' vegetative explants. We have isolated clones of genes transcribed in explants from floral medium, and not transcribed, or only weakly transcribed, in explants from vegetative medium. The transcription of these genes is induced by floral culture conditions, as homologous transcripts were not found in the starting tissue (i.e. 'time zero'). These floral specific cDNA clones are being classified into families by sequence homology, and analyzed by in situ hybridization to determine the timing and spatial distribution of their expression in the developing floral bud.

1. Tran Thanh Van, K. (1981) Ann. Rev. Plant Physiol. 32, 291-311.

F 111 IN VITRO MORPHOGENESIS OF ARRESTED EMBRYOS FROM LETHAL MUTANTS OF ARABIDOPSIS THALIANA, David W. Meinke and Linda H. Franzmann, Oklahoma State University, Stillwater, OK 74078.

Embryo-lethal mutants of Arabidopsis thaliana have been shown previously to contain arrested embryos that differ in size, color, lethal phase, pattern of abnormal development, response in culture, and accumulation of seed storage proteins. In our original study on the growth of mutant embryos in culture, we identified a putative auxotrophic mutant that developed into normal plants only when placed on an enriched medium, and a fused cotyledon mutant that developed into rootless plants, but arrested embryos from most other mutants failed to respond in culture. We have now found a much higher frequency of in vitro morphogenesis with arrested embryos cultured on a shoot regeneration medium containing MS salts with 1 mg/l BAP and 0.1 mg/l NAA. Most of our mutants with lethal phases extending from the globular to cotyledon stages of development have now produced roots and shoots on this medium. Many of the resulting plants have altered leaf morphology, trichomes, pigmentation, and inflorescences, but other plants are surprisingly normal. Two homozygous mutant plants (63A-1A and 109F-1C) have been shown to produce siliques containing 100% aborted seeds following self-pollination and 100% normal seeds following pollination with wild-type pollen. These results provide further evidence that lethal mutations may disrupt embryogenesis but not other stages of the life cycle.

F 112 *ARABIDOPSIS THALIANA* MUTANTS DEFICIENT IN AN ENZYME OF THE PURINE SALVAGE PATHWAY ARE MALE STERILE, Barbara Moffatt and C.R. Somerville, MSU-DOE Plant Research Lab, Michigan State University, E. Lansing, MI. 48824

We have been analyzing the purine salvage pathway of *Arabidopsis* to identify genes that might be used as selectable markers in plants. Adenine phosphoribosyl transferase (APRT) converts adenine and PRPP to adenine monophosphate. It is possible to directly select for APRT-deficient mutants by their ability to grow in the presence of toxic analogues of adenine: APRT-deficient mutants cannot salvage exogenous adenine and therefore are not killed by the analogue. We have isolated APRT-deficient mutants of *Arabidopsis* by germinating the progeny of a mutagenized seed population on a nutrient agar plate supplemented with 0.1 mM 2,6 diaminopurine. Under these conditions, wild type seed does not germinate, and mutants can be recovered at a high frequency. approximately Three independently isolated mutants have been characterized. All three contain allelic nuclear recessive mutations, and all are deficient in APRT activity. The mutants grow more slowly that wild type and are male sterile. Pollen development appears to become aberrant during the meiotic divisions of the pollen mother cell. In addition the mutants are difficult to establish in tissue culture. Available heterologous genes from *Drosophila*, *E. coli*. and mouse did not cross-hybridize to *Arabidopsis* DNA. Therefore the enzyme has been purified to facilitate the production of an oligonucleotide probe. To test whether the bacterial gene can be used as a selectable marker in plants, it has been cloned into T-DNA vectors under control of plant expression signals, and transferred into *Arabidopsis* and tobacco. Results indicate that active bacterial APRT is being produced in the transformed callus.

F 113 TEMPORAL REGULATION OF SELF-INCOMPATIBILITY IN OENOTHERA ORGANENSIS, Karen M.T. Muskavitch, Sherri M. Brown, and Martha L. Crouch, Indiana University, Bloomington, IN 47405. Self-incompatibility is a genetic system which prevents self-fertilization in many plant species. If a pollen grain and the pistil on which it lands share an allele at the S-locus, germination and/or growth of the pollen is inhibited thus preventing fertilization. Many self-incompatible species can be bud-pollinated; that is self-pollinated immature buds will set seed although mature flowers will not. Since the initiation of work with Oenothera organensis, it has been reported that bud pollination is ineffective in this species. Dispite the efforts of many workers using techniques such as chemical treatment and pistil grafting, it has not been possible to achieve seed set following an incompatible pollination except for instances when mutations have occurred at the S-locus. Recently, we have observed that O. organensis can be successfully bud pollinated 3-4 days pre-anthesis to yield viable seed. The progeny of such a pollination have lower frequencies of germination and seedling survival, they exhibit a range of self-incompatibility phenotypes, and they display S allele specificities consistant with a self-pollination. Bud pollination is most effective at cool temperatures; the frequency of seed set is very low at high temperatures. Investigation of the temperature effect and characterization of the progeny of self-pollinations will be presented. The success of incompatible bud-pollinations indicates that self-incompatibility is temporally regulated during O. organensis bud development and we are exploiting this phenomenon in our efforts to identify and characterize the S-locus.

F 114 GIBBERELLINS (GAs) IN RELATION TO FLOWERING IN SHORT (SD) & LONG-DAY (LD) PLANTS. Richard P. Pharis, Roderick W. King, Lloyd T. Evans, and Lewis N. Mander, Univ. of Calgary, Calgary, AB, Canada T2N 1N4 (RPP), Plant Industry, CSIRO, Canberra ACT 2601, Australia (RWK & LTE), and Research School of Chemistry, Australian National Univ., Canberra. Work on Pharbitis nil (SD) and Lolium temulentum, (LD) suggests that certain GAs play an important role in flower initiation. In P. nil (dwarf) flowering is promoted under sub-threshhold SD by many GAs (2,2 dimethyl GA4>GA32>GA3>GA5) if given before dark induction. The most effective flowering dose is 1/10 to 1/50 that for maximum stem elongation (the latter is not influenced by time of application, relative to the inductive dark period). But, even the most promotive GAs given at high doses, or after inductive dark, can inhibit flowering. In Lolium, one 24 h LD induces flowering. Many GAs can substitute for the LD, most notably GA32, 2,2 dimethyl GA4, and GA5. These GAs were effective for flowering at doses too low to promote stem elongation. GA32 was most like inductive LD in flowering, while having little effect on stem growth; GA1 the known 'effector' of stem elongation in maize and pea, had the opposite effect. Four orders of magnitude separated the most effective GA (2,2 dimethyl GA4) from GA1 in flowering efficacy. Only two orders of magnitude separated them in stem elongation. Concentrations of endogenous GA-like substances of Lolium apices (up to 1000 apices; 3 mg d.w.) increased within 24-72 h of the single LD. Most notable was a 3 to 5-fold increase in putative polyhydroxylated components (e.g., similar in polarity to GA8 [3 hydroxyls] & GA32 [4 hydroxyls]). This is intriguing given the high efficacy of the polyhydroxylated GA32 (noted above). The total GA concentrations in apices from plants induced by 1 LD was high (up to 42 ug/g d.w., or 3 x 10^{-5}M of GA3 equivalents). Investigations into gene expression where flowering is promoted by a GA, or by an environmental change that influences GAs, should recognize that endogenous or exogenous GAs may influence either, or both of flowering and vegetative growth, depending on type of GA and/or its concentration.

F 115 STIGMA SURFACE SECRETIONS OF PENNISETUM AMERICANUM (L.) Leeke, Bonnie J. Reger, USDA/ARS, Russell Res. Ctr., Athens, GA 30613. Characterization of stigma surface secretions in the grasses is important for a better understanding of various aspects of pollen-stigma interaction, including pollen capture and recognition reactions. Cytochemical tests showed that the pollen receptive cells of the stigma of P. americanum secrete a thin superficial proteinaceous coating or pellicle which has esterase activity. Comparison by IEF of whole-stigma extracts with eluates of the proteinaceous pellicle indicated that the proteins on the stigma surface are different than the intracellular proteins of the whole stigma tissue. One particular component having esterase activity was much more dominant in the eluate than in the stigma extract and appeared to accumulate during stigma maturity. No function as yet can be attibuted to this particular surface protein but further characterization is underway. Glycoproteins have been shown to be involved in pollen adhesion and in recognition responses in species having self-incompatibility systems. Pennisetum species do not have a self-incompatible system; therefore, it seems probable that this pellicle protein functions in pollen capture and formation of the menisci for pollen hydration and subsequent cascading events.

F 116 THE AGROBACTERIUM RHIZOGENES ROOT-INDUCING SYSTEM.
F. RICHAUD, A.-M. FLEURY-GUEROUT, C. ESTRAMAREIX and O. REYES.
Institut de Microbiologie, Université de Paris-Sud, 91405 ORSAY, FRANCE

Agrobacterium rhizogenes inoculation in wounds result in the formation of hairy-root. The conversion of plant cells to hairy roots is induced by genes encoded in the T-DNA regions. Three strains of A. rhizogenes were studied : A4, 1855 (essentially identical to A4) and 8196, a less virulent strain.

Single and multiple mutations affecting the T-DNA loci of A. rhizogenes involved in the induction of hairy roots have been isolated and characterized by infecting Kalanchoe diagremontiana and Daucus carota.

The results led us to propose that hairy root induction by strain A4 may result from redundant, parallel, rhizogenetic inducer pathways. These (at least three) patways are such that inactivation of any of the three does not abolish completely hairy root induction. Moreover, one pathway may be sufficient to induce roots on an organt of a choosen plant.

Recent results led us to suppose that two of these inducer pathways may be related to "auxins" while the third one may be related to "cytokinins".

F 117 ANALYSIS OF THE PHYTOCHROME PROTEIN AND LIGHT-REGULATED GENE EXPRESSION IN AN aurea MUTANT OF TOMATO. Robert A. Sharrock, Brian M. Parks, Alan M. Jones, Peter H. Quail, University of Wisconsin, Madison, WI 53706. Martin Koornneef, Richard E. Kendrick, Department of Genetics and Laboratory of Plant Physiology Research, Agricultural University, Wageningen, The Netherlands.

A tomato mutant at the au locus (au^W) has yellow-green leaves, elongated hypocotyls and reduced anthocyanin content when grown in white light. In vivo difference spectroscopy of etiolated tissue shows that the mutant has <5% the level of spectrophotometrically detectable phytochrome present in the wild type. Western blot analysis shows that the mutant has only a trace of immunochemically detectable phytochrome polypeptide relative to the wild type. Northern blot analysis shows that phytochrome mRNA is present in the wild type and the au^W mutant at equivalent levels and in vitro translation of poly(A)$^+$ RNA from the wild type and the au^W mutant results in synthesis of apparently identical 116 kDa polypeptides which are immunoprecipitable by anti-phytochrome antibodies. These results indicate that the reduced amount of phytochrome polypeptide in the au^W mutant is not the result of lack of transcription of the phytochrome gene(s) in this strain nor of the production of a nonfunctional phytochrome mRNA. Northern blot analysis of chlorophyll a/b binding protein (cab) mRNA levels demonstrates that phytochrome-mediated transcriptional regulation of this message is defective in the au^W mutant.

F 118 PRODUCTION OF MUTANT LINES OF LEMNA GIBBA G_3 BY CELL CULTURE AND WHOLE PLANT MUTAGENESIS. Janet Pernise Slovin and Jerry D. Cohen. Plant Hormone Laboratory, USDA, Beltsville, Md. 20705

The primary interest of our laboratory is the metabolism of the plant hormone indole-3-acetic acid (IAA). Because of the importance of plant hormones for growth and development, we want to understand how a plant decides how, how much, where, and when to increase or decrease the level(s) of free IAA, which we believe to be the active form of the hormone. To this end we have developed a new system, using the aquatic monocot Lemna gibba G_3, with which we can easily do rapid kinetic labeling in sterile conditions, and which is now shown to be amenable to genetic manipulations.

We have obtained many different kinds of mutants of Lemna by techniques including tissue culture and whole plant mutagenesis. One variant obtained by mutagenesis has no apparent chlorophyll b and very low or no steady state levels of the light harvesting chlorophyll a/b binding protein. Another type of variant exhibits a developmental anomaly: the pollen tube grows while the pollen is still on the anther. We have also obtained several mutant lines by tissue culture. One line (R-1) is approximately 1.5 times the size of the parental line (PL) and has the same amount of DNA per nucleus as PL. R-1 contains up to 100 times the amount of free IAA and, unlike PL, contains no detectable IAA-conjugates. We have initiated experiments using this mutant to determine the pathway of IAA biosynthesis and to study the processes by which plants regulate the formation of IAA-conjugates, and the hydrolysis of such conjugates to yield free IAA.

Supported, in part, by US-Israel BARD grant No. US-842-84.

F 119 DIFFERENTIALLY EXPRESSED POLY A^+RNA IN CARROT SOMATIC EMBRYOGENESIS, Thomas H. Ulrich, Basil J. Nikolau, and Eve S. Wurtele. NPI, 417 Wakara Way, Salt Lake City, Utah 84108.

We are investigating the differential expression of specific poly A^+RNAs associated with carrot somatic embryogenesis in cultures grown in inductive (-2,4D medium) and in maintenance medium (+2,4D medium). A library of cDNA representing poly A^+RNA from induced cultures was constructed using the vector λgt10. Differential screening of this library with ^{32}P-labeled ss-cDNA made from poly A^+RNA from induced cultures and maintenance cultures identified several clones which were differentially expressed. Northern blot analysis of at least one of these clones indicates that its poly A^+RNA for the cloned sequence is about 20-fold more abundant in the induced cultured cells. Maintenance cultures were fractionated into embryogenic clusters (which make embryos when transferred into -2,4-D medium) and non-embryogenic cells. Induced cultures were fractionated into globular, torpedo, and germinating embryos and non-embryogenic cells. The expression of poly A^+RNA from the above fractions is being evaluated by Northern blot analysis utilizing the differentially expressed clones. The nucleotide sequences of these clones are being determined. The regulation of the expression, structure, and function of these differentially expressed genes are being studied in relation to embryogenesis.

F 120 CLONING OF DNA FRAGMENTS COMPLEMENTARY TO TOBACCO NITRATE REDUCTASE mRNA AND ENCODING FOR EPITOPES COMMON TO THE NITRATE REDUCTASES FROM HIGHER PLANTS, Michel Vincentz, Roger Calza, Eric Huttner, Fabienne Galangau, Herve Vacheret, Isabelle Cherel, Christian Meyer, Jocelyne Kronenberger and Michel Caboche, Laboratoire de Biologie Cellulaire, I.N.R.A. F 78000 VERSAILLES, FRANCE.

Messenger RNAs encoding the nitrate reductase apoenzyme from tobacco can be translated in a cell-free system. PolyA^+ mRNA fractions from the 23-32s area of a sucrose gradient were used to build a cDNA library in the expression vector gt11 with an efficiency of cloning of approximately 10^4 recombinants/ng mRNA. Recombinant clones were screened with a rabbit polyclonal antibody directed against the corn nitrate reductase which cross reacts specifically with the nitrate reductases from dicotyledones. Among 240,000 recombinant plaques eight clones were isolated containing inserts of sizes ranging from 1.6 kB to 2.1 kB and sharing sequence homologies. Seven of these clones contained a common internal 1.6 kB EcoRI fragment. The identity of these clones was confirmed as follows. A fusion protein of MW 170 kd inducible by IPTG and recognized by the rabbit NR antibody was expressed by a lysogen derived from one of the recombinants. The antibodies binding the fused protein were eluted and shown to be inhibitory to the catalytic activity of tobacco nitrate reductase. Two monoclonal antibodies directed against nitrate reductase were also able to bind the hybrid protein. When the 1.6 kB EcoRI fragment was used as a probe for northern blot experiments a signal corresponding to a 3.5 kB RNA was detected in tobacco and N. plumbaginifolia mRNA preparations but no cross hybridization with corn mRNAs was detected. The probe hybridized with low copy number sequences in genomic blots of tobacco DNA.

F 121 MOLECULAR ANALYSIS OF DEFECTIVE KERNEL MUTANTS DERIVED FROM LINES EXHIBITING ROBERTSON'S MUTATOR ACTIVITY. Christie E. Williams, John I. Stiles, James C. Deputy and Heinrich Albert. University of Hawaii, Honolulu, HI 96822.

Several defective kernel mutants have been recovered from maize lines showing Robertson's mutator activity. The defective kernel phenotype can first be detected from seven to fourteen days post-pollination, and is characterized at maturity by grosely abnormal kernels. Northern blot analysis revealed that defective kernel mutants have reduced levels of some developmentally regulated mRNA species, including zein and sucrose synthase, in addition to alterations in their temporal expression. In contrast, RNA expression appeared to be normal for alcohol dehydrogenase, a gene that is active throughout development in kernel tissues. Thus, defective kernel genes may differentially affect the expression of other genes involved in kernel development. We are currently investigating the affect of defective kernel mutations upon the expression of other developmentally regulated genes.

Some of the defective kernel mutations resulted from the insertion of a transposable element related to Mu1. When southern blots of certain segregating lines are hybridized with a cloned Mu1 probe, specific restriction fragments that segregate with the mutant phenotype are detected. We are currently attempting to clone several of these defective kernel genes that have been tagged with the transposable element Mu1.

Regeneration and Transformation of Cereals

F 200 INHERITANCE OF THE CULTURE INDUCTION RESPONSE IN MAIZE Kelly R. Close and Lynne A. Ludeman, Sungene Technologies, Palo Alto, Calif. 94304

The maize culture induction response elicited by 2-, 3-, and 4-chlorophenoxyacetic acid was compared between a Reid-type inbred (B73) and a Lancaster type (MS71). Inheritance of the ability to undergo somatic embryogenesis from immature embryos was evaluated in the F_1 and F_2 generations of reciprocal crosses between the two inbreds. The ability of a particular growth regulator to induce embryogenesis in each inbred was concentration-dependent and genotype-specific, and the response pattern of the F_1 explants was nearly identical to that of the maternal parent of each cross. In addition, the F_2 generation explants showed a relatively uniform response for each culture induction treatment with no evident Mendelian segregation ratios. These results indicate that a significant component of the *in vitro* response may be cytoplasmic in nature.

F 201 NUCLEAR GENES EXPRESSED IN YOUNG BARLEY LEAF CELLS. Kirsten Gausing, Jette Kreiberg Birgit F. Jensen, Rosa Barkardottir, Peter S. Nielsen, Catarina B. Jensen Dept. of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Denmark

From a cDNA library made from poly(A) RNA from the basal one third of seven day old barley leaves, clones encoding chlorophyll a/b binding protein, small subunit of RuBPCase, plastocyanin, and ubiquitin have been isolated. All the proteins, except plastocyanin are coded by multigene families with 8-15 genes. In addition we find that genes encoding proteins hitherto characterized as seed proteins, are highly expressed in leaves. One of these gene-families codes for purothionin, the other codes for a protein belonging to the Bowman-Birk protease inhibitor - castor bean storage protein superfamily.

Gene expression studies during leaf development and in different tissues show that our cDNA collection represents examples of genes subject to different types of regulation: weakly and strongly light induced as well as light repressed, leaf specific expression. Most of the genes in the ubiquitin gene family are constitutively expressed at fairly high levels but some are specifically expressed in tissue containing dividing cells. We are in the process of isolating genomic clones for ubiquitin (among others) with the purpose of using ubiquitin promotors in combination with selective markers for the construction of vectors for gene transfer in cereals.

F 202 TRANSFORMATION OF ZEA MAYS BY AGROBACTERIUM TUMEFACIENS: EVIDENCE FOR STABLE GENETIC ALTERATIONS. S.L. Goldman[+], A.C.F. Graves[+], and J.L. Roberts[*]. [+]University of Toledo, Toledo, OH 43606, [*]Lilly Research Laboratories, Greenfield, IN 46140.

It has been shown recently that when virulent strains of Agrobacterium tumefaciens are used to infect four day old maize seedlings in a region which includes the scutellar node and mesocotyl T-DNA specific enzyme activities are detected in homogenates (Graves and Goldman, 1986 PMB 7:43). Opine biosynthesis is not observed, if avirulent strains are substituted to inoculate the plant. From this data it is possible to conclude that Z. mays is subject to A. tumefaciens mediated T-DNA transfer.

In this communication, we present data suggesting that transformation of corn seedlings by Agrobacterium is frequent and stable. This conclusion rests on two pieces of evidence. Nopaline dehydrogenase activity has been found in the leaves and pollen of 56 and 67 day old plants, respectively, that were infected with C58 four days post-germination. Furthermore, bacteria free tissue cultures initiated from CA48 infected seedlings and selected from media supplemented with antibiotics demonstrate the lysopine dehydrogenase activity encoded on the T-DNA of this strain's Ti plasmid.

F 203 INDUCTION OF EMBRYOGENIC CALLUS FROM MATURE SEEDS AND SEEDLING EXPLANTS OF NATIVE FORAGE GRASSES, Robert A. Gonzales, Becky Johnson and Eva Franks, The Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73402.

Efficient production of meat, milk, and wood depends on the availability of high quality plant material provided as hay and silage or as forage. Two native grass species, Digitaria sanguinalis (crabgrass) and Bothriochola ischaemum (old world bluestem) are currently being developed as new alternative forage crops for semi-arid regions of the central and southwestern USA.

We report here the establishment of a tissue culture protocol for the induction of embryogenic callus from mature seeds and seedling explants. Distinct advantage in the use of these explant materials as opposed to the more commonly used immature inflorescences are a more rapid production of embryogenic callus and year-round availability.

F 204 STRATEGIES FOR GENETIC MANIPULATION OF CEREALS, Horst Lörz, Jose Cabrera-Ponce, Alicia de la Pena, Elke Göbel, Barbara Junker and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.

