THE MOLECULAR BASIS OF PLANT DEVELOPMENT

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The Molecular Basis of Plant Development

Keynote Address

L 001 MATERNAL AND ZYGOTIC DNA BINDING PROTEINS REQUIRED FOR SPATIAL ACTIVATION IN THE SEA URCHIN EMBRYO, Eric H. Davidson, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

This presentation will concern the molecular basis of the process by which differential patterns of regional gene activity are instituted in the embryo. The cytoskeletal actin gene CyIIIa provides an excellent molecular marker for the zygotic program of gene expression characteristic of the embryonic aboral ectoderm. According to our recent lineage tracer studies the aboral ectoderm derives clonally from 6 specific cleavage stage founder cells. An additional marker gene considered is the SM50 spicule matrix gene, expressed exclusively in skeletogenic mesenchyme cells, the clonal 5th cleavage descendants of the micromeres. Regulatory sequences of both genes have now been shown to accurately direct spatial and temporal expression of associated reporter genes, after injection of the appropriate fusion gene constructs into sea urchin eggs. By using this method of gene transfer the cis regulatory domain of the CyIIIa gene has been delimited. Coinjection of excess quantities of DNA fragments containing subregions of the regulatory domain results in in vivo competition, and thus it is possible to analyze the functional significance of individual regulatory elements. DNA-protein binding studies in vitro demonstrate eight sites where highly specific interactions occur within this domain (10^4-10^5 fold preference for CyIIIa site vs. random DNA sequence). Some of the factors that bind to these sites are probably zygotic gene products, since their concentration increases as development proceeds and they cannot be detected in extracts of unfertilized eggs. However, others are clearly maternal, and are stored in unfertilized egg cytoplasm. Analysis of the origin, the cytological distribution and possibly the activation of certain of these CyIIIa regulatory factors in the egg and early embryo, considered in conjunction with the lineage and location of the aboral ectoderm precursors, should, we believe, provide a molecular interpretation of how the CyIIIa gene becomes differentially expressed as the aboral ectoderm is formed. The same factors are likely to regulate other aboral ectoderm genes as well. This is indicated by in vitro intergenic competitions, carried out with regulatory regions of other genes expressed specifically in the same cells. An interesting result follows from introduction of the CyIIIa regulatory sequences into eggs of a different sea urchin species. Though the exogenous fusion construct is regulated temporally in a correct manner, spatial regulation is wholly deranged, thus providing an opportunity to identify these cis-trans interactions required normally for spatial control.

Molecular and Genetic Approaches to Animal Development

L 002 SPECIFICATION OF THE EMBRYONIC BODY AXIS IN THE AMPHIBIAN EGG. John Gerhart and Brian Rowning. Department of Molecular Biology, University of California, Berkeley CA 94720.

The amphibian (Xenopus) egg is a polarized cell with two cytoplasmic hemispheres, animal and vegetal, arranged with cylindrical symmetry about an animal-vegetal axis. In the period of the first cell cycle after fertilization, the egg cortex and interior cytoplasm behave as two rigid units which rotate 30° relative to each other, thereby eliminating the cylindrical symmetry of the egg and replacing it with bilateral symmetry. The direction of rotation, which is roughly related to the single point of random sperm entry in the animal hemisphere, predicts very accurately the opposed meridians on which the dorsal and ventral midlines of the vertebrate body axis will develop. Rotation depends on the formation and function of parallel microtubule (MT) arrays which align in the direction of rotation in the shear zone of the vegetal hemisphere between the cortex and interior. These arrays appear when rotation starts (40 min postfertilization) and disappear when rotation stops (80 min postfert.). They can be visualized by immuno-fluorescence and immunoperoxidase reactions (These observations were made in collaboration with Dr. R. Elinson, Univ. of Toronto). Artificially activated eggs, that is, ones lacking a sperm, can nonetheless form aligned MT arrays and engage in cortical-subcortical rotation. Thus, the sperm normally just biases the direction of alignment and rotation, but does not provide indispensable components.

Tubule formation is rapidly and completely blocked by colchicine, nocodazole, cold shock, hydrostatic pressure, and UV irradiation. Embryos can develop from eggs that have been blocked in their rotation by these agents and treatments. We find that the amount of rotation sets limits on the most anterior extent of development of the body axis. When rotation is slightly reduced, the head is not formed but the trunk and tail look normal; when rotation is inhibited still more, the head and trunk are not formed but the tail looks normal. When rotation is blocked entirely, the head, trunk, and tail are absent and the embryo is cylindrically symmetrical and composed entirely of ventral tissues such as gut, coelomic mesentary, red blood cells, and a ciliated epidermis. Thus, rotation is needed for the development of dorsal anatomical structures; these are the ones which constitute the vertebrate body axis. Larger amounts of rotation are needed for the development of the more anterior dorsal structures. The basis for this quantitative relationship between cytoplasmic rearrangement in the egg and the final embryonic anatomy will be discussed.

L 003 REGULATORY INTERACTIONS AMONG HOMEO BOX GENES IN DROSOPHILA, Michael Levine, Timothy Hoey, Manfred Frasch and Rahul Warrior, Department of Biological Sciences, Fairchild Center, Columbia University, New York NY 10027.
There is evidence that selective patterns of homeo box gene expression involve cross-regulatory interactions, whereby one homeo box gene can influence the expression of others. Our studies have focused on regulatory interactions with the homeo box gene even-skipped (eve). eve mutations cause the most severe developmental defects among the known zygotically active homeo box genes in Drosophila, in that eve^- embryos completely lack segmentation. The eve^- phenotype appears to result not only from the absence of eve^+ products, but also as an indirect consequence of altered activities of other homeo box genes. We present evidence that the eve protein controls morphogenesis by regulating the expression of the segmentation gene engrailed (en), and by autoregulating its own expression. A full-length eve protein binds with high affinity to specific sequences associated with both the eve and en transcription units. Many of the en binding sites contain at least one copy of a 10 bp consensus sequence: T-C-A-A-T-T-A-A-A-T. These binding sites are also recognized by other homeo box proteins, lending support to the proposal that cross-regulatory interactions among homeo box genes involve a competiton of different homeo box proteins for similar cis regulatory sequences. Binding of the eve protein to 5′ eve sequences appears to involve a second, more selective binding activity that is not shared by other homeo box proteins. This activity appears to depend on regions of the eve protein that map outside the homeo domain.

L 004 MOLECULAR BASIS OF CELLULAR RECOGNITION DURING EARLY MORPHOGENESIS, David R. McClay, Department of Zoology, Duke University, Durham, NC 27706. Primary mesenchyme cells (PMC) of the sea urchin embryo ingress through the basal lamina and invade the blastocoel to signal the beginning of morphogenetic movements at gastrulation. The PMC movement is accompanied by three simultaneous changes in cell adhesion. The cells lose affinity for the extraembryonic matrix and for other cells, while they gain an affinity for fibronectin and other components of the basal lamina. This study investigates the complexity of the cell-substrate interactions. Five molecules are shown to participate in an array of cellular functions beyond their role as adhesive substrates. These molecules are shown to participate in assembly of the extracellular matrix and their presence is shown to be necessary for the normal sequence of gene expression. Absence or removal of substrate components causes an alteration in gene expression unique to a particular substrate. Several components are shown to be released from cells in polarized fashion. Each of these bears the same oligosaccharide which may provide the correct traffic signal for polarized release. Finally, cells provide signals to other populations of cells directly affecting the developmental pathway of the responding population. Thus, extracellular matrix components have a variety of roles in early morphogenesis beyond serving as an adhesive substrate. Supported by NIH grant #HD14883.

L 005 NEUROPEPTIDES: MULTIPLE REGULATORY MECHANISMS AND THEIR ROLES IN MEDIATING SIMPLE BEHAVIORS, Richard H. Scheller, Department of Biological Sciences, Stanford University, Stanford, California 94305, U.S.A.

The central nervous system of the marine mollusk Aplysia is a useful model system for studies of the cellular basis of simple behaviors. We have isolated several genes encoding the precursors for biologically active peptides used as intercellular messengers by identified neurons in the abdominal ganglion. The large size of Aplysia neurons makes it possible to analyze the expression of microinjected genes in single cells. Antibodies to synthetic peptides define the anatomical and subcellular distribution of the molecules. The peptide products are expressed in subsets of central neurons and are packaged in multiple populations of dense core vesicles. The distribution of immunoreactive processes suggests three anatomical locations of neurosecretion, the neuropile, the connective tissue sheath and specific peripheral targets. HPLC in conjunction with protein microsequencing are used to define the precise proteolytic processing pathway. Once cleavage products of the precursors are defined, physiological analysis of their actions is possible. The peptides act directly as excitatory and/or inhibitory transmitters and also modulate the actions of other substances on both neuronal and muscle targets. Voltage and patch clamp analysis are being used to define the cellular mechanisms of action of the peptide products.

Contrasting Processes of Plant and Animal Development

L 006 NODE COUNTING IN TOBACCO: PHYSIOLOGICAL AND GENETIC ASPECTS, Carl N. McDaniel, Susan R. Singer, Joan S. Gebhardt and Karla A. Sangrey, Plant Science Group, Dept. of Biology, Rensselaer Polytechnic Institute, Troy NY 12180.

The number of leaves produced by the terminal apical meristem of Nicotiana tabacum cv. Wisconsin 38 before forming the terminal flower is precisely regulated by endogenous processes. Removal of apical or basal leaves does not influence the number of leaves produced providing several leaves remain on the plant. Thus, it is not leaves but nodes or something tightly correlated with nodes that is counted. When released from apical dominance, axillary buds exhibit a similar counting phenomenon. That is, apical axillary buds produce few nodes and then a terminal flower while basal buds produce many nodes and a terminal flower. When two axillary buds are permitted to grow from a single main axis, each produces the number of nodes it would have produced in the absence of the other axillary shoot indicating that each meristem counts independently of other meristems. Axillary buds of main axes that have been placed horizontally produce the same number of nodes as similar axillary buds on vertical main axes indicating that gravity does not play a major role in the counting process.

The USDA Nicotiana germ plasm collection at Oxford, NC has TIs (tobacco introductions) which produce few nodes as well as TIs which produce many nodes. About 35 of each type have been selected and crosses among and between the selected TIs have been made. Initial data indicate that node counting is not cytoplasmically inherited and that most crosses give F1 plants that produce a number of nodes which is closer to or identical with the number of nodes produced by the few noded parent.

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L 007 CELL DETERMINATION IN TOBACCO, Frederick Meins, Jr., Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

During the development of multicellular plants, organs and tissues become progressively determined for specific developmental fates. In some cases the determined state, once established, can even persist in populations of dividing cells. The basic question that arises is whether this stability results from cell interactions or from alterations in cellular heredity. Studies of tissue-specific variation in the cytokinin requirement of cultured tobacco cells show that in some cases determination can be inherited by individual cells and that cells can undergo a process akin to transdetermination. At least three genetic loci, Hl-1, Hl-2, and Hl-3 appear to regulate determination for cytokinin requirement. Complementation studies using cells transformed with Ti plasmids defective at the isopentenyl transferase (ipt) locus show that the HL-1 locus has an oncogenic function. Evidence is presented that the cell-heritable, cytokinin-autotrophic phenotype of pith cells is maintained by a positive-feedback loop in which cytokinins or related cell-division factors either induce their own synthesis or inhibit their own degradation.

L 008 HETEROCHRONIC MUTATIONS CONTROLLING SHOOT DEVELOPMENT IN MAIZE, *Scott Poethig, Biology Department, University of Pennsylvania,* Philadelphia, PA 19104-6018.

In maize, the heterochronic mutations *Teopod 1* (*Tp1*) and *Teopod 2* (*Tp2*) cause the prolonged expression of juvenile vegetative traits during shoot growth. The most unusual feature of their highly pleiotropic phenotype is the transformation of reproductive bracts into leaves. To define the function of these genes and to identify loci with which they interact, we are isolating suppressors of their semi-dominant phenotype. One mutation that suppresses *Tp1*, and two mutations that suppress *Tp2* were isolated from an M1 population produced by EMS pollen mutagenesis. All three suppressors appear to be intra-genic loss-of-function mutations of these loci. A description of the phenotypes of these new mutations and their interactions with *Tp1* and *Tp2* will be presented.

As part of a general screen to identify chromosomal regions that interact with *Tp1* and *Tp2*, we are examining the effect of hyperploidy for various chromosome arms on expression of these mutations. Hyperploidy for distal segments of 5S, 5L, 6L, 9S and 10L had little or no effect on the expression of either *Tp1* or *Tp2*. Hyperploidy for 1L significantly reduced the number of tillers produced by *Tp2*; hyperploidy for 4L enhanced the effect of *Tp1* and *Tp2* on tassel morphology; hyperploidy for 3L slightly enhanced the tassel phenotype of *Tp1* and *Tp2*. The effects of 1L and 4L are particularly interesting in light of the fact that the *teosinte branched* (*tb*) mutation on 1L and the *Tunicate* (*Tu*) mutation on 4L interact synergistically with *Tp1* and *Tp2*. These results suggest that *Tp1*, *Tp2*, *tb* and *Tu* are members of the same regulatory network.

Differentiation in Prokaryotes and Lower Eukaryotes

L 009 INTRACELLULAR COMMUNICATION REGULATES GENE EXPRESSION AND CELLULAR DIFFERENTIATION IN *DICTYOSTELIUM*, Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, M-034, Univ. of California, San Diego, La Jolla, CA 92093.

The cellular slime mold *Dictyostelium discoideum* grows as single-cell vegetative amoebae which then undergo a multicellular differentiation upon starvation. The multicellular organism is formed by the aggregation of ~10^5 individual amoebae in response to a pulsatile chemotactic signal initiated from and relayed by the amoebae themselves. The chemotactic agent for the aggregation is cAMP, which acts like an extracellular hormone by interacting with a cell-surface cAMP receptor. Interaction of cAMP with the receptor mediates chemotaxis and induces specific class of genes via a number of signal transduction pathways. In addition, another factor, CMF, a protein of ~70 kd, is secreted by cells and is also required for the expression of a number of genes. After the formation of a multicellular aggregate, prestalk and prespore cells, the precursors to the stalk cells and spores within the terminal fruiting body, are induced. Induction of a number of prestalk and prespore cell-type-specific genes, as well as cytodifferentiation of the two cell types themselves, is induced by cAMP produced within the multicellular aggregate mediated through a number of signal transduction pathways acting via G (GTP binding) proteins. In addition, at least two other factors, adenosine and DIF, are also involved both in the induction of cell-type-specific genes and the maintenance of the appropriate spatial organization of the individual cell-types within the differentiating multicellular organism. Thus, through the multicellular differentiation intracellular communications through a number of different secreted cellular factors regulates the aggregation, cellular differentiation, spatial differentiation, and cellular patterning within the multicellular aggregate as well as cell-type stage and cell-type-specific gene expression. The molecular mechanisms and signal transduction pathways involved in controlling development in this organism will be discussed with emphasis on the functions of two of the secreted factors, CMF and cAMP, and the gene products of the spatial transduction pathways that regulate their action. Special emphasis will be given to the function of two recently cloned developmentally regulated G_α proteins on the various signal transduction pathways regulating gene expression, work that has been done in collaboration with the laboratory of Peter Devreotes.

L 010 REARRANGEMENT OF NITROGEN FIXATION GENES DURING HETEROCYST DIFFERENTIATION IN THE CYANOBACTERIUM *ANABAENA* 7120. Robert Haselkorn, William J. Buikema, James W. Golden†, Peter J. Lammers‡ and Martin E. Mulligan, University of Chicago, Chicago, IL, †Texas A&M University, College Station, TX, ‡New Mexico State University, Las Cruces, NM.

Anabaena is a filamentous cyanobacterium that produces specialized cells called heterocysts at regular intervals along each filament when deprived of fixed nitrogen under aerobic conditions. Heterocysts are anaerobic factories for nitrogen fixation (*nif*) in which many structural and physiological changes have occurred. We have identified two DNA rearrangements that occur during the differentiation of heterocysts in *Anabaena* 7120. The *nifD* gene, encoding the a subunit of nitrogenase, is interrupted by an 11 kb DNA element. During differentiation this 11 kb element is excised by site-specific recombination between short, direct repeats present at the ends of the element. The excision results in the restoration of the *nifD* coding sequence and of the entire *nifHDK* transcription unit. A cloned piece of DNA that contains the entire 11 kb element and flanking sequences excises the element in *E. coli* at a low rate. We have identified a gene within the 11 kb element that is believed to encode the site-specific recombinase responsible for excision of the element during heterocyst differentiation. Abundant expression of this protein in *E. coli* results in a greatly enhanced rate of excision of the element. We are currently working on the characterization of this reaction *in vitro*.

A second developmentally regulated gene arrangement has also been observed in *Anabaena*, occurring next to the *nifS* gene. This rearrangement involves a different set of repeated sequences, thus implying a different site-specific recombination system. In this rearrangement a 55 kb DNA element is excised, resulting in the juxtaposition of two *nif* genes upstream to *nifS*. Sequence analysis of this region after rearrangement has identified four *nif* genes, probably arranged as a single operon. They are *nifB*, *fdxN* (a ferredoxin-like gene also found 3' to *nifB* in *Rhizobium)*, *nifS* and *nifU*. Efforts are currently aimed at identifying the gene(s) responsible for this rearrangement.

L 011 CELL INTERACTIONS GOVERN MYXOCOCCUS DEVELOPMENT: Dale Kaiser, Lee Kroos, Adam Kuspa, Yvonne Cheng and Seung Kim, Department of Biochemistry, Stanford University, Stanford, California 94305

When starved, 10^5 Myxococcus cells congregate to form a fruiting body within which some cells become spores. Many new proteins appear during fruiting body development. A transposable promoter probe, Tn5 lac, can express β-galactosidase (β-gal) if it inserts with its left end downstream of an active promoter. Among 2,374 independent insertions of Tn5 lac into Myxococcus, 37 were identified that make at least 3 times more β-gal after fruiting body development starts than during growth. Each of these transcriptional fusion strains increases β-gal at a particular time in development, ranging from before aggregation to the time of sporulation. This set of fusion strains serves as an indicator of progress through the developmental program.

How is the time sequence of morphological, biosynthetic, and gene expression events controlled? A set of mutants has been isolated that behave as if defective in cellular interactions necessary for development. Sporulation in fruiting bodies is restored to the mutants if they are mixed with developing wild-type cells. "Complementation" experiments with whole cells divide the mutants into 4 groups (A, B, C and D). Particular sets of lac fusions depend on the normal function of complementation groups, A, B, C and D. For example, A⁻ strains containing 18 among 21 different lac fusions tested fail to make β-gal, while strains containing 3 (of 21) other lac fusions do make β-gal. The pattern of dependences of β-gal expression on A, B, C and D is consistent with a branched linear regulatory pathway (1).

A-dependent and C-dependent lac fusions are being used to purify the molecules responsible for an early (A) and a late (C) developmental signal. In crude extracts of developing wild-type cells, both activities are heat labile and nondialyzable (2).

References: (1) Kroos and Kaiser (1987) Genes and Devel. 1, 840-854.
(2) Kuspa et al. (1986) Devel. Biol. 117, 267.

L 012 GENETIC REGULATION OF GERM/SOMA DIFFERENTIATION IN *VOLVOX*.
David L. Kirk, Department of Biology, Washington University, St. Louis, MO 63130

Volvox carteri is a simple, multicellular green alga exhibiting a complete division of labor between mortal somatic cells and immortal germ cells. In the asexual reproductive cycle, each *Volvox* gonidium (asexual reproductive cell) acts as a stem cell, cleaving in a stereotyped manner to generate a new individual containing ~2000 somatic cells and ~16 gonidia that are set apart by asymmetric cleavage divisions. Based on phenotypic analysis of mutants, we have developed the working hypothesis that three categories of control genes play a central role in establishing this developmental dichotomy between germ and soma: First the *gls* (gonidialess) locus acts to generate large and small sister cells by asymmetric division. Then the *lag* (late gonidia) loci act in the large cells to shut off cleavage prematurely and shunt these cells into the reproductive pathway. Finally, the *regA* (regenerator-A) locus functions in the small cells to prevent all aspects of reproductive development.

In *regA* mutants, somatic cells first appear to differentiate normally; but then, instead of undergoing preprogrammed senescence and death as wild-type somatic cells do, these cells redifferentiate as fully functional germ cells. For brief periods just before the beginning and just after the end of embryogenesis, the *regA* locus exhibits extreme, locus-specific hypermutability in response to agents that induce error-prone recombination--but not in response to conventional point mutagens. We have interpreted this stage-specific, locus-specific hypermutability as an indication that *regA* may be turned "on" in somatic cells and "off" in gonidia by cyclic DNA rearrangements. To test this hypothesis, we are attempting to clone *regA* and compare its sequence in somatic and germ cells. Because no molecular probes for the locus were available, we have identified DNA polymorphisms tightly linked to *regA*, have cloned one of these polymorphic elements, and have initiated a chromosome walk from that element to the *regA* locus. Chromosomal rearrangments that inactivate the *regA* function are being used to define the functional boundaries of the locus, and a transformation system is being developed to test putative *regA* clones for their capacity to rescue mutants. Once the locus has been cloned and the cyclic-rearrangement hypothesis has been tested, our goal is to determine how the *regA* product acts to prevent reproductive development in the somatic cells.

L 013 GENETIC REGULATION OF DEVELOPMENT IN ASPERGILLUS NIDULANS. William E. Timberlake, Iain L. Johnstone, Eileen B. O'Hara, Margaret T. Boylan, Rodolfo Aramayo, Peter M. Mirabito, Thomas H. Adams and Margaret A. Marshall, Departments of Genetics and Plant Pathology, University of Georgia, Athens, GA 30602.

The molecular genetic systems of some filamentous fungi, for example Neurospora crassa and Aspergillus nidulans, are nearly as sophisticated as that of their unicellular relative Saccharomyces cerevisiae (yeast). Unlike yeast, however, the filamentous fungi form complex reproductive structures, the sexual fruiting bodies (such as cleistothecia and perithecia) and asexual sporophores (such as conidiophores), and are therefore of considerable value for the study of mechanisms controlling elaboration of multicellular structures in eukaryotes. In Aspergillus nidulans, approximately 100 genes have been identified that when mutated result in the formation of abnormal asexual reproductive structures (conidiophores; Clutterbuck review, 1977). At least 900 additional genes have been identified that are not transcribed during vegetative growth and are activated during conidiophore development (Timberlake, 1980). We cloned and have begun to analyze the functions of four genes in the former class, designated brlA, abaA, wetA and stuA. These genes appear to have important roles in regulating conidiophore development, and our understanding of their regulation and functions will be described. We also cloned many genes in the second class and have begun to analyze the mechanisms regulating their expression and, by making directed mutations, have attempted to begin to understand their physiological functions. A number of genes that are activated during development appear to be non-essential for development, at least under laboratory conditions. Others are obviously essential for normal development. The implications of these observations will be addressed.

Clutterbuck, A.J. 1977. The genetics of conidiation in Aspergillus nidulans, In Genetics and Physiology of Aspergillus, J.E. Smith and J.A. Pateman, eds., Academic Press: New York, pp. 305-317.

Timberlake, W.E. 1980. Developmental gene regulation in Aspergillus nidulans, Dev. Biol. 78, 497-510.

Induction and Analysis of Developmental Mutants

L 014 ISOLATION AND CHARACTERIZATION OF TRYPTOPHAN AUXOTROPHS OF ARABIDOPSIS Robert L. Last, Mary Berlyn and Gerald R. Fink, Whitehead Institute, Cambridge MA 02142.

Although the aromatic amino acid biosynthetic pathway is one of the most thoroughly studied gene-enzyme systems in microorganisms, no *bona fide* tryptophan mutant that manifests an amino acid requirement at the whole plant level, and behaves as a Mendelian genetic trait, is known. This pathway is of particular importance in plants because of its role in the biosynthesis of auxin, flavanoids, lignin precursor and other phenolic compounds. We have begun a molecular genetic approach to dissecting the regulation of this pathway in plants by isolating tryptophan-requiring mutants of *Arabidopsis thaliana* from mutagenized plants. As a complementary approach, we have cloned an Arabidopsis gene with striking amino acid homology to known tryptophan synthetase B genes of microorganisms.

Tryptophan auxotrophs have been identified by selection of M2 plants resistant to 5-methylanthranilic acid. A subset of these mutants unable to convert 5-methylanthranilic acid to the toxic analogue 5-methyltryptophan are also unable to convert anthranilic acid to tryptophan, and therefore require tryptophan for growth. Three independently isolated tryptophan auxotrophs have been identified. Each auxotrophic trait segregates as a single recessive Mendelian mutation in crosses to wild-type. The mutants germinate and the resulting plants show slight leaf and root growth when grown on sterile medium lacking tryptophan. After 1-2 weeks the small seedlings stop growing, presumably because seed tryptophan reserves are exhausted. Transfer of these starved plants to tryptophan-supplemented medium allows the mutants to recover and grow to maturity.

The tryptophan biosynthetic precursor anthranilate and its derivatives emit blue fluorescence when excited with UV light. One tryptophan auxotroph accumulates high levels of anthranilate-derived compounds and is highly fluorescent under UV light. Consistent with the accumulation of these intermediates in tryptophan biosynthesis, crude enzyme extracts from this mutant are unable to convert anthranilate to the immediate products in tryptophan biosynthesis. Taken together, this evidence shows that this mutant is blocked early in the conversion of anthranilate to tryptophan.