Cell and tissue culture techniques and methods for the genetic manipulation of cereal species *have progressed considerably in recent years. Plant regeneration from in vitro cultures,* especially from multicellular explants, has been achieved with all the major cereal crops. Success in cereal protoplast culture is still limited and successful plant regeneration is reported so far for rice only. DNA-mediated stable transformation of cultured cells of wheat, maize and rice has been obtained by transforming protoplasts with isolated plasmid DNA which contained a selectable chimeric gene coding for kanamycin resistance. A serious limitation in the wider application of DNA-mediated direct gene transfer for Gramineaceous species remains the difficulty of regenerating plants efficiently and reproducibly from isolated protoplasts. Alternative transformation methods independent of protoplasts are therefore being sought by introducing foreign genes either via microinjection into multicellular embryogenic or morphogenic structures, or by delivering the isolated DNA directly in plants. Genetic transformation of rye has been achieved by DNA-injection into floral tillers at a defined stage prior to meiosis. Agrobacterium tumefaciens and A. rhizogenes, natural vector systems frequently used for transformation of dicotyledonous species, hold promise to be suitable also for genetic transformation of monocotyledonous species including Gramineae. Several techniques have been developed or being studied actively to introduce foreign genes into cereals.

THE GENETICS OF SOMATIC EMBRYOGENESIS IN *ZEA MAYS*, Melanie E. Lynn,
F 205 Christina Strube, Thomas K. Hodges, Purdue University, West Lafayette, IN 47906.
Previous work has indicated that only selected genotypes of *Zea mays* are capable of producing embryogenic callus and regenerating from immature embryos. We have selected for genetic studies two nearly isogenic genotypes (A632 and A634) which differ markedly in their ability to regenerate. One hundred percent of the callus which arises from A634 embryos plated 3 weeks on MS media supplemented with 1.5 mg/l 2,4-D is morphologically embryogenic while embryos from A632 produce a watery, totally nonembryogenic callus. Based upon an inheritance study involving these two genotypes, relatively few genes seem to be required for somatic embryo formation.

We have analyzed total protein patterns from callus obtained 2 - 20 days post-plating of immature embryos on both one-dimensional SDS-PAGE gel systems and two dimensional systems and the major differences appear quantitative rather than qualitative despite the marked morphological differences in callus for these two genotypes. In addition, we have analyzed mRNA populations of both genotypes using total RNA-directed translation in a rabbit reticulocyte *invitro* system. These protein patterns on SDS gels also appear remarkably similar. In view of the extreme molecular similarity and morphological dissimilarity of these two tissues, we hope to be able to screen cDNA libraries of these two genotypes for somatic embryo-specific gene expression.

A Novel Method for Transforming Arabidopsis thaliana: A Non-Tissue Culture Approach
F 206 M. David Marks and Kenneth A. Feldmann, Sandoz Crop Protection Corporation, Zoecon Research Institute, Palo Alto, CA 94304
Germinating seeds of Arabidopsis thaliana were cocultivated with an Agrobacterium tumefaciens strain (c58C1rif) carrying the pGV3850:pAK1003 Ti plasmid. This Ti plasmid contains the neomycin phosphotransferase II gene (NPT II) which confers resistance to kanamycin or G418. Seeds (T1 generation) imbibed for 12 hours before a 24 hour exposure to Agrobacterium gave rise to transformed progeny (T2 generation). Some of the T2 seedlings and T3 families have been characterized for genetic segregation of functional NPT II gene(s), NPT II activity, and the presence of T-DNA inserts (Southern analysis). Ninety percent of the T2 individuals transmitted the resistance factor to the T3 families in a Mendelian fashion. Of the T3 families segregating in a Mendelian fashion (n=111), 62% segregated for one functional insert, 29% for two unlinked or linked functional inserts, 5% for 3 unlinked inserts, 1% for 4 unlinked inserts, whereas 3% appeared to be homozygous for the insert(s). All of the kanamycin resistant plants that were tested were found to possess NPT II activity. Southern analysis revealed that all of the resistant plants contained at least 1 copy of the T-DNA and that the majority of the plants had multiple inserts. In addition to demonstrating that Agrobacterium-mediated transformation has taken place, the analyses also indicate that a simple transformation of the genetically effective cells in the embryo rarely occurred. The ramifications of the results are under investigation.
This research is supported in part by a grant from NSF (DCB-8609623).

SOMATIC EMBRYOGENESIS IN OAT CULTIVARS
F 207 Annette Nassuth, Cheryl Fife, George Fedak and Illimar Altosaar
University of Ottawa, Ottawa K1N 9B4, Canada

Successful transformation of plants depends mainly on two factors: 1) an efficient regeneration system and 2) a transformation procedure that introduces foreign DNA into a tissue that can be regenerated. Our experiments are aimed at the development of these procedures for cultivated oats.

1a) Calli were initiated from immature embryos of six different oat cultivars (A. sativa cv Hinoat, Woodstock, Tibor, Donald, Chevron and Ogle). Embryogenic (E) callus was obtained when 1-2 mm immature embryos were placed on MS medium containing 2 mg/L 2-4D and the resulting calli were transferred after 4 weeks to MS medium containing 0.5 mg/L 2-4 D (regeneration medium). E callus has a smooth, white, opaque, knobby appearance and comprises only a small fraction of a given callus. The frequency of E callus formation varied depending on the cultivar used. Somatic embryos have been observed in Woodstock and Hinoat cultures and could be regenerated to small plantlets on MS medium without 2-4D Plants were regenerated also via organogenesis.

1b) More readily available explants are leaf bases. Therefore 1.5 mm leaf base pieces were taken from shoots (3.5 cm) 6 days after germination and tested for callus formation on MS medium with 2 mg/L 2-4D . Calli have been obtained from pieces taken from the most basal 10 mm and they are currently being tested for their regeneration capability.

2) Before trying any transformation procedure, we tested which drug could be used as selectable markers. It was found that callus induction on basal leaf pieces was inhibited by Hygromycin B (10 ug/ml) but not by Kanamycin (up to 750 ug/ml). The hygromycin phosphotransferase gene can thus serve as a selectable marker gene for oats.

F 208 **SPECIES-SPECIFIC SUSCEPTIBILITY FOR DIRECT GENE TRANSFER, I. Negrutiu[1,2], M. Saul[1], J. Paszkowski[1], M. Horth[2], J. Potrykus[2], M. Jacobs[1]- Institute of Molecular Biology, Brussels (2) and Friedrich Miescher Institut, Basel (1)**

High transformation frequencies for the Tn-5 kanamycin- resistance gene (1), within the percentage range of surviving colonies have been obtained in SR_1 tobacco with protocols based on either electroporation or $MgCl_2$/high PEG treatment (2,3). Another Nicotiana species, namely N. plumbaginifolia, exhibited a much lower (1 order of magnitude or more) susceptibility to transformation under a variety of conditions including those shown to be very effective with SR_1 tobacco (3). In order to establish what major factors are involved in this lower responsiveness, we analysed the transformation process within this species at various levels: (1) uptake of transforming DNA; (2) integration pattern; (3) expression of the foreign gene at callus, plant, and progeny level; (4) stability of the foreign gene.

Results will be presented to show that in N. plumbaginifolia DNA uptake, integration and expression affect the transformation efficiency. Globally taken, the data indicated that there is a species-specifity for high efficiency direct gene transfer and a very complex determinism for a possible state of "competence" in plant protoplasts.

(1) Paszkowski et al. (1984) EMBO J. 3, 2717-2722.
(2) Shillito et al. (1985) Biotechnology 3, 1099-1103.
(3) Negrutiu et al. (1986) Plant Molecular Biology (submitted).

F 209 STABLE TRANSFORMATION OF PANICUM MAXIMUM AND TRITICUM MONOCOCCUM: MORE EFFICIENT SELECTABLE MARKERS FOR THE GRAMINEAE, Peggy Ozias-Akins, Randal Hauptmann, Vimla Vasil, Zohreh Tabaeizadeh, Rob Horsch, Steve Rogers, Robb Fraley and Indra Vasil, Univeristy of Florida, Department of Botany, Gainesville, FL 32611 and Monsanto Co., St. Louis, MO 63198 USA.

Transient expression of chloramphenicol acetyltransferase (CAT) was detected after electroporation of protoplasts derived from several gramineous suspension cultures. Expression of the chimeric CAT gene driven by the CaMV 35s promoter could be observed in Panicum maximum, Triticum monococcum, Pennisetum purpureum, Saccharum officinarum, and Pennisetum transpecific hybrid (P. americanum, P. purpureum, P. squamulatum). Optimum voltage and capacitance for protoplast viability and CAT activity were determined and used in experiments with other chimeric gene constructions in an effort to produce stable transformants. Methotrexate resistant cell lines of Panicum maximum and hygromycin and kanamycin resistant cell lines of Triticum monococcum were recovered. Southern analysis has confirmed that some of these cell lines are transformed. All the gramineous suspsension cultures used in our experiments were able to tolerate high levels of kanamycin; therefore, growth on media containing methotrexate or hygromycin proved to be a more efficient selection method.

F 210 HIGH-FREQUENCY PLANT REGENERATION FROM CULTURED COTYLEDON SEGMENTS OF ARABIDOPSIS THALIANA, David A. Patton and David W. Meinke, Oklahoma State University, Stillwater, OK 74078.

Transformation of Arabidopsis thaliana leaf segments with Agrobacterium tumefaciens strains carrying resistance to hygromycin has recently been reported by Lloyd et al. (Science 234: 464-466; 1986). Regeneration of transformed plants was observed at a moderate frequency on a standard petunia regeneration (PR) medium containing MS salts with 1 mg/l BAP and 0.1 mg/l NAA. We have found that the regeneration frequency of wild-type plants not exposed to Agrobacterium can be increased dramatically by using immature cotyledons as an explant. Isolated cotyledon segments at a mature green stage of embryo development expand rapidly on a PR medium and begin to produce numerous shoots after 2-3 weeks in culture. Approximately 40% of the cotyledon segments eventually produce shoots. Regeneration occurs in the absence of the original shoot apical meristem. Hypocotyl segments from these embryos produce root hairs and dense callus in culture but only rarely develop shoots. The growth responses of leaf discs, cotyledon segments, and germinating seedlings were also compared on media supplemented with different concentrations of hygromycin, kanamycin, and G-418. Rapid growth of the cotyledon segments in culture was inhibited by each of these antibiotics. Our culture system therefore yields large numbers of regenerated plants that may be useful in future transformation studies.

F 211 SEQUENCE ANALYSIS OF WGA GENES FROM WHEAT, Natasha V. Raikhel, Thea A. Wilkins and *W. Michael Ainley, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312 and *Botany Department, University of Georgia, Athens, GA 30602

Wheat germ agglutinin (WGA) accumulation occurs in a tissue-specific and cell-type specific manner in various organs of wheat embryo (e.g. coleoptile, coleorhiza, radicle etc.). In order to investigate the tissue-specific expression of WGA genes we prepared two synthetic oligonucleotides, each consisting of 20 nucleotides complementary to the 5' and 3' ends of the coding portion of WGA mRNA (Wright et al. 1984). Oligonucleotides were synthesized on an Applied Biosystem 380 DNA synthesizer by a solid phase method. Each set of probes was degenerate. Oligonucleotides were purified by acrylamide gel electrophoresis and end-labelled with kinase.

Two cDNA libraries derived from wheat embryos at 3- and 4-weeks post-anthesis were prepared according to Alexander et al. (1984) and was provided to us by Dr. Chris Brinegar (ARCO Plant Cell Research Institute). The cDNA libraries were annealed to plasmid pARC7 and used to transform E. coli strain DH5α (Hanahah 1985). The transformed colonies were transferred to nitrocellulose filters and hybridized with the oligonucleotide probes. We selected six clones which hybridized with either one or both probes. Plasmid DNA was prepared from presumptive clones and DNA was cleaved with restriction endonucleases. All six clones are probably unique because they exhibit different restriction patterns. To determine the size of the messages represented by each clone, poly(A) mRNA was purified according to Williamson et al. (1985), electrophoresed through agarose and blotted onto nitrocellulose. These blots of poly(A) mRNA were then probed with ^{32}P-labelled cDNA inserts.

We have now subcloned the cDNA fragments into Bluescript vectors (Stratagene) and are sequencing them using the dideoxysequencing method of Sanger et al. (1977). We have partially sequenced two of the six clones and shown that the predicted amino acid sequences have almost complete homology to the published sequence of WGA. It is also clear that we are dealing with a small multigene family, because two partially sequenced clones differ from each other at the amino acid level.

F 212 MOLECULAR ANALYSIS OF THE T-DNA TRANSFER PROCES. Benedikt Timmerman, Scott E. Stachel, Marc Van Montagu, and Patricia Zambryski, State University Ghent, Belgium

Upon genetic transformation of plant cells by Agrobacterium tumefaciens, the T-DNA border sequences (25 bp repeats) are recognized and they direct, in a polar fashion, the mobilization of the T-DNA from the Ti plasmid of the bacterium to the plant nuclear genome (1). T-DNA transfer is triggered by the plant exudate acetosyringone which activates Ti plasmid encoded virulence genes (vir) (2). Vir gene products are instrumental to the actual DNA transfer. We have identified several T-DNA associated molecular structures which most likely represent the primary steps of the T-DNA transfer proces : the generation of a nick in the bottom strand of both T-DNA 25 bp repeats, and the formation of a free single-stranded, unipolar, linear T-DNA molecule (T-strand) (3). A third T-DNA associated event dependent on Vir induction is the precise excision of the T-DNA from the Ti-plasmid (4) and the formation of T-DNA circles (5, 6). The frequency of this event is ± 100 times lower than T-strand synthesis. The presented data suggest that excision occurs via site specific recombination, dependent on a single-stranded nick at one recombination site, while homology to this sequence at the other recombination site suffices. The role and relevance of these phenomena in the T-DNA transformation mechanism will be discussed.

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F 213 DEVELOPMENTAL REGULATION OF A MAIZE LIPID BODY PROTEIN, Vicki Bowman Vance and A.H.C. Huang, Biology Department, University of South Carolina, Columbia, SC 29208

We have cloned and sequenced a maize cDNA for the major lipid body protein (L3) and examined the expression of this gene during seed maturation and germination. This protein is tightly associated with the "half-unit" membrane of the lipid bodies which are synthesized and stored in the scutella during seed maturation. Northern analysis indicates that L3 gene expression is developmentally and hormonally regulated. The L3-mRNA is present in the scutella early in maize seed maturation and reaches a maximal steady state level at mid-maturation. It is detected in relatively low levels one day post-imbibition, abruptly disappears at two days post-imbibition and is not present at later stages of germination. The disappearance of the L3-mRNA at this early stage of germination could be delayed by imbibing the seeds in the presence of exogenous abscisic acid, suggesting direct or indirect positive regulation of L3 gene expression by this hormone. Analysis of polysomal distribution of the L3-mRNA indicates that abscisic acid does not affect the translation of this mRNA during early germination. The amino acid sequence of the L3 protein was inferred from the nucleic acid sequence of the cDNA clone. The protein contains an unusually long hydrophobic region which may be associated directly with lipid. Further, like mammalian *apolipoproteins*, the L3 *protein contains an amphipathic α-helix structure* at its carboxyl terminus.

F 214 PROLONGED EMBRYONIC SENSITIVITY TO ABA IN DORMANT WHEAT GRAIN, M. Walker-Simmons, USDA-ARS, Wheat Genetics, Quality, Physiology and Disease Research Unit, Washington State University, Pullman, WA 99164-6420.

The role of embryonic ABA (abscisic acid) in regulating wheat grain dormancy and germination has been examined. Some wheat cultivars produce grain that retains seed dormancy throughout grain development and desiccation. Embryonic sensitivity to ABA in these cultivars was measured by the capability of exogenous ABA to block embryonic germination. ABA (0.5 - 5.0 uM), but not water controls, effectively (50-90%) inhibited embryonic germination of embryos dissected from dormant grain throughout development and desiccation. Grains eventually lost dormancy weeks after reaching final dry weight. At the same time as grains lost dormancy, dissected embryos lost sensitivity to applied ABA. In contrast, wheat cultivars that produce grains with low levels of seed dormancy had less embryonic sensitivity to ABA. ABA (0.5 - 50 uM) was ineffective in inhibiting embryonic germination once grains reached maximum fresh weight and grain desiccation started to occur. Endogenous embryonic ABA concentrations in each cultivar measured with an indirect ELISA monoclonal antibody assay were similar. Causes of the embryonic sensitivity differences such as variation in ABA-inducible proteins are now under investigation.

Stress-Induced Gene Expression

F 300 ROOT COLONIZATION BY PSEUDOMONAS PUTIDA, Anne Anderson, Craig Tepper, Yecihe Acun, Beverly Graetz and Pouran Tari. Utah State University, Logan, Utah 84322-4500.

Pseudomonas putida, a suppressor of certain soil-borne plant pathogens, aggressively colonizes root surfaces. Cells of P. putida can be agglutinated by a glycoprotein detected on root surfaces. The role of the agglutinin in P. putida root colonization is being examined by use of mutants obtained by Tn5 and EMS mutagenesis. These mutants displayed levels of agglutination that varied from non-agglutinable (agg^-) to super-agglutinable (agg^S), where the cells agglutinated to a greater extent than the parental isolate. Agg^- and agg^S mutants that grew on liquid minimal medium at wild-type rates were selected for colonization studies. Under sterile conditions, agg^- and agg^S mutants colonized bean roots at near parental levels. Southern blot hybridization analysis indicated Tn5 to be inserted into unique genomic fragments for 5 agg^-, and 2 agg^S mutants. Outer membrane protein preparations from the EMS and Tn5 agg^- mutants lacked a Coomassie-blue staining band that was present in parental preparations.

F 301 OLIGONUCLEOTIDE-SITE DIRECTED MUTAGENESIS OF A HEAT SHOCK ELEMENT IN THE PROMOTER REGION OF A SOYBEAN HEAT SHOCK GENE, Dulce Barros, Eva Czarnecka and William B. Gurley, Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611.

The 5' flanking region of the soybean heat shock gene Gmhsp17.5E contains two overlapping heat shock promoter elements (HSE) located 17 bp upstream the TATA element. The TATA proximal HSE matches the Drosophila consensus (CT-GAA-TTC-AG) in nine of the ten specified bases, whereas the distal HSE shows homology to six bases. Oligonucleotide site directed mutagenesis was applied to introduce single base changes in the TATA proximal HSE of Gmhsp17.5E gene in order to evaluate the contribution of specific bases of the HSE to the thermoinducibility of transcription in plants. These alterations were made in seven positions within the HSE. In some positions more than one type of base was substituted for the original. The mutated HSE's were inserted into the promoter of Gmhsp17.5E and were cloned into a T-DNA-based double gene vector. The thermoinducible transcriptional activity of the mutated DNA was assessed in sunflower tumors.

F 302 GENES EXPRESSED DURING DIFFERENTIATION OF THE RUST FUNGI, S. Bhairi[1], R.C. Staples[1], and O.C. Yoder[2], [1]Boyce Thompson Institute and [2]Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
Germ tubes of uredospore germlings of the bean rust fungus, Uromyces appendiculatus, differentiate to form appressoria over the stomatal opening of their host plant. To study this location process, we have been isolating the dr-genes, genes specifically expressed during differentiation. So far, we have obtained 20 clones of dr-genes using a modified cascade hybridization procedure. Restriction mapping data and Southern hybridization analyses have indicated that 14 of the 20 clones are homologous. Northern analyses, using different EcoRl restriction fragments of one of the 14 clones as probes, indicated the presence of a dr-gene on one of the fragments. The other fragment contained a gene expressed in both differentiated and nondifferentiated germlings. The remaining six clones contain unique sequences. Three of these six clones hybridize to the corresponding transcript specifically present in the differentiated stage of the fungus, while the remaining three clones hybridize weakly to a single transcript present in both differentiated and nondifferentiated spores. We conclude that at least four dr-genes are specifically expressed during differentiation of bean rust uredospore germlings.

F 303 Phenylalanine-ammonia-lyase(PAL)activity in soybeans in response to Phytophthora megasperma f.sp. glycinea(Pmg) or $AgNO_3$. M.K.Bhattacharyya and E.W.B.Ward,Research Centre,Agriculture Canada, Univ. Sub Post office, London, Ontario,Canada N6A 5B7.

Rapid induction of PAL activity was recorded in resistant responses of intact etiolated hypocotyls and leaves but not in susceptible responses following inoculation with zoospores of Pmg.In immature trifoliates of a resistant cultivar PAL activity was not induced and leaves were susceptible. Following $AgNO_3$ treatment glyceollin production in immature leaves was one fifth of that in mature leaves, suggesting host defence genes are not expressed in immature leaves.

Hypocotyls of cultivar Altona are resistant to Pmg race 4 at 25°C but susceptible at 33°C and susceptible to race 6 at both temperatures. At 33°C, PAL activity after inoculation with race 4 was comparable to that with race 6. Following $AgNO_3$ treatment, PAL activity was much less (ca.54%) at 33°C than at 25°C. A similar decrease was demonstrated in a second cultivar with temperature-induced susceptiblity to Pmg, but smaller decreases occurred in cultivars that are not temperature sensitive.In a separate experiment tolerance of Pmg race 4 to glyceollin I at 33°C was greater than at 25°C. Temperature -induced changes in host metabolism coupled with changes in pathogen metabolism may explain temperature-induced susceptibility of soybeans to Pmg.

F 304 THE FER GENE FOR FE-EFFICIENCY REACTIONS IN TOMATO.
H.Frits Bienfait, Laboratorium Genetika, Rijksuniversiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium.

Fe deficiency induces, in roots of dicotyledons and non-grass monocots, a number of morphological and biochemical changes: high root hair density, formation of rhizodermal transfer cells, strong proton extrusion and a high ferric chelate reduction capacity (Turbo reductase, ref 1) in the epidermal plasma membranes. The roots can produce these responses autonomously, i.e. no signal from the leaves is necessary (Bienfait et al., in press).

The tomato mutant T 3820 fer cannot develop all these 'Fe efficiency reactions'. The mutated gene obeys the Mendelian laws. T 3820 fer is fully capable to form transfer cells at other places in the plant, and it synthesizes the 'Standard' electron transfer system in the plasma membrane, both of which are independent on the Fe status.

The FER gene apparently determines the sensitivity of the root to lowered Fe levels and the concomitant development of a number of responses.

Our present aim is to identify the FER gene product.

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F 305 HEAT SHOCK PROTEINS ARE NOT REQUIRED FOR THE ESTABLISHMENT OF THERMOTOLERANCE IN MAIZE

Peta C. Bonham-Smith[1], M. Kapoor[1] and J. Derek Bewley[2], [1]Department of Biology, University of Calgary, Alberta, [2]Department of Botany, University of Guelph, Ontario, Canada

Pretreating maize seedlings with either a progressive water stress of -0.25 MPa/hr from 0MPa to -1.25 MPa over 6 hr or a 4-day growth in various concentrations (50 μm to 200 μm) of $CuSO_4$, $CdCl_2$ or $ZnCl_2$ induces a thermotolerance within these seedlings which now enables them to accommodate a subsequent 3 hr heat shock of 40°C or 45°C (the latter is lethal in non-treated seedlings). Neither pretreatment induces the synthesis of heat shock proteins (hsps) as shown by in vivo protein labelling. Further, northern analysis with the maize genomic hsp 70 probe (pMON 9502-Monsanto) and in vitro translations of bulk RNA from pretreated seedlings show no induction of hsp mRNA. Seedlings treated for 1 to 4 days with 10^{-4}M ABA, as well as those treated for 1 to 4 days with 10 μg/ml Triadimefon (a fungicide which results in elevated levels of ABA in treated plants) also show a thermotolerance to the 3 hr heat shock of 40°C or 45°C. These latter data suggest that changes in ABA levels, and not the induction of hsps, play a role in the establishment of thermotolerance. Finally, inducing hsps by pretreating seedlings with a progressive heat shock of 2°C/hr from 26°C to 36°C prior to subjecting seedlings to a severe water stress, (-1.5, -1.75 or -2.0 MPa PEG for 24 hr) does not better enable seedlings to withstand this water stress - suggesting that hsps do not constitute a general stress protection system.

F 306 PROTEIN IN CELL WALLS ARE ALTERED AT LOW WATER POTENTIALS, Connie S. Bozarth, John E. Mullet and John S. Boyer, Dept. of Soil and Crop Sciences (CSB,JSB) and Dept. of Biochemistry (JSB,JEM), Texas A&M University, College Station, Texas 77843.

Stem elongation was inhibited in dark-grown soybean (Glycine max [L.] Merr.) when the roots were exposed to vermiculite having low water content (low water potential, ψ_w). After a time, growth resumed but at a reduced rate relative to the controls. The extractable protein increased in the cell walls as ψ_w decreased, especially a 28 kDa protein in the young tissue. In contrast, a 70 kDa protein, mainly present in mature cell walls, decreased at low ψ_w. Both proteins were glycoproteins with little or no hydroxyproline; the 28 kDa protein contained 25 mol% glycine and the 70 kDa protein contained 32.5 mol% isoleucine and 23.4 mol% tyrosine. This amino acid composition suggests that these are structural proteins, but not related to each other or to extensin. The protein changes occurred soon after the initial growth inhibition, and the 28 kDa protein appeared in the elongating tissue at about the time growth resumed. The correlation between growth and protein changes in the cell walls suggests that the two events could be related. Affinity purified antibody against the 28 kDa protein bound strongly to 28 kDa and 31 kDa protein in the cytoplasmic fraction. Cell wall proteins of similar molecular weight in several other species also reacted positively with this antibody, indicating that the 28 kDa protein is not limited to soybean.

F 307 ALTERED GENE EXPRESSION DURING DROUGHT STRESS IN TOMATO MUTANTS THAT ARE DEFICIENT IN ABA BIOSYNTHESIS, Elizabeth A. Bray, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521

Drought stress triggers ABA biosynthesis resulting in ABA accumulation in stressed leaves. During drought stress, protein synthesis may also be altered. Polypeptide biosynthesis in wild type tomatoes (Lycopersicon esculentum c.v. 'Ailsa craig') and mutants *that fail to accumulate ABA during stress (flacca, notabilis, and sitiens) is being studied* to identify and characterize genes that are expressed during drought stress in response to ABA. Leaflets were removed from the plant and fed ^{3}H leucine through the petiole, and after treatments, proteins were extracted, separated by SDS-PAGE, and visualized by fluorography. The control leaflets were incubated in plastic bags or floated on water for 4 h. The stressed leaflets were wilted to 88% of their original fresh weight and then incubated in plastic bags for the remainder of the 4 h incubation period. The pattern of polypeptide synthesis in well-watered leaflets differed among the four different genotypes. During drought stress, polypeptide biosynthesis differed in comparison to the control within and between genotypes. Mutants treated with ABA during stress in comparison with the wild type are being used to identify polypeptides that are induced by stress and to distinguish those that are induced by ABA.

F 308 CHARACTERIZATION OF UBIQUITIN GENES IN ARABIDOPSIS THALIANA, Thomas J. Burke and Richard D. Vierstra, Dept. of Horticulture, University of Wisconsin-Madison, Madison, WI 53706.

Ubiquitin is a highly-conserved, 76 amino acid protein which covalently attaches to other cellular proteins as a key step in protein degradation. To determine what roles the ubiquitin dependent proteolytic pathway may play in plant development, a molecular approach is being used to study ubiquitin gene expression. Two clones have been isolated from an Arabidopsis thaliana lambda-gtll genomic library which contain uiquitin coding sequences. The genomic library was screened with immunopurified antibodies raised against both oat and human ubiquitin. Two plaques were isolated which showed reactivity to both anti-oat- and anti-human ubiquitin antibodies while several other plaques were isolated which were recognized only by the anti-oat ubiquitin antibodies. The presence of ubiquitin coding sequences in both genomic clones was confirmed by hybridization to a chicken ubiquitin cDNA clone and to a yeast ubiquitin genomic subclone. The Arabidopsis clones are being used to study genomic organization and developmental regulation of ubiquitin sequences and to determine if the ubiquitin proteolytic pathway may play a role in various plant processes.

F 309 GENES EXPRESSED DURING PEACH FRUIT DEVELOPMENT, Ann Callahan, Eve Walton, Diane Wydoski, and Peter Morgens, USDA and West Virginia University Appalachian Fruit Research Station, Kearneysville, WV 25430.

Cold hardiness and high fruit quality have been difficult to combine in peach using traditional breeding methods. This is presumably because they are both complex and multigenic traits. Cold hardy genotypes are especially difficult to identify due to strong environmental effects and the various modes of cold resistance including wood hardiness, flower bud hardiness, rate of acclimization, and bloom period. By comparison fruit quality genotypes are relatively easy to identify. Genotypes that have most of the desirable cold hardiness phenotypes exist (e.g. peach cvs. 'Bailey' and 'Boone County'), but they lack certain fruit quality characteristics necessary for successful commercial cultivars (i.e. fruit size and color). As part of a combined effort with a peach breeder and a tissue culturist, we are attempting to isolate fruit-specific genes whose expression differs between cultivars considered to be of high fruit quality and those considered to be of low fruit quality. We hypothesize that these genes will be good candidates for gene transformation experiments to increase some aspects of fruit quality in cold hardy cultivars. Preliminary data on the isolation of these genes will be presented.

F 310 INDUCTION OF TOBACCO GENES BY TMV-INFECTION, Ben J.C. Cornelissen#, Jeannine Horowitz*, Robert B. Goldberg*, Rob A.M. Hooft van Huijsduijnen and John F. Bol, Dept. of Biochemistry, State University of Leiden, # MOGEN International B.V., Leiden, The Netherlands, *Dept. of Biology, UCLA, Los Angeles, CA 90024.

Infection of Samsun NN tobacco with Tobacco mosaic virus (TMV) results in the de novo synthesis of pathogenesis-related (PR) proteins 1a, 1b, 1c, 2, N, O, P, Q, R, and S, which all accumulate in the intercellular space of the leaf. We have cloned cDNA to six classes of tobacco mRNAs, A to F, that are synthesized upon spraying the plant with 5 mM salicylic acid. By immune-precipitation of in vitro translation products of hybrid selected mRNAs it was shown that mRNAs B, D, E and F encode PR proteins. Cluster B clones correspond to the related proteins 1a, 1b and 1c; cluster D clones correspond to the related proteins P and Q. mRNA E encodes a protein that corresponds to PR-S; it shows extensive homology to the sweet tasting protein thaumatin. Cluster B clones correspond to at least five different mRNAs, four encoding acidic PR-1 proteins and one encoding a more basic PR-1 protein. Genomic blots indicate the presence of six genes for acidic, and six genes for basic PR-1 proteins in the Samsun NN genome. Three of the genes for acidic PR-1 proteins were isolated from a genomic library and have been sequenced. In addition, several genes corresponding to cluster C cDNAs are being sequenced. A comparison will be presented of the putative regulatory sequences involved in the induction of cluster B and C gene-families by TMV-infection and salicylic acid treatment of tobacco.

F 311 THE PHENYLALANINE AMMONIA LYASE SMALL MULTIGENE FAMILY: ISOLATION AND CHARACTERIZATION OF 2 GENES DIFFERENTIALLY INDUCED BY STRESS, Carole L Cramer, Keith Edwards [1], Michel Dron, Wolfgang Schuch [1] and Chris J Lamb, Plant Biology laboratory, The Salk Institute for Biological studies, P.O Box 85800 San Diego, CA 92138-9216 USA. [1] Imperial Chemical Industries, Corporate Science laboratory, the Heath, Runcorn, Cheshire WA7 4QE, UK.

Phenylalanine ammonia-lyase (PAL) is the first enzyme and a key regulatory step in the phenylpropanoid pathway which produces isoflavonoid phytoalexins (antibiotics), anthocyanin pigments, lignins, and cinnamic acid esters (wound protectants) in higher plants. In bean (Phaseolus vulgaris), PAL is induced as part of the plant defense response during fungal infection, treatment with fungal elicitor, or wounding. In addition PAL is under developmental regulation by light and hormones. Genomic Southern hybridization against a PAL cDNA clone, isolated using RNA from an elicitor induced cell culture, suggest that the bean genome contains 3-4 PAL genes. We have isolated and analyzed two of these genes from a bean "Tendergreen" genomic library, they share 75% homology. The two genes contain 1 intron but different in size and primary sequence, the second exon is much more conserved than the first one. Data concerning different induction of expression for these two genes after wounding will be also presented.

F 312 DISEASE RESISTANCE RESPONSE GENES IN PEAS, Catherine H. Daniels, Yvonne S. Cody and Lee A. Hadwiger, Washington State University, Pullman WA 99164.

The pea serves as host to the fungal pea pathogen Fusarium solani f. sp. pisi. Its resistance response is only partially effective against the fungus and breaks down over time. The pea is not a host to the fungal bean pathogen Fusarium solani f. sp. phaseoli (Fsph) and the resistance response is both completely effective and sustained over time. cDNA clones of nine genes active in the pea-Fsph interaction have been screened and the behavior of their homologous disease resistance response genes (DRRG) monitored under a variety of stresses. A heat shock treatment of 40°C for 1 hr which breaks non-host resistance to Fsph also blocks accumulation of some 20+ pea proteins associated with resistance. Behavior of the DRRG was followed during heat shock and results indicate the DRRG are suppressed during treatment. Pea recovery from heat shock is accompanied by both a renewed potential to resist the fungus and a renewed potential of the DRRG to be induced by Fsph challenge. The activity of these DRRG was also followed after challenge by a second plant pathogen Pseudomonas syringae pv. pisi (Psp). Pea cultivars carrying single dominant Mendelian genes are resistant to one or more races of Psp. Nine cDNA clones derived from the pea-Fsph interaction were hybridized with RNA from five pea cultivars differentially reacting to three Psp races. Enhanced accumulation of RNA homologous to five cDNA clones was temporally correlated with race-specific resistance. Some of the cultivars were also challenged with Psp isolated cell wall preparations of each Psp race. The same five cDNA probes showed enhanced accumulation of homologous RNA which correlates both with results using live Psp cells and plant expressed phenotypic resistance.

F 313 STRUCTURE AND EXPRESSION OF ELICITOR AND UV LIGHT INDUCIBLE 4-COUMARATE CoA:LIGASE GENES IN PARSLEY. Carl Douglas, Heidi Hoffmann, Jeff Dangl and Klaus Hahlbrock. Max-Planck-Institut fuer Zuechtungsforschung, D-5000 Koeln 30, Fed. Rep. Germany

Genes encoding enzymes of general phenylpropanoid metabolism, which leads to the formation of defense related flavonoids and phenolic compounds in parsley, are transcriptionally induced in cell cultures by fungal elicitor and UV light. Parsley appears to contain two genes encoding 4-coumarate CoA:ligase (4CL), an enzyme of general phenylpropanoid metabolism, and genomic clones of each gene have been isolated. Both genes have been sequenced, and the 5' transcription start points determined. Additionally, full length cDNA clones of each gene have been sequenced. The two 4CL genes encode two slightly different 4CL isozymes. Sequence data from cDNA clones derived from both UV and elicitor induced cells and hybridization to a gene specific probe suggest that both genes are induced by both fungal elicitor and UV light. We have established a transient expression system in parsley protoplasts for elicitor and UV induced genes, which allows the rapid assay of expression from introduced DNA. The 5' upstream sequences from the 4CL genes required for elicitor and UV induction are currently being determined using this assay.

MUTATIONAL ANALYSIS OF THE SMALL SUBUNIT OF RUBISCO,

F 314 John Fitchen and Lee McIntosh, MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

The small subunits (S) of form I (L_8S_8) Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) are required for catalytic activity. Their role in catalysis, however, is unknown. We have undertaken a mutational analysis of the small subunit using cyanobacterial genes expressed in E. coli.

Mutations were made throughout the coding region of Anabaena rbcS using the in vitro mutagenesis and mutant isolation technique of Myers et al. (Science 229:242 1985). The mutant genes were inserted into expression vectors and co-transformed into E. coli with another plasmid directing the expression of Anabaena large subunits (L). In each case, the sedimentation properties of the subunits or multisubunit complex from E. coli lysates are being analyzed on sucrose gradients to determine whether the mutation interferes with assembly. Lysates are also assayed for RuBP-dependent CO_2 fixation. By isolating and characterizing mutant small subunits which are competent to assemble with large subunits to form an L_8S_8 structure, but which do not allow the multisubunit complex to perform catalytically, we hope to define the functional contribution of the small subunits to the holoenzyme.

TWO PERIBACTEROID MEMBRANE NODULINS OF SOYBEAN ARE EXPRESSED AT DIFFERENT STAGES OF NODULE DEVELOPMENT IN SOYBEAN

F 315 Marc G. Fortin, Nigel Morrison and Desh Pal S. Verma, Centre for Plant Molecular Biology, Department of Biology, McGill University, Montreal, Canada H3A 1B1

The peribacteroid membrane delimits a new compartment in infected cells of soybean root nodules that arises by internalization of bacteria from the infection thread into the host cell. Immunoprecipitation of hybrid-released translation products of these clones and immunoelectron microscopy using nodule-specific peribacteroid membrane and nodulin-24-specific antisera suggested that nodulin-24 and nodulin-26 are nodule-specific proteins of the peribacteroid membrane. The predicted topology of these proteins in the peribacteroid membrane suggest that nodulin-24 is located at the surface of the membrane facing the bacteroids, whereas nodulin-26 traverses the membrane and has both cytoplasmic and extracytoplasmic domains. Bradyrhizobium japonicum Tn5 mutants arresting soybean nodule development at different stages showed that both nodulin-24 and -26 are expressed at near normal levels in nodules that have infection threads invading the root cortex without any release of bacteria into the root cells. However, nodulin-24 is not induced in nodules lacking infection threads, an early step of the infection process. This suggests that more than one level of regulation of nodulin induction is present and that nodulin-26 can be induced in the absence of the peribacteroid compartment and internalization of Rhizobium.

HEAT-SHOCK PROTEINS IN DEVELOPING MICROSPOROCYTES IN MAIZE,

F 316 Carla B. Frova and Giorgio Binelli, Dept. of Genetics & Microb., University of Milano, Milano, Italy.

Maize sporophytic tissues exhibit a "heat-shock" response following a significant temperature increase. Normal protein synthesis decreases while a new, specific set of polypeptides is synthesized (hsps). Although a certain degree of thermotolerance seems to be associated with hsp production, the data available are not conclusive. Mature pollen seems to lack hsp synthesis. In order to improve our understanding of the heat stress response in maize, we checked for hsp synthesis during pollen development, and compared gametophytic and sporophytic heat-shock responses. Our results show that the synthesis of hsp is active in the developmental stages of pollen grains from 7 days after meiosis until 2 days before anthesis. The response however widely differs from the sporophytic one both in quantitative and qualitative terms, and appears to be genotype and stage dependent. Of the sporophytic hsps only the 72 Kd polypeptide was consistently found throughout all stages.

F 317 PHOSPHATE STARVATION INDUCIBLE EXCRETION OF ACID PHOSPHATASE BY CELLS OF LYCOPERSICON ESCULENTUM IN SUSPENSION CULTURE, Alan H. Goldstein, Dawn M. Baertlein and Robert G. McDaniel, University of Arizona, Tucson AZ 85721

Phosphate starvation inducible (PSI) gene systems are well characterized in both bacterial and fungal systems. A major component of the PSI response is de novo gene expression that results in excretion of large amounts of phosphatases into the external medium. These enzymes act to cleave organic P to available Pi. We have studied the PSI response of tomato (Lycopersicon esculentum cv. VF36) cells growing in suspension culture. Cells were grown in continuous liquid culture. To initiate an experiment 5ml of middle log-phase cells (10 days old) were transferred into either- media containing either 170 mg/L KH_2PO_4 or media with no added mineral P. Plus Pi and (-) Pi cells grew at approximately the same rate for the first 8 days. During this time exogenous Pi fell from 25 to 10ppm in the (+) cells while the minus cells remained at < 2ppm. The (-) cells reached stationary phase at 10 days whereas the (+) cells grew until day 12, achieving a final biomas of appx. twice the (-) cells. Acid phosphatase (AcPase) was measured daily in the supernatant of both treatments. A parallel sample was disrupted to assay total AcPase. AcPase activity, on a dry wt basis, was higher in the (-) supernatant at all time points. Maximum differences were observed between days 8 and 10 which corresponds to the time when the (-) cells were showing PSI growth reductions. During this time the (-) cells had 150-250 units/mg dry wt vs 10-20 for the (+) cells. Total AcPase in the disrupted cells was proportionally higher for both treatments, indicating that all cells contained the same amount of an intracellular AcPase. Experiments are underway to determine if this increase in enzyme activity results from de novo gene expression.

F 318 **GENETIC AND MOLECULAR STRUCTURE OF SALT TOLERANCE OF ELYTRIGIA ELONGATA AS EXPRESSED IN BREAD WHEAT.** **Patrick J. Gulick, Mark Edge, Kathleen Ross, and Jan Dvorak, University of California at Davis, Davis CA 95616.** **We used the complete set of 7 disomic additions, 21 disomic substitutions and 21 double monosomic additions of chromosomes from halophytic E. elongata into bread wheat cv. Chinese Spring in determining the genetic structure of salt tolerance of E. elongata which is expressed in wheat. Genes controlling salt tolerance were identified on all seven chromosomes; moderate increases in salt tolerance were associated with four single Elytrigia chromosomes, whereas dramatic increases occured in a number of pairwise combinations of Elytrigia chromosomes. We concluded that salt tolerance in E. elongata is controlled by a polygenic system, but that many genes behave as "major" genes and are thus amenable to molecular manipulation. In vitro translation of mRNA populations from salt treated and control E. elongata x Chinese Spring wheat amphiploid plants showed that there are genes induced during salt treatment. A cDNA library was constructed from salt treated E. elongata plants and salt induced genes are being selected by colony hybridization. Genetic mapping of the clones to specific chromosome regions by Southern blot analysis and the characterization of induction of the genes by Northern blot analysis will be used to determine causality between the expression of the cloned genes and the enhanced salt tolerance of the plant.**

F 319 EFFECTS OF HEAVY METALS ON TOMATO CELL CULTURES, Subhash Gupta, Bin Huang and Peter Goldsbrough, Purdue University, West Lafayette, IN 47907.

When cell suspension cultures of tomato (Lycopersicon esculentum Mills. cv VFNT-Cherry) are exposed to 400 uM Cd^{2+} there is a synthesis of low molecular weight peptides, which are not detected in cells grown in the absence of Cd^{2+}. In a number of other plant species, cadmium induces the synthesis of a family of peptides termed phytochelatins. Phytochelatins bind cadmium and have the general structure (γ-Glutamyl-Cysteine)n-Glycine, (n=2-11); for glutathione (GSH), n=1. We have purified a number of peptides that are synthesized in tomato cells grown in the presence of Cd^{2+}. The amino acid compositions of these induced peptides are consistent with those of phytochelatins.

Two lines of evidence indicate that GSH is a substrate for phytochelatin synthesis: 1) The increase in phytochelatin amounts is accompanied by a concomitant decrease in the levels of GSH 2) When cellular GSH is depleted by inhibiting the first enzyme of GSH synthesis, phytochelatin formation is inhibited. Phytochelatin synthesis is restored by the addition of exogeneous GSH. We can not at present rule out the possibility that GSH is the only substrate for phytochelatin synthesis. Little is known about the metabolic pathway(s) of phytochelatin production. We are presently investigating the enzymes involved in the synthesis and degradation of GSH and how these are involved in the synthesis of phytochelatin.

F 320 TMV INDUCED GENE EXPRESSION IN TOBACCO, Jeannine Horowitz and Robert B. Goldberg. University of California, Los Angeles CA 90024.

We are studying the molecular basis of the hypersensitivity and induced resistance observed in tobacco as a result of infection by TMV.

We have isolated and characterized cDNA clones from an infected library. These clones represent mRNAs which are induced as a result of the TMV infection. We have been able to identify some of the cDNAs as representing mRNAs for the Pathogenesis-Related proteins (PRs). The identity of the mRNAs represented by most of the cDNA clones, however, remains unknown.

We have done *in situ* hybridizations using infected and healthy leaves to localized the position of PRs and viral coat protein transcripts in relation to the lesion. We have also isolated the genes for the thaumatin-like PR protein from a tobacco genome library and we are using the genes in transformation experiments and in DNA-binding protein studies to identify the cis-acting regions and the trans-acting factors involved in the regulation of the response to the viral infection.