Plants auxotrophic for tryptophan show a variety of morphological abnormalities: slow growth, reduced apical dominance and abnormal leaf coloration. The most severely affected mutant also has abnormal flower morphology, dramatically reduced fertility and abnormal leaf size and shape. Some of these morpological abnormalities are reminiscent of the 2,4-D resistant mutants of Arabidopsis reported by Estelle and Somerville. The tryptophan biosynthetic defect presumably affects biosynthesis, metabolism or localization of a derivative of tryptophan such as auxin. The tryptophan mutants coupled with cloned tryptophan genes should permit direct analysis of the interaction of the regulation of tryptophan and auxin biosynthesis.

L 015 DIFFERENTIATION IN THE TOBACCO FLOWER PROBED WITH MONOCLONAL ANTIBODIES

Russell L. Malmberg, Phillip T. Evans, Brian L. Holaway
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We have isolated a series of monoclonal antibodies that react to antigens in flowers of *Nicotiana tabacum* displaying specificity or preferentiality in their cell and tissue distributions. We immunized mice with extracts from tobacco flowers and then screened the hybridomas by ELISAs against extracts from leaf, sepal, petal, stamen and carpel. Twenty five were chosen from the total screened. The antigens detected by about half of the antibodies are periodate sensitive, implying that the epitopes are carbohydrate. Characterization of the antigens by western blots, microsomal extracts, and competition assays allows a further classification of the antibody set on the basis of antigen type, even where the initial tissue preferences seemed very similar. Analysis of the spatial pattern of antigen distribution within tobacco flowers by immunolocalization reveals some antibodies recognize epitopes that are limited to very specific cells and tissues.

Previously we used polyamine mutant selections to obtain tobacco flowers that have a variety of developmental switch morphologies. The immunolocalization technique permitted us to analyze a mutant with stigmoid-anthers in more detail: an antibody recognizing a carpel transmitting-tract antigen also reacted to cells in stigmoid-anthers, as did an anther specific antibody.

Our results with the antibody set thus imply that biochemical differentiation within the tobacco flower includes cell and tissue specific glyco- moeities, and also that homologies, at the biochemical level, exist between normal floral tissues and the abnormal tissues in a phenotype with a developmental switch.

L 016 MUTATIONAL ANALYSIS OF THE DEVELOPMENT OF FLOWER COLOUR, Cathie Martin, Jeremy Bartlett, Steven Mackay, Clare Lister and Rosemary Carpenter, Genetics

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The production of colour in flowers is a highly regulated process of which one of the most interesting components is the determinaion of spatial pattern. In Antirrhinum, the existance of a large number of mutations that modify the distribution of pigment production illustrates the importance of spatial aspects of gene regulation.

Detailed molecular and genetic analysis have divided these pattern mutants into two types. Some affect spatial patterning through mutation of the genes involved directly in biosynthesis of anthocyanins. Two biosynthetic genes in Antirrhinum contain transposable elements in their promoter regions. The activity of these transposons has produced many mutations in vivo, and the effects of some of these on the expression of the adjacent genes will be discussed. The second group of mutations modify the spatial pattern of pigment production in trans. The genes involved appear to regulate the expression of a number of the biosynthetic genes in particular areas of the flower. One approach to isolate these genes is to use differential cDNA hybridisation. This method will also clone out any other genes regulated by the primary gene and so provides a means of isolating regulatory genes and identifying the biochemical processes by which they work.

The complex interaction between cis-acting and trans-acting regulatory components in the determination of pattern in flower pigmentation provides a readily accessible model system on which to base approaches to more complex systems of pattern determination.

L 017 MOLECULAR ANALYSIS OF EARLY EVENTS IN MAIZE LEAF DEVELOPMENT WITH PATTERN MUTANTS, Timothy Nelson, Jane Langdale, Beverly Rothermel, Mary Metzler, Leyla Bayraktaroglu, and Ellen Miller, Biology Department, Yale University, New Haven, CT 06511.

The carbon fixation pathway found in maize and other C4 plants relies on the interaction of two neighboring cell types in the leaf. Carbon dioxide is initially fixed in mesophyll (M) cells into 4-C acids which pass to bundle sheath (BS) cells. In BS cells, fixed CO_2 is released by decarboxylation and refixed by RuBPCase. Each vein is surrounded by an inner ring of BS cells and an outer ring of M cells. This scheme protects BS cell-localized RuBPCase from O_2 and increases available CO_2 concentrations.

We have studied the ontogeny of this cellular interaction by exploiting several developmental and clonal mutants of maize. We have used RuBPCase, PEPCase, PPdK, NADP-MDH, and NADP-malic enzyme antibodies and cDNAs as cell specific markers for BS or M cells. By immunolocalization and *in situ* hybridization experiments, we have followed the appearance and activation of the cell neighbors in the leaf and other photosynthetic organs. Our analyses of the clonal mutants *sr1*, *sr2*, *sr3*, *j2*, and *v5* confirm that M and BS cells arise from separate cell lineages and are not sister cells. BS cells are clonally related to the veins they surround. The activity of cells at mutant/normal clonal boundaries indicate that neighboring BS and M cells can be activated independently. Patterns of M and BS differentiation in leaves of the striped mutant *ar* and in normal leaves suggest that vascular development is linked to the development of local BS and M cells, since cells of both types are activated in a radial pattern around maturing veins. Full differentiation depends on the presence of light, since some BS and M cell type markers are expressed in both cell types in darkness.

Rescue of Developmentally Regulated Plant Genes

L 018 **ISOLATION OF DEVELOPMENTALLY IMPORTANT GENES FROM ARABIDOPSIS**, Howard M. Goodman, Bart den Boer, Christian Fritze, Brian Hauge, Gabor Lazar, Bill Loos, Hong-Gil Nam, Kyung-Hee Paek and David Yett, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

We are interested in elucidating mechanisms of gene regulatory control during early development and embryogenesis in higher plants. The short life cycle, availability of mapping strains, and the small genome size of Arabidopsis thaliana, offer the potential for the identification and cloning of those genes which, based on their mutant phenotype, appear likely to be involved in developmental regulation. As a first major step toward cloning such genes we are constructing complete physical and RFLP maps of the Arabidopsis thaliana genome. The physical mapping of individual clones is being carried out using the methodology described by Coulson, A., et. al. *Proc. Natl. Acad. Sci. USA*, 83, 7821 (1986). Briefly, DNA is isolated from randomly selected clones, digested with HindIII, the ends labeled using reverse transcriptase, and then digested with Sau3A. The resultant fragments are of suitable size for separation on denaturing polyacrylamide gels. The position of each labeled band is entered into a computer and stored in a growing data base. Overlapping clones ("contigs") are identified using a computer matching program. To be useful this overlapping cosmid set ("the physical map") must be correlated with the genetic map of Arabidopsis. Unfortunately, very few cDNA and/or genomic probes are available for genes of known genetic map position. To circumvent this problem a RFLP map is being constructed using probes from the same library employed for the physical map. At present 140 RFLP probes have been identified and are being mapped against genetic markers residing on all five chromosomes. When completed, the combined RFLP/physical map will allow ready access to any region of the genome of known genetic map location. To determine when the desired gene has been cloned, it will be necessary to transform mutant plants and assay for the gene by functional complementation. Since only a limited number of clones in the vicinity of the gene of interest need be examined, this procedure does not require a high efficiency transformation system. Obviously all the molecular tools currently available can be used to investigate the structure and function of the gene product once the appropriate clone has been identified.

L 019 STUDIES OF TRANSPOSABLE ELEMENT AC IN ZEA MAYS L., Peter Starlinger, George Coupland, Christiane Plum, Reinhard Kunze and Heidi Fusswinkel, Institut fur Genetik, Universitat zu Koln, Weyertal 121, 5000 Koln 41, FRG.
Transposable element Ac differs from other transposable elements by the negative dosage effect: With an increasing dosage of Ac elements in the genome, transposition occurs later and less frequently during endosperm development.

The understanding of this regulation would be interesting and also helpful if Ac is to be used routinely for tagging of unknown genes.

We have begun a study of transcription and translation of Ac (Kunze et al., 1987), have overproduced the Ac protein with the help of baculovirus expression vectors in insect cells (in a collaboration with C. Hauser and W. Doerfler in Koln), and are also studyiing the effect of mutations produced in vitro, when Ac is introduced into tobacco cells, using a phenotypic test worked out in collaboration with J. Schell's laboratory (Baker et al., 1987). Certain mutations in Ac abolish transposition. Some internal deletions act in cis: the elements containing such mutations cannot be transposed, even if an active Ac element is present in the same cell (Starlinger et al., 1988). Constructs which carry two left ends of Ac instead of the left and the right end are also not transposable though they carry the terminal inverted repeats.

Baker, B., G. Coupland, N. Fedoroff, P. Starlinger and J. Schell (1987), EMBO J. 6:1547-1554.

Kunze, R., U. Stochaj, J. Laufs and P. Starlinger (1987), EMBO J. 6:1555-1563.

Starlinger, P., B. Baker, G. Coupland, R. Kunze, J. Laufs, J. Schell, U. Stochaj (1988), in: Plant Transposable Elements, ed. O.E. Nelson, in press.

Development of the Gametophyte

L 020 MOLECULAR ASPECTS OF SELF-INCOMPATIBILITY, M.A. Anderson, E.C. Cornish, S-L. Mau, A. Bacic 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 pollen tubes after they grow some distance down the style. Rejection is controlled by the product of the S-gene, which has multiple alleles, S_1, S_2, S_3 and S_4. We are investigating several aspects of self-incompatibility:

(1) Nature of the S-gene: We have isolated cDNA clones encoding the putative S_2- and S_3-allele products. Overall, the sequences are approximately 70% homologous at the nucleic acid level and 80% homologous at the amino acid level. The sequences are punctuated with three highly variable regions which encode hydrophilic amino acids which are predicted to be on the surface of the protein. Southern analysis of N. alata genomic DNA using the S_2 and S_3 cDNAs as probes indicates:

. the gene is restricted to a single locus;

. the gene is present in low copy number (probably single copy);

. characteristic restriction fragment length polymorphisms for the different S-alleles.

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

(2) 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.

(3) Structure of pollen tube walls: Pollen tube walls of N. alata have three major components: a (1→3)-linked glucan, a (1→4)-linked glucan and a (1→5)-linked arabinan. The components are organized into two layers with the inner zone containing the (1→3)-β-glucan demonstrated by immunocytochemistry using an antibody to laminaribiose (Glcp1→3βGlcp) and the outer zone containing arabinan, demonstrated using an anti-arabinosyl antibody.

L 021 **ANALYSIS OF FLORAL SPECIFIC GENES**, Charles S. Gasser, Kim A. Budelier-Sachs, Alan G. Smith, Maud A.W. Hinchee, Dilip M. Shah, and Robert T. Fraley, Plant Molecular Biology, Monsanto Company, 700 Chesterfield Village Pkwy., St. Louis, MO 63198.

We are investigating genes whose expression is specifically induced during the development of floral organs. A differential screening procedure has been used to isolate cDNA clones which show preferential expression in either pistils or anthers. *In situ* RNA hybridization to sectioned flowers shows that expression of the genes corresponding to these cDNAs is confined to specific tissue layers within the pistils or anthers. These experiments, and Northern transfer analysis have additionally shown that the genes are only expressed at specific times in the development of the reproductive organs. Genomic clones corresponding to several of the cDNAs have been isolated from a library of tomato genomic DNA. We have identified and sequenced the putative promoter regions from these clones. We are in the process of characterizing these regions by fusing them to the *E. coli* β-glucuronidase gene (GUS) and transferring the resulting chimeric genes into plants. In separate experiments using the CaMV 35S, mannopine synthase and rubisco small subunit promoters we show that the indigogenic assay for this enzyme is a sensitive and accurate method of localizing gene expression in transgenic plants.

L 022 THE URF13-T GENE CONFERS FUNGAL TOXIN SENSITIVITY TO MAIZE MITOCHONDRIA AND E. COLI, Carl J. Braun and C. S. Levings III, Department of Genetics, North Carolina State University, Raleigh NC 27695-7614.

The mitochondrial genome of maize carrying the Texas male-sterile cytoplasm (cms-T) contains a gene, designated urf13-T, which is associated with cytoplasmic male sterility (cms) and disease susceptibility. cms-T maize is uniquely susceptible to a fungal pathogen, Bipolaris maydis race T (Southern corn leaf blight). The fungus produces a pathotoxin, T-toxin, that specifically affects mitochondria of cms-T maize. The toxin inhibits mitochondrial respiration, uncouples oxidative phosphorylation and causes mitochondrial swelling and massive ion leakage. The insecticide methomyl, although structurally unrelated to toxin, causes similar effects on cms-T mitochondria. The urf13-T gene encodes a 13 kDa polypeptide that is located in the mitochondrial membranes. We have placed the urf13-T gene into inducible bacterial expression vectors and transformed the constructs into E. coli. After induction, the transformed cells express the 13 kDa protein, which localized in the bacterial membranes. E. coli cells expressing the 13 kDa protein are sensitive to T-toxin and methomyl. These reagents inhibit respiration and cause massive ion leakage and rapid spheroplast swelling. E. coli cells not expressing the 13 kDa are insensitive to both toxin and methomyl. The 13 kDa protein is able to confer toxin sensitivity to E. coli similar to that observed in cms-T mitochondria. Site-directed mutations in the urf13-T gene have defined regions necessary for toxin sensitivity. These results indicate that the 13 kDa polypeptide encoded by the urf13-T gene is responsible for the specific susceptibility of cms-T maize to B. maydis, race T.

L 023 CHARACTERIZATION OF GENES THAT ARE EXPRESSED IN POLLEN, Joseph P. Mascarenhas, Department of Biological Sciences, State University of New York at Albany, Albany, N.Y. 12222, USA

Pollen grains of Zea mays and Tradescantia paludosa at the time of anther dehiscence contain a store of presynthesized messenger RNAs (mRNAs) which appear to have functions during germination and early pollen tube growth. These mRNAs are the products of about 20,000 different genes. Using poly(A)RNA isolated from mature pollen of maize and Tradescantia, cDNA libraries have been constructed. The libraries contain several thousand clones most of which are expressed in both pollen and in one or more vegetative tissues. A small fraction of the clones are pollen specific and are not expressed in sporophytic tissues. Southern hybridizations show that the pollen specific sequences, as well as the shared sequences are present in one or a very few copies in the genome. The use of several of the clones as probes in Northern analyses has shown that the corresponding mRNAs are first synthesized after microspore mitosis and the concentrations of the mRNAs in the pollen grain increase up to maturity. This suggests a function for these mRNAs during the terminal portion of pollen maturation and during germination. One of the pollen specific cDNA clones pZmc13, which is almost full length has been sequenced. The 5'-flanking regions of the corresponding genomic clone are being sequenced in order to make a preliminary characterization of the pollen promoter region. In addition, one other pollen specific clone and a clone which is expressed in both pollen and in vegetative tissues are being sequenced in order to compare the promoter regions of the different pollen expressed clones. (Supported by NSF Grant DCB-8501461).

L 024 THE STRUCTURE AND FUNCTION OF SELF-INCOMPATIBILITY SEQUENCES OF BRASSICA, June B. Nasrallah, Section of Plant Biology, Cornell University, Ithaca, NY 14853.

Self-incompatibility responses prevent self-fertilization in many species of flowering plants and are genetically controlled by alleles of the S locus. A number of the multiple S-homologous sequences derived from the S locus of Brassica and from the related self-fertilizing Arabidopsis have been cloned and analyzed. Only one of these copies is apparently expressed in Brassica stigmas and is intronless. The analysis of products derived from the expressed copy from different S alleles indicate strong homology but also reveal substantial structural differences. The basis of these differences and the implications for the evolution of the S locus will be discussed.

Gene Expression during Embryogenesis - I

L 025 EXPERIMENTAL MANIPULATION OF GENE EXPRESSION IN EMBRYOS OF BRASSICA NAPUS L., Martha L. Crouch, Keith S. Blundy, Margaret A. Blundy, Steve R. Scofield, Alice J. DeLisle, and Ruth R. Finkelstein, Department of Biology, Indiana University, Bloomington, IN 47405.

The most abundant proteins in embryos of Brassica napus (rapeseed) are two families of storage proteins. One family (cruciferin) consists of legumin-like polypeptides[1]. The other family (napin) is a group of small, basic polypeptides, related to the 2S albumins in castor bean and brazil nut[2], and is encoded by about 14 genes. Although the synthesis of both families is confined to embryogeny, napin gene expression precedes cruciferin by several days[3]. Regulation is at the transcriptional level during development. We have been studying the effects of hormonal and environmental treatments on storage protein gene expression in isolated embryos cultured at different times in development[4]. Results depend on embryo age at excision. Embryos at the start of storage protein synthesis maintain low levels of expression on a basal medium, even though they show signs of germination. Abscisic acid (ABA) will restore high levels of storage protein synthesis, as will culture on high osmotica. However, older embryos eventually turn off storage protein gene expression in culture, and it cannot be restored by ABA, although high osmotica will maintain synthesis for most of development. The relationship between regulation by ABA and osmotica is complex. The hypothesis that one or both of these cues are required during embryo development for maturation to occur is being tested.

In order to study regulation of individual genes in more detail, a cloned napin gene shown to be expressed[5] has been modified and transformed back into rapeseed plants. These transgenic plants are being used to study sequences required for the observed pattern of expression.

[1]Simon et al. 1985, Plant Mol. Biol. 5:191-201; [2]Crouch et al. 1983, J. Mol. Appl. Genet. 2:273-283; [3]Finkelstein et al. 1985, Plant Physiol. 78:630-636; [4] Finkelstein et al. 1987, In: Molecular Biology of Plant Growth Control (ed. J.E. Fox, M. Jacobs), A.R. Liss, NY, p. 73-84; [5]Scofield and Crouch 1987, J. Biol. Chem. 262:12202-12208.

L 026 LETHAL MUTANTS AND THE GENETIC CONTROL OF EMBRYO DEVELOPMENT IN ARABIDOPSIS THALIANA. David W. Meinke, Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

Developmental mutants have been used in a variety of animal systems to study the genetic control of morphogenesis and differentiation. The experimental approach has generally been to: (1) establish an appropriate model system; (2) isolate mutants with abnormal patterns of development; (3) analyze mutants with interesting phenotypes; (4) induce additional mutations to saturate a specific locus or mutant phenotype; (5) characterize a series of hypomorphic and hypermorphic alleles; (6) clone and sequence mutant alleles to determine the molecular basis of abnormal development; and (7) isolate extragenic suppressors to identify other genes associated with the same developmental pathway. The purpose of my research has been to study the genetic control of plant embryo development through the isolation and characterization of embryo-lethal mutants of Arabidopsis thaliana. Analysis of these mutants has included a determination of segregation ratios and patterns of abnormal development, complementation tests, gametophytic expression of mutant genes, extent of cellular differentiation, and response of mutant embryos in culture. We have recently identified an auxotrophic embryo-lethal mutant that can be rescued by the addition of biotin to arrested embryos cultured in vitro and to mutant plants grown in soil. This bio1 mutant of Arabidopsis should provide clues not only to the biosynthesis and transport of biotin in plants, but also to the function of biotin during plant growth and development. We are also mapping the chromosomal locations of our most interesting mutant genes in order to eventually clone these genes through chromosome walking. We have established an efficient method of regenerating plants from cultured cotyledons and have demonstrated that immature cotyledons can be transformed with Agrobacterium strains carrying resistance to hygromycin. Whether insertion of T-DNA represents an efficient method of tagging and identifying genes with essential functions during plant embryo development remains to be determined. We are currently isolating additional embryonic lethals from M-2 populations derived from mutagenized seeds in order to characterize in more detail the diversity of mutant phenotypes before identifying the specific loci that are most likely to play a direct role in the regulation of development.

L 027 DEVELOPMENTAL GENETICS OF PLANT EMBRYOGENESIS, Z. Renee Sung, Gerald Franz, P. Hatzopoulos, R. Chorneau, Z. Jin, K. Kalman, and K. Jeong, Departments of Genetics and Plant Pathology, Universith of California, Berkeley, CA 94720.
The study of embryology enables the examination of fundamental plant developmental processes--embryogenesis and organogenesis. We used somatic embryos grown from carrot culture to isolate immunological and molecular probes that can detect genes regulated during embryo development, the embryogenic genes (1,2). To study the temporal and spatial regulation of these genes, we are investigating the ontogenetic events and cellular factors that regulate the expression of these genes, and localizing their proteins on embryo sections by immunocytochemical microscopy. To study their role in embryo development, we are screening the nonembryogenic cell lines for the expression of these genes. We found a mutant cell line that produces a smaller protein and mRNA for one of the embryogenic genes, gene #8, than that produced by the embryogenic cell lines. To demonstrate that the altered gene is responsible for the nonembryogenic phenotype, we have cloned the wild type gene which will be introduced into the mutant to see if the transformed mutant can undergo embryogenesis. The progress on the characterization of the mechanism of temporal and spatial regulation as well as on the nonembryogenic mutant will be reported.

1. Choi J., L.-S Liu, C. Borkird, and Z. R. Sung. 1987. Cloning of developmentally regulated genes. PNAS. 84:1906-1910.
2. Smith J., M. Krauss, C. Borkird, and Z. R. Sung. A nuclear protein associated with cell divisions in plants. Planta. in press.

L 028 PATTERNS OF GENE EXPRESSION CONTROL IN PLANT DEVELOPMENT. Terry L. Thomas, Carl A. Adams, Raymond S. Vonder Haar, William S. Nelson, Dayton S. Wilde, Sacco De Vries, Kathryn E. Koprivnikar, Randy D. Allen, William S. Seffens, Silvia Bustos, Molly A. Bogue, John C. Thomas, Andrew N. Nunberg, Elizabeth A. Cohen and Craig L. Nessler. Biology Department, Texas A&M Universty, College Station, TX 77843.
Over the last few years, we have analyzed the structure and expression of several genes whose expression is subject to either ontogenic control or to control by factors such as physical stress or phytohormones. The genes we have examined in some detail include members of sunflower gene families that encode albumin and legumin-like seed proteins and extensin-like extracellular proteins. In the latter case, the extensin-like gene family includes seven members; at least three of which belong to apparently independent and exclusive regulatory networks. One network responds to ethylene; a second responds to the physical stress of wounding; a third network probably responds to more general developmental cues. A simpler pattern of expression is observed for sunflower seed protein genes; these genes are under very tight developmental control so that seed protein transcripts are only synthesized in immature seeds. The role of abscisic acid (ABA) on the expression of these genes has also been examined. We have also studied genes that are expressed during the process of somatic embryogenesis in carrot as an approach to obtain molecular information on early events of plant development. The best characterized representative of these genes is a cDNA sequence designated *Dc*3; the expression of this gene correlates strongly with the acquisition and maintenance of embryogenic potential in carrot tissue cultures. All of the genes we have examined belong to small gene families, and sequencing of representative family members of each gene class revealed typical RNA polymerase transcription units containing relatively few and relatively short intervening sequences. Gene transfer experiments are now in progress to either understand the role of these genes in plant development or to identify *cis* and *trans*-acting factors involved in controlling the expression of these genes.

Gene Expression during Embryogenesis - II

L 029 EXPRESSION OF LEGUME SEED PROTEIN GENES IN THE SEEDS OF TRANSGENIC TOBACCO, Maarten J. Chrispeels, Toni A. Voelker, Arnd J. Sturm, C. Daniel Riggs and Corinne Dorel, Department of Biology C-016, University of California, San Diego, La Jolla CA 92093

Embryogeny and seed formation are accompanied by the expression of a number of genes whose products accumulate in the protein storage vacuoles of the reserve tissues. We are interested in understanding how the expression of such genes is regulated and how the products are targeted to the storage vacuoles. Much of our work has centered on phytohemagglutinin (PHA), the lectin of the common bean. We cloned and sequenced two PHA genes (Pdlec1 and Pdlec2) from the PHA-deficient cultivar Pinto, and studied the expression of these genes in developing beans. By comparing the sequences of the 5' upstream regions with two highly expressed genes (dlec1 and dlec2) from a normal cultivar, we identified a 100 bp deletion in the 5' upstream region of Pdlec2 which may be responsible for its down-regulation in the bean. Transfer of dlec2 to tobacco showed that the spatial and temporal regulation of its expression were conserved in tobacco. The expression of Pdlec2 in transgenic tobacco was down-regulated to the same extent as in the bean. Promoter resection experiments and the construction of chimeric genes are now in progress to determine if the 100 bp deletion of Pdlec2 contains the putative enhancer sequence present in the other PHA genes.

In the bean, PHA is synthesized on the rough ER, and its transport to the storage vacuoles is mediated by the Golgi complex, where one of its two high mannose glycans is converted to a complex glycan. In tobacco seeds, PHA also accumulates in protein storage vacuoles, and carries one high mannose and one complex glycan. This indicates that the PHA has passed through the Golgi complex of the tobacco cells. PHA genes in which the glycosylation sites have been deleted by site-directed mutagenesis have been introduced into tobacco and the effect of this alteration on targeting and accumulation of PHA is presently under investigation.

To determine whether transport to the storage vacuoles is a regulated pathway or a default pathway, we made a chimeric gene having the 5' sequence and signal peptide of PHA linked to the coding sequence and 3' untranslated region of a cytosolic albumin from Pisum sativum. This construction is expressed in tobacco seeds and the subcellular location of the gene product is being determined.

Supported by grants from the National Science Foundation, the United States Department of Agriculture, and the Department of Energy.

L 030 STRUCTURE AND REGULATION OF CEREAL GRAIN STORAGE PROTEIN GENES, Richard B. Flavell, Vincent Colot, Laurian Robert, Andrew Goldsbrough and Mark Thomas, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2LQ, England.