F 321 SALT-INDUCED CHANGES IN THE PATTERN OF PROTEIN SYNTHESIS IN BARLEY ROOTS, William J. Hurkman, Chester S. Fornari, and Charlene K. Tanaka, U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA 94710. Barley plants (*Hordeum vulgare* L. cv California Mariout 72) were grown hydroponically with or without 200 mM NaCl. Roots of intact plants were labeled *in vivo* and proteins were extracted and analyzed by fluorography of two-dimensional (2D) gels. The effect of NaCl on incorporation of ^{35}S-methionine into proteins was complex. The response to NaCl did not involve the synthesis of unique proteins, but rather, involved the modulation of the net synthesis of a wide range of constitutive proteins. Of the numerous changes, the amounts of two protein pairs with pIs of 6.3 and 6.5 and mol wts of 26- and 27-kD increased significantly in response to NaCl. When salt-stressed plants were transferred to nutrient solution without NaCl, labeling of the 26- and 27-kD proteins returned to near control levels. No cross-reaction was detected between the antibody to the 26-kD protein from salt-adapted tobacco cells and the 26- and 27-kD proteins of barley. When plants were transferred to partially hydrated vermiculite, the labeling of the 26- and 27-kD proteins did not increase. In addition, these proteins did not increase when plants were treated with polyethylene glycol, but did increase when plants were treated with mannitol. RNAs extracted from roots of control and salt-treated plants were translated *in vitro* and products analyzed on 2D gels. As observed *in vivo*, the polypeptide patterns for translation products of RNAs from control and salt-treated plants were qualitatively similar, but the net synthesis of some proteins was quantitatively different. Translation products of approximately 26-kD with pIs of 6.7 and 6.8 increased significantly with salt stress and decreased with recovery.

F 322 ISOLATION OF GENES ASSOCIATED WITH PROLINE ACCUMULATION IN *DISTICHILUS SPICATA* CELLS EXPOSED TO NaCl, *Roosevelt Y. Johnson, James W. Heyser, Pamela J. Anderson and Nigel J. Robinson. *Department of Botany, Howard University, Washington, DC 20059, and Genetics Group, Mail Stop M886, Los Alamos National Laboratory, Los Alamos, NM 87545. Halophytic *Distichilus spicata* cells accumulate proline when exposed to high concentrations of NaCl. [13-C] NMR has been used to show that exogenously supplied [13-C] glutamate is metabolized to proline in these cells. Conversion of [13-C] glutamate into proline is stimulated when cells are exposed to NaCl. However, this stimulation is repressed when proline is included in the growth medium. Polyadenylated mRNA has been isolated from *D. spicata* cells exposed to NaCl. This has been used to prepare a cDNA library using the plasmid vector pARC7 and a synthetic oligonucleotide polylinker fragment. A proportion of the library has been screened for sequences corresponding to genes whose expression increases after exposure to NaCl. These cDNA's are being screened for sequences whose expression in response to NaCl is repressed by exogenous proline.

This work was performed under the auspices of the U.S. Department of Energy and the Natural Environment Research Council of Great Britain.

F 323 HEAT SHOCK PROTEINS IN PIGEON PEA (CAJANUS CAJAN), Ram Kishore and Kailash C. Upadhyaya, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA

The proteins synthesized in response to higher temperature in a tropical legume plant, pigeon pea (Cajanus cajan) have been characterized using both single and two-dimensional polyacrylamide gel elecdtrophoresis. Roots or stem cuttings of three days' old seedlings were incubated at various temperatures in the presence of ^{35}S-methionine. The proteins, thus synthesized, were separated on SDS-PAGE and visualised by fluorography. When the incubation temperature is raised from 30° to 40°C, synthesis of seven polypeptides (79,70,57,55,48,32, 15 kD) is enhanced. Some of the heat shock proteins (hsps) show low level of synthesis at normal growth temperature of 30°C. The most abundant hsp in Cajanus appears to be of 57 kD. The hsps cannot be distinguished on silver stained gels possibly indicating that they do not accumulate during incubation at higher temperature. The detailed kinetics in terms of temperature and time-duration would be discussed and analysis of hsps on two dimensional gels would be presented.

F 324 GENETIC DIVERSITY OF HEAT-SHOCK PROTEIN SYNTHESIS AND ITS ROLE IN THERMAL TOLERANCE OF WHEAT. M. Krishnan, H.T. Nguyen, J.J. Burke, R.A. Vierling, and D.R. Porter. Texas Tech University and USDA-ARS, Lubbock, TX 79409.

Our objectives are to determine (1) the genetic diversity and relationship between heat-shock protein (HSP) synthesis and thermal tolerance in wheat, (2) cellular localization of HSP's, and (3) genetic control of thermal tolerance and HSP synthesis. Genetic diversity in thermal tolerance of diploid and hexaploid wheat was determined by 2,3,5-triphenyl tetrazolium chloride test. Genotypes differing in thermal tolerance were evaluated for HSP synthesis in leaf tissues at different growth stages, HS temperatures and duration using 1-D, 2-D gel electrophoresis and in vitro translation of HS mRNA's. In vitro and in vivo HSP synthesis by chloroplasts and mitochondria were studied to determine the role of organelle genome in the synthesis of HSP's and their localization. Quantitative genetic analysis will be used to determine the genetic control of thermal tolerance. The effect of the loss of specific chromosomes on HSP synthesis using aneuploid genetic stocks will provide an understanding of chromosomal control of HSP synthesis in hexaploid wheat. We also attempt to make cDNA from HS mRNA isolated from heat tolerant vs. susceptible genotypes. The clones will be biotin labelled and hybridized in situ to root tip chromosomes in order to determine the HSP genes cytological location. In general, results on the role of HSP synthesis in relation to genetic diversity in thermal tolerance as well as the genetic aspects of HSP gene expression will be presented.

F 325 CONSTRUCTION OF A GENETIC MAP OF LETTUCE USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS. Benoit S. Landry, Rick V. Kesseli and Richard W. Michelmore, Department of Vegetable Crops, University of California, Davis, CA 95616

A detailed genetic map of lettuce (Lactuca sativa L.) is being constructed using restriction fragment length polymorphism (RFLP) analysis as a precursor to cloning disease resistance genes. This involves three phases: (i) Developing a source of probes, (ii) Identifying polymorphisms between parental lines, (iii) Analyzing segregation of polymorphic probes and developing the linkage map.

cDNA and single/low copy number DNA sequences were compared for their efficiency to detect polymorphisms in nine restriction endonuclease digests of four lines. The enzymes were also compared for their efficiency to detect polymorphisms. The segregation of 41 RFLPs was analyzed in a single cross and a linkage map containing isozymes, three morphological markers and five downy mildew disease resistance genes was constructed. The data provide evidence of duplications and a translocation.

F 326 PHOTOSYNTHETIC DEVELOPMENT IN NORMAL AND ARGENTIA LEAVES OF ZEA MAYS, Jane A. Langdale and Timothy Nelson, Biology Dept., Yale University, New Haven, CT 06511. Photosynthesis in C4 type grasses such as maize involves the interaction of two cell types (bundle sheath [BS] and mesophyll [M]) which both contain cell-specific photosynthetic enzymes. Malate dehydrogenase (MDH), phosphoenolpyruvate carboxylase and pyruvate phosphate dikinase are located in the M cells and malic enzyme and ribulose bisphosphate are found in the BS cells. We have used antibodies against C4 enzymes to mark the products of fully differentiated BS and M cells in developing maize leaves. Using in situ immunolocalization techniques in conjunction with the temperature-sensitive greening mutant argentia (ar), we have determined the order in which BS and M cells in a maize leaf accumulate photosynthetic proteins. The observed pattern reflects patterns of vascular differentiation. In ar leaves C4 enzymes accumulate in the correct cell-type and in the same order as in normal leaves but their appearance is delayed. Furthermore, BS cell development is delayed with respect to M cell development. Vascular differentiation does not appear to be delayed in ar leaves. We have determined by Western blot analysis that overall levels of C4 enzymes, with the exception of MDH, are lower in ar leaves. MDH accumulates to the same level in both normal and ar seedlings. Patterns of corresponding mRNA expression have also been examined.

F 327 DIFFERENTIAL INDUCTION OF PHENYLALANINE AMMONIA-LYASE GENES BY IRRADIATION AND STRESS IN BEAN HYPOCOTYLS Xiaowu Liang[1,2], Vincent P. M. Wingate[1], Carole L. Cramer[1] and Christopher J. Lamb[1] [1]Plant Biology Lab, Salk Institute for Biological Studies, P.O.Box 85800, San Diego, CA 92138; [2]Graduate Program in Biology, University of California, San Diego, La Jolla, CA 92093

Phenylalanine ammonia-lyase (PAL) catalyzes the first reaction in the biosynthesis from phenylalanine of a wide variety of phenylpropanoid natural products. The enzyme is under rapid modulation in response to a number of environmental and stress factors including light, wounding and fungal infection, etc., which cause rapid increases in PAL activity concomitant with the onset of accumulation of specific phenylpropanoid end products such as pigments, UV-protectants, lignin, cinnamic acid esters (wound protectants) and phytoalexins. In Phaseolus vulgaris, there are multiple forms of PAL encoded by a small multigene family. We have been interested in how the expression of this multigene family is regulated in plants in response to these diverse environmental signals. PAL mRNA and enzyme activities are markedly induced by light, wounding and infection in developing hypocotyls. However light and stress (wounding and infection) induce the synthesis of different patterns of PAL forms such that while wounding and infection induce the complete set of subunit isoforms and native isoenzymes, irradiation induces only a small subset. Moreover, induction by light is developmentally regulated such that only cells in the lower (elongated) part of developing hypocotyls respond to irradiation with respect to PAL induction. Experiments are under progress to delineate light-induced and stress-induced isogenes in the family of 3-4 PAL genes per haploid genome. It has been observed that different forms of PAL native isoenzymes exibit different kinetic properties [1]. We suggest that the PAL gene family encodes a set of closely related polypeptides that have similar but subtly different functional properties. These genes are positioned in different regulatory networks, thereby allowing a flexible, integrative response to environmental change during plant development.

[1] Bolwell,G.P., Bell,J.N., Cramer,C.L., Schuch,W., Lamb,C.J. and Dixon,R.A. (1985) Eur. J. Biochem. 149, 411-419

F 328 ENVIRONMENTAL AND DEVELOPMENTAL REGULATION OF A SINGLE CHALCONE ISOMERASE GENE IN BEAN, Mona C. Mehdy and Chris J. Lamb, Salk Institute, San Diego, CA 92138

Isoflavonoid phytoalexins and anthocyanin pigments are synthesized by two branches of the phenylpropanoid pathway in legumes. Isoflavonoids accumulate in response to pathogen infection and wounding whereas anthocyanin synthesis is regulated during development and by exposure to light. Chalcone isomerase (CHI) catalyzes the formation of an intermediate common to both branch pathways and elevated CHI enzyme activity is correlated with increased levels of these compounds in many plants. We have found that a single CHI gene exists in the bean genome and that CHI mRNA accumulates in Phaseolus vulgaris cell cultures treated with fungal elicitor. In addition, CHI mRNA accumulates during both incompatible and compatible intact plant-fungus interactions with delayed kinetics in the latter case. The CHI mRNA also accumulates in excised hypocotyl segments and in white and ultraviolet light irradiated etiolated seedlings. The distribution of CHI mRNA in different organs was examined. Root contained the highest level of CHI mRNA and decreasing levels were found in stem, mature flowers, and leaves, respectively. CHI gene expression is being further studied by run-off transcription experiments. The structure of the gene is being investigated in order to elucidate the regulatory sequences conferring environmental and developmental inducibility.

F 329 cDNA CLONING AND CHARACTERIZATION OF mRNAs DIFFERENTIALLY EXPRESSED IN T-DNA TRANSFORMED VERSUS NORMAL TOBACCO PLANTS,Johan Memelink,J.Harry C.Hoge and Rob A. Schilperoort,State University,Leiden,The Netherlands.

In vitro cultured transgenic tobacco shoots containing an active T-DNA gene 4(T-cyt gene)are unable to form roots and lack apical dominance,resulting in numerous adventitious sprouts. This abnormal phenotype results from the cytokinin stress imposed on these shoots by the cytokinin-synthesizing enzyme encoded by the T-cyt gene.In search for plant genes which are regulated by the high endogeneous hormone level,and which are involved in the establishment or maintainance of the abnormal phenotype,we screened cDNA libraries of transgenic T-cyt shoots and normal tobacco plants by differential hybridization.Thus far we have isolated 5 cDNA clones(pCNT1 to 5)for mRNAs that have a higher steady state level in T-cyt shoots than in the shoot part of normal tobacco plants.Surprisingly,pCNT1,4 and 5 mRNA levels are particularly high in the roots of in vitro cultured normal plants.pCNT1,2 and 3 mRNAs in normal shoots can be induced by culturing the shoots on cytokinin-containing medium,to levels which are comparable to those found in T-cyt shoots.cDNA clones pCNT3,4 and 5 correspond to virus-inducible genes belonging to PR(=Pathogenesis-Related)gene families(Hooft van Huijsduijnen, R.A.M. et al(1986)EMBO J.5:2057-2061),respectively B,coding for the PR-1 protein group,F, coding for chitinase,and a novel class,G.In contrast,pCNT1 and 2 mRNA levels in tobacco leaves are not induced by infection with TMV-virus.The possible involvement of these mRNAs in plant growth and differentiation processes is currently under investigation.

F 330 MUTANTS IN SOYBEAN UREASE EXPRESSION, E. Meyer-Bothling and J. C. Polacco University of Missouri, Columbia MO 65211

Seven soybean urease mutants were recovered from 2000 bulked M2 seed (J.E. Harper) derived from ethylmethanesulfonate or N-methynitrosourea-treated Williams seed. All contain single gene, recessive mutations except e3 which is codominant, producing 10% standard seed urease activity as a heterozygote and 0.1% activity as a homozygote.

Class I mutants (n4,n6,n7,n8) are allelic with sun, a naturally occurring mutation eliminating accumulation of seed (embryo-specific) urease mRNA. Class I mutants produce normal ubiquitous urease, the isozyme found in all soybean tissues. n4 and n7 resemble sun in that they fail to accumulate seed urease mRNA. Mutants n6 and n8 are leaky; n6 produces embryo-specific urease protein altered by several criteria. Thus class I mutants appear to fall within a single functional coding locus (sun) for the embryo-specific urease. Eu-a and Eu-b encoding slow and fast-running embryo urease, respectively, are apparently sun alleles although they map 1 unit from the original sun lesion.

Class II mutants e1, e2, and e3 fall into at least 2 complementation groups. All have aberrant seed (embryo-specific) and leaf (ubiquitous) urease activities. e1 lacks both activities altogether; e2 lacks ubiquitous urease activity but has reduced seed activity; e3 has coordinately reduced leaf and seed activities. All contain normal levels of seed urease mRNA and protein with normal electrophoretic mobility. However, alterations in holomeric state (e1,e2,e3), temperature-lability (e2,e3) and pH optimum (e3), often observed in both isozymes, suggest altered maturation common to both ureases (e.g. Ni emplacement).

F 331 CLONING AND EXPRESSION OF THE CHALCONE SYNTHASE MULTIGENE FAMILY FROM PETUNIA HYBRIDA. J.N.M. Mol, R.E. Koes, C.E. Spelt and A.R. van der Krol.
Dept. Applied Genetics, Free University, Amsterdam, The Netherlands.
We have recently shown that floral tissue of Petunia hybrida (variety V30) expresses only one member of the chalcone synthase (CHS) multigene family (Koes et al. Nucl. Acids Res. 14, 1986, 5229-5239). From an EMBL-3 library 7 distinct complete CHS-related genes (A-G) could be isolated including the expressed one (gene A). All 7 genes show a similar overall architecture: one small and one larger exon separated by a highly variable intron. Homology analysis indicates that three of the genes (C, E, G) are very similar in both intron and exon sequence (90% homology). Homology among the other genes in the coding sequence is less (70-80%) whereas introns and 3'-untranslated regions have diverged completely, providing a basis for differential recognition. None of our clones contains more than one CHS gene indicating absence of intimate linkage. Sequence analysis carried out sofar shows that genes B-G lack the typical characteristics of pseudogenes and may therefore be functional and expressed in different tissues or under different environmental conditions. We are testing this hypothesis on the basis of our observation that the Group I enzyme PAL is UV-inducible in cell suspensions of the Petunia line V30.
We have transferred the A-gene to tobacco and demonstrated its flower-specific expression. Experiments are in progress to identify sequence motifs involved in the regulation of this gene.

F 332 COMPARATIVE SEQUENCE ANALYSIS OF HEAT SHOCK PROTEIN GENE FAMILIES OF SOYBEAN, Ronald T. Nagao and Joe L. Key, Department of Botany, University of Georgia, Athens, GA 30602. An increase in growth temperature (heat shock) causes soybean seedlings to stop normal protein synthesis and synthesize a limited number of heat shock proteins (HS-proteins). In contrast to animal systems where the high molecular weight HS-proteins predominate, in soybeans the low molecular weight (15 to 18, 21 to 24 and 27 kD) families of HS-proteins are most abundant. To help understand the regulation of HS gene expression and HS-protein function, genes from different molecular weight families have been isolated and their nucleotide sequences have been determined. The 5'-ends of most of these genes contain multiple sequences with homology to the heat shock consensus element (HSE) originally described from Drosophila. The most proximal to the TATA box has two overlapping HSEs. Further upstream from this proximal HSE, the genes from soybean also contain additional distal HSE homologies. One gene contains only a single HSE in the HS promoter region with no additional secondary distal HSEs within 750 nucleotides. Sequence differences are noted in the presumed HS promoter regions from different HS gene families. Additional sequences which may be involved in affecting HS gene expression including steroid binding elements, metal responsive elements, SV40-like enhancer elements, regions of potential Z-DNA configuration, upstream dyads and various repeat structures have been located in many of the HS genes. A comparative analysis of the predicted amino acid sequences encoded by these genes shows conservation of hydrophilic and hydrophobic domains among the different gene families. Further comparisons with HS-proteins of Drosophila and other organisms suggest conservation of functional domains among the HS-proteins from very diverse organisms.

F 333 CHARACTERIZATION AND REGULATION OF TOBACCO CHITINASE GENES, Jean-Marc Neuhaus, Hideaki Shinshi, Debra Mohnen and Frederick Meins, Jr., Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland. The pathogenesis-related enzyme, chitinase, can be regulated by the hormones auxin and cytokinin. When tobacco pith tissue is subcultured on hormone-free medium, its chitinase content increases by ca. 5-fold to upto 8% of the soluble protein over a 7-day period. This induction is inhibited 90% by mixtures of auxin and cytokinin added to the culture medium. A partial cDNA clone of tobacco chitinase (pCHN50) was isolated and sequenced. Chitinase mRNA measured by Northern blot analyses and chitinase measured by electroimmunoassay show similar patterns of regulation by auxin and cytokinin, indicating that the hormones act at least in part at the mRNA level. Sequence analysis of 8 cDNA clones shows that there are at least 2 expressed genes. Southern blot analysis of genomic DNA provides evidence for 3 related chitinase genes. Two different genomic clones were isolated. At least one of these genes is expressed.

F 334 SALT STRESS INDUCES TRANSCRIPTION OF PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) IN MESEMBRYANTHEMUM CRYSTALLINUM. Jim A. Ostrem, Christine B. Michalowski, Jurgen M. Schmitt, Steven W. Olson, Hans J. Bohnert, University of Arizona, Tucson, AZ 85721

We are using the succulent M. crystallinum as a model for investigating the molecular biology of stress induced changes in plant gene expression. This species responds to salt stress by switching from C3 photosynthesis to Crassulacean acid metabolism (CAM). When plants are irrigated with 0.5 M NaCl the activity of PEPC, a key enzyme in CAM, increases in leaf extracts. We used this increase in PEPC activity to monitor CAM induction in salt-stressed plants at different stages of development. Our results indicate that the ability of the plant to respond to salt stress is influenced by the age of the plant. While PEPC activity increases within 2 to 3 days when 6 week old plants are salt stressed, no increase in PEPC activity is detectable in younger plants irrigated with 0.5 M NaCl. Immunoblot analysis of extracts from leaf and axillary shoot tissue of salt-stressed, 6 week old plants indicates that the synthesis of PEPC is induced in all plant parts. The increase in PEPC protein in plant tissues is paralleled by an increase in PEPC mRNA as assayed by in vitro translation of RNA in rabbit reticulocyte lysates and immunoprecipitation with anti-PEPC antibodies. We have isolated PEPC cDNA clones from a CAM cDNA library in the expression vector lambda gt 11. Northern hybridization and quantitative slot blot hybridization suggest that salt stress induces the transcription of a CAM specific PEPC gene or gene family.

F 335 IN VIVO DETECTION OF REGULATORY FACTORS AND CHROMATIN STRUCTURE OF MAIZE ADH1, Anna-Lisa Paul and Robert Ferl, University of Florida, Gainesville, FL 32611.

We have examined the chromatin structure of the Alcohol dehydrogenase-1 gene from maize to target those regions of the 5' flanking sequence that interact with regulatory factors. The distribution and characteristics of the DNAse-1 hypersensitive sites are consistant with a model in which the major regulatory events happen within the first few hundred bases 5' to the start of transcription. In vivo DMS footprinting and genomic sequencing have identified the binding sites of several factors in the region, and a model for the regulatory properties of Adh1 is proposed.

F 336 DIFFERENTIAL EXPRESSION OF SEVERAL GENES DURING TOMATO FRUIT FORMATION Birgit Piechulla, Wilhelm Gruissem; Dept. of Botany, UC Berkeley, CA 94720, USA

Several physiological and structural changes occur after pollination and fruit set during tomato fruit development and ripening. However, the mechanisms that regulate these alterations are presently not well understood. We have therefore analysed the expression of various nuclear and organelle encoded genes during fruit formation and correlatedchanges of transcript levels to changes of the physiological status of tomato fruits.

During the growth of tomato fruits (cell division and enlargement), which requires organized synthesis of structural components, highest levels of tubulin A and B transcripts are measured in 20 day old tomato fruits, and no such mRNAs are detectable in mature green fruits. Genes coding for photosynthesis-specific stromal and thylakoid membrane proteins (lsu and ssu of RuBPC/Oase, chl a/b binding protein, P700 RC protein of PSI, Q_B-binding protein of PSII) reach highest steady-state mRNA levels 2-3 weeks after pollination. Inactivation of these genes occurs early during fruit development, although photosynthetic proteins and components are detectable in mature green fruits. Significant changes in mRNA levels of cab, rbcS and rbcL are measured in fruits harvested at night and day time, which indicates diurnal rhythmicity of gene expression in tomato fruits. Transcript levels of the ct and mt ATPase subunits are high in 2-3 week old fruits. Later during fruit development and ripening, the ct ATPase subunits III and A decrease to non-detectable levels, while the mt ATPase B and A subunits remain relative high, indicating ATP synthesis predominately by the mt ATPase during the ripening process. During ripening, when high levels of reducing sugars are present, the cytoplasmic fructose-1,6-bisphosphate aldolase mRNAs increase dramatically.