During development of a wheat seed, the genes encoding gliadins and glutenins are activated and the proteins are deposited in protein bodies in the endosperm. The genes are encoded by multigene families and representatives of the multigene families have been cloned and sequenced. The structure of the genes includes a central region composed of variants of repeating units. This structure gives rise to considerable variation within and between families which will be discussed. The endosperm-specific expression of these genes is determined by a relatively short region upstream from the CAAT box. This was established by fusing the promoter and upstream regions of high and low molecular weight glutenin genes to the coding region of β-glucuronidase and introducing the chimaeric genes into tobacco by Agrobacterium. Activity of the chimaeric genes was detected only in the endosperm of the tobacco seeds. The sequence responsible for endosperm-specific expression was deduced from studying chimaeric genes containing different segments of the region upstream from the TATA and CAAT boxes.

Analysis of the upstream regions suggest that their evolution involved amplifications of short DNA sequences. The possible significance of this in gene regulation systems will be discussed.

L 031 **EXPRESSION OF PEA SEED GENES IN HETEROLOGOUS HOSTS,** Thomas J. Higgins, Edward J.. Newbigin, Hartmut E. Schroeder, Philip A. O'Brien, Donald Spencer, D.J. Llewellyn and Stuart Craig, CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, A.C.T. 2601, Australia.

We have isolated and characterized genes for the pea seed storage proteins, vicilin and pea albumin 1 (PA1). Vicilin is a major protein (50% of total pea seed protein) which is totally devoid of sulfur-containing amino acids. PA1, on the other hand, is a relatively minor component (5-10% of total seed protein) but is rich in the sulfur-containing amino acid, cysteine (11%) and accounts for about 60% of the protein sulfur in pea seeds. The two genes, in both modified and unmodified forms, have been transferred to two new hosts, namely, tobacco and alfalfa, with a view to altering the total protein amino acid composition, especially in seeds and leaves. When the unmodified genes were transferred to tobacco, their expression was detected in seeds but not in other organs of the plant. Within the seed, vicilin protein was found in cotyledonary protein bodies but not in endosperm cells. Sequences on the 5' flanking side of the genes were removed and replaced with 5' flanking regions of two other genes, the cauliflower mosaic virus (CaMV) 35S gene and the octopine synthase (OCS) gene from *Agrobacterium tumefaciens.* Expression levels were low when the OCS 5' region was placed upstream of the PA1 gene. However, high levels of mRNA for vicilin and PA1 were detected in stems and young leaves of both tobacco and alfalfa when the 5' region of the CaMV 35S gene was used as the promoter sequence. Lower levels were detected in other organs. Vicilin protein was detected in leaves, stems, roots, seeds and callus of tobacco and there was an approximate correlation between protein level and mRNA level in each organ or tissue type. The vicilin protein was processed to different extents and into polypeptides of different sizes in the various organs of tobacco. The vicilin processing products in leaf were also different between tobacco and alfalfa. Further processing of vicilin occurred as tobacco leaves aged. Current research is aimed at elucidating factors involved in protein stability and at targeting of protein to different subcellular compartments in the transgenic plants.

L 032 GENETIC AND DEVELOPMENTAL VARIATION IN ZEIN GENE EXPRESSION DURING MAIZE ENDOSPERM DEVELOPMENT, Brian A. Larkins, John C. Wallace, Robert J. Kodrzycki and Gary A. Thompson, Department of Botany and Plant Pathology, Purdue University, West Lafayette IN 47907.

The storage proteins of maize seed are a group of alcohol-soluble polypeptides called zeins. These protein are synthesized on rough endoplasmic reticulum (RER) membranes and aggregate within the RER to form protein bodies. Four types of protein body proteins (which we call alpha-, beta-, gamma-, and delta-zeins) can be distinguished. The alpha-zeins predominate in most genotypes and account for around 70% of the total; the beta- and gamma-zeins each account for 10% to 15% of the total, while the delta-zein may contribute around 5% of the total protein. These different types of zeins have distinct organizations within the protein body. The alpha-zeins make up the core of the protein body and the beta- and gamma-types form a cross-linked matrix on its surface. Although there do not appear to be significant differences in the developmental regulation of genes encoding these proteins, the zein composition of protein bodies is variable. This results, at least in part, from differences in gene expression among cells of the endosperm. Protein bodies in cells just below the aleurone contain a higher proportion of beta- and gamma-zeins and less alpha-zein, whereas protein bodies from cells deeper in the endosperm contain a higher proportion of alpha-zein and less beta- and gamma-zeins.

Genes encoding the alpha-zeins are present in a large multigene family of 75 to 100 members, while genes encoding the beta- and gamma-types are present in one or two copies. Although the expression of these genes is coordinately regulated during seed development, there is little homology in their 5' flanking regions. Furthermore, mutations that affect the expression of the alpha-types do not significantly alter the expression of the gamma-type. We have characterized a conserved sequence in the 5' flanking regions of genes encoding the alpha-zeins that is important for their transcriptional regulation. This sequence corresponds to a 200 bp region that precedes the mRNA cap site by 100 bp. This region enhances epxression of heterologous genes when electroporated into maize or carrot protoplasts; however, it does not efficiently direct tissue-specific expression in transgenic petunia plants. Results of these experiments suggest that there are differences between the sequences programming seed-specific expression in monocots and dicots.

Activation of Nuclear and Organelle Genes after Germination

L 033 EXPRESSION OF ABA-INDUCIBLE GENES IN WATER STRESSED CEREAL SEEDLINGS. Peter M. Chandler[1], M. Walker-Simmons[2], R.W. King[1], M. Crouch[3] and T.J. Close[1], [1]Division of Plant Industry, CSIRO, Canberra ACT 2601 Australia, [2]USDA-ARS Washington State University, Pullman WA 99164-6420, [3]Department of Biology, Indiana University, Bloomington IN 47405.

cDNA clones for mRNAs which are up-regulated by treatment with abscisic acid (ABA) were isolated from barley aleurone. These same mRNAs are down-regulated by gibberellic acid (GA), and so constitute a set of mRNAs in aleurone whose hormonal regulation is exactly the opposite to that of the extensively studied α-amylase mRNAs.

Expression of this set of mRNAs under natural conditions was observed in aleurone from (i) grain losing water in late development, and (ii) young seedlings exposed to non-lethal dehydration. In these situations aleurone ABA levels are either expected to be elevated (developing grain) or have been observed to be elevated (dehydrating seedlings), suggesting that endogenous ABA is regulating gene expression.

One family of mRNAs defined by the aleurone cDNA clone pHVA39 was expressed at high levels in shoots and roots of seedlings or older plants subjected to water deficiency but not in the watered controls. Evidence that expression of the A39 mRNAs is being regulated by endogenous ABA comes from three lines of investigation. Firstly, ABA application stimulates mRNA appearance in the absence of water stress. Secondly, there is an excellent correlation between mRNA levels and ABA levels in shoot, root and aleurone from either well-watered, dehydrated, or dehydrated then rehydrated seedlings. Furthermore the level of ABA in aleurone from dehydrated seedlings exceeds that which results in the appearance of A39 mRNAs in isolated aleurone treated with exogenous ABA. Finally, corn seedlings homozygous for mutations leading to vivipary, which fail to accumulate ABA when exposed to water stress, also fail to elevate levels of mRNA homologous to A39 following water stress, but do respond to applied ABA.

Although the functions of the corresponding proteins regulated by ABA are presently unknown, this is an area of great interest as indirect evidence suggests that the presence of these proteins is correlated with survival of water stress.

L 034 REGULATION OF GENE EXPRESSION DURING SEED GERMINATION AND POSTGERMINATIVE DEVELOPMENT, John J. Harada, Catherine S. Baden, Lucio Comai, and Robert A. Dietrich, Department of Botany, University of California, Davis, CA 95616.

Germination is a major transition period in the sporophytic life cycle during which quiescent mature embryos undergo a series of differentiation events resulting in the formation of viable young seedlings. We are studying the regulation of genes which are expressed primarily during seed germination and early seedling growth in Brassica napus to gain insight into the developmental control of germination-related processes. mRNAs which become abundant in seedling within one day after the start of imbibition and are present at low or undetectable levels in immature embryos, dry seeds, and leaves have been identified. We have shown that these mRNAs are unequally distributed in seedlings. One set of mRNAs is prevalent in seedling axes while a second group is abundant in cotyledons. Cotyledon-abundant mRNAs which include those encoding the glyoxylate cycle enzymes isocitrate lyase and malate synthase are also detected in distinct regions of the axis. To determine when these genes are activated, cloned mRNA accumulation was studied during embryogeny and germination. We found that although these mRNAs are most prevalent in seedlings, they also are detected in embryos; each spatially-regulated mRNA set accumulates at distinct embryonic stages. Our findings suggest that the spatial and temporal regulation of genes encoding postgermination-abundant mRNAs reflects similarities and differences in seedling axis and cotyledon physiology and that these genes are activated during embryogeny. Furthermore, accumulation of cotyledon-abundant mRNAs is remarkably similar, suggesting the possibility that this gene set is coordinately regulated. Current studies are directed towards understanding mechanisms involved in regulating postgermination-abundant genes in embryos and seedlings. (Supported by NSF Grant No. DCB-85 18182).

L 035 EXPRESSION AND REGULATION OF LIGHT-HARVESTING CHLOROPHYLL A/B-PROTEINS AND THE SMALL SUBUNITS OF RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE, **E.M. Tobin, J. Buzby, G.A. Karlin-Neumann, D. Kehoe, M. Naderi, P. Okubara, J. Silverthorne, L. Sun, and T. Yamada, Biology Department, University of California, Los Angeles, CA 90024**

We have characterized the expression of members of two gene families in Lemna gibba and Arabidopsis thaliana. The first family encodes the small subunit (SSU) of ribulose 1,5-bisphosphate carboxylase/oxygenase. In Lemna, using six different gene specific probes, we have examined the expression of these genes, all of which can be responsive to phytochrome action, albeit to differing extents. Two of these members also exhibit a large degree of organ specificity, and comparison of "run-on" transcription with mRNA levels suggests that this regulation occurs at a post-transcriptional level. Post-transcriptional regulation also has been found for another Lemna gene whose RNA is more abundant in dark-treated than in constant white light grown Lemna [1]. A cDNA clone corresponding to this RNA has been sequenced, but the protein it encodes has not been identified. The promoters of two of the Lemna SSU genes (5A and 5B) have been sequenced and fused to a reporter gene; the fusions have been used to transform tobacco in order to see if the differential expression is maintained in another species. Specific regions of these promoters can bind nuclear proteins, but thus far no differences have been seen in binding to nuclear extracts from light-grown or dark-treated plants. In Arabidopsis, we have studied the expression of three linked genes which have been sequenced and found to encode identical mature forms of a light-harvesting chlorophyll a/b-protein (LHCP) [2]. Phytochrome action can result in a large and rapid increase in the LHCP RNA in six-day old etiolated seedlings. The three characterized genes are very similar at the nucleotide level, but one (AB140) diverges from the other two (AB165 and AB180) sufficiently to enable the transcripts to be distinguished by S1 nuclease protection experiments. The two kinds of mRNAs are present in nearly equal amounts in mature white light-grown plants. However, in etiolated seedlings 1 min red light causes a much greater increase in the level of the AB140 RNA than in the combined levels of the AB165 and AB180 RNAs. Additional evidence suggests that that the AB165/180 genes might be exhibiting organ-specific regulation. Fusions of a major portion of these genes and their promoters [3] can also be demonstrated to respond to phytochrome action in transformed tobacco seedlings.

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[2] Leutwiler, LS, Meyerowitz, EM, and Tobin, EM (1986) Nucl. Acids Res. 14: 4051-4064.
[3] An, G (1987) Mol. Gen. Genet. 207: 210-216.

Gene Expression in the Mature Sporophyte

L 036 GENE REGULATION DURING TUBERISATION IN SOLANUM TUBEROSUM, Michael Bevan, Richard Jefferson, Andrew Goldsbrough and Elaine Atkinson, IPSR Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

We have used fusions of the gene encoding patatin, a major tuber protein, with the reporter gene β-glucuronidase to study the regulation of transcription of this gene during tuber induction. Deletion analysis of the promoter revealed domains involved in tuber specificity, general enhancement of transcription, and repression of patatin expression in the inappropriate organ and in uninduced tissues. The general enhancer of transcription was found to operate in tobacco when fused to a truncated viral promoter. Further experiments involving the "repressor" or silencer region, the regulation of patatin by daylength, and a histochemical analysis of patatin transcription will be presented.

L 037 SPATIALLY REGULATED PATTERNS OF GENE EXPRESSION IN THE TOBACCO PETAL, Gary N. Drews and Robert B. Goldberg, Department of Biology, University of California, Los Angeles, CA 90024. We have chosen the tobacco petal as a system to study spatial and temporal gene regulation because a developmentally regulated pigmentation pattern is established during the ontogeny of this organ resulting in the accumulation of anthocyanin pigments in a restricted group of cells. This pigmentation pattern defines two regions that we refer to as the pink region and the white region. We constructed a cDNA library of mRNAs contained within the pink region and identified four cDNA clones that represent mRNAs present at a higher level in the pink region than the white region. These mRNAs are also present at a higher level in the petal than any other organ. In addition, we isolated a tobacco genomic clone containing sequences encoding chalcone synthase (CHS), an enzyme involved in anthocyanin production. As with the other mRNAs, CHS is present at a higher level in the pink region than the white region. However, CHS mRNA is present at high levels in both petal and anther but at lower levels in all other organs. Using *in situ* hybridization, we observed that these five mRNAs display two distinct cell accumulation patterns within the pink region. The first pattern is epidermis-specific and the second is mesophyll-specific. Thus, the spatial expression of the genes encoding these mRNAs is regulated with respect to organ system, region, and cell type. We demonstrated that these mRNAs are also temporally regulated by analyzing their accumulation programs during petal development. We utilized an *in vitro* transcription assay to determine the level at which these genes is regulated. We found that both the organ-specific and region-specific expression patterns are regulated to a first approximation at the transcriptional level.

L 038 GENES INVOLVED IN FLORAL INITIATION. D. R. Meeks-Wagner (1), E. S. Dennis (2), and W. J. Peacock (2). (1) Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, and (2) Division of Plant Industry, CSIRO, G.P.O. Box 1600, Canberra, ACT, 2601 Australia.

We have begun to identify and characterize gene expression involved in the initiation of flowering in higher plants with the goal of understanding of the mechanisms of gene regulation and function during plant development. Our general approach for isolating genes involved in floral initiation is to use cDNA cloning and differential sceening between select vegetative and floral mRNA populations; this approach is being pursued both with several plant systems. At present the majority of our work is focused on a molecular analysis of floral initiation in tobacco facilitated by the Thin Cell Layer (TCL) tissue culture system (1). Thin Cell Layers are small tissue explants which can be induced to form vegetative or floral buds without intermediate callus formation. Organogenesis occurs over a 20 day period with three major cytological "landmarks" observable: Day 7: maximal rate of cell division; Day 11-13: meristems formed; Day 20: differentiated organs visable. Genes expressed at these stages of organogenesis are being isolated and characterized for their pattern of transcription both in TCL explants and in plants grown from seed. This analysis has been started by isolating cDNA clones of genes expressed in Day 7 floral TCL explants and not expressed in Day 7 vegetative explants. The expression of several of these genes is being compared with information on the mechanism of floral determination in tobacco, and a molecular analysis of these genes is being undertaken to investigate their function in plant development.

1. Tran Thanh Van, M. (1973) Planta 115: 87-92.

L 039 THE ROLE OF WALL PROTEINS IN THE STRUCTURE AND FUNCTION OF PLANT CELL WALLS, J.E. Varner, Washington University, St. Louis, MO 63130.
The plant cell has a complex cell wall with characteristics unique for each cell type at each developmental stage. Of the wall components--cellulose, hemicelluloses, pectins, lignins, enzymes, proteins, suberins, minerals, metabolites and water--only in the case of cellulose is it possible to claim that we know with precision much about disposition and function in the wall. Our most complete information comes from studies of the walls of Acer pseudoplatanus--cells that do not have an easily definable developmental status. This presentation will focus on the structural proteins of the wall. These include the extensins, the glycine-rich proteins, the proline-rich proteins and the threonine-rich proteins. It will emphasize the importance of knowing about the developmental expression of the genes for these proteins and the precise sites in the wall of accumulation of the proteins. It will examine postsecretion--in muro-modification of these structural proteins, and the most likely interactions of these proteins with the other wall components. It will consider possible gel properties of the wall. And finally it will emphasize the advantages of considering the wall as an organelle whose duties change throughout the lifetime of the cell.[1]

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[1]Cassab, Gladys I. and Varner, J.E. Cell Wall Proteins. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1988. 39:321-53.

Gene Expression during Fruit Development

L 040 REGULATION OF GENE EXPRESSION BY ETHYLENE DURING TOMATO FRUIT RIPENING, Jill Deikman, Sabine P. Cordes, James E. Lincoln and Robert L. Fischer, Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720.
The plant hormone ethylene plays an important role in the regulation of fruit ripening. For many fruits (including tomato) the onset of ripening coincides with a burst of ethylene biosynthesis, ripening can be induced to occur prematurely by exposing unripe fruit to exogenous ethylene, and inhibitors of ethylene action or biosynthesis greatly retards ripening. The regulation of gene expression by the plant hormone ethylene has been investigated by cloning mRNAs that accumulate both during fruit ripening and in unripe fruit exposed to exogenous ethylene. Genes that encode two of these mRNAs have been studied in detail. Nuclear run-on transcription experiments indicate that their relative transcription rates increase during fruit ripening and in response to exogenous ethylene. We are currently beginning to analyze the DNA and protein elements that might regulate their transcription.

L 041 PHYSIOLOGICAL AND GENETIC FACTORS GOVERNING THE EXPRESSION OF THE POLYGALACTURONASE GENE IN RIPENING TOMATOES, Donald Grierson, Christopher Smith, Colin Bird*, John Ray*, Wolfgang Schuch*, Peter Morris, Julie Knapp, Steven Picton, Philippe Moureau*, Graeme Hobson** and Kevin Davies, Department of Physiology and Environmental Science, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, U.K., *ICI Plant Biotechnology Group, PO Box 11, The Heath, Runcorn, Cheshire, U.K. and ** Institute for Horticultural Research, Littlehampton, BN17 6LP, U.K.

A number of mRNAs accumulate during tomato ripening that are believed to encode proteins that catalyze different aspects of the process. DNA and protein sequencing indicates that pTOM 6, a cDNA clone for one ripening-related mRNA, encodes the fruit softening enzyme polygalacturonase (PG). We have used this cDNA to probe the organization and expression of genomic sequences encoding PG. Only one PG gene has been found. A 7,500 bp sequence containing the complete coding region, which is identical to the cDNA sequence, and 5' and 3' flanking regions has been obtained. The PG mRNA is not present in significant amounts in leaves, roots, or green fruit, but accumulates during ripening. Inhibition of C_2H_4 perception with Ag^+ prevents the appearance of PG mRNA in green fruit. Addition of Ag^+ after ripening as stated causes a dramatic decline in PG mRNA. At 35°C, which inhibits ripening, PG mRNA is greatly reduced, and there is little production of the mRNA during prolonged exposure at this temperature. This and other results indicate the ripening programme is temperature sensitive. The rin ripening mutant contains a PG gene but does not accumulate PG and shows little softening. Hybridization experiments indicate that less than 1% of the normal PG mRNA levels are produced in the mutant, which also shows altered production of other ripening-related mRNAs. Experiments are in progress to analyze the function of putative cis-acting control elements of the PG gene in transgenic tomato plants.

L 042 **REGULATION OF NUCLEAR AND PLASTID GENE EXPRESSION DURING FRUIT DEVELOPMENT**
Wilhelm Gruissem, Thianda Manzara, Jon Narita, Birgit Piechulla and Leslie Wanner, Department of Botany, University of California, Berkeley, CA 94720.

Following pollination and fruit set, several physiological and structural changes occur during tomato fruit development and ripening, including cell division, enlargement, cell wall softening, and chloroplast differentiation. As a first step to understand the mechanisms that regulate these alterations, we have analyzed the expression of several nuclear and organelle genes during fruit formation, and correlated changes of transcript and protein levels to the physiological status of tomato fruits and the differentiation of chloroplasts into chromoplasts. The nuclear and plastid mRNAs for photosynthesis-specific stromal and thylakoid membrane proteins accumulate during the early phase of fruit development. At least for the ribulose-1,5-bisphosphate caboxylase small subunit gene family, however, only two of the five members are expressed in fruit pericarp tissue. The level of these and other photosynthetic mRNAs are controlled by a diurnal cycle, which correlates with the photosynthetic activity of the chloroplast during this developmental period. The mRNA levels for most photosynthetic proteins are undetectable at the onset of chromoplast differentiation during fruit ripening. The cytoplasmic and chloroplast form of hydroxymethylglutaryl coenzyme A reductase, which catalyze the first committed step in isoprenoid synthesis, are both highly active at very early fruit development stages, but activity is undetectable at the time of lycopene synthesis during fruit ripening. The levels of other mRNAs also fluctuate during fruit development and ripening. For example, highest levels of tubulin A and B are detected early in fruit development, but not in mature fruit. This correlates with cell division and enlargement in small fruit. During ripening, when high levels of reducing sugars are synthesized, accumulation of mRNAs for the fructose-1,6-bisphosphate aldolase can be detected. In contrast, the mRNA for the β-subunit of the mitochondrial ATPase appears to be constitutively expressed, since no significant changes are observed in its level during fruit development and ripening. We will present an overview of these results, and discuss our most recent work on control mechanisms that may be involved in the regulation of mRNA levels.

L 043 EXPRESSION OF SELECTED GENES DURING TOMATO FRUIT MATURATION AND RIPENING, William R. Hiatt, Raymond E. Sheehy, Catherine M. Houck, Julie R. Pear, Rik L. Rasmussen, Christine K. Shewmaker and Ann R. Pokalsky, Calgene, Inc., 1920 Fifth St., Davis, CA 95616.

Tomato cDNA and genomic clones for elongation factor-1α (EF-1α), 2A11, and polygalacturonase (PG) have been isolated and characterized. EF-1α is responsible for the insertion of aminoacyl-tRNAs into the A site of the ribosome during the elongation phase of protein synthesis. 2A11 was identified by differential hybridization as a gene expressed at extremely high levels specifically during fruit development. PG is thought to be a primary factor in fruit softening via pectin degradation. DNA/protein structure for each gene will be discussed. The patterns of expression of these genes during fruit development will be described by Northern analyses and in situ hybridization.

Gene Expression in Novel Developmental Situations

L 044 ISOLATION OF TELOMERIC DNA SEQUENCES FROM ARABIDOPSIS THALIANA

Eric J. Richards and Frederick M. Ausubel, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Telomeres are the structures that form the termini of linear eucaryotic chromosomes. Telomeres stabilize the ends of the chromosomal DNA molecules and facilitate the complete replication of the DNA strands at the extreme termini. Detailed study of telomere structure and function is limited to lower eucaryotic organisms from which telomeric DNA sequences have been isolated. Cloning of telomeres from higher eucaryotic organisms is hampered by the large size of the chromosomes and the attendant dilution of telomeric sequences relative to non-telomeric sequence in the genome.

We have developed a method for constructing genomic libraries highly enriched for telomeric sequences enabling the isolation of telomeres from higher eucaryotic organisms with large chromosomes. The method was used to clone telomeric DNA sequences from A. thaliana. Two independent genomic libraries were constructed by ligating a plasmid cloning vector onto the repaired ends of high molecular weight A. thaliana DNA, followed by subsequent restriction digestion and an additional ligation step to form circular recombinant molecules. The inserts in these libraries should be a mixture of telomeric inserts derived from true chromosomal ends and random, non-telomeric inserts isolated from broken ends created during chromosomal DNA isolation. In order to enrich for telomeric clones only those clones with inserts found in both libraries were saved thereby discarding most clones containing inserts derived from random sites in the chromosome arm. Telomeric clones were identified by screening the remaining clones for their ability to hybridize to exonuclease sensitive restriction fragments. Using this procedure an A. thaliana telomeric clone was isolated allowing study of telomere structure in a higher eucaryote.

The structure of A. thaliana telomeres is similar to that seen in lower eucaryotes; the cloned A. thaliana telomere is composed of tandemly repeated, C-rich, simple sequence blocks. In addition, the telomeres of A. thaliana are heterogeneous in size as are most lower eucaryotic telomeres. Genomic sequences homologous to the A. thaliana telomeric repeats are present in the genome of other higher plants and some animal species. In the case of corn and humans, these genomic sequences are located at the telomere.

L 045 REGULATION OF CHITINASE GENE EXPRESSION IN TRANSGENIC PLANTS, Richard Broglie and Karen Broglie, Agricultural Products Department, E. I. Du Pont de Nemours & Co. (Inc.), Wilmington, DE 19898.

The production of chitinase by higher plants is thought to be part of a natural defense mechanism against chitinous pathogens. In bean this enzyme is encoded by a small multigene family and its synthesis is strongly regulated at the level of gene transcription by a number of inducers including the phytohormone, ethylene. We have isolated three members of this multigene family and have determined the complete nucleotide sequence of one of these genes, designated λ CH5B. This gene is situated on a 4.7kb EcoRI-HindIII fragment and is encoded by a single, uninterrupted open reading frame. The open reading frame, which encodes a 26 amino acid signal peptide as well as the 301 amino acid mature protein, is flanked by approximately 2kb of DNA sequence upstream and 1.7kb of sequence downstream. As a starting point towards identifying cis- and trans- acting regulatory elements responsible for transcriptional control of the chitinase gene, we have inserted the entire 4.7kb EcoRI-HindIII fragment of bean DNA, as well as DNA fragments containing progressive deletions in the upstream DNA sequences, into tobacco plants. Northern blot analysis of transgenic plants containing the entire 4.7kb DNA fragment indicate that expression of the bean chitinase 5B gene in tobacco plants is dependent upon exogeneously supplied ethylene. Thus it is possible that transgenic tobacco plants may be used to study the molecular basis of ethylene-mediated gene expression.