F 337 DIFFERENTIAL mRNA TRANSCRIPTION DURING SALINITY STRESS IN BARLEY, Subbanaidu Ramagopal, USDA-ARS, Pacific Basin Area, Aiea, HI 96701

The molecular and genetic bases of salinity tolerance in plants are not understood. Gene expression at the mRNA level was investigated in a salt-tolerant and a salt-sensitive genotype of barley. Seedlings were exposed to NaCl stress and translatable mRNAs isolated from root and shoot tissues. A reticulocyte cell-free system was programmed with barley mRNAs and the in vitro products were resolved on two dimensional polyacrylamide gels following isoelectric focusing (IEF) or nonequilibrium pH gradient gel electrophoresis (NEPHGE) in the first dimension. The functional mRNAs in unstressed seedlings were almost qualitatively indistinguishable between the two genotypes. However, salinity stress triggered differential transcription of specific mRNAs depending upon genotype and tissue. In roots, twelve new mRNAs were induced which encoded proteins of 21 to 34 Kd with a pI range of 6.1 to 7.7. In shoots, the nine new mRNAs coded for proteins of 18 to 50.5 Kd with a pI range of 5.4 to 7.8. These new stress mRNAs represented one of two main classes. Class I consisted of mRNAs shared by both genotypes. Class II represented mRNAs specific to each genotype; unique mRNAs of roots accumulated preferentially in the salt-tolerant genotype whereas those of shoots accumulated in the salt-sensitive genotype. The findings suggest that transcriptional as well as post-transcriptional mechanisms regulate gene expression in barley during salinity stress.

F 338 EXPRESSION OF BACTERIAL GENES IN PLANTS, S. Riazuddin, T. Parveen, Centre for Advanced Molecular Biology, University of the Punjab, Lahore, Pakistan. Seedlings of chick pea (Cicer Arietinum L.) grown under sterile conditons have been used to obtain in vitro cell cultures. At the age of 2-3 weeks, leaves were peeled off their lower epidermis, plasmolyzed with sucrose and treated with a mixture of known enzymes. After 14 h incubation, protoplasts were purified by filtration and low speed centifugation to give a final yield of 5×10^5 protoplasts per gram of leaf material. Experimental conditions have been defined to permit cell-wall synthesis in mesophyll protoplasts and their development into calli. Expression of foreign DNA in chick pea plantlets obtained through suspended cell cultures or using leaf disk method are being investigated. Data will be presented to demonstrate regeneration of whole plant from single cell isolates and expression of bacterial genes in chick pea.

F 339 GENETIC DIVERSITY IN PHOTOSYNTHESIS AND WATER USE EFFICIENCY OF WHEAT AND WHEAT RELATIVES, Steven W. Ritchie, Henry T. Nguyen, and A. Scott Holaday, Texas Tech University, Lubbock, TX 79409.

Improving water use efficiency in wheat through plant breeding or biotechnology will require a greater understanding of the biochemical mechanisms and genetic regulation of photosynthetic efficiency. Our long-term objectives are to 1) determine the genetic diversity of photosynthesis and water use efficiency (WUE) in winter wheat and wild species of wheat, 2) determine the biochemical basis and genetic control of high WUE in wheat, and 3) search for a biochemical screening technique to be used for genetic manipulation of WUE in wheat. WUE in this study will be reported as the ratio of photosynthetic rate to transpiration rate as measured under saturing light conditions. Genetic diversity for photosynthesis and WUE will include a comparison between diploid and tetraploid wild species of wheat to cultivated hexaploids under various water stress regimes and developmental stages. Data for leaf water potential will also be presented as a quantitation of the water stress level. Preliminary data comparing the genotypes for ribulose-1,5-bisphosphate carboxylase/oxygenase activation under well-watered and increasing water-stress conditions will also be presented. The role of the organelle genome in the expression of these gene systems will be evaluated using reciprocal crosses of selected hexaploid genotypes.

F 340 EVIDENCE THAT POLY(γGLUTAMYLCYSTEINYL)GLYCINES ARE NOT PRIMARY GENE PRODUCTS, Nigel J. Robinson, Robert L. Ratliff, Pamela J. Anderson and Paul J. Jackson. Genetics Group, Mail Stop M886, Los Alamos National Laboratory, Los Alamos, NM 87545.

Cadmium resistant Datura innoxia cells produce poly(γglutamylcysteinyl)glycines, $(\gamma EC)_nG$, when exposed to high concentrations of cadmium. These polypeptides sequester cadmium, and appear to be acting as functional analogues of metallothionein. The presence of γ-carboxamide linkages in these compounds has been demonstrated using [^{13}C] NMR. This suggests that $(\gamma EC)_nG$ are not primary gene products. To confirm this, synthetic oligonucleotide sequences were used as probes to hybridize to any mRNA sequences which could encode the polypeptides. These probes did not hybridize to mRNA isolated from cells grown either in the presence or absence of cadmium. This data again suggests that these compounds are not translational products of mRNA but are the products of a biosynthetic pathway. The structure of the polypeptides suggests that they may be synthesized from glutathione molecules with the concomitant release of glycine residues. Furthermore, $(\gamma EC)_2G$ appears to be the substrate for $(\gamma EC)_3G$. This biosynthetic pathway has been examined in vivo using double isotope pulse-chase labelling followed by reverse phase HPLC to identify, and quantify, products of the pathway.

This work was performed under the auspices of the U.S. Department of Energy and the Natural Environment Research Council of Great Britain.

F 341 POST-TRANSCRIPTIONAL REGULATION OF NUCLEAR-CYTOPLASMIC PARTITIONING OF PHYTOCHROME-SENSITIVE TRANSCRIPTS. Anurag D. Sagar, Winslow R. Briggs and William F. Thompson. Carnegie Institution of Washington, Department of Plant Biology, Stanford CA 94305

The relative abundance of several nuclear and cytoplasmic RNAs known to be under phytochrome regulation were investigated in order to assess the possibility of gene regulation at the level of messenger RNA transport from the nucleus to the cytoplasm. Buds from etiolated pea seedlings kept in darkness or subjected to one of several different light treatments were harvested and RNA extracted from the nuclear and cytoplasmic fractions. RNA was analyzed by slot-blot assay, using as probes several cDNA clones known to code for light-regulated mRNAs (including those for chlorophyll a/b binding protein, the small subunit of ribulose bisphosphate carboxylase, and ferredoxin). Each transcript exhibited a different response to the various light treatments, but for each clone, a characteristic linear relationship was observed between nuclear and cytoplasmic transcript levels. For certain clones, large increases in cytoplasmic mRNA were accompanied by only small changes in nuclear RNA, while for others, nuclear RNA increased dramatically, along with cytoplasmic message. Despite these differences in partitioning between clones, the linear relationship between nuclear and cytoplasmic message levels for any given clone suggests that each gene is characterized by a specific rate of transport out of the nucleus, regardless of the level of expression.

F 342 CAN POLLEN SURVIVE HEAT STRESS WITHOUT HSP J.A.M. Schrauwen, M.M.A. van Herpen and W.H. Reijnen, Catholic University, Nijmegen, The Netherlands

The protein synthesis of mature pollen in vitro from Lilium longiflorum, is affected moderately by the exposure time to high temperatures. After 2-D electrophoresis the protein pattern derived from heat-treated (39 °C) pollen can be distinguished from the non heat-treated. Some proteins disappeared while others were synthesized. The latter proteins have features which are not identical with the so called heat shock proteins (HSPs).
The overall change in the pattern is independent of the moment of heat-treatment. The same 2-D pattern was obtained when pollen was incubated at 39 °C first for 3 min immediately after imhibition and followed 3 h later for 30', compared to pollen grown at 27 °C for 3 h and then incubated at 39 °C for 27'
In contrast the physiological response on heat treatment is completely different. Pollen germination and pollen tube growth are affected as well by the moment as the exposure time of the heat treatment.

F 343 The Gene Yl1 Marks Germination in Soybean, Susan M. E. Smith and Michael L. Christianson, Sandoz Crop Protection Corp, Zoecon Research Institute, Palo Alto, CA 94304

Germination comprises gene actions that cause cells to switch from quiescence to active growth and division. The gene Yl1 is a codominant chlorophyll mutation in soybean which is expressed in the seedling and mature plant but not in the embryo; self-pollination of heterozygotes produces seed which segregate 1:2:1 for yellow: yellow-green: green seedlings, although the embryos are uniformly green. The switching on of Yl1 is a directly observable genetic event that can be used in tests of the timing and controls on germination. Immature embryos can be precociously germinated and will segregate for color; the change in color, however, develops well after such traditional markers of germination as change in cotyledon angle and the beginning of root elongation. Confident interpretation of color change in these precocious germlings is hampered by the spindly appearance of the plantlets. Mature soybean seeds are tan. In the green-seeded mutant, d1d1d2d2, embryos do not lose color as they mature. Combining Yl1 with this mutant line will allow direct observation of color change during normal germination; we are particularly interested in when Yl1 switches on relative to such physical markers of germination as root and shoot growth.

F 344 SDS SOLUBLE PROTEIN PATTERNS IN SALT (NaCl) TREATED SUSPENSION CULTURES OF THREE HALOPHYTES, Peter F. Straub and John L. Gallagher, College of Marine Studies, University of Delaware, Lewes, DE 19958.

A unique 26 Kd protein and numerous quantitative changes in protein patterns have been detected in tobacco cell cultures in response to salt (NaCl) treatment. This study looked for analogous proteins in native halophytes. Callus cultures were initiated in Distichlis spicata, Sporobolus virginicus and Kosteletzkya virginicus from seed, rhizome, and seedling hypocotyl, respectively on a Murashige and Skoog medium with 1 mg/l of each 2,4-D and IAA. Callus cultures started on solidified media were transferred to a liquid formulation of the same medium and agitated at 100 rpms on a gyratory shaker. Cell suspensions were maintained by serial transfer (2:5) to fresh medium at 7-10 day intervals. Cells were harvested by vacuum filtration on #2 paper filters, washed, homogenized with 0.05 M tris-HCl pH-6.8 and 1% SDS. Protein concentration was measured with the Bradford assay after TCA precipitation. Proteins were run on a 12% SDS discontinuous polyacrylamide system and stained with coomassie blue. Protein bands were recorded and quantitated by densitometry. No unique protein in the 26 Kd region was detected although there were some quantitative differences in protein patterns noted in each of the species.

F 345 CHARACTERIZATION AND EXPRESSION OF CHLOROPLAST HEAT SHOCK PROTEINS IN PEA AND PETUNIA. Elizabeth Vierling & Qiang Chen, Department of Biochemistry, University of Arizona, Tucson, AZ 85721.

During high temperature stress, or heat shock, specific nuclear-encoded heat shock proteins (HSPs) are transported into chloroplasts. The function of these proteins is unknown. We have previously described the isolation of a cDNA encoding chloroplast HSP22 from soybean. We have now used the soybean HSP22 cDNA to isolate homologous cDNAs from pea and petunia, and are using these clones to investigate the structure and expression of these proteins. The hybridization criteria used to identify the pea and petunia clones indicates that nucleic acid homology between the chloroplast HSPs is greater than 70%. Hybrid-selection translation experiments demonstrate the proteins are synthesized as precursor polypeptides of 28 kDa in soybean, 27 kDa in pea and 30 kDa in petunia; the molecular weight of the mature proteins within the chloroplast are 22 kDa, 20-21 kDa and 22-23 kDa, respectively. DNA sequence analysis has indicated there is amino acid homology in the carboxy terminal region between the soybean chloroplast HSP and low molecular weight HSPs from soybean and other organisms. These data suggest possible functional homologies as well. We have isolated cDNAs for cytoplasmic HSPs from pea and petunia, and a comparative analysis of these with the corresponding chloroplast HSPs will further define the homologous regions. Preliminary studies indicate expression of chloroplast HSP mRNAs is not restricted to photosynthetic tissues; roots from heat shocked plants contain similar mRNA levels as leaves. We hypothesize that chloroplast HSPs function in all types of plastids and may play a role similar to that of small HSPs found in other parts of the cell.

F 346 RNA TRANSCRIPT INDUCTION AND REPRESSION IN CELLULAR SALT TOLERANCE OF MEDICAGO SATIVA, Ilga Winicov*, Jakob H. Waterborg*, Rodney E. Harrington* and Thomas J. McCoy+, Department of Biochemistry, University of Nevada, Reno, NV 89557* and Department of Plant Sciences, University of Arizona, Tucson, AZ 65721+

Diploid cell lines of Medicago sativa HG2 grow in callus or suspension cultures. Medium containing 85 mM NaCl is lethal to greater than 95% of the HG2 cells. Continuous subculture on 170 mM NaCl containing medium has developed cell lines which grow normally at the high NaCl concentration. This salt tolerant phenotype is stable even after several months subculture on normal Schenk and Hildebrand medium. Cellular salt tolerance has been characterized by analysis of polyadenylated RNA from salt tolerant and salt sensitive HG2 lines by in vitro translation. Wheat germ translation of the isolated polyadenylated RNA has yielded 10-130 kd [^{35}S] methionine labeled polypeptides. Comparisons of the translation products show a marked decrease of some and increase of other specific mRNA species in the salt tolerant variant. Salt stress of the salt sensitive HG2 line results in the appearance of several specific induced polypeptides. The specific mRNA products which increase in the salt tolerant variant, do not appear under salt stress of the salt sensitive HG2 cell line.

THE INFLUENCE OF CROWN GALL ONC -GENES ON THE DEVELOPMENT OF PLANTS

F 347 George J. Wullems, Rindert Peerbolte, Johan Memelink, Harry H.C.Hoge and Rob A. Schilperoort. Department of Plant Molecular Biology, University of Leiden, The Netherlands.

Transformation of plant cells with Agrobacterium tumefaciens via co-cultivation or with Ti-plasmid DNA via direct gene transfer results in the occurence of transformed plants. Variation in the expression of the T-DNA onc-genes gives variation in tumor and plant morphologies. In particular the expression of T-DNA gene 4 (tmr or cyt locus, in the absence of expression of the tms or aux-locus, esults in a wide range of morphological and physiological abnormalities like the absence of root formation, absence of apical dominance, morphological malformations of the leaves and the occurence of male sterility. The relation between these traits and the activity of the onc-genes is studied at the molecular level. Specal attention is paid to the relation between male sterility, induced by A.tumefaciens and the activity af the T-DNA gene 4.

Promoters, Enhancers, Regulators, Transgenic Expression

F 400 A Transient Expression System for the Assessment of Plant Promoters. S.C.H. Alfinito, P.S. Dietrich, L.E. Murry and R.M. Sinibaldi, Sandoz Crop Protection, Zoecon Research Institute, Palo Alto, CA 94304

Recent developments in molecular biology have facilitated the introduction of novel genes into plants. Achieving their proper expression has become a major effort in many laboratories. To assess expression we have developed a promoterless reporter gene system whereby promoter sequences can be rapidly tested. This plasmid contains a polylinker into which different promoter sequences can be cloned, followed by a chloramphenicol acetyl transferase (CAT) reporter gene and a 3' poly A addition site from the nopaline synthetase gene. The promoterless plasmid exhibits very low CAT activity when electroporated into carrot protoplasts. We have made several constructions with the 35S promoter from CaMV, one of which contains self complementary polylinker sequences in front of and behind the CAT gene. This plasmid exhibits much lower CAT activity when compared to an identical plasmid where the polylinker sequences have been removed. These fore and aft polylinker sequences may inhibit translation by some sort of fold back mechanism. We are in the process of characterizing promoter sequences from an 18 Kd and two 85 Kd heat shock genes isolated from corn. The promoter regions of these genes will be cloned into our promoterless expression plasmid and assayed for CAT activity following electroporation into plant protoplasts. Utilizing sets of nested deletions in the promoter region we hope to determine sequences responsible for heat inducible and constitutive expression of these genes. We have been making protoplasts from a line of black mexican sweet corn cells, and these will be used in homologous electroporation expression studies with the corn promoters.

F 401 TRANSFER OF A SULFUR-RICH PROTEIN GENE FROM BRAZIL NUTS TO OTHER PLANTS. Susan B. Altenbach, Karen W. Pearson, and Samuel S. M. Sun, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

An abundant seed protein is found in Brazil nuts (*Bertholletia excelsa*) which is unusually rich in the sulfur-containing amino acids, methionine (18%) and cysteine (8%). This sulfur-rich protein is synthesized initially in Brazil nuts as a 17 kDa precursor polypeptide which undergoes three cleavage steps before reaching its mature form. First, a signal peptide of about 2 kDa is cleaved from the precursor, leaving a 15 kDa polypeptide which is then trimmed to a 12 kDa polypeptide and finally to the 9 kDa and the 3 kDa subunits of the sulfur-rich protein. We are studying the expression of this developmentally-regulated seed protein in transgenic plants with the hopes of altering the amino acid composition of seed proteins. A cDNA encoding the 17 kDa precursor of the Brazil nut sulfur-rich protein has been attached to the promoter region and the 3' untranslated region of phaseolin, a seed protein from French beans, and this construct has been transferred to other plants using a binary vector system of *Agrobacterium tumefaciens*. In order to study the processing, transport and stability of the sulfur-rich protein in transgenic plants, we have also transferred regions of the Brazil nut cDNA clone which would encode the 15 kDa, 12kDa and 9 kDa forms of the sulfur-rich protein under the control of the promoter and signal peptide region of phaseolin. We are currently analyzing the expression of these constructs in transgenic plants.

F 402 Control of Gene Expression and Protein Synthesis in Cereal Endosperms
I. ALTOSAAR, S. Fabijanski, A. Nassuth, S. Chang and S. Dukiandjiev
Biochemistry Dept., University of Ottawa, Ottawa, K1N 9B4 Canada

Translation of mRNAs in developing endosperm may be controlled by several factors:
1. We have shown that the abundant prolamin-coding 12S mRNA in wheat, barley and rye is also present, but not translated, in oat. Rather, the less-abundant globulin-coding 18S mRNA is preferentially translated causing globulins to constitute >70% of the mature grain protein in oats, whereas wheat etc. contain <5% globulins. Polysomal protein factors enhance the synthesis of 18S globulin mRNA in oat endosperm (Plant Molec. Biol. 4:211-218,1985).
2. 5'-Flanking sequences of prolamin and globulin mRNAs may also exhibit differential affinities to endosperm ribosomes. We have sequenced a full-length prolamin cDNA clone specific for oat 12S mRNA. When compared to an oat globulin cDNA sequence, there is a striking high degree of homology in the 3' flanking sequences. In vitro translation of hybrid cDNA constructs, where the 5'- globulin and prolamin flanking sequences have been switched, indicate the level of translational control imparted by each type of leader sequence.
3. Specific modifications of ribosomes in endosperm tissue also allow specific mRNAs to be recognized and translated. Polysomal proteins from developing endosperm and etiolated shoots are similar on 2-D gels but their in vivo phosphorylation patterns are very different. One of the heavily phosphorylated proteins from endosperm is similar in charge and M_r to S-6, a ribosomal protein phosphorylated in other eukaryotes. In vitro phosphorylation studies suggest that this S-6 like protein in endosperm may be involved in translational control of oat protein synthesis.

F 403 ISOLATION, ANALYSIS AND REGULATION OF THE NITRITE REDUCTASE GENE FROM SPINACH
Eduard Back, Will Burkhart, Mary Moyer, Laura Privalle and Steven Rothstein ,CIBA-GEIGY, Research Triangle Park, NC 27709.
A cDNA expression library from nitrate induced spinach leaves was screened for the nitrite reductase (NiR) gene with NiR antibodies and with several oligonucleotide probes derived from NiR partial aminoacid sequence. From one of the isolated cDNA clones the DNA sequence was established. It contains the complete coding region of the NiR precursor protein. From Northern blot analysis and primer extension experiments this clone was shown to represent a nearly full length cDNA copy of the NiR mRNA. Using this cDNA clone as a probe a genomic NiR clone was isolated from a genomic DNA library of spinach. Its DNA sequence analysis shows three introns in the NiR coding region. Southern blot hybridizations proved that there is one gene per haploid genome in spinach. The induction of the NiR gene by nitrate was studied by measuring the levels of both RNA and protein.

F 404 PROMOTER MAPPING OF THE AGROPINE SYNTHASE GENE OF AGROBACTERIUM TUMEFACIENS,
Ram Bandyopadhyay and William B. Gurley, Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611.

Deletion mutagenesis studies were carried out to identify the cis-acting regulatory sequences in the promoter region of the agropine synthase gene from Ti plasmid (pTi15955) of Agrobacterium tumefaciens. Promoter activities of the mutants were evaluated at the transcriptional level using a double-gene reference vector that contains a copy of the test gene as an internal standard. Our results with 5'-deletions indicated two distinct sequence motifs having activator-like properties. One of these two elements is located between positions -218 and -206 and the other between -178 to -149. Removal of these elements reduced the transcript level to 15% of wild type. Deletions further downstream, covering two "CAAT"-box elements did not show significant changes in the transcript level. The "TATA"-motif when deleted, left about 5% of wild type promoter activity. Internal deletions are being constructed in order to characterize the two activator elements more precisely and to further delineate the involvement of other sequences in promoter function.

F 405 ISOLATION OF GENES THAT COULD SERVE AS TRAPS FOR TRANSPOSABLE ELEMENTS IN HORDEUM VULGARE, Ellen Barzen, Wolfgang Rohde, Adriano Marocco, Margit Wissenbach and Francesco Salamini, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.
We are interested in employing the method of transposon tagging to isolate genes that control developmental processes in barley. However, no transposons have yet been identified from this plant species. Therefore we have started to isolate and characterize barley genes which may serve as traps for transposons, i.e. genes whose inactivation by transposon insertion can be easily monitored phenotypically. Genes involved in anthocyanin and starch biosynthesis may serve such a purpose. Transposon insertion into any of these genes might lead to unstable mutable alleles and consequently to a variegated phenotype with respect to color expression (anthocyanin genes) or starch composition. Our approach is based on the use of heterologous probes for molecular hybridization.
So far we have isolated and partially characterized barley genes homologous to the a1, bz1, bz2, c1 and c2 (chalcone synthase) genes of Zea mays. Furthermore, a full-length wx cDNA encoding starch synthase has been isolated and sequenced.
Analysis of cDNA and genomic clones at the molecular level will be presented for some of the genes and compared to those from other plant species.