L 046 HEAT SHOCK IN SOYBEAN: SOME PHYSIOLOGICAL AND REGULATORY OBSERVATIONS ON THE PHENOMENON, Joe L. Key, Michael Mansfield, Lenny Edelman, C. Y. Lin, Janice Kimpel, Elizabeth Vierling, James K. Roberts, Julie Li and Ronald T. Nagao, Department of Botany, The University of Georgia, Athens, Georgia 30602.

A number of aspects of the heat shock (HS) response in soybeans has been investigated over the past several years: thermotolerance and the possible importance of HS proteins; the differential stability of HS mRNA at HS and control temperatures; the differential stability of mRNAs during HS representative of several classes of non-HS genes; the differential recovery of gene expression following HS at different temperatures ("optimal" and "supra-optimal"); expression of HS genes under various growth regimes; and aspects of regulation of expression of HS genes. Results of these studies will be summarized, and an attempt will be made to place these in an overview perspective of heat shock.

L 047 EXPRESSION OF GENES ELECTROPORATED INTO RICE AND MAIZE CELLS, Virginia Walbot, Judy Callis, Jeffrey R. deWet, Gabriele Dietrich, and Philippe Tacchini, Stanford University, Stanford CA 94305.
Electroporation is an efficient method for introducing DNA (1) or mRNA (2) into monocot protoplasts. We are interested in determining whether the electroporated protoplasts retain the physiological characteristics of the tissues from which they were derived, to allow experimental tests of promoter function, post-transcriptional events, and mRNA usage in transient assays. For heat shock, protoplasts do parallel the response shown by intact plant tissue by slowing transcription of most genes while inducing transcription from the *hsp*70 promoter.

We will also report on recent studies to define aspects of mRNA structure important in the final level of product. Using mRNA poration (2) we have demonstrated that a "cap" structure is essential and that a poly(A) tail is very important. Using deletion mutants of the *Adh1* gene, we have demonstrated that introns result in a profound increase in product levels (of either ADH1S isozyme, CAT or luciferase as reporter genes, 3). Alteration of the 3' end of constructs can also greatly influence the amount of reporter gene product made; we will report on comparisons of the histone H3 3' region, NOS and monocot genes. We have also tested the effects of translational enhancers derived from viral sources on gene expression in monocot cells. Several technical advances in reporter gene detection and in the electroporation protocol will also be described.

1) Fromm, M. E., Taylor, L. P. and V. Walbot. 1985. Proc. Natl. Acad. Sci. 82: 5824.
2) Callis, J., Fromm, M. and V. Walbot. 1987. Nucl. Acids Res. 15: 5823.
3) Callis, J., Fromm, M. and V. Walbot. 1987. In press, Genes & Development.

Effect of Hormones on Plant Development

L 048 EFFECT OF OLIGOSACCHARINS ON PLANT GROWTH AND DEVELOPMENT, Peter Albersheim, Christopher Augur, Alan G. Darvill, Nancy Doubrava, Stefan Eberhard, David J. Gollin, Teresa Gruber, Victoria Marfa-Riera, Maria Elena Mayorga, Debra Mohnen, Roger O'Neill, Audrey Southwick, and William S. York, Complex Carbohydrate Research Center and Dept of Biochemistry, Univ. of Georgia and USDA Russell Lab, Box 5677, Athens, GA 30613.

Fragments of plant cell wall polysaccharides have been shown to be able to regulate, in bioassays, a variety of processes relating to growth and development. Xyloglucan fragments, at 10^{-9} M, inhibit auxin-induced growth of pea stem segments while an alkali-solubilized mixture of wall fragments stimulates elongation growth of wheat coleoptiles and inhibits auxin-induced growth of the coleoptiles (Stephanie Mustaftchiev, personal communication). The activities of enzymes that release and process xyloglucan fragments appear to be under auxin control. We have evidence that a pectic polysaccharide is able to induce vegetative shoots in tobacco explants; the same or a related cell wall polysaccharide inhibits root formation in the explants. This lecture will describe these biological systems and the structures of the cell wall-derived regulatory molecules--the oligosaccharins. (Supported by U.S. Dept. of Energy DE-FG09-87ER13425 and DE-FG09-87ER13426)

L 049 GENETIC ANALYSIS OF HORMONE RESPONSE IN ARABIDOPSIS THALIANA, Cynthia Lincoln, Bryan Pickett, Allison Wilson, Tony Bleecker*, and Mark Estelle, Dept of Biology, Indiana University, Bloomington IN. 47405 *MSU-DOE Plant Research Lab, Michigan State University, East Lansing, MI 48824.

We are using a genetic approach to identify some of the genes involved in metabolism and action of IAA in Arabidopsis. By isolating mutants which are resistant to exogenous application of various auxins we have defined two loci which appear to play a role in some aspect of auxin function. These two genes are called Axr1 and Axr2. The axr1 mutants show a different level of resistance to IAA, 2,4-D and 1-NAA suggesting that these mutants are altered in a cellular component which is interacting directly with auxin. In addition to auxin resistance, mutations at both Axr1 and Axr2 confer a distinctive morphological phenotype. Mutations at Axr1 are recessive and cause changes in leaf shape, internode length, flower development, apical dominance, root geotropic behavior and growth in culture. The axr2 mutation is dominant and produces a very different morphological phenotype than the axr1 mutants. The most dramatic aspect of this phenotype is a disturbance in the tropic behavior of the inflorescence. In order to investigate the functional relationship between the Axr1 and Axr2 genes we are constructing double mutants. The results of these experiments will be presented.

We are also using a similar kind of analysis to study the mode of action of the plant hormone ethylene. A number of mutants have been isolated that are resistant to exogenous application of ethylene. The best characterized of these mutants is a dominant mutation called er1. By a number of criteria this mutant shows no response to exogenous ethylene even at very high concentrations (100 ppm). Despite the loss of any measurable ethylene response the er1 mutants are vigorous and fully fertile suggesting that under controlled growth conditions ethylene action is not required for near normal growth and development in Arabidopsis.

The biochemical defect has not yet been determined for any of these mutations. However we feel our efforts to develop a genetic approach to the problem of auxin and ethylene action in Arabidopsis will lead to the identification of some of the proteins required for hormone function.

L 050 AUXIN-REGULATED TRANSCRIPTION OF SOYBEAN GENES, Tom J. Guilfoyle, Gretchen Hagen, Bruce McClure, Christopher Brown, Melissa Gee, and Richard Wright, Department of Biochemistry, University of Missouri, Columbia, MO 65211.

We have isolated and characterized a number of cDNA clones to auxin-responsive polyadenylated RNAs in soybean (1, 2). The accumulation of these mRNAs is regulated, at least in part, at the level of transcription. Induction of nuclear transcription and accumulation of polyadenylated RNAs are observed within a few minutes after auxin stimulation of excised hypocotyl. Three of the auxin-induced polyadenylated RNAs reach half maximal steady state levels 10 minutes after auxin application and maximal steady state levels 30-60 minutes after hormone addition. Five of the cDNA clones correspond to mRNAs that are specifically induced by auxins while nonauxin analogs, other phytohormones and stress agents fail to induce the auxin regulated genes. Some of the auxin-responsive mRNAs or their corresponding polypeptides (3) are induced most strongly in elongating regions of hypocotyls and epicotyls while others are most highly expressed in root/hypocotyl transition regions. Three of the polyadenylated RNAs are transcribed from a locus containing three auxin-regulated genes within a 5 kb genomic region. The clustering of these three genes appears to have arisen by a gene duplication event(s).

1. Hagen, G. and T.J. Guilfoyle (1985) Mol. Cell. Biol. 5:1197.
2. McClure, B.A. and T. Guilfoyle (1987) Plant Mol. Biol. 9:611.
3. Wright, R.M., G. Hagen, T. Guilfoyle (1987) Plant Mol. Biol. 9: 625.

L 051 MANIPULATION OF ENDOGENOUS AUXIN AND CYTOKININ LEVELS IN TRANSGENIC PLANTS, Harry J. Klee and June I. Medford, Plant Molecular Biology Group, Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, MO 63198.

Auxins and cytokinins are essential for plant growth and development. Alterations in the normal levels of these compounds can have profound effects on the patterns of growth and differentiation. We are studying the roles of these hormones more precisely by altering their levels *in vivo*. To do this, we have used the genes from an Agrobacterium tumefaciens Ti plasmid that lead to synthesis of auxin and cytokinin. These genes have been fused to several transcriptional promoters and introduced into plants. Transgenic plants that express the Ti auxin or cytokinin genes both constitutively and in a regulated manner have been obtained. The effects of constitutive expression of auxin in transgenic plants have been described [1]. Regulated expression has been accomplished both in a tissue and temporally specific manner using either a seed storage protein promoter or a maize Hsp70 (heat shock) promoter. The Hsp70 promoter has been successfully used to produce transient bursts of auxin or cytokinin. The effects of these hormone manipulations on plant growth and development will be discussed.

1. Klee, et al. Genes and Development 1:86-96 (1987).

L 052 CONTROL OF CEREAL EMBRYOGENESIS AND THE REGULATION OF GENE EXPRESSION BY ABSCISIC ACID (ABA)*, Ralph S. Quatrano, William R. Marcotte, James C. Litts, Christopher C. Bayley and Sonja A. Schmitz, Central Research & Development Department, Experimental Station, E402, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

Our major goal is to understand the controls operative in the expression of genes and gene blocks during cereal embryogenesis. A set of defined culture conditions allow isolated 10-15 day old cereal embryos to either precociously germinate, or to undergo normal maturation and accumulation of stored reserves. The growth regulator ABA controls this developmental switch *in vitro* and there is strong evidence for a similar role of ABA *in vivo*. Over a 3-5 day culture period in the presence of ABA (1-100μM), immature wheat embryos increase in fresh and dry weight and undergo an accelerated but normal morphogenesis and accumulation of mature embryo proteins. In the absence of ABA, this maturation program is not observed and the embryo germinates into a normal seedling. Some proteins that accumulate in the presence of ABA have been identified and include the lectin wheat germ agglutinin, an abundant protein found in the mature embryo (Em protein) and the globulin storage proteins. Characterization of these genes and the pattern of accumulation of their respective proteins and mRNA's during grain development, and in culture with ABA, revealed that regulation of these genes by ABA occurs at the level of transcription and mRNA stability. Comparison of genomic sequences of these genes revealed that the globulin sequence from wheat shared limited but distinct amino acid sequence homology with the 7S globulins from bean (phaseolin), soybean (conglycinin) and pea (vicilin). However, a strong similarity was noted throughout the protein when hydropathy plots were compared. Sequences 5' to the transcription start site for these three genes that are regulated by ABA did not reveal any region of homology. Constructs of the intact and deleted 5' as well as the 3' regions with the glucuronidase (GUS) reporter gene have been tested in transient assays using protoplasts from monocots and dicots. The same constructs, as well as others containing the entire gene were used in Agrobacterium-mediated gene transfer to selected dicots. Results from both the transient and stable transformation assays will be discussed in relation to the cis-acting sequences involved in the ABA regulation.

* A portion of this research was completed by Ralph S. Quatrano and James C. Litts at Oregon State University (Department of Botany) with support from the USDA Competitive Grants Program (84-CRCR-1-1380).

Effect of Light on Plant Development

L 053 GLUTAMINE SYNTHETASE GENE FAMILY: DIFFERENTIAL REGULATION BY LIGHT AND OTHER EXTERNAL FACTORS. Gloria M. Coruzzi, Janice W. Edwards, Elsbeth L. Walker, Fong-Ying Tsai, and Scott V. Tingey*. The Rockefeller University, 1230 York Ave., New York, N.Y. 10021-6399. *Present address, E.I. DuPont, Exp. St. Bldg 402/4223, Wilmington DE 19898.

We have begun to examine the structure and in vivo expression of the family of genes encoding the chloroplast and cytosolic forms of glutamine synthetase (GS) in Pisum sativum. Three full length GS cDNA clones have been characterized and shown by in vitro transcription and translation to encode chloroplast GS2, cytosolic GS1, or cytosolic GSn polypeptides. Southern blot analysis and genomic clone characterization has identified four GS genes in pea: one for chloroplast GS2, one for cytosolic GS1, and two for cytosolic GSn. In vitro uptake studies show that chloroplast GS2 is synthesized as a precursor polypeptide (containing an amino terminal transit peptide), which is imported into the chloroplast stroma, and processed to a mature size of 44 kd. The GS2 polypeptide and mRNA are expressed most abundantly in leaves, in a light-dependent fashion. We have examined the kinetics of light-induction as well as the possible roles of phytochrome, blue-light, or photorespiratory ammonia in mediating GS2 expression in vivo. The DNA sequence of the GS2 promoter and transcription start site have been examined. The genes for the cytosolic forms of GS (GS1, GSn) are highly homologous but distinct. We have characterized the promoters of the two GSn genes by nucleotide sequence analysis. 3' S1 nuclease analysis was used to examine GSn gene-specific expression during a time course of nodule development. Analysis of GSn transcript levels in Fix$^-$ nodules, has shown that metabolic induction by ammonia is not involved in the expression of GSn mRNA in root nodules. We have also examined the expression of GS genes in other organs during pea development. Our studies have shown that chloroplast and cytosolic forms of GS are encoded by highly homologous nuclear genes. This data provides evidence that one molecular mechanism for chloroplast evolution involves nuclear gene duplication and subsequent specialization at each locus. A series of 5' promoter deletion mutants in the individual GS genes will tested for functionality in vivo in transgenic plants. Our continuing studies are aimed at defining the cis-acting elements of the GS gene promoters which are responsible for the diverse patterns of expression in vivo.

L 054 FACTORS WHICH EFFECT THE EXPRESSION OF PHOTOSYNTHETIC GENES, Pamela Dunsmuir, David Gidoni, Mark Stayton and John Bedbrook, Advanced Genetic Sciences, 6701 San Pablo Avenue, Oakland, CA 94608.

Two aspects of the Petunia Cab gene family will be discussed. Firstly the analysis of a divergent pair of promoter regions, and secondly the diurnal fluctuations which occur for genes from different Cab gene subfamilies.

We have used systematic mutation analysis and gene transfer technology to define specific sites within the Cab22 promoter region which effect high level expression. Our results indicate Cis acting elements both downstream and upstream to the CAAT box that are involved in the regulation of Cab gene expression.

We have characterized the expression of the genes which encode different light harvesting peptides throughout a defined day/night program in petunia. There are significant differences in the diurnal expression patterns for genes encoding different proteins. We have examined whether these properties are conserved after gene transfer.

Cis-elements and Trans Factors Controlling Plant Gene Expression

L 055 MULTIPLE REGULATORY ELEMENTS OF THE PEA rbcs-3A GENE, Maria Cuozzo, Cris Kuhlemeier, Pamela J. Green, Steve A. Kay, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021-6399, USA

The upstream region of the pea rbcS-3A gene contains multiple sequence motifs, boxes I-V, which are conserved among five members of the pea rbcS multigene family. These boxes have been the target of deletion analysis and site-directed mutagenesis to study light regulation and organ specificity of the altered genes in transgenic plants, and binding properties of the DNA fragments in vitro. Analysis of the upstream region from -410 to -50 has revealed that two conserved motifs near -150, boxes II and III, have homologous copies further upstream near -220, boxes II* and III*. Each set of boxes can function in the absence of the other: a fragment from -410 to -170 can confer light regulation and organ specificity, as can a fragment deleted of sequences upstream of -166. However, the expression levels of the -166 deletion are reduced at certain developmental stages of the plant. Full expression may require cooperation between the sets of boxes, or the boxes may have different developmental specificities. In the absence of the upstream boxes, small substitution mutations in boxes II and III, including a GG to CC transversion in the SV-40 core enhancer-like box II sequence, abolish expression. Positive regulators boxes II and III overlap with previously defined negative regulatory elements[1]. A protein factor from pea nuclear extracts, GT-1, specifically interacts with these upstream regulatory sequences[2]. Gel retardation assays and DNaseI footprinting have shown that GT-1 binds to DNA fragments containing boxes II and III, as well as to fragments containing II* and III*. Diminished factor binding to fragments containing substitutions in conserved boxes parallels the data from transgenic plants on positive regulation of rbcS-3A expression.

[1]Kuhlemeier, C., Fluhr, R., Green, P., and Chua, N.-H. 1987. Genes and Devel. 1:247-255.

[2]Green, P. J., Kay, S. A., and Chua, N.-H. 1987. EMBO J. 6: 2543-2549.

L 056 SELECTION FOR *TRANS*-ACTING REGULATORY MUTATIONS IN *ARABIDOPSIS*, Elliot M. Meyerowitz, John Bowman, Caren Chang, Patty Pang and Martin Yanofsky, Division of Biology, California Institute of Technology, Pasadena CA 91125 USA.

We are attempting to obtain mutations in genes whose normal products are necessary in *trans* for the high-level expression of abundantly transcribed seed-specific genes in *Arabidopsis*. To do this we have cloned the *Arabidopsis* alcohol dehydrogenase gene. Reintroduction of this gene into the genome of *Adh*$^-$ mutants causes the resulting transgenic plants to express ADH activity in embryos, and as a consequence they can be killed by exposure to allyl alcohol during imbibition. The *Adh* gene coding sequence has been fused to regulatory sequences for genes specifically and highly expressed in embryos, and these fusion constructs inserted into the genome of *Adh*$^-$ plants. These constructs cause *Adh*$^-$ embryos to show ADH activity, thus allowing genetic selection against the expression of the seed gene promoters. Additional experiments have established methods for molecular cloning of *Arabidopsis* genes about which no more is known than their genetic map position. This should allow a molecular approach to any *trans*-acting regulatory mutations that are obtained.

L 057 STRATEGIES OF GENE EXPRESSION FOR SURVIVAL OF ANAEROBIC STRESS IN PLANTS, W.J. Peacock, CSIRO Division of Plant Industry, Canberra, A.C.T., Australia.

Plants, like many other organisms, respond to conditions of low oxygen tension, such as occur during flooding, by switching carbohydrate metabolism from an oxidative to a fermentative pathway. Initially lactate is produced and cellular pH drops. There is then a shift to the production of ethanol catalysed by pyruvate decarboxylase and alcohol dehydrogenase. This stabilises cytoplasmic pH. The ability of plants to use mainly ethanolic fermentation rather than be reliant solely on lactic fermentation enables them to withstand hypoxic conditions for periods of up to several days.

Alcohol dehydrogenase and other enzymes involved in either the lactic or ethanolic fermentation pathways are polypeptides which are specifically produced under anaerobic conditions. Most other protein synthesis ceases with oxygen deprivation. For a number of the anaerobically-induced genes there is selective transcription and subsequent selective translation of their mRNAs.

Analysis of the upstream regions of a number of the anaerobic genes has enabled us to identify key sequence elements and to nominate a specific hexanucleotide (TGGTTT) as being involved in the coordinate expression of genes responding to anaerobic stress. The anaerobic hexanucleotide interacts with a protein that binds specifically to that sequence. The anaerobic response is common to most plant species and we have found using both sequence analyses of promoter regions and functional analyses in heterologous species, that the response mechanism is conserved. The interaction between anaerobic promoter regions and the TATA sequence is less efficient in monocot/dicot transfers than within either of these two major plant groups. We are able to overcome the relative inefficiency of the monocot promoter in a dicot by the addition of an enhancer element from the octopine synthase gene. We have characterised the enhancer in its interactions with a number of promoters and also with a specific binding protein.

Developmental Mutants; Rescue

L 100 Volvocales GP2, a Highly Conserved Cell Wall HRGP. W.S. Adair, Washington University, St. Louis, MO 63130

Unlike higher plants, algae in the order Volvocales have cell walls constructed entirely from hydroxyproline-rich glycoproteins (HRGP's). These algae, which range in complexity form unicellular forms such as Chlamydomonas reinhardtii to multicellular organisms like volvox carteri, have wall morphologies that are thought to reflect evolutionary relationships. We recently examined these taxonomic assignments directly, employing an in vitro assembly system to construct hybrid cell walls. In this functional test, C. reinhardtii was found to be more closely related to colonial (Gonium pectorali) and multicellular (Volvox) species than to other Chlamydomonads. Analysis of HRGP's involved in interspecific assembly revealed that Gonium and Volvox each has a major wall component homologous to C. reinhardtii GP2. Preliminary evidence suggests that each GP2 homologue is a molecular complex of at least 6 distinct polypeptides, each highly conserved within these species. Ability to carry out homospecific and heterospecific cell wall assembly in vitro, from purified HRGP's, provides a novel approach to the identification and dissection of important functional domains.

L 101 ISOLATION AND SEQUENCE CHARACTERIZATION OF A cDNA FROM A DEVELOPMENTALLY REGULATED GENE IN NICOTIANA, Claudio L. Afonso, John Brumbaugh, Chris A. Wozniak and David W. Galbraith, University of Nebraska-Lincoln, Lincoln, NE 68588

A cDNA library was constructed in the expression vector lambda gt11 using mRNA from actively dividing N.tabacum cell suspension. A polyclonal antibody directed against a callus associated protein was used to select two clones of 2.5 and 1kb respectively. Both clones hybridize to a 6 kb message that is highly expressed in cell suspension of N.tabacum and N.sylvestris but nearly absent in differenciated leaf tissue. The gene is present as a single copy in N.tabacum and in N. sylvestris and Petunia hybrida. The expression of the message seems to be modulated during development as it is more abundant in immature seeds and flowers than in leaf tissue. Partial sequence of the isolated clones has revealed one continuous open reading frame.The deduced amino acid composition suggest that the message encodes a highly hydrophilic protein. Stretches of similarity to eukaryotic and prokaryotic DNA binding proteins suggest a related function.

L 102 ANALYSIS OF WHEAT GLIADIN PROMOTER REGION FOR ITS TRANSCRIPTIONAL EXPRESSION IN ELECTROPORATED PROTOPLASTS. Arun P. Aryan, Douglas J. Leisy, Gynheung An, and Thomas W. Okita. Institute of Biological Chemistry, Washington State University Pullman, WA 99164-6340.

Structural and functional analysis of the gliadin (glia) promoter was conducted by transient expression assays of a series of vectors, introduced electroporatically into tobacco protoplasts. The promoter region (-592 to -72 bp from the translational start) of this developmentally regulated gene, when fused upstream to the chloramphenicol acetyl transferase (CAT) reporter cartridge, was unable to direct CAT expression in tobacco cells unless some nopaline synthase (nos)-activating sequences were present at 5'-end of glia-promoter. However, this glia-promoter alone was found to be functional in rice protoplasts. For structural analysis, the glia-promoter was progressively deleted from the 3'-end and attached upstream to a non-functional nos-promoter (-101) or a functional nos-promoter (-155) fused to CAT gene (An et al; 1986, Mol. Gen. Genet. 203: 245-250). The transient expression of CAT activity in tobacco cells showed that the extent of recovery in promoter-function in the first set of plasmids was proportional to the enhancement of nos-promoter function in the second set. The transcriptional activity of glia-promoter (-592 to -72 bp) in these constructs was reduced by ≅ 40% on deletion of sequences -140 to -72 bp, which included both TATA (-104 bp) and CAAT (-136 bp) boxes, but was drastically decreased (≅ 80%) when further regions containing CACA box (-196 bp; Reeves and Okita; 1987, Gene 52: 257-266) were also deleted. This observation supports a transcriptional role of the CACA region in glia-promoter function.

L 103 THE EFFECT ON POTATO AND TOMATO DEVELOPMENT OF THE INTEGRATION OF AGROBACTERIUM ONCOGENES, Patrizia Bogani, Carla Montanelli[1], Priscilla Bettini, Marcello Buiatti, David Inzè[2], Gennarino Nascari[1] and M. Gabriella Pellegrini, Dipartimento di Biologia Animale e Genetica, Università di Firenze; (1) Istituto Agronomico per l'Oltremare, Firenze; (2) Laboratorium voor Genetika, Rijksuniversiteit, Gent.

A series of infection experiments on tomato cotyledons and potato tuber disks was carried out with the aim of studying the effect on development of the interaction between plant genomes and A. tumefaciens oncogenes. In tomato, plant regeneration was obtained after infection with A. tumefaciens lacking one or two oncogenes. In potato the same results were obtained only when one oncogene for auxin synthesis was deleted. Differences in transgenic plants development were more evident in the case of potato. Moreover the effect of alien gene integration was different according to the cultivar infected and at variance with changes in development induced by exogenous hormone treatments. These results suggest the possible usefulness of integration of A. tumefaciens oncogenes for the directed modification of plant development.

L 104 SPATIAL PATTERN OF LUCIFERASE ACTIVITY IN TRANSGENIC PLANTS HARBORING DIFFERENT CaMV 35S RNA PROMOTER DELETIONS, Joy Chien, Gretchen Scheel and David W. Ow, Plant Gene Expression Center, USDA/ARS, Albany, CA 94710.

The 35S RNA promoter from cauliflower mosaic virus is one of the best studied promoters in plants. Transcriptionally active in a wide range of plant species, it has also been found, recently, to have tissue-specific (Ow and Howell, in Biotechnology in Agriculture and Forestry, in press) and cell cycle-specific (Nagata et al., MGG 207:242-244, 1987) regulation. Previously, we have reported on the functional domains that determine promoter strength as defined by a set of promoter deletions in transient expression assays (Ow et al., PNAS 84:4870-4874, 1987). In this study, we have taken this same set of promoter deletions and introduced it into tobacco plants. As the promoters are fused to the firefly luciferase reporter gene, we were able to assay conveniently for gene activity using both enzyme assays and photographic exposure of plant organs. In this poster, we will present our current findings on the tissue distribution of luciferase activity among plants transformed with different CaMV 35S promoter constructs.