F 406 EXPRESSION OF A SYNTHETIC SPINACH ACYL CARRIER PROTEIN-I GENE IN ESCHERICHIA COLI. Phillip D. Beremand,* Daniel J. Guerra,* David J. Hannapel,* David N. Kuhn,† and John B. Ohlrogge.* *Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604; and †Biochemistry Department, Purdue University, West Lafayette, IN 47907

Oligonucleotides were synthesized, annealed, and ligated to construct two partial ACP-I genes. These partial genes were separately inserted into M13mp19 and subsequently joined to form a full-length gene encoding the entire amino acid sequence of spinach ACP-I. This synthetic gene was subcloned into a plasmid expression vector downstream from the trc promoter and a ribosome binding site. Cells carrying this plasmid produced a protein that was similar in electrophoretic mobility to and immunologically cross-reactive with spinach ACP-I. Furthermore, E. coli cells producing this protein were able to attach the prosthetic group phosphopantetheine, as evidenced by in vivo incorporation of $^{32}PO_4$ and by the activity of the partially purified protein in an acyl-ACP synthetase reaction. Radioimmunoassay results indicated that under inducing conditions this protein accounts for approximately 1% of the total host cell protein.

F 407 TRANSLATIONAL CONTROL OF RIBULOSE 1,5 BISPHOSPHATE CARBOXYLASE SYNTHESIS IN AMARANTH. James O Berry, John P. Carr, and Daniel F. Klessig, Waksman Institute, Rutgers University, Piscataway, New Jersey 08854.

We have recently shown that rapid and dramatic alterations in the synthesis of the RuBPCase LSU and SSU polypeptides occur in response to changes in illumination and that the expression of these genes appears to be regulated, in part, at the translational level. As an initial step towards deciphering the mechanism(s) of this translational regulation, the association of LSU and SSU mRNAs with polysomes was examined by dot blot analysis of sucrose gradient fractions and by in vitro polysome run-off assays. In light-grown cotyledons and in dark-grown cotyledons transferred into light for 5 hours (when synthesis of RuBPCase in vivo is detected) the LSU and SSU mRNAs were found in association with polysomes. In dark-grown cotyledons (when RuBPCase synthesis in vivo is not detected) these two mRNAs were found mainly in the monosome/free mRNPs fractions. These results suggest that light-induced LSU and SSU synthesis in dark-grown seedlings may be regulated at the level of translation initiation. In contrast, no changes in the association of LSU and SSU mRNAs with polysomes was detected during the rapid shutdown of RuBPCase synthesis which occurred when light-grown seedlings were transferred to darkness. These results suggest that light regulates RuBPCase gene expression at the level of translational elongation as well as initiation.

F 408 CONSTRUCTION OF LARGE LINEAR PLASMID LIBRARIES FROM HIGHER EUKARYOTE GENOMES. D.T. Burke, G.F. Carle, and M.V. Olson. Washington University School of Medicine, St. Louis, MO 63110.

A system for the cloning and restriction mapping of DNA molecules 100 kilobases or greater in size has been developed using Saccharomyces cerevisiae as the host organism. The functional elements required for the propagation of heterologous DNA sequences as artificial chromosomes in yeast (Murray and Szostak, Nature 305, 189-193, 1983) are supplied by the vector pACV6. The vector provides telomeric ends and selectable yeast genes bracketing the cloning site, as well as centromere and autonomous DNA replication functions. A genetic screen permits identification of yeast transformants containing cloned DNA.

A library of cloned high-molecular-weight mammalian DNA has been prepared and analysed for average insert size, cloning efficiency, and clone stability. Representative artificial chromosome clones over 100 kb in length have been indirect end-label mapped for BamHI restriction enzyme sites. The propagation of large DNA fragments from the genomes of higher eukaryotes in the less complex host Saccharomyces cerevisiae may facilitate the molecular analysis of these genetic systems.

F 409 REGULATION OF MAIZE LEAF NADH:NITRATE REDUCTASE m-RNA BY NITRATE AND LIGHT
Wilbur H. Campbell and G. Gowri, Michigan Tech Univ, Houghton MI 49931
Regulation of the appearance of nitrate reductase (NR) activity in higher plant leaves and other tissues has been widely studied. Recently, we applied an immunological approach for studying the appearance of both the NR activity and protein in maize leaves. The protein appeared prior to the activity when nitrate was applied in either light or dark. In order to determine if nitrate and light are regulating the level of NR m-RNA, c-DNA clones for this enzyme have been prepared from maize poly-A RNA. Total RNA was isolated from 5-day-old maize leaf, 4 Hr after applying nitrate in the light in order to selectively enrich NR m-RNA. c-DNA was synthesized using the purified, maize poly-A RNA as a template and a c-DNA library was constructed in lambda gt-11 expression vector. The library was screened using monospecific rabbit IgG prepared against maize NADH:NR. The positives from the primary screen were rescreened with polyclonal, monospecific mouse IgG anti-NR, which yielded 4 positive clones out of 270,000 total recombinants. These 4 putative NR clones are under further investigation using a heterologous probe from squash NR c-DNA provided by N. Crawford and R. Davis. The fusion proteins produced by these clones will also be investigated using a series of monoclonal antibodies prepared against NR to determine if the NR polypeptide component can be recognized in an ELISA. Ultimately, the cloned NR c-DNA will be nick-translated and the probes used in Northern and dot blots to evaluate the levels of NR m-RNA in maize leaves as influenced by nitrate and light.
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F 410 SYNTHESIS OF ORGANELLAR DNA BY PERMEABILIZED SOYBEAN CELLS, Gordon C. Cannon, Sabine Heinhorst and Arthur Weissbach, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.
Soybean suspension cells were permeabilized by treatment with L-lysophosphatidyl choline (lysolecithin). The extent of permeabilization was dependent on the length of exposure and concentration of lysolecithin. The interior of the lysolecithin treated cells was freely accessable to molecules as large as DNase I (M_r = 40,000). Incorporation by the cells of radiolabeled nucleoside triphosphates into DNA proceeded for at least 90 minutes, and the initial rate of incorporation was equal to 50% of the theoretical rate of DNA synthesis *in vivo*. Analysis of product DNA revealed that only mitochondrial and plastid DNA was synthesized by the permeabilized cells.

The permeabilized soybean cells were capable of RNA and protein synthesis as indicated by the incorporation of radiolabeled UTP and leucine, respectively, into acid precipitable material.

F 411 THE GAPDH GENE SYSTEM IN MAIZE, R. Cerff (a), F. Quigley (a), H. Brinkmann (a), P. Martinez (a), W. Martin (b), (a) Laboratorie de Biologie Moleculaire Vegetale, Universite de Grenoble I (b) Max-Plank-Institut fur Zuchtungforschung

Glyceraldehyde-3-phosphate dehydrogenases (GAPDH) of the chlorplast and the cytoplasm represent an interesting model system to study the expression and evolution of plant nuclear genes (1,2). We have identified and sequenced two cDNA clones encoding the entire catalytic subunits of chloroplast and cytosolic GAPDHs from maize. The two enzymes share only 45% of their amino acid residues and show distinct differences in their codon choice patterns. In addition, we have cloned and partially characterized the corresponding genes by restriction mapping and hybridization of Southern blots with various cDNA fragmants. The results show that the two genes vary dramatically in size: While the gene for chloroplast GAPDH is only slightly longer that its corresponding mRNA (anout 2 kb), the gene for cytosolic GAPDH comprises more that 10kb of DNA.

1. Cerff, R., Hundrieser, J., Freidrich, R. (1986) Mol. Gen. Genet. 204, 44-51.
2. Martin, W., Cerff, R. (1986) Eur. J. Biochem. 159, 323-331.

F 412 Abnormal chloroplast morphology and leaf variegation in a transgenic plant.

Amy Chan, Lisa Macht, Dave Irwin, Alison Buchan & Joan McPherson.
Department of Botany, The University of British Columbia, Vancouver, B.C.

Tobacco leaf discs were transformed with vectors derived from modified Agrobacterium tumefaciens Ti plasmids. Plants carrying constructs including the chimaeric Cauliflower mosaic virus 35S promoter - neomycin phosphotransferase gene were regenerated from the transformed cells. From about one thousand transgenic plants one showed distinct variegation of pigmentation in the leaves and slight leaf shape modification. Chloroplasts were examined by E.M. in individual leaf segments, which ranged from white to dark green pigmentation. Electron microscopy showed a distinct lack of chloroplasts in the white leaf segments and these showed abnormal morphology, i.e. they had distended shape and lacked grana. Chloroplasts from other regions of the leaf showed varying types of morphology. Nucleic acid isolated from nuclei and chloroplasts of the white and green regions of the leaves and of callus from these tissues is being investigated. It will be of interest to determine the site/s of integration of the inserted foreign DNA sequences, and their putative effects on the abnormal plant morphology.

F 413 REGULATION OF RUBISCO ASSEMBLY IN PEA CHLOROPLAST EXTRACTS. P. Chaudhari, S. Cannon, A. Hubbs, H. Roy, Plant Science Group, Biology Department, Rensselaer Polytechnic Institute, Troy, NY 12180-3590.

Our laboratory has shown that large subunits of ribulose bisphosphate carboxylase/oxygenase (RuBisCO) are incorporated into the hexadecameric enzyme in pea chloroplast extracts. It may be questioned whether this represents assembly of RuBisCO or "mere" exchange of large subunits into the hexadecameric enzyme. We now show that such incorporation is stimulated by addition of purified pea or spinach RuBisCO small subunits. Thus, it appears that true assembly does take place. The large subunits which assemble into RuBisCO are not free, but appear to be associated with binding protein monomers and/or dodecamers. Antibodies to the binding protein inhibit assembly. Thus, the binding protein appears to be involved in the mechanism of assembly of RuBisCO. It has been suggested (Hemmingsen and Ellis, Plant Physiol. 80, 269) that the dodecameric form of the binding protein may not exist at <u>in vivo</u> ATP concentrations. We find that the dodecameric form of the complex is sensitive to dilution in the presence and in the absence of ATP. It is, therefore, likely to exist <u>in vivo</u>, in an equilibrium which is regulated by light, such that increased light leads to increased availability of large subunits associated with monomers of the binding protein.

F 414 SITE-SPECIFIC MUTATIONS IN THE D2 POLYPEPTIDE OF PHOTOSYSTEM II IN THE CYANOBACTERIUM *SYNECHOCYSTIS* 6803, Dexter A. Chisholm and John G. K. Williams, E. I. Du Pont de Nemours & Co., E402/2229, Wilmington, DE 19898.

We are exploring structural and functional features of the Photosystem II reaction center complex by constructing site-specific mutations in the D2 polypeptide component. The D2 polypeptide, when paired with the D1 polypeptide (the 32-kDa herbicide-binding protein), is believed to bind members of the photosynthetic electron transport chain, including the reaction center chlorophyll, the quinone Q_A, and the non-heme ferrous iron. This idea is based on the three-dimensional structure of the bacterial photosynthetic reaction center, which has some functional characteristics and amino acid sequences in common with Photosystem II (H. Michel et. al.(1986) EMBO J. 5, 1149-1158). To explore the validity of this idea, and to learn about the roles of specific amino acids in the Photosystem II protein complex, we have developed a genetic transformation system in the cyanobacterium *Synechocystis* 6803 which makes possible the construction of site-directed mutations in Photosystem II genes. The D2 polypeptide is encoded by the psbD gene. *Synechocystis* 6803 has two copies of psbD, called psbDI and psbDII. Both copies have been deleted from the cyanobacterial chromosome by gene replacement techniques. The psbD double-deletion strain has no Photosystem II function and it requires glucose for growth. Photosynthesis is restored by transformation with the wild-type psbDI gene. To make mutant Photosystem II centers, the double-deletion mutant is transformed with psbDI genes containing site-specific mutations. Several mutations are being constructed and characterized: a) a mutation that introduces an acidic amino acid residue into the putative binding pocket for Q_A, b) a mutation that "straightens" a kink in an important trans-membrane alpha helix by replacing a proline residue with alanine, and c) mutations that replace a histidine residue at the carboxy terminus of the D2 protein, to look for involvement of the D2 polypeptide in water oxidation.

F 415 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF PHYTOCHROME MRNA ABUNDANCE IN OAT SEEDLINGS. James T. Colbert (1), Stephen A. Costigan (1), Patricia Avissar (1), Alan H. Christensen (2), N. Kent Peters (3), and Peter H. Quail (2). (1)Department of Botany, Colorado State University, Fort Collins, CO, 80523, (2) Department of Botany, University of Wisconsin, Madison, WI, 53706, and (3) Department of Biological Sciences, Stanford University, Stanford, CA, 94305.

Irradiation of 4-day-old etiolated oat seedlings results in a >90% reduction of phytochrome mRNA abundance by 5 hours after a light treatment. In vitro run-off transcription assays indicate that the amount of run-off transcript homologous to the phytochrome cDNA clone pAP-3.2 declines to approximately 30% of dark level by 15 minutes post-irradiation and remains at the new level for at least 10 hours. These data imply that the cytoplasmic concentration of phytochrome mRNA is regulated by both transcriptional and post-transcriptional events. Analysis of our current data is consistent with a dramatic increase in the rate of phytochrome mRNA degradation after a light treatment. We are currently investigating the role of mRNA degradation in phytochrome gene expression.

F 416 IDENTIFICATION OF DNA SEQUENCES REQUIRED FOR SEED-SPECIFIC ACTIVITY OF A WHEAT STORAGE PROTEIN GENE PROMOTER IN TOBACCO, Vincent Colot, Mike Bevan and R. Thompson, Plant Breeding Institute, Cambridge CB2 2LQ, U.K.

The regulation of gene expression during cereal grain development is a process with major economic consequences. The isolation of clones encoding the most highly expressed genes involved, those encoding the grain storage proteins, has now been achieved for several cereals. In the absence of a transformation technique for these monocotyledonous species, however, it has not yet been possible to define DNA sequences conferring seed-specificity of expression in the homologous plant cell. We have therefore investigated whether sequences from a wheat storage protein gene can direct specific transcription in seeds of transgenic tobacco plants. A deletion series of fragments extending from -940 bp down to 160 bp with respect to the transcription start of a low molecular weight (LMW) glutenin gene of wheat were fused to the coding sequence of a bacterial chloramphenicol acetyl transferase (CAT) gene, and the fusions introduced into tobacco via Ti-plasmid-mediated transformation. CAT activity was detected in developing seeds, but not in leaves, from transformed tobacco plants with constructs having as little as 327 bp upstream of the LMW glutenin transcription start. A DNA fragment extending from -940 to -150 bp of the LMW glutenin promoter also conferred seed-specific expression on an otherwise inactive truncated CaMV 35S promoter, lacking CaMV sequences upstream from the TATA box (O'Dell et al., Nature 313, 810). We therefore conclude that seed-specific gene regulation in wheat and tobacco possess interchangeable components, and that the study of monocot gene expression in dicot tobacco seeds is of biological significance.

F 417 REGULATORY DOMAINS OF THE PROMOTER OF THE SOYBEAN HEAT SHOCK GENE, hsp 17.5 E., Eva Czarnecka, J. L. Key* and W.B. Gurley. Microbiology and Cell Science, University of Florida, Gainesville, FL 32611; *Botany, University of Georgia, Athens, GA 30602.

A series of 5' and internal deletions was created through the promoter region of soybean heat shock gene hsp 17.5 E (E2019) which codes for a small hsp. All constructs were introduced by a low copy member integration into the sunflower genome via A. tumefaciens and a T-DNA-based double gene reference vector. The reference gene was comprised of the wild type heat shock promoter and a 15bp insertion in the leader. Control and heat shocked poly (A^+)RNA were obtained from transformed sunflower tumors and analyzed to assess transcriptional activity of the mutated promoter by the primer extension and S1 nuclease protection assays. Removal of the eight heat shock elements (HSE's) decreased the transcriptional activity by 25%. Further deletion of the secondary TATA/dyad motif resulted in a 50% reduction of promoter activity. Deletion 5' to position -80 retains TATA and an overlapping HSE pair showed 22-24% of wild type transcriptional activity. DNase T footprinting experiments indicated the strong protection of the first and second TATA proximal HSE's and an AT-rich region further upstream. Additionally, DNase T hypersensitive sites corresponded with the primary TATA and other AT-rich regions further upstream.

F 418 FUNCTIONAL ANALYSIS OF THE NOPALINE SYNTHASE DUAL PROMOTER IN TOBACCO AND AGROBACTERIUM, Paul R. Ebert and Gynheung An, Washington State University Pullman, WA 99164-6340

The Agrobacterium nopaline-type Ti plasmid pTiT37, contains the nopaline synthase gene within the TDNA. This gene is transcribed and translated in both plants and bacteria as measured by the reporter gene, chloramphenicol acetyl transferase. The constitutive bacterial promoter exists between nucleotide positions -63 and -16 relative to the plant transcript initiation site. Strong homologies are found in this region to the E. coli -35 and TATA box consensus sequences. Since the gene is 10x more active in Agrobacterium than in E. coli, there must exist Agrobacterium specific signals as well. The plant promoter, according to both transient and stable expression assays, exists between nucleotide positions -130 and -16 relative to transcript initiation. The eukaryotic TATA box is responsible for 10x activation of the plant promoter and appears to overlap the prokaryotic TATA box. Deletion of the sequence between -82 and -62, which is homologous to the animal CAAT box consensus sequence, results in a 4x decrease in promoter activity. A larger region appears to be active in plants, however, as the sequence between -92 and -62 is responsible for 20x promoter activation. An upstream activator , between -130 and -99, is absolutely required for promoter activity in tobacco. This element functions marginally when inverted and increases the promoter activity by 50% when duplicated. Precise spacing between the various promoter elements is not a prerequisite for near wild type levels of promoter activity.

F 419 UPTAKE AND EXPRESSION OF A FOREIGN GENE LINKED TO DIFFERENT PROMOTERS IN PLANT PROTOPLASTS AND IN ALGAL CELLS, Teng-yung Feng, Institute of Botany, Academia Sinica, Taipei, Taiwan 11529, ROC.

The chloramphenicol acetyltransferase (CAT) gene from bacteria and the aminoglycoside phosphotransferase (NPT II) gene from E. coli Tn5 were expressed in plant protoplasts and in algae after introduction of these genes by electroporation. When the CAT or NPT II gene was fused to nopaline synthetase (NOS) promoter of Ti plasmid or the 35S promoter of cauliflower mosaic virus, gene expression was in constitutive fashion. Whereas, when the gene was fused to the promoter of ribulose-1,5-bisphosphate carboxylase large subunit gene (rbcL), gene expression was controlled by light. Using unicellular green alga Chlamydomonas reinhardtii as a model, a life cycle-dependent gene expression was demonstrated. Furthermore, an artificial plasmid, containing expression cassette and an autonomously replicative sequence from chloroplast DNA of Chlamydomonas, was found to be replicative in the transformed cell and to be differentially methylated in its cytosine residues when the plasmid DNA was examined during different life stages. However, the transcriptional activity of the gene in the transformed cell seemed to be unrelated to the DNA methylation.

F 420 ANALYSIS OF THE MAIZE STREAK VIRUS (MSV) RIGHTWARD PROMOTER IN TRANSIENT EXPRESSION ASSAYS IN CORN CELLS, Carmen Fenoll and Stephen H. Howell, Dept. of Biology C-016, University of California, San Diego, La Jolla, CA92093

Geminiviruses are a group of plant viruses with one- or two-component genomes of circular, single-stranded DNA. Geminivirus genomes which have been sequenced are organized in a similar manner, with ORFs diverging rightward and leftward from a central intergenic region. In a few cases, including MSV, bidirectional transcription originating from the intergenic region has been demonstrated. We have chosen to study the possible role of the central noncoding region in the regulation of transcription of MSV genome. To study the ability of this region to drive virion-sense (rightward) transcription, we have fused the intergenic region to a cat-nos3' reporter gene . The construct supports transient expression of the cat gene when transfected by a polyethylene glycol procedure into Zea mays protoplasts derived from cell suspension cultures. This shows that the MSV intergenic region contains, at least, the minimal transcription signals required for expression in embryo-derived, corn suspension culture cells. Unidirectional and internal deletions were used to localize an upstream region essential for promoter activity. The region contains inverted repeats and sequences resembling other eukaryotic cell promoter elements, such as a CAAAT box and several Sp1 binding-like sites. Work is in progress to elucidate in greater detail the role played by this region in MSV rightward transcription.

F 421 **GENOME CHARACTERIZATION OF *BREMIA LACTUCAE*.** **Francis, D. M. and Michelmore, R. W., Department of Vegetable Crops, University of California, Davis, CA 95616.**

A serious limitation in controlling the pathogenic Oomycete fungi is the lack of information concerning the genetic changes that occur when host resistance genes are rendered ineffective. The *Bremia lactucae-Lactuca sativa* system represents one of the better characterized gene-for-gene interactions, and may provide a model for studying the molecular basis of pathogenic variation. As a prerequisite to cloning avirulence genes, we are presently characterizing the genome of *B. lactucae*. Genome size was estimated using RFLP markers which segregate as single loci to probe quantitative dot blot reconstructions. Analysis of random pUC clones and random Lambda clones indicated that *B. lactucae* has a large amount of repeated DNA in its genome, and that the repeated fraction is interspersed with low copy sequences. These findings are in contrast to several well characterized saprophytic fungi.

F 422 A TRANSCRIPTIONALLY ACTIVE MULTIGENE FAMILY ENCODES TWO DIFFERENT Q_B PROTEINS IN *ANACYSTIS NIDULANS* R2, Susan S. Golden*, Judy Brusslan§, and Robert Haselkorn§, *Dept. of Biology, Texas A&M University, College Station, TX 77843, and §Dept. of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637 .