L 105 PHOSPHORYLATION OF TUBULIN BY CARROT CELLS TEMPERATURE-SENSITIVE FOR SOMATIC EMBRYOGENESIS, Jung H. Choi, Georgia Institute of Technology, Atlanta, GA 30332

TS59 is a variant carrot cell line selected for its inability to undergo embryogenesis at 32 degrees (Breton and Sung, 1982). The rate of growth of TS59 cells appears unaffected by high temperature; however, the resulting cell clusters lack discernible organization or embryonic morphology. At lower temperatures, such as 25 degrees, recognizable globular embryos form at 50% efficiency. Even at the lower temperatures, however, the embryos which do form rarely elongate, and giant spherical embryoids accumulate in these cultures. Preliminary two-dimensional gel studies indicated that several proteins in TS59 differed from wild type cells by an altered post-translational modification (Loschiavo, 1985). Analysis of phosphoprotein patterns in TS59 and in the parental wild type cells revealed that TS59 cells overphosphorylate a specific set of proteins at the elevated temperature. Two of these phosphoproteins comigrate with purified tubulin on two-dimensional gels. This overphosphorylation of tubulin can be demonstrated in vitro with TS59 cell extracts and purified tubulin as well as in vivo. We hypothesize that overphosphorylation of tubulin by TS59 cells at high temperatures may result in disruption of cortical microtubule arrays, ultimately disrupting morphogenesis by randomizing planes of cell division and directions of cell elongation.

L 106 GENETIC ANALYSIS OF PHOTORECEPTOR ACTION PATHWAYS. J. Chory and F. Ausubel, Dept. of Molecular Biology, Mass. Gen. Hospital, Boston, MA.

The photoconversion of photoreceptors by light induces the diverse morphogenic responses which result in greening. We are pursuing a dual approach for identifying the factors involved in transducing the developmental signals which trigger greening. The first approach is directed at the identification of regulatory gene(s) which affect the transcription of nuclear-encoded photosynthesis genes, specifically the cab genes. The strategy involves fusing a cab promoter to both a selectable and screenable marker and transferring these chimeric constructions back into A. thaliana to find mutants which aberrantly express the marker genes. We have transformed A. thaliana with these constructions and are in the process of bulking seed to select mutant plants. The second approach involves the direct selection for plants with a predicted mutant photoreceptor phenotype. Mutants which do not show the phytochrome-mediated hypocotyl elongation inhibition response due to light have been isolated by Koornneef et al. and by ourselves. We have characterized these mutants with regard to pigment content, expression of photosynthesis genes and proteins, and chloroplast structure. One of these mutants shows a dramatic increase in the cell number per leaf. We have also screened for mutants with a "de-etiolated" phenotype in the dark, i.e., seedlings which show leaf expansion and stem inhibition in the dark and are studying 8 of these. We have screened for mutations in the blue-light photoreceptor pathway by looking for plants which are phototropic-minus and have obtained 13 such mutants. Of these 13, 3 show reduced apical dominance and are self-infertile due to lack of stamen elongation. These are similar to the IAA-resistant mutants described by Estelle et al. and may implicate a role for auxin in phototropic bending in Arabidopsis.

L 107 MOLECULAR BASIS OF PLANT CELL WALL LOOSENING, Daniel Cosgrove, Department of Biology, Penn State University, University Park, PA 16802

Plant cell growth requires that the pre-existing wall be loosened and then stretched irreversibly by the mechanical forces arising from cell turgor. The molecular basis of wall loosening is not yet known. Recently we reported that wall specimens from growing stem tissue will undergo long term extension (creep) when placed under tension in an acidic pH. This study reports on efforts to determine the molecular nature of such wall creep, and in particular whether creep is enzymatically-mediated. Many treatments which interfere with enzyme activity were found to inhibit wall creep, for example, treatment with protein denaturants such as 8 M urea, high salt, 50% methanol, mM concentrations of heavy metals, and boiling in water. Creep rate showed a Q10 of 2 to 2.5 in the temperature range of 20-30°C, and was irreversibly eliminated by preincubation with proteases. Sulfhydryl reducing reagents such dithiothreitol and cysteine enhanced creep, as did calcium chelators. Creep activity was stable at pH's below 5.5, but was unstable at higher pH. These results suggest that a wall-bound enzyme is needed for creep activity. Comparison of endogenous creep with creep elicited by addition of various polysaccharide hydrolases indicates that hydrolytic activity is not the basis of wall creep. In addition, sugar analogs which are effective inhibitors of various glucosidases, did not affect wall creep. Attempts to extract and reconstitute creep activity are currently in progress.

L 108 REGULATION OF A TRANS-ACTING FACTOR BY PHOSPHORYLATION

Neeraj Datta and Anthony R.Cashmore, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

We have isolated and partially purified a trans-acting factor from pea nuclei which binds specifically to the pea Rubisco SS3.6 promotor. The binding activity of this factor is reversibly regulated by phosphorylation. It is phosphorylated by NII kinase and is inactive in the phosphorylated form. Reconstitution experiments with purified phosphorylating enzymes are in progress.

L 109 ISOLATION OF TISSUE-SPECIFIC PROMOTERS OF RICE, B.S. de Pater, L.A.M. Hensgens and R.A. Schilperoort, Department of Plant Molecular Biology, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The aim of this project is to isolate tissue-specific promoters of rice to study developmentally regulated gene expression. A cDNA library has been constructed of two weeks old seedlings of the Indica variety IR36. The cDNA library was screened for leaf/stem and root specific sequences. Nine cDNA clones were selected: two correspond to root-specific mRNAs, four to leaf/stem specific (and light-inducible) mRNAs and three to constitutively expressed mRNAs. Genomic blots of rice showed that some of the cDNA clones hybridized with one restriction fragment (single copy genes) and that others hybridized with four to ten restriction fragments (multigene families). Sequencing of the cDNA clones is in progress and the corresponding genomic clones will be isolated in the near future from a rice genomic library that we constructed.

L 110 **REGULATION OF CHIMERIC GENE EXPRESSION IN MAIZE PROTOPLASTS BY INTRON-1 OF Adh1.** Gabriele Dietrich and Virginia Walbot, Department of Biological Sciences, Stanford University, Stanford, CA 94305

The function of introns in eukaryotic genes is still not fully understood. However, the finding of enhancer-like elements in introns of some animal and viral genes has suggested an important role of introns in the regulation of gene expression. It has recently been shown that the presence of the first intron of maize **Adh1** amplifies the expression of the **Adh1** gene by 50 to 100 fold after transformation of maize protoplasts. Similar enhancement was also observed using the CaMV- or NOS-promoter and various reporter genes by transient expression in maize protoplasts. In contrast to enhancer-elements, the function of **intron-1** was strongly dependent on its orientation and position in the gene construct (Callis, Fromm and Walbot, Genes and Development, in press).
Deletion experiments have shown the need of both intron borders, indicating a requirement for correct splicing of **intron-1**. Deletions within **intron-1** are being tested to characterize the borders of a possible enhancing region within the intron. To further characterize the function of **intron-1**, nuclear runoff experiments will be used to determine if transcriptional or posttranscriptional mechanisms are involved in the regulation of chimeric gene expression.

L 111 A MOLECULAR GENETIC APPROACH TO THE IDENTIFICATION AND CLONING OF ETHYLENE-INSENSITIVE MUTATIONS IN ARABIDOPSIS USING YEAST ARTIFICIAL CHROMOSOME (YAC) LIBRARIES, Joseph R. Ecker and Plinio Guzman, Plant Sciences Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Ethylene is a regulator of plant growth and development. In addition, ethylene is known to mediate many host responses to pathogen attack. Very little, however, is understood about the molecular mechanism of ethylene recognition and action in plants. We have undertaken a molecular genetic approach to the analysis of ethylene-regulated processes by identifying ethylene-insensitive *Arabidopsis* mutants. A number of independent mutations in ethylene-induced inhibition of hypocotyl elongation have been isolated. Ethylene insensitive (ein) mutants also show little or no ethylene-induced inhibition of root growth. Interestingly, several ein mutants display no apical hook; a structure whose formation and maintenance in etiolated seedlings is thought to occur in response to ethylene. Genetic complementation analysis of ein mutants is in progress. Genetic mapping studies using both genetic and RFLP markers will allow positioning of ein mutations on the *Arabidopsis* linkage map. Ultimately, our goal is the molecular cloning of ein genes. Using present methods, the cloning of plant genes about which nothing more is known than the genetic map position is technically difficult. Because of either large amounts of repeated DNA sequences or large gap regions in current RFLP maps, standard chromosome "walking" or even "jumping" techniques in most plants are untenable. Our approach is to clone large segments of plant DNA into yeast. A library of carrot DNA-containing yeast artificial chromosomes (YACs) was cloned into yeast. Individual YACs of sizes 100-260 kb were resolved by pulse-field gel electrophoresis using a CHEF apparatus. Progress toward construction and analysis of *Arabidopsis* YACs will be discussed.

L 112 INSERTIONAL MUTAGENESIS IN **ARABIDOPSIS THALIANA**: A DWARF MUTANT INDUCED BY A T-DNA INSERT. Kenneth A. Feldmann[1], Michael L. Christianson[2] and M. David Marks[3]. E.I. duPont, Wilmington, DE 19898[1], Sandoz Crop Protection, Palo Alto, CA[2], Univ. Nebraska, Lincoln, NE[3].
A population of 500 transformed lines of **A. thaliana** generated by **Agrobacterium**-mediated transformation of germinating seeds, have been examined for segregating phenotypic alterations. Numerous variants have been observed. One such mutant, T-31, is characterized by shortness in stature (7 cm), increased number of axillary shoots (>10), epinastic dark green rosette leaves, decreased seed set (due to failure of the stamens to elongate), and delayed flowering. Physiological tests to determine which pathway the alteration occurred in are in progress. The marker used in this transformation protocol was nptII, a dominant selectable marker, conferring resistance to kanamaycin. Plants of normal stature segregate 2R:1S when transferred to Kan-containing medium. Dwarf plants are resistant to Kan. Reciprocal crosses of dwarf (Kan^R) plants to wildtype plants (Kan^S) produced F1 plants which are Kan^R and normal for height. F2 families from selfed F1 plants segregate 1 dwarf,Kan^R:2 normal,Kan^R:1 Kan^S,normal. A lambda library was constructed from genomic DNA isolated from non-segregating dwarf lines. Clones containing T-DNA were selected and purified. Isolation and characterization of the T-DNA insert and plant gene are in progress. The characterized wildtype gene will be transformed into dwarf mutants to test for complementation.

L 113 PHOTOPERIOD INDUCED CHANGES IN THE mRNA POPULATION OF THE FLOWERING PLANT PHARBITIS NIL, Roderick Felsheim and Anath Das, Biochemistry Department, University of Minnesota. St.Paul, MN 55108.
To investigate the mechanism of floral induction in plants we chose to study the Japanese morning glory, Pharbitis nil strain Violet. Pharbitis can be induced to flower with a single inductive dark period. Six day old seedlings, grown in continuous light, were subjected to a 14 hour inductive dark period. Uninduced control plants received a 10 minute night break 8 hours into the dark period. Poly A (+) RNAs were isolated from the cotyledons of both induced and uninduced plants and were translated in an in vitro rabbit reticulocyte system. Translation products were analyzed by two dimensional gel electrophoresis. Significant changes in the level of synthesis of several proteins were observed upon induction. Most notable was the appearance of three new proteins with apparent molecular masses of 28,000, 33,000 and 46,000 daltons, and the disappearance of one protein of about 16,000 daltons.

L 114 ANALYSIS OF ABA-INSENSITIVE MUTANTS OF ARABIDOPSIS, Ruth R. Finkelstein and Chris Somerville, Michigan State University, East Lansing, MI 48824
Abscisic acid has been implicated in regulating many aspects of plant growth and development, but the molecular mechanism of ABA action is still not understood. For example, we do not know if a single class of receptor mediates all responses or if there are tissue-, stage-, or response-specific receptors. We are characterizing a variety of ABA-inducible responses in ABA-insensitive mutants of Arabidopsis to determine the extent of their insensitivity. None of the three ABA-insensitive loci are required for all ABA-inducible responses, but each is required for a subset of those tested. Mutations at all three loci reduce dormancy and ABA-inhibition of germination. However, of the responses tested to date, the remaining effects of abi1 and abi2 appear confined to vegetative growth, while abi3 action primarily affects seed development. This suggests that, if there is a single class of ABA receptor, it is not encoded by any of these loci. These data neither rule out nor distinguish between the possibilities that these mutations affect stage-specific receptors or transduction into a cellular response. Eventually,we hope to clone one of these loci using either a transposon-tagging system we are currently building or chromosome walking.

L 115 NODULIN GENE EXPRESSION AND NODULIN FUNCTION, Francine Govers, Corné Pieterse, Jeroen Wilmer and Ton Bisseling, Dept. of Molecular Biology, Agricultural University, Wageningen, The Netherlands.

In leguminous root nodules and during the formation of these nodules several plant genes, the so-called nodulin genes, are specifically expressed. Nodulins appear differentially during the development of the symbiotic interaction with Rhizobium. Early nodulin genes are expressed during the stage that nodule structures are formed and their gene products can have functions in the infection process or the nodule morphogenesis. Late nodulins are first detectable when nitrogen fixation starts and they can have a role in supporting the nitrogen fixing bacteroids or in the assimilation of fixed nitrogen. In our laboratory several cDNAs representing early nodulin genes from soybean and pea have been cloned (Franssen et al., PNAS 84, 4495-4499, 1987; Scheres et al., to be published). Based on the expression pattern during root nodule development early nodulin genes can be divided in subclasses. Moreover, expression of some early nodulin genes is only observed in nodules that have infection threads or infected cells while others are also expressed in nodules lacking intracellular bacteria and infection threads. DNA sequencing has revealed that one early nodulin, ENOD2, is extremely proline rich and we have indications that ENOD2 has a role in the nodule morphogenesis. One of the approaches that we use to elucidate the functions of early nodulins in nodule morphogenesis is transformation of leguminous and non-leguminous plants with early nodulin genes and subsequently analyse the effects of, for example, overproduction of a particular early nodulin during nodule development or expression of a nodulin gene in cells that normally do not express this gene. Results of these experiments will be presented.

L 116 GENOMIC ORGANIZATION OF A CLASS OF SMALL, AUXIN INDUCIBLE SOYBEAN SEQUENCES, Gretchen Hagen, Melissa Gee, Chris Brown and Tom Guilfoyle, University of Missouri, Columbia, MO 65211.

A class of small (550 bp) soybean RNAs has been identified and shown to be rapidly induced following auxin application (McClure & Guilfoyle, PMB, in press). Southern blot analysis of soybean genomic DNA has revealed that the genes coding for these RNAs are clustered within about 5 kb (McClure, unpublished). Using cDNAs corresponding to these RNAs as probes, a soybean genomic library has been screened. A clone containing this locus has been purified, partially mapped by restriction enzyme analysis and subcloned into DNA sequencing vectors. Results of transcript mapping, DNA sequence comparison of cDNAs and the corresponding genes and analysis of the 5' and 3' flanking regions of these genes will be presented.

L 117 GENETIC ANALYSIS OF THE KNOTTED LOCUS, Sarah Hake, PGEC USDA, 800 Buchanan St., ALbany, Calif. 94710.

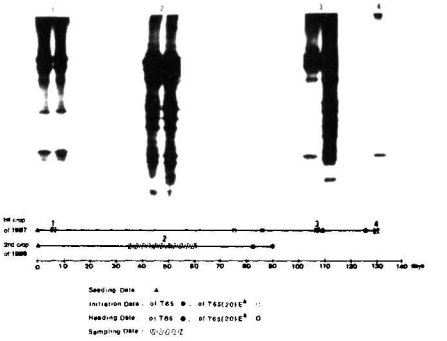
The knotted mutation of maize, Kn1, is defined by five dominant mutants that specify unexpected divisions of cells near lateral veins of the leaf blade (Freeling and Hake, 1985). The resulting phenotype includes displaced and de novo ligule formation, and hollow protrusions or knots along the lateral veins. Dosage analyses using T-B translocations and reciprocal translocations suggest that the Kn1 mutation is a neomorphic mutation; either a vast overproduction of a normal product, an altered normal product or an entirely new product. To determine how the Kn1 locus acts, we have attempted to transposon tag the knotted mutation using the transposons, Ac and Mu. The strategy taken is to inactivate the dominant mutant phenotype and recover a recessive normal plant. In addition to inactivating the Kn1 locus; we have a putative Ds induced Kn1 mutation and a new Mu1-induced knotted mutation. The former is linked to the original knotted locus and the expression of knots in this particular mutation is dependent on the presence of Ac. We do not yet know whether the Mu-induced Kn mutant is at the original locus. (Freeling, M. & S. Hake (1985) Genetics 111, 617-634)

L 118 GENETIC REGULATION OF FLORAL MORPHOGENESIS IN ARABIDOPSIS, George W. Haughn[1], Jose Martinez-Zapater[2], Rebecca Rasooley[2] and Ljerka Kunst[2], 1) Biology Department, University of Saskatchewan, Saskatoon, Sask., Canada, S7N 0W0, 2) MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.
Molecular and genetic analyses of homeotic mutants of Drosophila melanogaster have revealed much about the genetic regulation of developmental pathways in insects. We are attempting to study the regulation of flower development in Arabidopsis thaliana using a similar approach. Several Arabidopsis mutants have been isolated which are homeotic with respect to flower morphology (1). We have begun to study two of these mutants; Flo2 and Flo9. Flo2 has carpels in place of sepals while Flo9 has petals in place of stamens. Both phenotypes show variability in penetrance and expressivity. Preliminary genetic evidence indicates that both phenotypes are due to single recessive nuclear mutations. These data suggest that FLO2 and FLO9 are loci involved in establishing the identity of developing sepal and stamen primordia.

1. Haughn, G.W. and C.R. Somerville 1988. Genetic Control of Morphogenesis in Arabidopsis. Dev. Genet. in press.

L 119 DEVELOPMENTALLY REGULATED CELL WALL PROTEIN GENES OF SOYBEAN, JONG CHAN HONG, Ron T. Nagao and Joe L. Key, Botany Dept., Univ. of Georgia, Athens, GA 30602.
Previously we have reported the isolation and sequence of a putative soybean cell wall protein gene, SbPRP1 [Hong et al., JBC 262, 8367-8376 (1987)]. This gene is a member of a family of related cell wall protein genes as identified by hybridization to an auxin-responsive clone, pTU04. This family of genes is differentially expressed during the growth of the hypocotyl and dramatic changes are observed in the size of poly(A) RNA hybridizing to pTU04 depending on the stage of hypocotyl development. A 1050 nt RNA is primarily transcribed in the meristematic region vs 1220 (SbPRP1 gene) and 650 nt RNAs in the mature quiescent region of the hypocotyl. The presence of the 1220 nt RNA is associated with cell maturation and this RNA encodes a highly basic protein containing a signal sequence. The mature protein has an amino acid repeat structure primarily of Pro-Pro-Val-Tyr-Lys. We have isolated and sequenced genes for the 650 (SbPRP3) and 1050 (SbPRP2) nt transcripts. The sequence of SbPRP3 is similar to SbPRP1 in repeat structure but contains a shorter coding region. The expression of the SbPRP2 gene (1050 nt mRNA) is meristematic region-specific in the hypocotyl and encodes another novel proline-rich protein with essentially an alternating tandom repeat of Pro-Pro-Val-Tyr-Lys-Pro-Pro-Val-Glu-Lys. Northern analyses using gene specific probes indicate developmental expression of this gene family. Our data strongly suggest that these genes may encode cell wall proteins that play an important role in plant growth and development. We are currently comparing the developmental expression of these cell wall protein genes with extensin-related cell wall protein sequences.

L 120 SPECIFIC PROTEINS ARE PHOSPHORYLATED BY PROTEIN KINASE(S) PRESENT IN DEVELOPING RICE PANICLES, Nien-tai Hu, Ching-Min Cheng and Kuo-Hai Tsai, National Chung Hsing University, Taichung, Taiwan, 40227, R.O.C.
Initiation of rice panicles marks the turning point from vegetative growth to reproductive growth. From then on, cells at the shoot apex start differentiating into various parts of rice flowers. Rice panicles initiation date varies with crops and lines. In this study, shoot apexes before initiation and developing panicles after initiation were examined for protein kinase activity. T65 (T) and its isogenic line of early-heading, T65(20)E^a (A), were studied in parallel. Specific proteins were phosphorylated by the protein kinases present in the developing rice panicles. Autoradiograms of samples collected on different days during the rice panicles development are presented.



L 121 CHARACTERISATION OF AUXIN VARIANTS OF Nicotiana plumbaginifolia

Yvan Fracheboud, Martin Suter, Anne D. Blonstein, Patrick J. King
Friedrich Miescher-Institut, Postfach 2543, 4002 Basel, Switzerland

Auxin auxotrophs, temperature-sensitive auxin auxotrophs, and variants resistant to toxic concentrations of auxins have been isolated either by a cloning procedure applied to haploid protoplasts, or by screening M2 families after EMS seed mutagenesis.
This poster presents preliminary physiological and biochemical characterisation of the variants both at the cell culture and at the plant level. (See also the poster by Jurg Oetiker).

L 122 REQUIREMENTS FOR THE INSERTION INTO THYLAKOIDS OF AN INTEGRAL MEMBRANE PROTEIN, LHCP. Bruce D. Kohorn, David L. Murray, and Andrea Auchincloss, Duke University, Durham, NC 27706. The light harvesting chlorophyll binding protein, LHCP, is a member of an integral membrane complex of pigment and protein. LHCP is synthesized in the cytoplasm and transported to the chloroplast where it must pass a double outer membrane, and then insert into the thylakoid. We have identified a carboxy-terminal α-helix of LHCP that is required for the insertion of the protein into thylakoids. The attachment of this α-helix to soluble proteins causes the fusion proteins to associate with thylakoids. We are investigating the role that this α-helix has in causing a protein to integrate into membranes. Other studies are in progress to determine the order in which the membrane spanning α-helices of LHCP insert into the thylakoid. We are also attempting to isolate nuclear mutations in the cellular machinery that interacts with LHCP as it is transported to the chloroplast. We have fused a chloroplast transit peptide to an enzyme whose activity is absolutely required in the cytoplasm. A positive selection for the inability to transport this fusion protein to the chloroplast in transformed plants is being used to identify mutants in Arabidopsis.

L 123 TISSUE SPECIFIC REGULATION OF THE WHEAT GLIADIN GENE PROMOTER IN TRANSGENIC TOBACCO. Douglas J. Leisy, Christopher D. Reeves, and Thomas W. Okita, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340. A region of DNA extending from +18 to -1782 from the translational startpoint of the wheat gliadin gene was connected in frame to a chloramphenicol acetyl transferase (CAT) reporter gene and transferred to tobacco via the *Agrobacterium tumefaciens* based binary vector system developed by G. An (*Plant Physiology* (1986) 81, 86-91). Insignificant levels of CAT activity were detected in the seeds and leaves of 3 transgenic plants tested. Dot blot analysis showed, however, that one plant did exhibit significant levels of CAT specific mRNA in midstage seeds (8-12 days after flowering, DAF), but not in early seeds (6-8 DAF), or leaves. This suggests that the CAT gene in this plant is regulated in a tissue specific manner at the transcriptional level. We are currently assaying extracts from transgenic tobacco plants by Western blot using a CAT specific antibody probe to determine whether or not an inactive or unstable form of the CAT enzyme is produced. We are also constructing plants containing deletions into the 3' and 5' regions of the wheat gliadin gene promoter in order to define cis-acting elements that are needed for its expression and tissue-specific regulation in transgenic tobacco. In addition, a series of 3' deletions is also being connected to both an intact nopaline synthase (NOS) promoter and a disabled NOS promoter containing only the TATA and CAAT motifs but lacking essential upstream elements, to see if: 1) gliadin promoter elements can substitute for the essential upstream NOS promoter elements, and 2) gliadin promoter elements can confer tissue specific expression to the NOS promoter.

L 124 HORMONE-REGULATED GENE EXPRESSION, William R. Marcotte, Jr., Sonja A. Schmitz, Christopher C. Bayley, and Ralph S. Quatrano, E. I. du Pont de Nemours and Co., Inc., Experimental Station, Wilmington, DE 19898.

Two cDNA clones, representing ABA-regulated genes, were isolated from *Triticum aestivum* L. (cv. Chinese Spring) using differential hybridization. The products of these genes were shown to be induced by addition of ABA and the induction was shown to be, at least in part, at the level of transcription. Genomic clones corresponding to these cDNAs were isolated and are now being used for chimeric constructs utilizing a reporter gene which encodes the enzyme β-glucuronidase (GUS). The functionality of these constructs is being tested first in transient assays using both monocot and dicot protoplasts prior to transgenic plant studies. The chimeric genes will be used to delineate the promoter regions necessary for ABA regulation in transgenic plants. The analyses will consist of promoter deletion and site-directed mutagenesis studies. Results from these analyses will be presented.

L 125 **COMPLEMENTATION OF A STABLE, TEMPERATURE-SENSITIVE AUXIN AUXOTROPHIC VARIANT OF HYOSCYAMUS MUTICUS WITH THE AUXIN GENES OF AGROBACTERIUM TUMEFACIENS.** Jurg Oetiker and Patrick J. King, Friedrich Miescher-Institut, Postfach 2543, 4002 Basel, Switzerland

A variant of Hyoscyamus muticus, isolated by a haploid protoplast cloning procedure, has an absolute requirement for auxin for survival when incubated above ca. 29°C. This temperature-sensitive phenotype is expressed both at the ceil culture and at the regenerated shoot level, within four days of the change to the higher temperature. This variant presents a new experimental situation for examining auxin action where a critical change in auxin metabolism/activity can be brought about by a simple temperature switch. The temperature-sensitive trait is complemented by transformation *with genes 1 and 2 of Agrobacterium tumefaciens*. Biochemical analysis of the variant should increase our knowledge of IAA metabolism in plant cells, and the transformation experiments should allow us to define more precisely the relationship between A. tumefaciens and plant cells at the IAA level. (See also the poster by Yvan Fracheboud.)