A gene in the chloroplast genome of plants, *psbA*, encodes the protein component of the second stable electron acceptor for photosystem II, Q_B; this protein is also the site of action of the herbicides atrazine and diuron. The genome of the cyanobacterium *Anacystis nidulans* R2 contains three distinct *psbA* genes which are all transcriptionally active in wild-type cells. We have cloned the genes *psbAI*, *psbAII*, and *psbAIII* and determined the nucleotide sequence of each. All three of the genes have open reading frames which encode polypeptides of 360 amino acids. The proteins encoded by *psbAII* and *psbAIII* are identical; they differ from the predicted amino acid sequence of the protein encoded by *psbAI* at 25 amino acid positions, 12 of which are within the first 16 amino acids of the protein. We identified mRNA species transcribed from each of the genes, and determined the transcriptional start sites. A sequence which is homologous to the consensus promoter sequence for genes in *Escherichia coli* precedes the site of transcription initiation from *psbAII* and *psbAIII*. The *psbAI gene*, whose message is the most abundant of the *psbA* transcripts, lacks an *E. coli*-like promoter. We have selectively inactivated each of the *psbA* genes in the *Anacystis* chromosome by insertion of antibiotic resistance gene cassettes into the coding regions. Individual cloned genes were inactivated *in vitro*, then recombined into the *Anacystis* chromosome in place of the normal gene. Strains which have any one or two of the *psbA* genes inactivated are viable and evolve oxygen at normal rates in photosystem II electron transport assays; oxygen evolution is blocked in all of these strains by the addition of 10^{-6}M diuron.

F 423 APPLICATION OF *AGROBACTERIUM* -MEDIATED TRANSFORMATION TO THE MOLECULAR ANALYSIS OF TOBACCO VEIN MOTTLING VIRUS, Robert Graybosch and Arthur G. Hunt, University of Kentucky, Lexington, KY 40546

We are using *Agrobacterium* -mediated transformation of tobacco as a means of studying the expression of viral genes and the replication of viral RNA in TVMV-infected plants. TVMV is a plus sense, single stranded viral pathogen of tobacco. Using "cDNA" copies of the viral RNA genome, we have constructed chimeric genes capable of producing RNAs containing the 5' and 3' sequences of the viral genome, as well as RNAs complementary to these regions. In addition, we have built genes designed to express various viral gene products. We will discuss the effects of our various "RNA" constructions on viral replication and pathogenicity in transgenic plants. We will also detail our strategies for expressing various gene products, and describe our progress in expressing the cytoplasmic inclusion protein, the viral coat protein, and a 28kD polypeptide of unknown function.

F 424 THE PEP CARBOXYLASE GENE FAMILY OF ZEA MAYS, John W. Grula, Carlotta A. Glackin and Richard L. Hudspeth, Phytogen, 101 Waverly Dr.,Pasadena, CA 91105.

We have isolated genomic clones from a B73 Zea mays library that contain four different genes encoding phosphoenolpyruvate (PEP) carboxylase. Three of the four genes share substantial sequence homology as revealed by restriction map similarities and by using the clones to probe genome blots and Northern blots of RNA from green leaves, etiolated leaves and roots. These three more closely related genes are divergent from the PEP carboxylase mRNA in green leaves and exhibit relatively strong reactions to the PEP carboxylase mRNA in etiolated leaves and roots. In contrast, a fourth gene hybridizes intensely with PEP carboxylase message in green leaves, but weakly to the PEP carboxylase mRNA in etiolated leaves and roots. Thus, this gene probably encodes the form of PEP carboxylase involved in C_4 photosynthesis, and it appears to have diverged from the other members of the family. Finally, the blot data also indicate the presence of at least one other PEP carboxylase gene in Zea mays that is divergent from the four we have cloned.

F 425 ISOLATION, CHARACTERIZATION AND SEQUENCE OF SOYBEAN BETA-TUBULIN GENES MJ Guiltinan, D-P Ma, D Barker, MM Bustos, and DE Fosket, University of California, Irvine, CA 92717 and Texas A&M University, College Station, TX 77843-3258. A soybean genomic library was screened with a Chlamydomonas cDNA probe to isolate thirty-five clones with beta-tubulin sequence homology. Analysis of these clones by restriction mapping suggested that 2 classes of beta-tubulin genes were present in the soybean genome. This conclusion was supported by data obtained from Southern blots of restricted soybean genomic DNA hybridized with a labeled soybean beta-tubulin coding sequence probe. Representatives of these 2 classes of beta-tubulin genes were subcloned and sequenced. A comparison of the deduced amino acid sequence of these genes with published sequences of beta-tubulin genes from phylogenetically diverse organismsdemonstrated that they diverged primarily near their carboxy terminus, but that they exhibited from 69 to 81% sequence homology overall. The two beta-tubulin genes were divergent from each other as well at both the nucleotide and amino acid level. Although there was no homology to the intron sequences of the beta-tubulin genes of other organisms, the 2 introns of the soybean beta tubulin genes each contained an 18 nucleotide sequence with 80% homology. The SB1 soybean beta tubulin gene also exhibited a high degree of homology to a 36 base pair region of the 3' untranslated region of one of the chicken beta-tubulin genes.

F 426 DISEASE RESISTANCE RESPONSE GENES INDUCED IN PEAS BY FUNGAL WALL CHITOSAN: PROPOSED MODE OF INDUCTION, L. A. Hadwiger, D. F. Kendra and B. W. Fristensky, Washington State University, Pullman WA 99164. A root rotting fungus Fusarium solani f. sp. phaseoli (Fsph) isolated from infected beans is not able to grow and infect pea plants. This "non-host resistance" is associated with the synthesis of specific mRNAs and proteins. The walls of Fsph and a pathogen infecting peas F. solani f. sp. pisi (Fsp) both contain predominantly chitin and β-glucan but only 1% chitosan (polyglucosamine). Pea tissue contains β-glucanase and chitinase and can degrade Fsph and Fsp walls releasing chitosan from both fungi. Commercial crab shell chitosan both induces disease resistance response genes (DRRG) and total immunity in pea pods to Fsp. Therefore, the nature of the chitosans inherent to both fungi were characterized in an attempt to discover how the pea successfully resists Fsph but eventually succumbs to Fsp. Chitosan (which is synthesized via chitin in fungi) derived from cell wall Fsph differs structurally and functionally from that derived from the walls of Fsp. Fsp chitosan contains lower M.W polymers and requires higher concentrations to inhibit spore germination, induce resistance and elicit pisatin accumulation than Fsph chitosan. Models will be presented of both the proposed in vivo induction of DRRG via DNA complexing by chitosan, and of the suppression of the resistance response caused by treatments with smaller polyamines.

F 427 DIFFERENTIAL GENE EXPRESSION IN DEVELOPING TOMATO SEEDS, M.J. Harrison and T.A. Brown, Dept of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD, UK. Tomato has been chosen as a model experimental system with which to study the genetics and molecular biology of seed development. The first aim has been to identify genes expressed only in the developing seed. A seed cDNA library has been prepared and seed-specific clones identified by probing with leaf, root and fruit RNA. These clones are now being grouped into families and the translation products characterised. A second aim has been to identify β-amylase and chymotrysin inhibitor (CI) clones from the cDNA library, using barley cDNAs as probes. Two β-amylase and six CI clones have been identified. The β-amylase clones hybridise strongly to seed and leaf RNA and weakly to root RNA. The CI clones hybridise to either seed and leaf or seed and root RNA. Further experiments will determine the homology between these clones and identify the DNA sequences responsible for mediating differential expression of the equivalent genes.

F 428 INTERSPECIFIC TRANSFER OF SULFONYLUREA-HERBICIDE RESISTANCE BY PLANT TRANSFORMATION
George W. Haughn and Chris R. Somerville, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing MI 48824.
Barbara J. Mazur, Chok-Fun Chui and Julie K. Smith, Central Research and Development Dept., Experimental Station, Du Pont and Co., Wilmington DE 19898.
The sulfonylurea herbicides strongly inhibit the branched-chain amino acid biosynthetic enzyme acetohydroxy acid synthase(AHAS). We have isolated mutants of Arabidopsis thaliana resistant to the sulfonylurea herbicide chlorsulfuron. Genetic and biochemical analyses of one such mutant line(GH50) have suggested that chlorsulfuron resistance in the mutant is due to a dominant mutation within the AHAS structural locus(we designate the mutant allele Csr-1). Because of the potentially-broad utility of herbicide resistance as a selectable marker for molecular-genetic studies, we cloned the AHAS gene from line GH50 using as a probe the corresponding gene from wildtype Arabidopsis. DNA sequence analysis showed that Csr-1 differs from the wildtype by a single nucleotide substitution. Transgenic tobacco plants carrying Csr-1 are approximately two orders of magnitude more resistant to chlorsulfuron than wildtype. Preliminary experiments have indicated that Csr-1 can function as an efficient selectable marker for plant transformation.

F 429 USE OF RFLPS TO ANALYZE GENOMIC STRUCTURE AND GENE FUNCTION IN PLANTS, Tim Helentjaris, NPI, Salt Lake City, Utah 84108.

Both randomly-isolated DNA clones and clones for genes with known identities have been used to detect genetic loci through restriction fragment length polymorhisms (RFLPs). Using these RFLPs, detailed linkage maps have been assembled for both maize and tomato and in maize, this map has been correlated with the described chromosomes through the use of monosomics, translocations, and both isozyme and morphological marker loci. These sets of RFLPs have then been used to analyze both genomic structure and individual gene function. For instance in maize, large duplicated segments (greater than 50 map units) have been found as contiguous regions on different chromosome pairs, i.e. chromosomes 2 and 7 share large homologies as do 3 and 8, which is informative as to the origin of modern maize. These RFLPs have also been used to analyze and dissect traits with complex genetic makeup, such as plant heighth and yield, which reveals the number and location of genetic loci involved, relative importance, and gene action and in some cases may allow one to assign component loci to known genes.

F 430 USE OF THE FIREFLY LUCIFERASE GENE TO DETECT GENE ACTION IN TRANSGENIC PLANTS. Stephen H. Howell, David W. Ow, Keith V. Wood, Marlene DeLuca, Jeffrey R. de Wet and Donald R. Helinski, Departments of Biology and Chemistry, University of California San Diego, LaJolla CA 92093

Light emitted from luciferase encoded by firefly luciferase-plant gene fusions has been used to monitor plant gene expression in transgenic plants. Luciferase catalyzes the light emitting, ATP-dependent oxidation of luciferin. Luciferase can be detected in extracts using a luminometer and a standard luciferase assay. The assay is more sensitive and rapid than a typical CAT assay. Luciferase activity can also be detected *in situ* in a variety of plant tissues. Luciferin is taken up by plant cells in culture, through the vasculature of explanted tissue and through the roots of whole plants watered with luciferin containing solutions. Light emitted from the luciferin-infiltrated tissue can be detected on film or by image-intensifying video equipment. For example, we have observed that rapid uptake of luciferin through the petiole of an explanted leaf from a transgenic tobacco plant. The vasculature progressively emits light from the base to the tip when the petiole is dipped in a luciferin-containing solution. Most observations have been made on transgenic plants bearing the luciferase gene driven by the CaMV 35S promoter (Ow et al., Science, in press), however, transgenic plants transformed with other luciferase constructs, such as ones involving the RUBP carboxylase small subunit promoter, have also been studied.

F 431 RFLP ANALYSIS OF *BREMIA LACTUCAE* Hulbert, S. H. & Michelmore, R. W. Department of Vegetable Crops, U.C. Davis.

Bremia lactucae is a biotrophic pathogen causing downy mildew of lettuce. Extensive genetic studies on both the host and pathogen have shown that at least 13 matching gene pairs determine specificity in this gene-for-gene interaction. A detailed genetic map of the pathogen is being developed using restriction fragment length polymorphisms (RFLPs). Low copy number, genomic clones and cDNAs were used as probes to identify RFLPs on Southern blots made with DNA from several isolates of *B. lactucae* digested with several enzymes. More than 50 polymorphisms have been identified with the first 220 probes that were hybridized. Mendelian inheritance has been demonstrated for most of these RFLPs in crosses between isolates of *B. lactucae*. Cosegregation of RFLPs and virulence genes is being analysed in highly polymorphic families.

RFLPs in mitochondrial DNA will enable studies on the sexuality of this heterothallic fungus and will indicate whether there is a relationship between sexuality and mating type. However, no mitochondrial polymorphisms were identified when one Californian and two European isolates were compared. The search for a mitochondrial polymorphism is being continued with a more diverse group of isolates.

F 432 ANALYSIS OF PLANT POLYADENYLATION SIGNALS, Arthur G. Hunt and Robert Graybosch, University of Kentucky, Lexington, KY40546

We are dissecting the polyadenylation signals of two plant genes. We have defined upstream and downstream sequences that are required for the function of the poly(A) signal from a pea rbcS gene in transgenic tobacco. Moreover, we have uncovered two cryptic poly(A) sites in close proximity to the "normal" poly(A) site in this gene. We are conducting a similar study of the poly(A) site of the cauliflower mosaic virus 19S/35S "genes". We will describe the system used for assessing poly(A) site function in transgenic tobacco, discuss the molecular anatomy of plant poly(A) signals, and relate them to possible mechanisms of polyadenylation in plants.

F 433 BETA-GLUCURONIDASE (GUS) AS A SENSITIVE AND VERSATILE GENE FUSION MARKER IN PLANTS. Richard A. Jefferson, T. A. Kavanagh and Michael W. Bevan. Department of Molecular Genetics, Plant Breeding Institute, Cambridge, England.

We have developed the E. coli beta-glucuronidase gene (GUS) as a gene fusion marker for analysis of gene expression in transformed plants. Most higher plants completely lack intrinsic beta-glucuronidase activity, thereby enhancing the sensitivity with which measurements can be made. We have constructed gene fusions using the cauliflower mosaic virus (CaMV) 35S promoter, the promoter from the small subunit of ribulose bisphosphate carboxylase (Rubisco) or the promoter of a tobacco chlorophyll a/b binding protein gene to direct the expression of beta-glucuronidase introduced into tobacco plants by Agrobacterium - mediated transformation. Expression of GUS can be accurately and quantitatively measured using fluorometric assays of trace amounts of transformed plant tissue. Plants expressing GUS are normal, healthy and fertile. GUS is very stable, tolerating large amino-terminal fusions, retaining activity after electrophoresis on SDS polyacrylamide gels and after prolonged storage of extracts. Histochemical analysis has been used to demonstrate the localization of gene activity in tissues of transformed plants. The use of the GUS fusion system for measurements of chimeric gene activity in single cells, for in vivo analysis of gene expression and for fusion genetics will be discussed. In addition, results showing the use of GUS fusions to sensitively measure "transient" expression of chimeric genes introduced into cultured plant cells by electroporation or other means will be presented.

F 434 REGULATED EXPRESSION OF A CHIMERIC PATATIN-GLUCURONIDASE FUSION IN TUBERS AND INDUCED INTERNODE CUTTINGS OF TRANSFORMED POTATO. Richard A. Jefferson and Michael W. Bevan, Plant Breeding Institute, Cambridge, England.

We have used the GUS fusion system to investigate the regulation of a tuber-specific gene in transformed potato (Solanum tuberosum var. Desiree). Expression vectors were constructed in a binary Agrobacterium vector, pBIN 19, using the putative promoter regions of a gene encoding patatin, the major "storage" protein of potato tubers, to direct the expression of the E. coli beta-glucuronidase gene (GUS). These vectors were introduced into potato plants by Agrobacterium - mediated transformation of tuber disks, and regeneration of plants from the resulting kanamycin resistant shoots. About two hundred independent transformants resulting from transformation with one of five promoter deletion derivatives of the patatin-GUS fusions were analyzed for GUS activity. Single leaf stem cuttings were cultured on normal hormone-free medium, or on medium containing high sucrose levels and additional cytokinin (tuber-induction medium). In most of the transformants, GUS activity was dramatically induced under the conditions that induced tuberization. Sections of developing tubers were stained for beta-glucuronidase activity using histochemical techniques. GUS activity was confined to cells of the "ground" tissue of the tuber, and was not detected in cells of the periderm or nodes (eyes). All of the deletion derivatives tested, ranging in size from 3500 bp to 360 bp upstream of the patatin initiator codon were functional in directing the inducible synthesis of beta-glucuronidase. Detailed analysis of the histochemical localization and the hormonal conditions of induction will be presented.

F 435 MOLECULAR CLONING OF SPECIFIC FRAGMENTS OF ctDNA RELATED TO THE CYTOPLASMIC MALE STERILITY IN RAPE, Gao Jie, Gong Fanrui, Li Jigeng, Institute of Genetics, Sinica, Beijing, China

Choloroplast DNA of a sterile line from a rape variety with a radish cytoplasm and its fertile counterpart was isolated and digested with EcoRI, BamHI, HindIII, PstI and XhoI respectively. Significant differences both in the number and relative positions of restricted segments were observed between sterile line and its fertile except in PstI pattern. Three specific fragments from the restricted pattern digested by BamHI were obtained and DNAs were recovered. Then these fragments were incubated with plasmid pBR322 digested by BamHI and T_4 -DNAligase respectively. The obtained recombinants were used to transform E. coli HB101. Through resistant screening, clone hybridization and eletrophores analysis, we got three clones carrying the specific fragments respectively. Now the characters of these are being investigated.

F 436 DEVELOPMENT OF A TRANSFORMATION VECTOR FOR BREMIA LACTUCAE (LETTUCE DOWNY MILDEW) USING A HOMOLOGOUS ACTIN PROMOTER. Howard S. Judelson, Benoit S. Landry, William E. Timberlake, and Richard W. Michelmore. University of California, Davis, CA 95616

We are developing a transformation vector for the plant pathogen Bremia lactucae that incorporates a homologous actin promoter. The actin gene was cloned from a Lambda EMBL4 DNA library using heterologous probes. Southern blot analysis shows that there is a single actin gene in the fungus. DNA sequence data from the clone demonstrated that it contains 85% amino acid homology with yeast actin. The B. lactucae gene lacks introns in the coding sequence and contains canonical transcription initiation and termination signals. The 5' flanking region is AT rich (ca. 400 of the 500 upstream bases are A or T). Enhancer-like sequences are found 300 bases upstream and 60 bases downstream of the initiation codon. Transcriptional and translational fusions are being made between the promoter from this gene and neomycin phosphotransferase and hygromycin B phosphotransferase genes. Our long-term goal is to use the transformation system to clone avirulence genes in B. lactucae by identifying clones from a cosmid library which confer avirulence on otherwise virulent isolates.

F 437 PLASTOCYANIN SYNTHESIS IN PEA LEAVES David I. Last and John C. Gray, Botany School, University of Cambridge, Cambridge CB2 3EA, U.K.

Plastocyanin is a 10.5 kDa copper-containing protein found in the thylakoid lumen of chloroplasts where it plays an essential part in photosynthetic electron transfer. Translation of pea leaf poly A+ RNA in a rabbit reticulocyte lysate produces a protein of mobility 22 kDa which is immunoprecipitated by antibodies to pea plastocyanin. A pea leaf cDNA library has been constructed in the Eco K selection vector M13K8.2 and several plastocyanin cDNA clones have been identified by hybridisation with a mixed oligonucleotide probe. The nucleotide sequence of the cDNAs indicate that pea plastocyanin is synthesised initially with a 67 amino acid extension at the N-terminus of the mature protein. This transit peptide shows considerable similarities with that of Silene Pratensis, as well as some differences. The cDNA sequences suggest the existence of a single plastocyanin mRNA species in pea leaves. Hydridisation of ^{32}P-labelled cDNA with a Southern blot of restricted pea nuclear DNA indicates that the plastocyanin gene exists as a single copy in the haploid genome.

F 438 REGULATION OF GENE EXPRESSION BY ETHYLENE IN RIPENING TOMATO FRUIT James E. Lincoln, Sabine P. Cordes, Jill Deikman, and Robert L. Fischer, Div. Molecular Plant Boilogy, U.C. Berkeley, CA 94720

The regulation of gene expression by the plant hormone ethylene has been investigated by cloning mRNAs which accumulate in unripe tomato fruit exposed to exogenous ethylene. The response to exogenous ethylene is rapid; within 30 to 120 minutes an increase in the cloned mRNA concentrations can be detected. Measurements of ethylene production during fruit development detect low basal levels in unripe fruit and much higher levels in ripening fruit. Blot hybridization experiments show that expression of the cloned genes is developmentally regulated during fruit ripening and their accumulation is repressed by a specific competitive inhibitor of ethylene action, norbornadiene. Expression of the cloned genes during fruit ripening is temporally separated into two classes; early gene expression is induced when the ethylene concentration is basal, while late gene expression is activated coordinately with the increase in ethylene concentration. These results suggest that early genes are induced by increases in tissue sensitivity to ethylene, while late genes respond to increases in ethylene concentration. Nuclear run-on transcription experiments show that cloned gene transcription is activated during fruit development. In addition to the cloned mRNAs which respond rapidly to exogenous ethylene, a polygalacturonase cDNA clone has been studied. The mRNA level for polygalacturanase does not accumulate rapidly in response to exogenous ethylene, but increases dramatically during fruit ripening. However, nuclear run-on transcription experiments indicate that post-transcriptional processes play an important role in regulating the polygalacturonase mRNA level during fruit ripening.

F 439

Abstract Withdrawn

F 440 REGULATION OF THE SYNTHESIS AND ACCUMULATION OF PHOTOSYSTEM II POLYPEPTIDES AND THEIR mRNAs IN CHLAMYDOMONAS REINHARDTII. Stephen P. Mayfield, Michael Kuchka, and Jean-David Rochaix. University of Geneva, Department of Molecular Biology, Geneva, Switzerland

Chloroplast mutations which affect the accumulation of one PS II core component result in a deficiency of all core PS II polypeptides. Loss of the core PS II particle does not necessarily result in a deficiency of the peripheral PS II proteins associated with oxygen evolution. Two nuclear mutants affecting oxygen evolution have been characterized. One of these is missing a 20 kD nuclear encoded polypeptide (OEE2) and its corresponding mRNA. The other is missing a 26 kD nuclear encoded polypeptide (OEE 1, 33 kD of spinach) and its corresonding mRNA. The latter mutant has reduced levels of all of the core PS II polypeptides. We have seen no affect of any of these mutations on the accumulation of other PS II mRNAs, nor on the synthesis of any of the PS II polypeptides, except for those directly affected by the mutation. We have recently isolated another class of nuclear PS II deficient mutants in which we find that the synthesis of chloroplast encoded PS II polypeptides is arrested. The mRNAs encoding these proteins are at levels equal to or above those in wild type cells, while other chloroplast encoded polypeptides are synthesized at normal levels in the mutant cells. Thus we may have identified a class of nuclear genes which specifically regulate the expression of chloroplast encoded PS II genes post-transcriptionally.