L 126 EMBRYO-LETHAL MUTANTS OF ARABIDOPSIS THALIANA. David Patton, Joe Shellhammer, Linda Franzmann, Tama Schneider, Ann Reynolds, Ken Robinson, and David Meinke, Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

Arabidopsis thaliana has been described previously as a model system for the isolation and characterization of recessive embryo-lethal mutants. Arrested embryos from 30 mutant lines isolated following EMS seed mutagenesis have been shown to differ with respect to their size and color, pattern of abnormal development, expression of mutant genes prior to fertilization, response in culture, development of homozygous mutant plants, ultrastructure, and extent of cellular differentiation. Complementation tests between mutants with similar phenotypes have demonstrated that 2 mutants (87A and 117N) are allelic. F-2 mapping with standard morphological markers is being used to determine the chromosomal locations of the 115D (green blimp), 112A (rootless), and 122GE (biol) mutant genes in preparation for chromosome walking and molecular cloning. Double mutants have also been constructed to determine epistatic interactions. Additional mutants have recently been isolated from M-2 populations. Several of these mutants have distinctive phenotypes not represented in our initial collection. The frequency of embryonic lethals among M-2 plants appears to exceed that of all other mutant classes combined.

L 127 CHARACTERIZATION OF A TOMATO FRUIT SPECIFIC GENE AND ANALYSIS OF THE TAGGED GENE IN TRANSGENIC PLANTS, Julie R. Pear, Burt Rose, Neal Ridge and Catherine M. Houck, Calgene, Inc., 1920 Fifth St., Davis, CA 95616.

A tomato fruit cDNA bank was sequentially screened using radioactive cDNA made from leaf, immature green fruit and mature red fruit mRNAs. Five clones were selected to determine their expression pattern during fruit development. Northern hybridizations were performed on mRNA from root, stem, leaf and various stages of fruit maturation using each clone as a probe. Each clone was also used as a probe to a Southern blot of tomato genomic DNA cut with several restriction enzymes. One clone, 2A11, was selected as being expressed at all stages of fruit development but not in root, stem or leaf tissue and as being present in the tomato genome as a single copy gene.

A genomic clone corresponding to the 2A11 cDNA was isolated from a tomato genomic library constructed in Charon 35. Sequence comparison of the cDNA clone and the genomic clone showed them to be identical over the region they share in common with the genomic clone possessing a single large intron near the 5' end of the message. The genomic clone was tagged with several different DNA sequences at two sites in the region corresponding to the 3' nontranslated portion of the 2A11 mRNA and reintroduced into tomato plants through cocultivation. Analysis of the transgenic plants is presented.

L 128 TAGGING DEVELOPMENTAL MUTANTS CONTROLLING MORPHOLOGICAL CHARACTERS IN MAIZE, Peter A. Peterson, Iowa State University, Agronomy Dept., Ames, Iowa 50011.
The tagging of developmental mutants by transposons in maize is a most intriguing approach to the study of developmental mutants. Heretofore, a number have been identified and have been properly tagged. The Knotted (Kn) locus in maize with Mu has been a case in point. Other mutants have been tagged and these represent interesting developmental mutants. Also included is the Vp locus tagged by the vp-rcy receptor (Cy:Mu).--Two clear morphological mutants include a developmental mutant controlling the development of kernel size. This is called mn8662248u and was discovered by Y. B. Pan. This mutant has the following characteristics: i) It is not male transmitted; ii) it gives a miniature seed; iii) it is cosegrating with the Uq element. This mutant arose following 5-Aza-2'-deoxcytidine treatment in an attempt to activate Uq. Another mutant arose that was tagged with the receptor (rcy) of the Cy-element. This mutant affects the development of the plant causing many tillers to be formed, and the maize ear to form miniature-type ears or no ears at all, single kernel ears, and varied types of ear development. This arose in the population containing Cy and is dominant. This mutant was discovered by Pat Schnable in a population containing Cy.--Another type of approach is to tag existing mutants. These include the Tassel Seed allele (Ts) that converts tassels to pistillate components, and Teopod (Tp) that grossly alters the morphology with narrow leaves and podded kernels and Tunicate (Tu) that extends glumes on the ear. These experiments are under way.

L 129 DEVELOPING EFFICIENT TRANSPOSON GENE TAGGING SYSTEMS IN PLANTS, Steven R. Scofield and Mike W. Bevan, IPSR Cambridge Laboratory, Cambridge, England CB2 2LQ.
Transposon gene tagging holds great promise for making possible the isolation of genes known only by phenotypes associated with their mutant condition. This approach has made possible the isolation of many interesting genes in organisms with characterized transposon systems. The demonstration that maize Ac and Antirrhinum Tam 3 transposable elements are active when introduced into heterologous plant systems suggests that it may be possible to apply transposon tagging strategies to any plant amenable to transformation. However, the experience of tagging maize genes with Ac and Spm suggests an average frequency of 10^{-5} for mutating target genes. We believe that this low frequency will tremendously limit the general application of these methods in heterologous systems and are using genetic engineering to develop transposon systems designed specifically for efficient gene tagging. Key features of our system are excision markers, making possible selection against non-transposed and therefore irrelevant material and inducible or organ-specific promoters for timely expression of transposase.

L 130 AN EMBRYO-LETHAL MUTANT OF ARABIDOPSIS THALIANA IS A BIOTIN AUXOTROPH. Joe Shellhammer and David Meinke, Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.
Very few auxotrophic mutants have been isolated in higher plants. Most of the genes required for the biosynthesis and utilization of amino acids and vitamins in plant cells remain to be identified. The molecular basis of abnormal development in most developmental mutants of plants also remains to be established. We describe here an embryo-lethal mutant of Arabidopsis thaliana that can be rescued by the addition of biotin to arrested embryos cultured in vitro and to mutant plants grown in soil. This biol mutant of Arabidopsis should provide clues not only to the biosynthesis and transport of biotin in plants, but also to the function of biotin during plant growth and development. Lethal mutants have been used in a variety of animal systems to study the genetic control of morphogenesis and differentiation. Abnormal development has been shown in some cases to be caused by defects in basic cellular processes. The biol mutant of Arabidopsis is to our knowledge the first example of an embryonic lethal with a defined biochemical defect early in embryogenesis that can be corrected by adding a specific nutrient. Whether developmental arrest in other embryonic lethals is caused by similar disruptions in housekeeping functions or by defects in genes that regulate development directly remains to be determined.

L 131 MUTATIONAL ANALYSIS OF THE OCTOPINE SYNTHASE ENHANCER ELEMENT, Karambir Singh, Jeff Ellis, Liz Dennis and Jim Peacock, CSIRO, Division of Plant Industry, Canberra 2601, Australia.
We have identified a 16 base pair, upstream regulatory element of the octopine synthase gene (OCS) which behaves as an enhancer in transient expression assays using plant protoplasts. The element, which is a perfect palindrome, shows no tissue specificity and functions well in both maize, a monocot and tobacco, a dicot. To define presicely the bases responsible for the enhancing effect we have performed a saturation mutagenesis of the 16 bp element by synthesising a collection of degenerate oligonucleotides. Point mutations have been generated throughout the element and their effect on expression assayed by placing them upstream of a crippled maize alchol dehydrogenase 1 (Adh1) promoter linked to the glucoronidase (GUS) gene. The phenotype of these mutants will be presented. The majority of the single base mutants have wild type activity, whereas many of the mutants with 2 or more base changes are 5-30 fold less active than wild type. We have also found that the sequence requirements for OCS enhancer activity differ in transient versus stable assays. Although the 16 bp element is sufficient for full enhancer activity in transient assays, additional sequences are required for full activity in transgenic plants. A 42 bp fragment which contains the 16 bp palindrome and some flanking sequences is sufficient for full activity in stably transformed plant cells.

L 132 REGULATORY CIS-ELEMENTS AND TRANS-ACTING FACTORS INVOLVED IN THE REGULATION OF NODULE SPECIFIC SOYBEAN GENES. Jens Stougaard, Erik Ø. Jensen, Jan-Elo Jørgensen, Niels Sandal, Frans de Bruijn, Jeff Schell and Kjeld A. Marcker. Department of Molecular Biology and Plantphysiology, DK-8000 Aarhus C, Denmark; Max Planck Institut für Züchtungsforschung, 5000 Köln 30, F.R.G.

Nodulins are a group of plant polypeptides specifically synthesized in the nitrogen fixing nodules on leguminous plants. Among the predominant nodulins are the leghemoglobins (Lb). Nodule specific expression of a soybean lbc_3 gene and a nodulin N23 gene is mediated by DNA elements located within the 5' upstream sequences of the genes. This was established by analysing the expression of chimaeric genes in transgenic legumes. The positions of several positive regulatory elements in both the lbc_3 and N23 upstream sequences were mapped by Bal31 deletion series. Furthermore a 37 bp. region containing sequences conserved in other nodulin genes was shown to be required for the nodule specific expression of the lbc_3 gene.

Nuclear extracts from soybean nodules, leaves and roots were used to investigate protein-DNA interactions in the lbc_3 upstream sequence. Two distinct sequences were identified, which strongly bind a nodule specific factor. The position of the two binding sites coincide with one of the positive elements. Competition experiments suggest that both elements bind the same nodule specific factor, although their A-T rich sequences differ substantially. Factors with the same binding properties have also been identified in nuclear extracts from nodules of two other legumes.

L 133 DETECTION AND CHARACTERIZATION OF AN ISOFLAVONE REDUCTASE IN $CUCL_2$-STRESSED GARDEN PEA (*Pisum sativum*), Yuejin Sun, Carol L. Preisig, David E. Matthews, Hans D. VanEtten and Geza Hrazdina, Cornell Univerisity, Geneva, NY and Ithaca, NY.

We are studying enzymes that may be important in the biosynthesis of the pea phytoalexin pisatin. An NADPH-dependent isoflavone reductase, catalyzing the synthesis of the isoflavanone sophorol from 2',7-dihydroxy-3',4'-methylenedioxyisoflavone, was detected in extracts of $CuCl_2$-treated pea seedlings. Extraction and assay protocols were based on those for the analogous enzyme from chickpea (Tiemann et al., 1987, FEBS Letters 213:324-328), which produces sophorol as an intermediate in maackiain biosynthesis. Pea is unusual among legumes in producing the (+) isomer of pisatin, while chickpea synthesizes (–)maackiain. The enzymic basis of their stereospecific biosyntheses is not known; the first potentially decisive step is the isoflavone reductase. In garden pea, the reductase activity appeared to be inducible, and was detected in extracts of 24 and 48 h treated seedlings. Ammonium sulfate fractionation (40-80%) of the crude extract yielded 1.3-fold greater total detectable enzyme activity. Subsequent desalting on Sephadex G-50 yielded a preparation stable for one week at -20°C.

L 134 THE STUDY OF CELL LAYER INTERACTIONS USING GRAFT CHIMERAS OF LYCOPERSICON ESCULENTUM, Gene Szymkowiak and Ian Sussex, Dept. of Biology, Yale University, New Haven, CT 06511

Chimeric plants, whose shoot meristems are composed of cell layers that are genetically different from each other, can be generated by grafting two genetically marked plants and regenerating shoots from the graft junction. In L.esculentum, an abscission zone or joint normally forms at the middle of the pedicel. After fruit maturation abscission occurs at the joint so that the calyx and half of the pedicel remain attached to the fruit. In plants expressing the jointless (j) mutation the abscission zone is not formed so that after the fruit is harvested the calyx and all of the pedicel remain attached to the plant. A chimera has been generated consisting of an epidermis that is wild type for j and the internal tissue that is carrying the jointless mutation. In the chimera the joint is formed at the normal point on the pedicel and abscission occurs as in the wild type plants. The wild type epidermis is interacting with the mutant internal tissue to produce a normal abscission zone. Graft chimeras are being used to investigate a wide variety of cell layer interactions in the development of the tomato plant.

L 135 ANTI-SENSE RNA AS A TOOL TO MANIPULATE METABOLIC PATHWAYS IN HIGHER PLANTS

Alexander van der Krol, Ingrid M. van der Meer, Ronald E. Koes, Anton G.M. Gerats, Joseph N.M. Mol. Antoine R. Stuitje, Free University, Dept. of Genetics, P.O. BOX 7161, 1007 MC Amsterdam

In *Petunia hybrida,* flavonoid genes are expressed primarily in floral tissue and play an important role in flower pigmentation. To study the possibilities of using anti-sense RNA in manipulating metabolic pathways in higher plants we have chosen the flavonoid biosynthesis pathway as a model system. As a consequence a block in enzyme activity in this pathway by the anti-sense RNA approach is expected to influence the flower color phenotype. Chalcone synthase (CHS), the key enzyme in the flavonoid specific pathway was chosen as the model enzyme in this study. In petunia, the CHS genes comprise a multi-gene family of which only 1 member (gene A) is predominantly transcribed in epidermal floral tissue. To ensure the synthesis of relatively high amounts of anti-sense RNA, we have used the constitutive CaMV 35S-promoter to direct anti-sense transcription of a CHS gene A cDNA copy. This construct was introduced in colored *Petunia hybrida* strains and *Nicotiana tabacum* using the *A. tumefaciens* leafdisk transformation system. Upon flowering, the transgenic *Petunia* plants showed a varying degree of flavonoid biosynthesis resulting in almost completely white flowers, flowers with novel color patterns as well as normal colored flowers. Northern blot analysis of RNA isolated from white floral tissue showed an extensive reduction of CHS m-RNA steady-state levels. Preliminary experiments failed to detect double stranded CHS RNA molecules. In the heterologous system, reduction of CHS mRNA steady-state levels was also observed, however transgenic Tobacco plants showing phenotypically the anti-sense effect were found at a lower frequency. Further analysis of these transgenic plants is in progress.

L 136 ABSCISIC ACID IS REQUIRED TO MAINTAIN NORMAL LEVELS OF EMBRYO-SPECIFIC mRNAs IN ZEA MAYS, Bruce A. Williams and Adrian Tsang, Department of Biology, McGill University, 1205 Dr. Penfield Ave., Montreal, H3A 1B1, PQ, Canada.

The involvement of plant growth substances in normal embryonic gene expression in Zea mays has been examined using cloned cDNA as markers. A cDNA library was constructed using poly(A)$^+$RNA from mature embryos as template. Embryo-specific sequences were identified via differential hybridization with end-labelled poly(A)$^+$RNA from embryo or seedling tissues. Northern blot analysis indicates that the levels of mRNA for several of these embryo-specific genes are very low early in embryogenesis, increase dramatically during mid-development, then decline during maturation of the seed. Since a similar profile for abscisic acid accumulation has been described by others in the maize embryo, we examined the effects of this and the other major plant growth substances on the levels of these embryonic genes. When cultured overnight in the presence of 1 µM abscisic acid, mRNA levels are maintained. In the absence of abscisic acid, cultured embryos exhibit a rapid decrease in levels of embryo-specific mRNA sequences and commence precocious germination. Thus, it appears that the expression of these genes may be modulated by abscisic acid.

L 137 MOLECULAR CHARACTERIZATION OF POLLEN SPECIFIC cDNA CLONES FROM TOMATO (LYCOPERSICON ESCULENTUM), Rod Wing, Susan Larabell, Judy Yamaguchi and Sheila McCormick, Plant Gene Expression Center, USDA-ARS, Albany, CA 94710.

Seven pollen specific cDNA clones have been isolated from tomato by differential hybridization (McCormick et al. 1987). Preliminary evidence suggests that some of these cDNAs are expressed in meiotic stage microspores. These clones are being characterized further with respect to: timing of gene expression (Northern analysis), function (DNA sequencing and promoter analysis), genetics (RFLP mapping), and evolution (cross hybridization to genomes of other plant genera). The results of these characterizations will be presented and discussed.

McCormick, S., A. Smith, C. Gasser, K. Sachs, M. Hinchee, R. Horsch, and R. Fraley (1987). Identification of Genes Specifically Expressed in Reproductive Organs of Tomato. In: "Tomato Biotechnology", D.J. Nevins and R.A. Jones, Eds. pages 255-265.

Gametophyte Development

L 200 ANALYSIS OF GENES EXPRESSED DURING POLLEN DEVELOPMENT IN *OENOTHERA ORGANENSIS*, Sherri M. Brown and Martha L. Crouch, Indiana University, Bloomington, IN.

We are analyzing several genes expressed in the mature pollen grain of Oenothera organensis, the evening primrose. Several cDNA clones were isolated by differentially screening a library constructed from mRNA present in mature Oenothera pollen. Northern analysis showed that genes expressed in pollen have a variety of temporal and spatial patterns of mRNA accumulation. One class of cDNAs detected mRNAs only in pollen. mRNAs in this class accumulate in a variety of patterns throughout pollen development, but none were detectable before the mitotic division that produces the vegetative and generative cells. A second class of cDNAs detected mRNAs in other parts of the plant as well as in pollen. These mRNAs, unlike the first class, were detectable at all gametophytic stages tested. Similarly, mRNAS encoding actin, tubulin, and histone were detectable in all parts of the plant, including all stages of pollen development. Experiments in progress address what happens to mRNAs present in mature pollen when the pollen germinate.

We have identified the protein encoded by one of the cDNAs by using antibodies to β-galactosidase/ pollen cDNA fusion proteins. Western blot analysis using these antibodies revealed a protein of 40-45Kd present in mature pollen, suggesting that this mRNA is not stored for translation after pollen germination. We are currently using the antibodies to characterize this protein in more detail by determining its accumulation pattern in pollen development and fate upon pollen germination.

L 201 TOWARD THE CLONING OF GENES REGULATING DEVELOPMENT IN ARABIDOPSIS. Caren Chang, John Bowman, Martin Yanofsky, and Elliot Meyerowitz, Division of Biology, Caltech, Pasadena, California 91125.

Our goal is to isolate genes which control specific developmental steps during floral development in *Arabidopsis*. We have constructed a restriction fragment length polymorphism (RFLP) map in *Arabidopsis* providing molecular markers interspersed throughout its genome. Currently this map consists of more than 80 markers within the five linkage groups with an average of less than 1000 kb between cloned markers. The average distance from a known cloned marker to many genes of interest should be considerably less. These markers provide starting points for chromosome walking and should eventually allow the isolation of any gene for which a phenotype exists. We have aligned this map, or are in the process of doing so, with the positions of a number of floral-specific mutations. These include apetala-2, apetala-3, pistillata, clavata-1, and agamous, some of which may be termed homeotic mutations since they result in the conversion of one floral part to another. In addition, we have constructed a plant transforming cosmid vector which facilitates the isolation of overlapping clones by including SP6 and T7 bacterophage promoters flanking *Arabidopsis* insert DNA. Progress on the RFLP map and on the isolation of clones which complement floral mutations will be presented.

L 202 USE OF MOLECULAR MARKERS FOR THE IDENTIFICATION OF S. LYCOPERSICOIDES ALIEN ADDITION LINES, Roger T. Chetelat, Kevin Alpert and Joseph W. DeVerna, Campbell's Institute for Research and Technology, Route 1 Box 1314, Davis, CA 95616.

A triploid hybrid, comprised of two genomes of L. esculentum and one genome of S. lycopersicoides (LA1964), was crossed as pistillate parent to L. pennellii (LA716). The resulting progeny included aneuploids, which carried extra S. lycopersicoides chromosomes. The tomato molecular map, including isozyme and genomic clone markers, was used to identify the extra chromosomes. Isozyme markers were found for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12. Previously isolated and mapped tomato genomic clones (Tanksley, Miller, Paterson and Bernatzky, Stadler Genetics Symposium, 1987, in press) were used to identify chromosomes for which no isozyme markers were available as well as to provide additional markers for all chromosomes. This application of the tomato molecular map provides a means of estimating the homology between S. lycopersicoides and the Lycopersicon species. Extensive homology was indicated by the presence of linked markers for several addition lines and by the morphological resemblance of all lines to the corresponding tomato primary trisomics. These alien addition lines represent excellent material for mapping new molecular markers and dominant genes governing horticultural traits.

L 203 PRODUCTION OF MONOCLONAL ANTIBODIES TO A VERY LOW ABUNDANCE PROTEIN: PHYTOCHROME FROM GREEN OAT LEAVES, Marie-Michèle Cordonnier,* Sandy Stewart,* Yukio Shimazaki,† Yu-Chie Wang,† and Lee Pratt,† *CIBA-GEIGY Biotechnology, Research Triangle Park, NC 27709, and †Botany Department, University of Georgia, Athens GA 30602.

Phytochrome from 10-day-old, greenhouse-grown oat (Avena sativa L., cv. Garry) leaves, estimated to be no more than about 0.002% of the extractable protein, was purified in the presence of iodoacetamide to minimize its preferential proteolytic degradation. Crude homogenates were clarified by centrifugation and then enriched in phytochrome by sequential polyethylenimine and ammonium sulfate fractionation, followed by hydroxyapatite chromatography. The absorbance ratio at 667 nm and 280 nm after saturating irradiation with far-red light was 0.006-0.009. Assuming that phytochromes from green and etiolated oats have identical extinction, these samples would be 0.6-0.9% pure. Phytochrome for immunization was purified further by sodium dodecyl sulfate, polyacrylamide gel electrophoresis. Following electrotransfer to nitrocellulose, phytochrome bands were excised, and the nitrocellulose was washed with distilled water and dried. The phytochrome-containing nitrocellulose was dissolved in dimethylsulfoxide, emulsified with Freund's adjuvant, and injected into mice. Hybridomas obtained from subsequent fusions were screened both by immunoblotting and ELISA. Five monoclonal antibodies directed to phytochrome from green oats have been obtained. Each recognizes several-fold better phytochrome from green than from etiolated oats, which is the inverse observed with antibodies to phytochrome from etiolated oats. Results obtained with these new antibodies are consistent with the hypothesis that phytochrome from green and etiolated oats are products of different genes.

L 204 SEX-SPECIFIC EXPRESSION OF PROTEINS FROM MICROSPORES AND MEGASPORES, James D. De Camp, Jr. and Augustus E. De Maggio, Dartmouth College, Hanover, NH 03755.
A thorough understanding of the reproductive processes of higher plants requires a knowledge of the mechanisms that control and direct the development of the male and female gametophyte. In flowering plants comparative biochemical analysis of the events in microsporogenesis, megasporogenesis and subsequent maturation of male and female gametophytes is difficult because of the structural inacessibility of the megaspore and megagametophyte. The free-sporing, heterosporous, quillwort, ***Isoetes riparia*** provides an alternative, simpler system for investigating biochemical and molecular aspects of gametophytes. The first step in our investigation is a study of the sex-specific and cell-specific expression of the globulin proteins of microspores, megaspores and corm tissue. Sucrose density centrifugation demonstrated that corm and megaspore globulin extracts contain two proteins with mean peak sedimentation coefficients of approximately 11S and 2S. One- and two-dimensional SDS-PAGE has revealed that microspore, megaspore and corm globulin polypeptides differ in size and charge. Polyclonal antibodies raised against corm globulin protein and affinity-purified to the corm 11S polypeptides cross react with microspore and megaspore polypeptides on immunoblots. Reversed-phase HPLC chromatograms of microspores and megaspores further demonstrated heterogeneity of the polypeptides based on their hydrophobicity. We conclude that the globulins represent a family of proteins expressed in relation to programs of cell differentiation and sex determination. We anticipate that characterization of the genes for these proteins will uncover important regulatory aspects of microspore and megaspore development and the regulation of sex in a higher plant.

L 205 ELECTROPORATION OF MAIZE AND RICE PROTOPLASTS, Jeffrey R. de Wet and Virginia Walbot, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.
Nucleic acids can be introduced into cells by the process of electroporation, the induction of transient pores in membranes by an electric field. This technique can be used to introduce cloned genes into plant protoplasts where they are expressed transiently or in some cases stably. Optimizing the process of electroporation involves a trade-off between factors influencing cell survival and the efficiency of gene transfer. We have introduced the firefly luciferase gene into maize and rice protoplasts by electroporation under a variety of conditions. Some of the factors we have examined for their effects on gene expression after electroporation are: the strength of the electric field, the length of the electric pulse, the ionic composition of the buffer in which the cells are porated, and the temperature at which cells are maintained both before and during electroporation. We found that all cell types are porated most efficiently with a field strength between 500 and 750 volts/cm and with a pulse length of about 10 msec; smaller protoplasts generally required a higher field strength than large protoplasts. Cells survival required that the poration take place in a high ionic strength buffer; cells remained intact during poration in low ionic strength buffers but die soon thereafter. The temperature of the protoplast suspension during electroporation had little effect on the expression of the transferred gene. However, pretreatment of the protoplasts with a heat shock significantly increased the level of gene expression. As a part of this work we tested expression vectors containing various transcriptional enhancers and the 5' untranslated leaders of viral genes that have been shown to affect the efficiency of translation. The results of the experiments with the monocots rice and maize have been compared to the results of similar experiments with protoplasts from carrot cells, a dicotyledonous plant.

L 206 DIFFERENTIAL EXPRESSION OF THE FAMILY OF GENES ENCODING 220KD SUBUNIT OF RNAP II IN SOYBEAN. Margaret Dietrich* and Tom Guilfoyle. *University of Minnesota, St. Paul, MN 55108; University of Missouri, Columbia, MO 65211. A small multigene family of at least 4 members encodes the largest subunit of RNAP II in soybean. All 4 cross-hybridize in DNA hybridization experiments. Two of these genes (A,J) appear to be pseudogenes. Sequence analysis of the 3'end of these genes indicates in frame stop codons in both genes. Restriction fragments containing the 3' end of the pseudogenes hybridize to a 6.5 pA^+RNA, but the signals are weak and may be due to cross-hybridization with pA^+ RNA expressed from the other two genes. Expression from the remaining two genes (B,C) is being examined. The two expressed genes are very similar on the DNA level, including the 3' untranslated region. Using restriction fragments (which are known to cross-hybridize) from the 3' end of these genes as probes on Nothern blots, it appears that B is expressed at a higher level than C in control basal hypocotyl tissue, while C appears to be induced morethan B by 2,4-D treatment in this same tissue. These same probes from B and C hybridize at approximately the same levels to control and treated plumule pA^+RNA, both showing the same level of induction. B is known to be expressed in the treated plumule as a cDNA to B from that tissue has been identified. Synthetic DNA oligomers are being used to examine specific expression.

L 207 STRUCTURE AND FUNCTION OF THE EN/SPM TRANSPOSABLE ELEMENT SYSTEM OF ZEA MAYS

Alfons Gierl, Zsuzsanna Schwarz-Sommer, Peter A. Peterson and Heinz Saedler

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Gene 1 encodes the predominant mRNA of the transposable element En/Spm. The transcript of gene 1 is initiated at a promoter in the left end of the element and spans almost the entire length of the element. The product of gene 1 has been expressed in E. coli and antibodies have been raised against it. The DNA-binding characteristics of this protein to the En-1 element have been analysed. The structure of two mutant En derivatives was also analysed and its relation to the functions of these elements will be discussed. One of the elements is genetically characterized by a drastically reduced mutator (exision) activity, but retains the suppressor activity. The second element encodes a product which inhibits the mutator function of an autonomous element.

L 208 ISOLATION AND CHARACTERISATION OF DEVELOPMENTALLY EXPRESSED GENES FROM MAIZE TASSELS, Andrew J. Greenland, Marie-Marthe Suner, Susan Y. Taylor and Mark Vaudin, Plant Biotechnology Group, ICI International Seeds Business, Jealott's Hill, Bracknell, Berkshire RG12 6EY, U.K.

To analyse gene expression during during male flower development in maize we have prepared cDNA libraries from 5-10 cm tassels bearing premeiotic anthers and 12-20 cm tassels bearing anthers undergoing meiosis (predominantly diad and early tetrad stages).

Each library contains 10^5 clones from which 10^4 clones were selected for differential screening using radiolabelled single-strand cDNA probes prepared from the homologous tassel and from green-leaf poly(A)$^+$ RNAs. The temporal and tissue-specific expression of several cDNA clones arising from the differential screen has been examined by Northern and dot blot hybridisation. We show that these cDNAs represent mRNAs present in maize tassels but which are absent or present at considerably lower levels in the other major organs of the plant. In addition these mRNAs show varying patterns of accumulation during development of the tassel. Currently we are using in situ hybridisation to localise gene expression within developing tassels.

L 209 CHARACTERIZATION OF TUBULIN ISOTYPES IN *Zea mays*. Catherine Joyce and Carolyn Silflow. Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota.

Alpha and beta tubulin are encoded by multigene families in most organisms studied to date. Tubulin isotype expression has been studied in a number or organisms including Drosophila, chicken, rat, and more recently, higher plants. Corn is an attractive system for studying tubulin isotype expression in higher plants because growth and development in this plant are well characterized. Two dimensional gel and western blot analysis of tubulin isotypes in corn(B73) tissues has shown a minimum of three alpha and six beta tubulin isotypes. All three of the alpha tubulin isotypes were observed in all tissues examined, although the relative amounts of the different isotypes showed variation between tissues. α-1, the most acidic isotype, was the major α-tubulin in all tissues examined. α-2 was present in a much greater abundance in pollen than in any other tissue. α-3 was present in much greater amounts in younger/meristematic tissues such as young cobs(1-3.5 cm), embryos, and root tips. A sample composed of root hairs showed that all three of the α-tubulins could be observed in a single cell type. The work on the beta tubulins shows that the most basic isotype is present in much greater abundance in pollen and much lesser abundance in younger/meristematic tissues such as young cob. The abundance levels of this beta tubulin isotype shows a correlation with α-2 levels and an inverse correlation with α-3 levels.

L 210 INTRODUCTION OF NUCLEIC ACIDS INTO ISOLATED MAIZE MITOCHONDRIA, Patricia Leon, R. Michael Mulligan and Virginia Walbot, Department of biological Sciences, Stanford University, Stanford CA 94305

Transcription initiation of mitochondrial genes from different organisms exhibits considerable variation. For example, human mitochondrial genes have a very simple pattern with only two regions where transcription initiates. In contrast, yeast mitochondrial genes have at least nineteen different initiation sites scattered throughout the genome. The transcription of maize mitochondrial genes shows a complex pattern in which both processing and multiple initiation sites are involved (Mulligan et al. submitted MGG). Transcription initiation have been mapped for the genes for subunit 9 of the ATP synthase (atp9) and for the subunit 3 of cytochrome oxidase (cox III), and numerous initiation sites exist for each gene (Mulligan et al. submitted).

The identification of important cis-acting regions involved in transcription initiation of maize mitochondrial genes has been hindered by the lack of an expression system where such genes can be tested. We are presently attempting to establish such an expression system. We have established electroporation conditions to introduce specific DNA molecules into maize mitochondria. Resistance to DNase treatment demonstrates that exogenous nucleic acids have been introduced into the organelle. We have calculated that between 1 to 10% of the input DNA is protected by the organelle. The stability of the DNA introduced into the mitochondria and the metabolic capacity of the organelle over time have also been studied. Finally, we discuss the possibilities of using such a system to study the process of transcription in maize mitochondria.

L 211 MOLECULAR GENETIC ANALYSIS OF THE DEVELOPMENTAL PROGRAM FOR FLOWERING IN TOMATO. Eliezer Lifschytz, Shifra Ken-Dror, Dana Hareven and Nava Hennig, Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel.

For the sake of experimental working hypothesis only floral development is envisaged as being controlled by three major groups of genes. (A) Genes that control the response to the inductive cues. (B) Genes that constantly maintain the induced meristematic cells and their derivatives in the "flowering" state. (C) Genes that in a sequential fashion regulate the differentation of the various floral organs We wish to identify and study the "maintenance" genes (analogous perhaps with Drosophila hemeotic genes) and their interaction with the special organ-regulating genes. We are trying therefore to identify genes and gene products characteristic of the "flowering" as opposed to the "vegetative" state. It is expected from such a "flowering" gene that its product is found in: 1) the earliest possible floral meristems, 2) throughout development, 3) in all floral organs, but, 4) not in the vegetative parts of the plant. The first step therefore is to look for common denominators of the flowering process. For this purpose protein fractions of tomato leaves were compared with fractions from normal flowers before anthesis and flowers of the anantha mutant which serves as a source for presumptive floral meristems. Proteins were divided into soluble, insoluble and nuclear fractions. Soluble fractions were processed on DEAE-cellulose and PC columns, and fourteen proteins representing several hypothetical groups of gene activities were isolated and antibodies raised in rabbits. Polyclonal antibodies were in turn used to isolate cDNA clones from expression libraries of anantha and normal flowers. The analysis of proteins and genes that characterize the flowering process in tomato is illustrated.

L 212 ALTERATIONS ASSOCIATED WITH THE MALE-STERILE CYTOPLASM OF OGURA RADISH. Christopher A. Makaroff, Ingrid J. Apel and Jeffrey D. Palmer, Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Cytoplasmic male-sterility provides a useful system to study nuclear-mitochondrial interactions and their role in plant development. The male-sterile cytoplasm of Ogura radish has been characterized and compared to that of normal radish. Although the two mitochondrial genomes are similar in size and tricircularity, at least ten inversions must be postulated to align the two genomes. Altered transcriptional patterns were identified for three mitochondrial genes, atp6, atpA, and coxI. Radish nuclear genes that restore fertility to the Ogura cytoplasm have no affect on the atp6 and coxI transcripts, but do influence the atpA transcriptional pattern. Detailed characterization of atp6 from normal and Ogura radish indicates that rearrangements have resulted in gene disruption. Rearrangement breakpoints have been identified in Ogura radish both within the atp6 coding region and 100 bp 3' of the gene. The DNA segment comprising the first 9 amino acids and 5' flanking region of the normal atp6 is located 100 kb proximal to the rest of the gene in Ogura radish. Substituted for this segment upstream of the Ogura atp6 is a 105 amino acid ORF that is absent from the mitochondrial genome of normal radish. Short repeated sequences have been identified that may have been involved in generating these rearrangement events.

L 213 MOLECULAR STRUCTURE, AND EXPRESSION OF THE VIVIPAROUS-1 LOCUS IN MAIZE, Donald R. McCarty and Chris Carson, Vegetable Crops Dept. IFAS, Univ of Florida, Gainesville, Florida. 32611.

Viviparous-1 is a regulatory gene required for the expression of multiple genes late in maize seed development. Most notably *vp* mutations block anthocyanin synthesis in the aleurone and developmental arrest in the embryo. In addition, *vp* blocks accumulation of numerous enzymes from unrelated metabolic pathways in the aleurone. *vp* embryos have reduced sensitivity to ABA (Robichaud et al., Dev. Gen. 1:325). We have cloned the *vp* locus by transposon tagging with Robertson's Mutator (McCarty, Carson, Stinard and Robertson, 1987, in preparation). Sequences flanking the cloned Mutator element detect three poly A transcripts (approx. sizes 2500, 1350, and 900 nt) in wildtype developing maize kernels. The largest transcript (2500 nt) is absent in kernels homozygous for the *vp-mum1* and *vp-R* mutant alleles, and expression of the 900 nt transcript is apparently reduced in these stocks. However, the *vp-mum1* and *vp-R* alleles differ in expression of the 1350 nt transcript. This transcript is diminished in *vp-mum1*, but is found at wildtype levels in the *vp-R* stock. While the 2500 nt transcript is clearly implicated with *vp*, the significance of the smaller RNA's and the basis for the allelic differences in their expression remains obscure. We have also analyzed several mutant *vp* alleles at the DNA level. In the *vp-W2* allele, at least 2 kbp of gene are deleted. One breakpoint of the deletion occurs with in the cloned region. In addition, at least four alleles bear evidence of transposable element insertions.

L 214 SMALL AUXIN REGULATED mRNAs IN SOYBEAN, Bruce McClure and Tom Guilfoyle, Dept. Biochemistry, University of MO, Columbia, MO 65211.

We have characterized a group of four small auxin-inducible RNAs using cloned cDNAs. The RNAs display especially rapid induction kinetics. Using RNA blot analysis, accumulation of the RNAs may be detected after a 2.5 min treatment of elongatinghypocotyl sections with 50 M 2,4-D. The accumulation response appears to be half maximal after 10 min and reaches steady-state after 30-60 min. Since other plant growth regulators, metabolic inhibitors and stress treatments do not cause accumulation of the RNAs, we conclude that the response is specific to auxins. In vitro nuclear run-on experiments have shown that the RNA accumulation response is at least in part due to increased transcription. Genomic mapping experiments have shown that the genes encoding the RNAs are tightly clustered in the soybean genome. Sequence analysis suggests that the RNAs encode a family of small proteins of M_r=10kd.

L 215 FUNCTIONAL ANALYSIS OF UPSTREAM ELEMENTS NECESSARY FOR NOPALINE SYNTHASE GENE EXPRESSION, Amitava Mitra and Gynheung An,
Institute of Biological Chemistry,
Washington State University, Pullman, WA 99164.

The regulatory elements controlling the nopaline synthase (nos) gene expression are being identified by stable and transient transformation assay. The upstream control region is composed of a potential Z-DNA element and two pairs of 8 base pairs (bp) and 11 bp repeats. We have previously reported that downstream 8 bp repeat and the Z-DNA element are essential for the promoter activity. We have recently found that the upstream 8 bp repeat is also important for the promoter strength. Deletion of the 11 bp repeats did not affect the promoter activity in transient assay system. Further, deletion of the first three base pairs of the Z-DNA element along with the upstream 8 bp repeat abolished the promoter activity. The effect of the mutations on the promoter characteristics is being studied in stable transformants to identify elements for developmental regulation and tissue specific expression.

L 216 ACETOLACTATE SYNTHASE GENE FROM IMAZAPYR-RESISTANT ARABIDOPSIS THALIANA.

Sathasivan Kanagasabapathi [1], George W. Haughn [2] and Norimoto Murai [1]
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Imidazolinones (imazapyr) and sulfonylureas (chlorsulfuron) are effective herbicides. Though structually unrelated, both herbicides inhibit acetolactate synthase (ALS), or acetohydroxy acid synthase (AHAS), the first enzyme in the biosynthetic pathway of branched chain amino acids. The mechanisms of inhibition of ALS activity by these herbicides differ in both the rate responses and enzyme kinetics. An ALS gene from chlorsulfuron-resistant mutant of Arabidopsis thaliana was shown to express sulfonylurea-resistant phenotype. In order to understand the mechanism of action of imidazolinone herbicides, we have isolated imazapyr-resistant homozygous mutant Imr-3 from A. thaliana (L.) Heynh cv. Columbia. Genetic analysis indicated that resistance is due to a single dominant nuclear mutation and maps to the ALS locus of chromosome 3. Inhibition of ALS activity by imzapyr suggests that the mutant ALS allele encodes the altered enzyme form more resistant to the herbicide inhibition. A genomic DNA library was constructed from the Imr-3 DNA in the lambda vector EMBL3 and screened by an ALS gene probe. A positively hybridizing clone was isolated and purified. ALS gene is subcloned into pUC18/19. The DNA sequence of the ALS gene is determined by the Sanger chain termination method in M13 vectors. The sequences that differ from the wild-type and chlorsulfuron-resistant ALS gene will be identified and implication of such mutation on the protein structure analyzed.

L 217 INVOLVEMENT OF PHOSPHOINOSITIDES IN GIBBERELLIN ACTION,

Pushpa Murthy and Jill Renders, Chem. and Chem. Engrg., Michigan Technological Univ. Houghton, MI 49931

The importance of calcium ions for gibberellin-induced synthesis and secretion of α-amylase in aleurone cells of barley seeds is well-established. The general mechanism by which Ca^{2+} modulates a response is through a change in its concentration. In a large number of animal tissues, cell-membrane phospholipids mediate signal transduction by phospholise-C hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to myo-1,4,5-inositoltrisphosphate (IP_3) which, in turn, increases intracellular Ca^{2+} concentration.

The presence of phosphatidylinositol (PI) in barley aleurone cells is known but there is no report on the presence of phosphatidylinositol-4-monophosphate (PIP) or PIP_2. We have conclusively established the presence of PIP and PIP_2 in aleurone cells by radiolabeling phospholipids with $^{32}P_i$ and ^{3}H-inositol, isolating labeled phospholipids and comparing their chromatographic behavior to standard compounds.

The molecular mechanism by which Ca^{2+} ion concentration is regulated in aleurone cells is unknown. We propose that cell-membrane phospholipids mediate gibberellin action by releasing IP_3 from PIP_2, which, then, increases intracellular Ca^{2+} concentration. To establish the above sequence of events, we pre-labeled phospholipids with ^{3}H-inositol, exposed the cells to gibberellin, entracted phospholipids and inositol phosphates and separated them by chromatography. Preliminary evidence suggests the involvement of phospholipids in gibberellin action (supported by NSF).

L 218 GENE TAGGING IN TOMATO USING THE TRANSPOSABLE ELEMENTS Ac AND Tam 3.

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

Lycopersicon esculentum, the cultivated tomato, is genetically well described, as documented by a linkage- and RFLP map and it is therefore possible to use molecular techniques for the isolation of plant genes. Using an endogenous transposable element for gene tagging in tomato is not yet possible, although instable lines are presently under investigation. Therefore we plan to use a heterologous gene tagging system based on the knowledge that the maize Ac-element can be activated in tomato. To be able to recover putative mutated genes of interest directly, a replicon and resistance marker for E.coli have been inserted into the transposable elements Ac and Tam 3. To monitor transpostion of Ac and Tam 3, avoiding laborious DNA-analysis, we have constructed special vectors. We cloned Ac and Tam 3 transposon cassettes between the CaMV 35 S promoter and the HPT II gene to inactivate this plant selectable marker. Transposition events in transgenic plants will result in excision of the element and restore resistance to hygromycin. Transposition will be assayed both at protoplast and seed level. Integration into nearby located genes can be assayed because this construct is flanked by the NPT II gene and the tms-2 gene.

L 219 SEED STORAGE PROTEIN GENES IN *ARABIDOPSIS THALIANA*, Patty P. Pang, Elliot M. Meyerowitz, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Seed storage proteins are very abundant proteins stored in mature seeds for use during seed germination. The expression of seed storage protein genes is highly regulated and the messages can only be detected in developing embryos, thus make it an ideal model system for the study of gene regulation during embryogenesis. In *Arabidopsis thaliana*, there are at least three 12S globulin storage protein genes which do not cross-hybridize to one another at normal hybridization stringency conditions. Complete nucleotide sequence for two of these genes and partial sequence of the third have been determined. They are about 75% homologous to each other. These genes are present either in single copy or in two copies in the *A. thaliana* genome depending on the ecotype. This low copy number of seed storage protein genes is very unusual since seed storage protein genes in other plants are present in large gene families consisting of very closely related (>90% homology) genes.

The 12S cruciferin genes in *Brassica napus*, which belongs to the same family as *A. thaliana*, are under the control of the plant hormone abscisic acid (ABA). The increase in expression is at the transcription level. The expression of the three 12S globulin genes have been studied in wild type strain, a strain which is deficient ABA and a strain which is has a lowered response to ABA of *A. thaliana*. There is no significant difference in the level of transcripts of these genes in the three strains and the onset of expression is slightly delayed in the mutants. This suggests that there are other factors controlling the expression of 12S genes besides ABA.

To understand the mechanism of regulation of the 12S genes, I have made translational fusions of the alcohol dehydrogenase (*Adh*) gene of *A. thaliana* in order to isolate mutations in trans-acting factors regulating the expression of the seed storage protein genes. We can select against the expression of *Adh* gene by allyl alcohol at the germination stage. These constructs have been introduced into a *Adh* deficient strain of *A. thaliana* using *Agrobacterium* Ti-mediated transformation. *Adh* deficient strains of *A. thaliana* are resistant to allyl alcohol. Preliminary histochemical staining for *Adh* activity suggests that the product of the translational fusion has *Adh* activity. The level of *Adh* activity in different transformed lines of the same construct varies. Characterization of these transformed lines are under way. Since there are different levels of *Adh* activity, the various transformed lines may be sensitive to different doses of allyl alcohol. According to this criteria, seeds from the appropriate transformed lines will be mutagenized and seeds from these mutagenized plants will be tested for germination in the presence of allyl alcohol.

L 220 PURIFICATION, CHARACTERIZATION AND ANTIBODY PRODUCTION OF ACC SYNTHASE FOR CLONING ITS GENE IN ZUCCHINI FRUIT TISSUE. Takahide Sato and Athanasios Theologis, Department of Molecular Plant Biology, U.C. Berkeley, Berkeley, CA 94720, and Plant Gene Expression Center, ARS, USDA, Albany, CA, 94710.

Ethylene, one of the simplest organic molecules with biological activity, regulates various aspects of plant growth and development. Its biosynthesis is enhanced by a variety of plant hormones and environmental stimuli. This enhancement is mediated by regulating the synthesis of ACC synthase, the key regulatory enzyme in the biosynthetic pathway of ethylene. In order to understand the transcriptional regulation of the ACC synthase gene by these various inducers our first goal is to isolate a cDNA clone by antibody screening of expression libraries. In this effort we have purified ACC synthase 6,000-fold from zucchini slices treated with IAA, BA and LiCl, by conventional and high performance liquid chromatography. The enzyme has a specific activity of about 35,000·nMoles·mgr^{-1} hr^{-1}, a pH optimum of 9.5, an isoelectric point of 5.0 and a Km of 17 μM with respect to S-adenosylmethionine. By disk-gel electrophoresis followed by SDS-PAGE the ACC synthase corresponds to a 46 kd polypeptide. However, the mol. wt. of the native enzyme is about 86 kd, estimated by gel filtration, suggesting that the enzyme is a dimer of two almost identical subunits (46 kd each). Concomitantly with the purification and characterization of the enzyme we raised antisera to partially purified ACC synthase. These antisera were purified by affinity chromatography with proteins from the uninduced tissue. Both unpurified and purified antisera immunoinhibit ACC synthase activity in crude enzyme preparations. More importantly, the purified antiserum recognizes only a 46 kd polypeptide which is absent in the uninduced tissue and greatly enhanced in treated tissue. The purified antiserum is currently being used for screening λgt11 expression libraries.

This work was supported by grants from NSF (DCB 84-2157) and NIH (GM-35447) to A.T.

L 221 GAAAAAT IS A SEQUENCE WHERE BENDS OCCUR IN CHROMOSOMAL DNA, Dale M. Steffensen, University of Illinois, Urbana, IL 61801. The genome of Zea mays is organized with highly coiled segments (knob DNA) in the midst of opened-up DNA in the interphase nucleus. The upstream sequences of several developmentally regulated genes have sequence similarities to knob DNA, that is GAAAAAT. Several recent studies with trypanosome and mouse DNA suggest that these GAAAAAT sites (usually in a cluster of four) will bend if complexed with a DNA binding protein. Also these regions won't bend if complexed to A:T binding compounds or dyes. This study will display the uncoiling of knob DNA in maize caused by the A:T binding dye, Hoechst 33258 and by proteases. At the gene regulation level the same methods are applied to Adh1 in presence and absence of the dye. The Adh1 gene has several GAAAT or GTTTGG regions upstream of the TATA box. Other tests of the bend hypothesis for regulation control will be presented. The in vivo methods will allow us to test the role of "the bend" in gene regulation with seedlings. Various other genes with known upstream sequences can be tested for the response to bend inhibition of inducible loci.

L 222 EXPRESSION OF POLLEN-SPECIFIC GENES DURING DEVELOPMENT OF THE MALE GAMETOPHYTE IN NORMAL AND MALE STERILE TOMATOES, Virginia M. Ursin, Judy Yamaguchi and Sheila McCormick, Plant Gene Expression Center, USDA-ARS, Albany Ca. 94710.

Anther-specific cDNA clones were isolated from a tomato cDNA library made from mature anthers and screened with seedling cDNA. Through Northern analysis it was determined that 7 cDNAs corresponding to single copy genes were pollen specific; preliminary evidence suggests that some may be expressed as early as tetrad stage in microspore development. Expression of each of these cDNAs during microspore development, pollen maturation, pollen tube growth and fertilization is being characterized by in situ hybridization. The expression of these clones in several male sterile tomato lines with lesions in various stages of pollen development will be presented.

L 223 INVESTIGATIONS INTO THE ROLE OF CALCIUM IN THE POLLEN - STYLE INTERACTION IN LILIUM LONGIFLORUM THUNB. USING CHLOROTETRACYCLINE AND LANTHANUM. Peter H. Velguth, and Wesley P. Hackett. Dept. of Horticulture, Univ. of Minn., St. Paul, MN 55108

In vivo pollen tubes stained with CTC exhibit densely granular florescence through long portions of either self or cross pollination derived tubes. This pattern differs greatly from reports of CTC induced gradient of florescence in in vitro pollen tubes and our observations of tubes grown in lily stigmatic exudate. This argues for the possibility of a later mode of calcium action in in vivo pollen tube development (than the above cases) which is programmed in the pollen grain given a suitable environment or induced by the style. The style canal cells of lily exhibit florescence in response to CTC which does not change in response to two style treatments which erase incompatibility recognition or upon passage of self or cross pollen tubes. Our studies reveal no differences specific to incompatibility status of either pollen or style tissues in membrane bound Ca and suggest membrane bound Ca is not important in establishing differential growth rates of pollen tubes. Lanthanum (La) is noted as an antagonist of calcium functions and is also employed to trace apoplast space in plant cells due to its electron density and inability to cross plasma membranes. La injected into the hollow style canal of lily immediatly pre-pollination allows self pollen tubes to achieve the growth rates normally exhibited by cross pollen tubes. Although the exact mechanism of La erasure of incompatibility recognition is difficult to establish, the ability of La to erase recognition along with its inability to cross membranes may mean that a component of the reaction occurs in the apoplast. Implications of this result will be discussed in regard to models of incompatibility and different roles of symplast/ apoplast located functions

L 224 MOLECULAR ANALYSIS OF DEVELOPMENTAL PROCESSES DURING PLANT REPRODUCTION. M.M.A. van Herpen, J.A.M. Schrauwen and G.J. Wullems, Department of Molecular Plant Physiology, University of Nijmegen, The Netherlands.

Synthesis of proteins during both germination and pollentube growth, in binucleate pollen is essential for both processes to proceed and runs at a very high rate. It has been shown however that transcription does not take place during pollen germination. Heat stress however is believed to have an effect in pollen both on the level of transcription and on the level of translation and is therefore a good tool to study the molecular factors that regulate gene expression in germinating pollen. It has been shown that following heat shock both survival of pollen of lily and tobacco and a change in protein synthesis after heat shock could be astablished. In Lily transcription during pollen germination can be turned on, in tobacco it is accellerated by heat shock. However, both species show some common proteins as a reaction on heat shock which might serve on identical purpose, i.e. the survival of the pollen and the restart of the growth of the pollen tube. The actual functional relation between these HSP's and the reaction on heat stress will be further investigated.