GENETIC TRANSFORMATION AND FOREIGN GENE EXPRESSION IN GRAPEVINE

F 441 Carole P. Meredith[1], Lori A. Martin[2], James A. Stamp[1], and Abhaya M. Dandekar[2], Department of Viticulture and Enology[1] and Department of Pomology[2], University of California, Davis, CA 95616.

We have shown that commercially important cultivars of Vitis vinifera are infectable with Biotype I Agrobacterium tumefaciens. In vitro grown plants of Vitis vinifera cvs. Cabernet Sauvignon, Cardinal, and Zinfandel were inoculated with Agrobacterium tumefaciens containing tumorigenic recombinant derivatives of the plasmid pTiA6 carrying chimeric constructs of the bacterial aminoglycoside phosphotransferase (APH(3')II) gene conferring resistance to kanamycin. Tumor incidence was 60-90%. Tumors obtained from Cardinal and Zinfandel were hormone autotrophic in vitro, but tumors of Cabernet Sauvignon required the addition of both auxin and cytokinin. Tumor cell lines produced octopine and expressed APH(3')II activity. Expression of APH(3')II was greatest in constructs that contained the cauliflower mosaic virus 35S promoter. The nopaline synthetase promoter produced greater APH(3')II activity than the octopine synthetase promoter. APH(3')II activity was also demonstrated by the growth of the cell lines on different levels of kanamycin. With the goal of obtaining transgenic plants, embryogenic callus of Vitis longii was co-cultivated with Agrobacterium containing a disarmed pTiA6 derivative carrying the same kanamycin resistance gene. Kanamycin-resistant proliferating somatic embryos were isolated after co-cultivation; control embryogenic callus was sensitive to kanamycin. Individual somatic embryos were isolated and induced to germinate. An analysis of the regenerated plants from these kanamycin-resistant somatic embryos will be presented.

THE GENES ENCODING THE QUINONE AND HERBICIDE BINDING PROTEINS OF BARLEY THYLAKOIDS

Eva Neumann, Sandy Berry-Lowe

F 442 Carlsberg Laboratory, Dept. of Physiology, Copenhagen, Denmark

The herbicide binding protein D-1 and the related D-2 protein are integral components of the PSII complex in thylakoids. The homology of these proteins with the reaction center polypeptides of purple bacteria suggests them to participate in binding the reaction center chlorophyll of PSII. We have localized the psbA gene - coding for D-1 - in a 20 kbp SalI fragment of barley chloroplast DNA (cpDNA) by hybridization with a) a synthetic oligomer, consisting of 18 bases, b) a probe of the psbA gene of spinach (kindly provided by H.J. Bohnert). In addition, we received a 3.44 kbp BamHI probe of the barley cpDNA (kindly provided by T. Berentzen), containing the psbD gene (coding for D-2) and part of the psbC gene (coding for chlorophyll a protein 3 of PSII). These two genes are also localized on the 20 kbp SalI fragment, which extends into one of the inverted repeats. Thus the psbC and the psbD genes are localized close to the psbA gene as has been described for wheat by Courtice et al., an arrangement which differs from the chloroplast genome organization of the dicots spinach and tobacco. We are sequencing the barley psbA gene. Our results reveal differences in the 3rd base position of many codons but close homology to the amino acid sequence of the spinach and tobacco psbA genes. The psbA and the psbD genes have been ligated into the expression vector pDS6. This expression plasmid permits the in vitro transcription and translation of these genes. We shall present our attempts to express the psbA and the psbD genes with the ultimate purpose of reconstituting in vitro the D-1 and the D-2 polypeptides with quinone and chlorophyll.

A 35S-P FRAGMENT EXPRESSES SOME ENHANCER PROPERTIES. Joan T. Odell, C. Jeffrey

F 443 Mauvais, and Susan Knowlton. Du Pont Experimental Station, Wilmington, DE 19898

The cauliflower mosaic virus (CaMV) 35S promoter directs a high level of constitutive expression of adjacent coding regions in transformed tobacco calli and plants. Previous analysis of 5' deletion fragments identified sequences between -46 and -105 as critical for directing high 35S-P activity (J.T. Odell et al. Nature 313, p810, 1985). An isolated 35S-P fragment containing this sequence region was cloned into the SstII site at -150 of the nopaline synthase promoter in a NOS-P-CAT construction. CAT activity levels were compared to those from NOS-P-CAT and 35S-P-CAT chimeric genes following transient expression in tobacco protoplasts. The fragment was able to substantially increase the expression level from the NOS-P when placed in either orientation. The same affect was observed in stably transformed tobacco calli. This 35S-P fragment is being tested for other enhancer properties.

F 444 EXPRESSION OF A MOUSE METALLOTHIONEIN GENE IN PLANTS, Véronique Pautot and Mark Tepfer, Laboratoire de Biologie Cellulaire, INRA, 78000 Versailles, FRANCE.

Using the Ri plasmid of Agrobacterium rhizogenes strain A4 as a vector, we have introduced into tobacco plants several chimeric genes based on either genomic or cDNA clones of a gene coding for a mouse metallothionein, MT-1. In all cases the nopaline synthase promoter and the 3′ regions were used. Analysis of RNA synthesized from these genes in plants makes possible the study of the effectiveness in a plant cell context of MT-1 signals regulating mRNA polyadenylation and intron splicing. We have also studied the resistance to heavy metals of root cultures expressing MT-1 mRNA.

F 445 GLUTAMINE SYNTHETASE GENES OF ARABIDOPSIS THALIANA: ORGAN-SPECIFIC EXPRESSION OF A SMALL GENE FAMILY, T. Kaye Peterman, Klaus Trinks, and Howard M. Goodman, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, 02114.

Glutamine synthetase (GS) plays a critical role in the nitrogen metabolism of higher plants. GS is essential for the primary assimilation of ammonia produced by nitrate reduction as well as the reassimilation of ammonia resulting from photorespiration and amino acid degradation. Given the different physiological roles of glutamine synthetases in roots and leaves we predicted that root- and leaf-specific glutamine synthetases would exist and that the glutamine synthetase gene family of *Arabidopsis* would be a good model system for studying organ-specific gene expression in higher plants. We are interested in the molecular basis and regulation of this organ-specificity. To this end we constructed a cDNA library in λgt11 from *Arabidopsis* leaf polyA+ RNA. Using GS antibody as probe we isolated cDNA clones which fall into different classes. Northern blot analysis indicated that these are expressed in an organ-specific fashion. Further characterization of these cDNA clones and the corresponding genomic clones is in progress. Also hybrid-select translations will allow us to correlate the cDNA clones with specific GS polypeptides.

F 446 EFFECT OF 35S LEADER MODIFICATIONS ON PROMOTER ACTIVITY, D. A. Pierce, I. J. Mettler, A. R. Lachmansingh, L. M. Pomeroy, E. A. Weck, and D. Mascarenhas, Stauffer Chemical Company, Richmond, CA 94804.

Several versions of the 35S promoter from CaMV have been attached to CAT or NEO marker genes and tested for transient promoter activity in maize and tobacco. Three versions were created by use of available restriction sites and differ only in the length of the leader region (4, 131 or 198 nucleotides) following the CAP site. These constructions showed striking differences in activity between maize and tobacco. The analysis was extended to include a series of Bal31 deletions which generated a wider range of leader lengths. The differences in expression shown by this series are not strictly a function of leader length. The results point to an interesting region of the 35S leader which contains two oligonucleotide repeats. This region seems to correlate with the higher level of expression of several 35S promoter versions in maize. The activity of two of the leader constructions in growth-selected maize transformants showed differences in activity similar to that detected by transient gene expression.

F 447 PEA AND WHEAT CHLOROPLAST GENES FOR THE ALPHA SUBUNIT OF RNA POLYMERASE. Saul Purton, Sean M. Hird, Tristan A. Dyer and John C. Gray. Botany School, University of Cambridge, Cambridge CB2 3EA, U.K. and Plant Breding Institute, Trumpington, Cambridge, CB2 2LQ, U.K.

The chloroplast genome of higher plants codes for a number of components of the chloroplast transcription and translation apparatus. Nucleotide sequence analysis downstream of the gene (petD) for the 15kDa subunit of the cytochrome b-f complex in both pea and wheat has revealed a large open reading frame of 330 and 338 codons, respectively. The deduced amino acid sequences both show significant homology to the alpha subunit of DNA-dependent RNA polymerase from Escherichia coli. The pea and wheat genes (rpoA) for the alpha subunit are transcribed in the opposite direction to petD and in both cases the intergenic region contains an inverted repeat sequence which could form a stable stem-loop structure. This may function as a termination signal for both rpoA and petD. Upstream of rpoA in pea chloroplast DNA and transcribed in the same direction, is an open reading frame of 137 codons which codes for a protein homologous to ribosomal protein S11 from E. coli.

The distribution and function of the rpoA gene product in pea chloroplasts is being explored with antibodies raised against fusion proteins produced from a series of expression vectors in E. coli.

F 448 CHLORELLA ALGAL VIRUSES, Anne M. Schuster[a], James L. Van Etten[b], and Russel H. Meints[a,b], School of Biological Sciences[a] and Department of Plant Pathology[b], University of Nebraska, Lincoln, NE 68588.

Exsymbiotic, Chlorella-like, eukaryotic green algae act as hosts for a group of DNA containing viruses. As an example, PBCV-1 is a large, dsDNA (300 kbp) virus which infects Chlorella strain NC64A. It is easily assayed since it will form plaques on lawns of alga. Viral attachment and infection is rapid and the entire lytic cycle of the virus is complete within 6 to 8 hours. This is the first example of a plant-virus system which lends itself to investigation using the established technology adapted from studies of bacteriophage.

We have isolated more than 75 unique plaque forming viruses from fresh water sources in the U.S. The viruses are all similar to PBCV-1 in that they are large polyhedrons, contain dsDNA genomes and are cholroform sensitive. However, the viruses can be distinguished from PBCV-1 and from each other by at least one of the following characteristics: plaque size, reaction to PBCV-1 antiserum, DNA restriction patterns, sensitivity or resistance to restriction, percent methylated DNA and protein patterns. Based on combinations of these criteria, the viruses were grouped into 11 classes. Viral DNAs showed some homology to PBV-1 DNA, however, those viral DNAs which were highly resistant to restriction and also highly methylated showed a much reduced homology.

When the viruses ultimately lyse their algal hosts the resulting lysate is a rich source of cell wall degrading enzyme(s), which we have called 'lysin'. Lysin can be used to produce Chlorella protoplasts. Presently we are attempting (i) to isolate and characterize the gene(s) for lysin and (ii) to develop a transformation system for Chlorella protoplasts.

F 449 MOLECULAR STUDIES ON THE DOTTED TRANSPOSON SYSTEM OF MAIZE, J.J. Sorrentino[1], N.S. Shepherd[1], Zs. Schwarz-Sommer[2], and C. O'Reilly[2], [1]DuPont Experimental Station, E328/246, Wilmington, DE 19898, and [2]Max-Plank-Institut für Züchtungsforshung, 5000 Köln 30 FRG.

The Dotted transposon system of maize is a two element system which controls mutability at the A_1 (Anthocyaninless) locus. The autonomous element, Dotted, controls mutability of two recessive alleles a-st and am-1:Cache, among others. These alleles are said to contain the nonautonomous element, rDt.

Cloning of a wildtype A_1 locus (O'Reilly et al., 1985 EMBO J 4: 877-882) provided a homologous probe for the molecular cloning of a-st and am-1:Cache. When compared to the wildtype A_1 gene structure, both of these alleles contain an 0.7 kb insertion which has imperfect terminal inverted-repeats. The nucleotide sequence of the two insertions is nearly identical. Because of these characteristics, we believe the insertions are the rDt element. Since a-st and am-1:Cache differ in the frequency and developmental timing of mutation under Dotted control, the structure of the rDt insertions and flanking sequences have been compared. The rDt insertions occur at different positions in the A_1 gene and are inserted in opposite orientations. Local secondary structure in the surrounding A_1 sequences may affect rDt excision frequency, and thus mutability.

F 450 **Probing the *Agrobacterium tumefaciens* plant transformation mechanism with Ti plasmids containing multiple left T-DNA borders,** Eric R. Ward and Wayne M. Barnes, Department of Biological Chemistry, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

A. tumefaciens cells harboring Ti plasmids with multiple (2 to 20) left T-DNA borders specify integration of a T-DNA bounded by the right border and the innermost left border. Following acetosyringone induction and native Southern transfer of total bacterial DNA from the same strains, we observe not only a T-strand of the size predicted from the T-DNA, but also a many-fold excess of single-stranded molecules representing DNA between the repeated copies of the left border. These reiterated single strands apparently arise from nicking at every border and replacement strand synthesis that tracks through the many left borders. However, Southern analysis of DNA from uncloned tumors induced by these strains shows only a T-DNA carrying the tumor-causing genes and no T-DNA that would result from reading through the innermost left border:

	----------LB-LB---LB---LB---LB---LB---LB---LB---LB---LB---LB--------tu. or genes---------RB
Induced single strands:	+ + + + + + + + + + +
T-DNA in tumors:	- - - - - - - - - - +

That the T-DNA apparently arises from only the rightmost T-strand can be accounted for by two mechanisms, either: i) only T-strands with *bona fide* right border ends are transferred to the plant cell, or ii) all T-strands are sent to the plant cell, and the observed stable integration of only the rightmost one is a trivial consequence of selecting for the tumor genes which lie on it. By placing a selectable marker between outlying left borders and determining the frequency of its transfer to plant cells, we should be able to distinguish between these alternative hypotheses.

F 451 MULTIPLE TRANSACTING FACTORS MAY BE INVOLVED IN THE REGULATION OF THE SHRUNKEN GENE IN ZEA MAYS L., Wolfgang Werr, Boris Springer, Christoph Maas, Hans-Jürgen Joos, Wolf-Bernd Frommer and Peter Starlinger, Institut für Genetik, D-5000 Köln 41, Federal Republic of Germany.
The shrunken gene is expressed in several maize tissues and encodes an anaerobic protein (Springer et al., MGG in press). We have started to identify regulatory important sequences within the promotor of the shrunken gene. Our first approach is mapping of promotor deletion mutants in a transient gene expression system. The second experimental line is the identification of transacting factors in nuclear extracts prepared from nuclei isolated from different maize tissues. We detect multiple protein-DNA- interactions with small promotor fragments spanning sequences up to - 600. At the moment we are in the process of footprinting these interactions within the shrunken promotor.

F 452 WOUND-INDUCED EXPRESSION OF POTATA PROTEINASE INHIBITOR II GENE ON TRANSGENIC TOBACCO PLANTS, L. Willmitzer, M. Keil and J. Sanchez-Serrano, 1000 Berlin, FRG.
A potato proteinase inhibitor II gene was transferred into tobacco plants using Agrobacterium/Ti-plasmid mediated gene transfer techniques. Whereas no or little expression of the gene could be detected on non-wounded leaves, high levels of proteinase inhibitor II mRNA were detected in leaves of several transgenic tobacco plants after mechanical wounding as well as after treatment of detached leaves with oligosaccharides. No signal could be detected in non-wounded stem, root- or flower tissue.
Chimaeric genes containing different 5'- resp. 3'-parts of the proteinase inhibitor gene and the CAT gene have been constructed and transferred into plant cells and are presently analyzed for their expression.

F 453 TRANSCRIPTION OF PEA CHLOROPLAST DNA, N. Woodbury and L. Roberts, Carnegie Institution of Washington, Stanford CA, M. Dobres and W. Thompson, Botany Dept., North Carolina State University, Raleigh, NC, J. Palmer, Div. of Biological Sciences, University of Michigan, Ann Arbor, MI
74 clones covering nearly the entire pea chloroplast genome were used to probe Northern blots of total pea RNA. Transcripts were detected covering approximately two-thirds of the chloroplast genome. A number of theses transcripts encoded two or more genes, and multiple, overlapping transcripts were found for many genes. We then attempted to determine the approximate number and location of transcription initiation sites in the chloroplast by hybridizing in vitro capped chloroplast RNA to each of the 3' end of capped RNAs. At least 25 of the 74 clones were shown to contain transcription initation sites.

Late Additions - F454 - F457

F 454 ANALYSIS OF THE CONTROL OF EXPRESSION OF BETA-GLUCURONIDASE IN THE DIFFERENT CELL TYPES FOUND WITHIN THE LEAVES OF TRANSGENIC PLANTS, David W. Galbraith, Richard A. Jefferson, Kristi R. Harkins, T.A. Kavanagh and Michael W. Bevan, University of Nebraska-Lincoln, Lincoln NE 68588-0118 USA and Plant Breeding Institute, Cambridge CB2 2LQ, England.

We have used a new gene fusion system and fluorescence-activated cells sorting to study the expression of chimaeric genes in single cells of transformed plants. Protoplasts were prepared from leaves of plants containing transcriptional fusions of beta-glucuronidase (GUS) to promoters of Cauliflower Mosaic Virus (CaMV 35S), the chlorophyll a/b binding protein (CAB) or the small subunit of ribulose bisphosphate carboxylase (rbcS). The protoplasts were sorted according to diameter and according to chlorophyll content in order to separate those derived from mesophyll tissue from those of epidermal and perivascular origin. GUS activity in single protoplasts was measured. GUS was active within all types of protoplasts isolated from plants containing the CaMV-GUS chimaeric gene. In contrast, protoplasts lacking chlorophyll contained low levels of GUS expressed from the light-regulated rbcS-GUS and CAB-GUS gene fusions. The expression of GUS in the cells of those plants that contained chlorophyll was related to the cellular chlorophyll content. The degree of correlations differed between the different gene fusions, expression of the CAB-GUS fusions being tightly coupled to chlorophyll levels, whereas the control of expression of the rbcS and CaMV fusions was less stringent.

F 455 IMPORT OF PLASTOCYANIN TRANSIT PEPTIDE DELETION MUTANTS INTO CHLOROPLASTS, Johan Hageman, Carolien Baecke, Sjef Smeekens and Peter Weisbeek, University of Utrecht, Padualaan 8, NL 3584 CH Utrecht, The Netherlands.

Plastocyanin, a thylakoid lumen protein that is synthesized in the cytoplasm has to cross three membranes, two envelope membranes and the thylakoid membrane to arrive at its site of function. The 66 amino acids long transit peptide of the plastocyanin precursor can be divided into two functional domains, a chloroplast import domain and a thylakoid transfer domain.

The chloroplast import domain is involved in the transport over the chloroplast envelope and is cleaved off in the stroma by a specific processing peptidase. The thylakoid transfer domain part is involved in the routing of the intermediate to the thylakoid lumen. It is removed by a thylakoidal processing protease that recognizes the mature processing site in the intermediate but not in the precursor.

An ordered set of deletion mutants of the thylakoid transfer domain is constructed, starting from the mature processing site upwards into the transit peptide to determine which parts are important for routing and processing. *In vitro* import of the mutant proteins into chloroplasts shows that the thylakoid transfer domain is essential for targeting of the proteins inside the chloroplast, but not for the import into the chloroplast.

F 456 NUCLEAR LOCI THAT AFFECT TIMING OF LHCII ASSEMBLY IN MAIZE

Mary Polacco[1] and Carolyn Vann [2], [1]Dept. of Biochemistry, University of Missouri, Columbia, MO and [2]Dept. of Biology, Ball State University, Muncie, IND

We have identified two unlinked nuclear loci in maize that alter the timing of the assembly of LHCII during chloroplast morphogenesis. Normally, LHCII assembly is a late event in chloroplast development. Most delayed greening (virescent) mutants in maize and other higher plants do not perturb the sequence of membrane differentiation in developing thylakoids. One of the mutations we are studying causes a delayed greening that is accompanied by early assembly of LHCII. This mutation has unmasked another unlinked allele that corrects the assembly order, without affecting the delayed greening phenotype. Northern analysis reveals that the size of the steady state pools of *cab* mRNA vary in the mutants parallel to the timing of LHCII accumulation: early assembly is accompanied by a large *cab* mRNA pool while normal assembly order is associated with a significantly reduced *cab* mRNA pool. Thus, the variant alleles directly affect LHCII assembly, possibly at the level of gene expression

F 457 DEVELOPMENTAL EXPRESSION AND STRUCTURAL ORGANIZATION OF SUNFLOWER STORAGE PROTEIN GENES, R.A. Vonder Haar, R.D. Allen, E.A. Cohen, M.M. Keifer, C.A. Adams, C.L. Nessler, K.A. Koprivnikar and T.L. Thomas, Texas A&M University, College Station TX 77843.

The two major storage protein classes in sunflowers are the 2s albumins and the 11s helianthinins. These proteins are synthesized exclusively in embryonic tissues during sunflower seed development. The albumins and their transcripts are detectable by 5 days post-fertilization (DPF), and the helianthinins by 7 DPF. cDNAs encoding both storage protein classes have been isolated and characterized. Genomic blot analyses indicate the albumin storage proteins are encoded by a small gene family including at least 2 genes, one of which is represented by the cDNA clone *Ha*5. Helianthinin storage proteins are encoded by a gene family comprised of at least two subfamilies represented by the cDNA clones *Ha*2 and *Ha*10. Genomic DNA recombinants complementary to Ha5, Ha2 and Ha10 have been isolated from a genomic DNA EMBL3 library. Representative recombinants encoding legumin (*Ha*G3) and albumin (*Ha*G5) storage proteins have been sequenced. The gene *Ha*G5 contains one short intron and 2 exons as well as the usual consensus eukaryotic *cis* regulatory elements. Similar consensus sequence elements are found in *Ha*G3, a legumin-encoding genomic sequence; this gene, however, contains 2 introns and 3 exons. The third exon of *Ha*G3 includes the α,β subunit cleavage site common to all known legumin storage protein genes.

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