Gene Expression during Embryogenesis

L 300 PROCESSING OF A BRAZIL NUT SULFUR-RICH SEED PROTEIN IN TRANSGENIC PLANTS. Susan B. Altenbach, Karen W. Pearson, Lisa C. Staraci, Gabrielle Meeker and Samuel S. M. Sun*, The Plant Cell Research Institute, Inc., 6560 Trinity Court, Dublin, CA 94605 and *Department of Plant Molecular Physiology, University of Hawaii, Honolulu, HA 96822.

A 2S protein which is unusually rich in the sulfur-containing amino acids is synthesized in the seeds of Brazil nut. This sulfur-rich protein consists of 2 subunits of 9 kDa and 3 kDa, joined together by disulfide linkages. *In vitro* and *in vivo* labelling studies have demonstrated that both subunits result from the stepwise cleavage of a 17 kDa precursor polypeptide and that the processing involves two intermediate precursors of 15 Da and 12 kDa.

Using a cDNA clone which encodes the sulfur-rich protein, we have constructed several chimeric genes which encode either the 17 kDa precursor or fusion proteins in which the signal peptide from phaseolin has been linked to the 15 kDa or 12 kDa intermediate precursors or to the 9 kDa subunit of the sulfur-rich protein. In an attempt to determine whether all three processing steps are required for the stable expression of the sulfur-rich protein gene, we have transferred these chimeric genes to tobacco and have analyzed the expression of these genes in the transgenic plants. A protein of 9 kDa is accumulated in large quantities in the seeds of plants transformed with the chimeric genes encoding either the 17 kDa precursor or the fusion proteins of the 15 kDa or the 12 kDa intermediate precursors. The sulfur-rich protein did not accumulate in the seeds if only the 9 kDa subunit fused to the phaseolin signal peptide was introduced into the plants. The results suggest that the presence of a signal peptide is sufficient to direct the sulfur-rich protein to the site of subsequent processing steps and that, in tobacco, the absence of the second processing step does not preclude the final processing step. Finally, both subunits of the protein are required for the accumulation of the protein in the seeds.

L 301 EXPRESSION AND ARRANGEMENT OF ALCOHOL-SOLUBLE STORAGE PROTEIN GENES DURING EMBRYOGENESIS IN OAT, Steve Fabijanski, Erik Hansen, Resham Bhella, Marc Giband and Illimar Altosaar, Biochemistry Department, University of Ottawa, Ottawa, Ontario, K1N 9B4, Canada.

CDNA clones have been isolated for an alcohol soluble prolamin from oat developing endosperm tissue. A complete cDNA clone has been sequenced and the corresponding amino-acid sequence deduced. The cDNA clone shows the presence of a 28 amino acid leader sequence that is cleaved at an unusual Tyr↓Glu cleavage site. N-terminal sequencing of the first 19 amino acids of the mature protein verifies this site as the site of cleavage as well as confirming the identity of the protein encoded for by this cDNA. The protein is rich in sulfur containing amino acids, and does not have a typical repeated amino acid sequence arrangement that has been seen in some classes of prolamins from other cereals. One interesting feature of this clone is that it contains in the 3' end of the clone regions of near-exact homology with sequences found at the 3' end of a cDNA clone for the major salt-soluble globulin protein. Southern blot analysis of cultivars demonstrates that this gene is part of a multigene family, and in a cultivar that has been shown to synthesize elevated amounts of this prolamin during seed maturation, the number of genes as well as the levels of mRNA for this protein are greater than in cultivars with normal levels of this protein. These results are discussed in regards to regulation of protein content and packaging in cereal endosperm tissue during embryogenesis.

L 302 COMPARATIVE EXPRESSION ANALYSIS OF LATE EMBRYOGENESIS ABUNDANT (**Lea**) COTTON GENES IN TRANSGENIC TOBACCO AND **ARABIDOPSIS**, Gary T. Anderson, D. Wayne Hughes and Glenn A. Galau, Botany Dept., Univ. of Georgia, Athens, Georgia 30602.

Two cotton genomic clones which encode transcripts expressed specifically in late embryogenesis have been transformed into tobacco and **Arabidopsis** by the **Agrobacterium** binary vector system. Previously, detailed accumulation kinetics studies indicated that the mRNA's encoded by these genes were coordinately regulated during late embryogenesis in cotton, and that their abrupt accumulation during embryo maturation corresponded temporally with embryo abscission from the maternal plant. One of the genes ($\mathbf{Lea}_4$) is present in tobacco and is regulated similarly to that in cotton; the other ($\mathbf{Lea}_{12}$) is not detectable during tobacco embryogenesis. Although we are currently developing a homologous transformation system in cotton, heterologous transformation provides the opportunity to compare the expression of cotton sequences abundant in normal embryogenesis with that in a different nuclear environment. The data presented compares the previously characterized expression of two **Lea** genes with their expression in developing and mature seeds of tobacco and **Arabidopsis.** The regulation of introduced **Lea4** compared with the native tobacco counterpart, and of the introduced D34 which has no native counterpart will be discussed. To identify which controlling regions confer developmental regulation, various putative promoter sequences have been ligated to beta-glucuronidase and transformed into tobacco and **Arabidopsis** and are currently being analyzed.

L 303 GLYCININ EXPRESSION IN TRANSGENIC PETUNIA AND TOBACCO

Barbara L. Ballo, Albert Spielmann, and Robert B. Simpson
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We have inserted an 11S (glycinin) gene from soybean (*Glycine max* L.) into both tobacco and petunia genomes using a disarmed *Agrobacterium tumefaciens* binary vector. Regenerants contain 1-6 copies of the integrated gene. During seed formation transformants accumulate correctly processed glycinin subunits (38.5 kDa acidic plus 19.5 kDa basic; hexamer of paired subunits = 11S), as confirmed by Western blotting and two-dimensional analysis of seed proteins. Additionally, some petunia transformants amass a 55 kDa (7S) protein not found in control seed. This protein was identified as a glycinin precursor by N-terminal sequencing. Although synthesis, modification and storage of the major classes of seed reserve proteins appear to be highly conserved mechanisms in dicotyledonous plants, cleavage of this 7S precursor to the acidic and basic subunits and formation of the hexameric 11S complex does not appear to be critical to successful storage or utilization as a seed reserve. The 55 kDa species comprises up to ca. 6% of the globulin fraction in mature transformed seed and is degraded during germination concomitant with petunia seed proteins.

L 304 PARAMETERS INFLUENCING THE SYNTHESIS OF WHEAT α-GLIADIN IN YEAST, Ann E. Blechl, John I. Stiles* and Frank C. Greene, WRRC-ARS-USDA, Albany, CA 94710 and *Dept. of Plant Molecular Physiology, University of Hawaii, Honolulu, HI 96822.

We are interested in optimizing expression levels of wheat storage proteins in yeast in order to determine the effects of sequence modifications on their properties. Under control of the yeast CYC1 promotor, wheat α-gliadin accumulates to about 0.1% of log-phase cell proteins (Neill et al., Gene 55:303-317, 1987). To achieve higher levels of expression, the gliadin coding region has been placed under control of the strong inducible yeast GAL10 promotor. The construction has been introduced into yeast both as a multi-copy plasmid and as an integrated form in the genome. To attain an anti-codon balance more complementary to gliadin mRNA codons, the same yeast strain has been co-transformed with the gliadin construction and plasmids bearing various yeast tRNA genes. The effects of transcription from the GAL10 promotor, of anti-codon availability, and of cell growth phase on α-gliadin mRNA and protein production in this heterologous system will be discussed.

L 305 DEVELOPMENTAL REGULATION OF CHLOROPHYLL A/B BINDING PROTEIN GENES IN SOYBEAN. Chang, Y.C., Demmin, D.S., Stockinger, E., and Walling, L. L. Department of Botany and Plant Sciences. University of California, Riverside, California. 92521

The genes that code for the chlorophyll a/b binding proteins in soybean comprise a multi-gene family. These genes are regulated at the trancriptional level during development. We have isolated the entire soybean Cab gene family. To date, we have characterized 6 of the Cab gene family members. Two genes are pseudogenes with unique characteristics that have not been described before for other plant Cab genes. Nucleotide sequences for genes 1 through 5 will be presented. Gene specific probes for Cab genes 1 through 5 have been isolated and are being used to determine if Cab gene mRNAs accumulate in an independent or coordinate fashion during embryogenesis, and in the organs of the mature plant. S1 nuclease mapping studies indicate that genes 3,4, and 5 are expressed to high levels in leaves.

L 306 IMMUNO-HISTOLOGICAL LOCALIZATION OF TWO SUCROSE SYNTHASES IN MAIZE, P. S. Chourey[1,2] & Y-C. Chen[2], USDA-ARS[1] & Univ. of Florida[2], Gainesville, FL 32611.
The spatial and temporal distributions of the two sucrose synthaeses, SS1 and SS2, encoded by the Sh and the Ss2 genes respectively have been analyzed in the shrunken (sh) and the wild type genotypes by immunogold silver staining (IGSS) reaction. The SS1 protein was readily detectable in the Sh sectioned endosperm cells at both 8 and 16 days after pollination (dap) stages (much greater abundance at the latter stage). The early expression in the endosperm was localized to the aleurone layer, the crown region and in the area directly above the embryo; the latter expression was seen in both the central and the crown regions. In the embryo, a significant level of SS protein was detectable as a well marked cell specific layer which overlapped with well differentiated pro-vascular cell types. At the cellular level, SS1 protein in endosperm cells was localized adjacent to the cell wall and on the starch grains. In the sh genotype, the IGSS reaction with the SS1 anti-serum detected positive reaction in the aleurone layer and a very weak reaction in the endosperm cells. Use of the SS2 antiserum in sh endosperm sections, however, detected a unique spatial pattern of Ss2 expression: low levels in the crown but moderately abundant levels in the basal region while the embryo staining was similar to the wild type genotype. The central region of the sh endosperm seems to break down (leading to the shrunken phenotype) prior to any deposition of starch grains or the SS protein in these cells. We conclude that there are at least two cell types separable on the basis of spatial as well as temporal expression of the Sh and the Ss2 genes in developing endosperm and embryo of maize.

L 307 EXPRESSION OF KAFIRIN PROTEIN FROM AN UNMODIFIED MONOCOT GENE SEQUENCE IN TRANSGENIC TOBACCO SEED, Richard T. DeRose, Janice L. Anthony and Timothy C. Hall, Department of Biology, Texas A&M University, College Station, TX 77843-3258.

Previous studies that have analyzed the function of monocot promoters in transgenic dicot plants have indicated that dicot plants do not express monocot gene sequences. No monocot gene sequence under the control of its own promoter, including zein from maize, has to our knowledge been successfully expressed as protein in a dicot host. The results presented here represent the first description of protein expression from an unmodified monocot gene sequence. We have previously characterized the 22 kilodalton kafirin gene family, the major storage prolamin in sorghum grain, and found it to be approximately 80% homologous at both the nucleotide and amino acid levels to zein from maize. Two genomic kafirin sequences (clones λGK.1 and λGK.4) have been inserted into separate tobacco plants via *Agrobacterium tumefaciens* mediated DNA transfer. One clone (λGK.4) contains a stop codon within the coding region and was not expressed in transgenic tobacco seed. A second clone (λGK.1), which possesses all the hallmarks of a functional gene and has 98% sequence homology with a kafirin cDNA clone (pSK8), was found to be expressed as protein in transgenic tobacco seed. No kafirin protein was found in transgenic tobacco leaves. The temporal and developmental expression for kafirin protein in transgenic tobacco seeds is currently under investigation and the results of these experiments will be presented.

L 308 HISTOLOGY AND PROTEIN PROFILES DURING SOMATIC EMBRYOGENESIS IN CALLUS CULTURES OF ALFALFA. A.A. El Bakry, D.F. Hildebrand and E.G. Williams. Agronomy Department, University of Kentucky, Lexington, KY 40546-0091.

Full sibs from a cross of alfalfa, Medicago sativa L. cv. 'Regen S', were screened for ability to regenerate by somatic embryogenesis (SE) from mature ovaries explanted onto a modified Blaydes medium containing 2.0 mg/l each of 2,4 dichlorophenoxy acetic acid (2,4D) and Kinetin (K). Genotypes were identified which produced embryos at high and low frequency (30-0 per explant) after 6 weeks in culture. In cultures regenerating at high frequency, proembryonal complexes of 4-8 cells were observed in epidermal and subepidermal tissues at 4-5 days after explanting. Maximum meristematic activity was achieved by 6-7 days, and well formed globular embryos were observed after 2 weeks in culture. Low-frequency genotypes showed prolific callus growth with little organization of compact embryogenic nodules. Proteins from genotypes showing high-and low-frequency SE were radiolabelled for 2 hours in vivo with ^{35}S-methionine after 3, 6 and 14 days in culture, and analysed by two-dimensional gel electrophoresis (1, IEF, 2, SDS-PAGE). Differences were observed between the high- and low-frequency genotypes at day 6 and day 14 in culture. Analysis of SE frequency and protein profiles, of the high regenerating genotype, at a range of 2,4D and K concentrations up to the standard level (2.0 mg/l of each hormone), gave a response curve showing some SE at the lowest auxin level tested (0.1 mg/l).

L 309 SEED-SPECIFIC EXPRESSION OF A PEA LEGUMIN GENE IN NICOTIANA PLUMBAGINIFOLIA,
J Raymond Ellis, Anil H Shirsat, Andy Hepher, Jenny N Yarwood, John A Gatehouse, Ron R D Croy and Don Boulter, University of Durham, Durham, DH1 3LE, UK.

A 3.4-kilobase genomic DNA fragment from Pisum sativum containing the legA gene, which encodes a major legumin storage protein, was transferred to Nicotiana plumbaginifolia using an Agrobacterium tumefaciens strain containing the Bin19 binary vector system. Northern hybridisation analysis of legA-transformed plants demonstrated that legumin-specific RNA was present in developing seeds but not in developing leaves. Legumin protein was immunologically detected in the mature seeds of legA-transformed plants, and was present as the correct-size protein composed of disulphide-bonded polypeptides. It is concluded that the transferred pea genomic fragment contains all the information necessary for seed-specific expression of the legA gene, and for correct processing of the primary transcript and the precursor legumin protein.

L 310 BIOCHEMICAL ASPECTS OF SOMATIC EMBRYOGENESIS IN CULTURED PLANT CELLS
Ravindra N Chibbar, Fawzy Georges, Jerry P Shyluk, Clifford S. Mallard and Friedrich Constabel, Plant Biotechnology Institute, NRC Saskatoon S7N 0W9 Canada.

Two cell lines (Ca-4 and Ca-7) of carrot (Daucus carota L. var Royal Chantaney) derived from hypocotyl tissue were identified which differ in their response to induction of somatic embryogenesis. The line Ca-7 formed somatic embryos when plated on 2,4-D free medium, but not the cell line Ca-4. Two major groups of esterase isozymes were identified on non-denaturing PAGE. In the slow moving group an isozyme 'x' migrated faster in the embryogenic cell line Ca-7 than in the non-embryogenic cell line Ca-4 (Plant Physiol 83(4S):76, 1987). By using the specificity for substrates (1-naphthyl acetate and 2-naphthyl acetate) it has further been shown that the isozyme 'x' belongs to the pseudocholine esterase (E.C.3.1.1.8) group. Work on the characterization and immunological cross reactivity with choline esterases from other sources will be presented.

Cell line Ca-4 hydroxylated more proline than cell line Ca-7 as compared to total proline incorporation in to proteins as determined by an in vivo assay (Plant Physiol 66:1044, 1980). The observation suggests differences in basic biochemical processes in these two cell lines and corroborates the existence of identifiable biochemical differences between carrot cell lines responsive and non-responsive to induction of embryogenesis.

L 311 ISOLATION OF DEVELOPMENTALLY AND EVOLUTIONARILY IMPORTANT GENES IN *VOLVOX*. Jeff Harper, Lai Wa Tam , Judy K. Miller, Sue Goetinck and David Kirk. Washington University, St. Louis Mo. 63130.

The Order Volvocales (with unicellular *Chlamydomonas* at one end, multicellular *Volvox* at the other and several colonial genera in between) is being used to explore the molecular basis for the evolution of three features that characterize development in *Volvox* and many other multicellular organisms: establishment of a germ-soma dichotomy, organization of a complex extracellular matrix (ECM) based on hydroxyproline-rich proteins and stereotyped morphogenetic cleavage patterns.

One of the key genes controlling the*Volvox* germ-soma dichotomy is *regA* which functions to suppress reproductive development in somatic cells. We have initiated a chromosome walk to clone the *regA* gene. One unexpected early finding is that DNA linked to the gene is surprisingly deficient in sites for 4 bp restriction enzymes.

A lambda GT10 cDNA library is being screened in 3 ways for other genes of interest. First, cDNAs with homology to a major *Chlamydomonas* hydroxyproline rich ECM gene have been cloned and a sequence comparison with the *Chlamydomonas* prototype initiated.

Second, a differential screen for developmentally specific cDNAs has generated 120 cleavage-specific clones and several hundred additional clones specific for a developmental stage dominated by ECM production.

Third, a differential screen for evolutionarily novel cDNAs (i.e. genes present in *Volvox* but not *Chlamydomonas*) has been initiated. Our first estimate is that 3% of the clones in our *Volvox* cDNA library fall in this class. To date, 6 of 7 *Volvox* specific clones examined in detail show homology to different repetitive gene families, many of which are missing from all but the most advanced genera in the order. One clone is a single copy gene and shows no homology to the more primitive Volvocalean DNAs analyzed. The class of clones of special interest are those that are both developmentally regulated and specific to the more advanced Volvocales--3 clones in this class have been identified.

L 312 TISSUE SPECIFIC EXPRESSION OF A WHEAT α/β GLIADIN STORAGE PROTEIN GENE IN TOBACCO, Patrick H. Higgins and J. Antoni Rafalski, E. I. Du Pont de Nemours & Co., Inc., Wilmington, DE 19898

pW1215 is a genomic clone representative of the α/β gliadin family of wheat storage protein genes. These genes are expressed in a tissue specific manner and the products are packaged into membrane bound protein bodies located in the endosperm of the grain. The 5' promoter region of 1215 contains a putative "upstream regulatory element" differing from the consensus element by 1 bp. The 1215 gene was expressed in E. coli. Transformant bacterial extracts have been shown to be immunoreactive with gliadin rabbit antisera. The 1215 native gene and 1215 structural region regulated by the CaMV 35S promoter and nos 3' polyadenylation signals were separately used to transform tobacco via the Ti plasmid. Plants were regenerated and assayed for the tissue specificity of the 1215 gene expression. The 1215 gene product was not detected immunologically in tissues of transformed plants. RNA was isolated from whole plantlets, leaves, and tobacco seeds, 20 days post-pollination. The transgenic plants with the native gene have 1215 mRNA in developing seeds but not in leaves or whole plantlets, whereas, transgenic plants with the 1215 gene regulated by the 35s promoter have 1215 mRNA in the leaves. Thus the native 1215 wheat gene appears to be developmentally regulated in tobacco. To increase the level of expression of the 1215 gene while retaining the tissue specificity, we are altering the promoter and examining the expression of other storage protein genes.

L 313 MODULAR ACCUMULATION OF TRANSCRIPTS IN MATURING COTTON EMBRYOS, D. Wayne Hughes, Robert E. Wyatt, and Glenn A. Galau, Botany Dept., Univ. of Georgia, Athens, Georgia 30602.

Forty random cDNA clones isolated from cotton dry mature and immature embryo cDNA libraries were used in three ways. First, developmental changes at the molecular level during the maturation period of cotton embryogenesis were examined. The associated transcripts were grouped into four major classes according to the kinetics of accumulation. These represented a maturation class (**Mat**), a late embryogenesis abundant class (**Lea**), a maturation repressed class (**Mar**), and a germination class (**Grm**). The superimposed patterns of all forty transcripts indicated that critical points in the accumulation occurred at common times and that the accumulation curve for any given class of transcripts could be constructed by combining one or more of a minimal number (6) of basic component curves. Second, the array of clones was applied to analysis of embryos excised at different stages and incubated on various media. Data are presented to show that embryos excised before the last 10% of embryogenesis precociously germinate vigorously, but do not terminate several existent embryo programs and prematurely move into the late embryo maturation phase. Mature dry embryos, immature embryos within the last 10% of embryogenesis, or younger embryos predried before incubation all behave similarly in that embryo programs are turned off and germination programs are turned on. Finally, these data will also invoke the necessary existence of a maturation factor not abscisic acid, and will underscore the critical effect of removal of the embryo from the maternal environment.

L 314 PREPARATION OF VIABLE PROTOPLASTS FROM GRAPEVINE CALLUS SUSPENSION CULTURE CV.CABERNET SAUVIGNON AND SUBSEQUENT REGENERATION TO PLANTS, Pavel G.Kovalenko,Anatoly P.Galkin,Institute of Bioorganical Chemistry,Acad.Sci.of the Ukrainian S.S.R,Kiev-94,252660,USSR

For study of any genetical manipulation in Grapevine most effectively use suspension culture and isolated protoplasts.This is very good model for study cell-parasite interactions.Protoplasts were prepared from cell suspension cultures-derived from cv.Cabernet Sauvignon callus,which had been initiated from the young stems and leaves on modfied MS(Murashige-Skoog) medium supplemented with mg/l:BAP(0.5-2.0),NAA(2.0-4.0).Callus was subcultured during 4 months,then from it was obtained suspension culture which was treated following enzyme formulations which showed that the more rich percent of suspension cells could be converted to viable protoplasts:(% %) Cellulase 0.5,Pectinase 0.5,Driselase 0.5;0.6Msorbitol,0.5mM MES,0.05M glicine,and salts as $MgCl_2$ $7H_2O$ and $CaCl_2$ the concentration of which was raised to 2mM. Protoplasts purification required only simple washing without damaging centrifugation and its was cultured on modified H(Nitsch) medium with addition of mg/l:BAP(o.2-2.0),NOAA(2.0-5.0),BSA(5000),CH,0.5M glucose and low concentration of sucrose and mannitol.On the 10 day of cultivation cell clusters were washed in fresh medium and plated in 0.45% agarose.The cultured protoplasts produced numerous procallus colonies after 4 weeks. Yet 5 weeks ago was obtained regenerants on modified 1/3 MS medium.

L 315 OPTIMIZATION OF TOBACCO PROTOPLASTS TRANSFORMATION BY RECOMBINANT DNA,P.G.Kovalenko,N.N.Domansky,A.P.Galkin,Institute of Bioorganical Chemistry,Acad.Sci.of the Ukrainian S.S.R. Kiev-94,252660,USSR Stable transformation of tobacco protoplasts at frequencies of up to 10^{-3} -10^{-2} has been obtained.The plasmid,containing kanamycin resistance gene with tobacco nuclear gene promoter was constracted and introduced into pla nt cells by direct gene transfer.Transformation was carried out as follows Aliquots of freshly isolated protoplasts at a density of 2 10^5per ml,were suspended in W5 medium and subjected to a heat shock followed by a cold shock for mitotic synchronization.Than 20 mg plasmid DNA and 10mg calf thy mus carrier DNA with suplemented of 45% w/v PEG solution,in 0.45 M manni- tol,5% DMSO,17mM $MgcI_2$ and 0.05M glycine,were added stepwise.Twenty minu- tes after addition DNA and PEG ,protoplasts were washed with Ca^{2+} -glycine buffer at high pH(10) and plated.The trasformation frequency was calculate from the number of micro-colonies entering selectin with lethal dose of kanamycin.

L 316 SEED-SPECIFIC GENE EXPRESSION IN TRANSGENIC RAPESEED REGULATED BY A NAPIN PROMOTER, Jean C. Kridl, David W. McCarter and Beth M. Andrews, Calgene, Inc., 1920 Fifth St., Davis, CA 95616.

Napins are a family of seed storage proteins of *Brassica napus* (rapeseed) expressed exclusively in the embryo of developing seeds. The genes encoding napin proteins would be expected to have regulatory sequences that direct embryo-specific expression. A cloned napin gene containing 300 nucleotides of 5'-noncoding sequence was modified by insertion of DNA tags in both the coding and 3'-noncoding regions. The tagged napin gene was reintroduced into rapeseed via *Agrobacterium tumefaciens* transformation of *B. napus* hypocotyl explants. The engineered napin gene was expressed in developing embryo tissue of regenerated plants and not in leaf tissue, but at a level much lower than the endogenous napin gene. The addition of 1.8 kb of 5'-noncoding sequence did not alter the tissue specificity or level of expression of the engineered gene. The longer napin promoter and 3'-noncoding sequences were used to express a spinach leaf acyl carrier protein (ACP) gene in transgenic rapeseed. The napin/ACP gene is expressed seed-specifically and during the appropriate developmental stages for the napin promoter. A comparison of the expression levels of the napin/ACP gene and the tagged napin genes will be presented and discussed in relation to mRNA stability.

L 317 ACCUMULATION AND DEGRADATION OF STORAGE PROTEINS IN MAIZE EMBRYOS, Alan L. Kriz, Department of Agronomy, University of Illinois, Urbana, IL 61821.

Two types of storage proteins are present in maize embryos: the saline-soluble, water-insoluble globulins are the major protein reserves and the alcohol-soluble prolamins (zeins) are the minor reserves. This situation is the reverse of that in the endosperm, where zeins predominate and globulins are present at low levels. In the embryo, both types of proteins accumulate in specialized structures called protein bodies and are rapidly degraded during germination. As a first step to understanding the differential expression of genes encoding storage proteins in the embryo, we have determined the accumulation patterns of globulins and prolamins during seed development. We have also examined embryo storage protein profiles in maize mutants that are deficient in the production of either globulins or prolamins and found that, in some cases, a decrease in amounts of one type of protein is compensated by an increase in levels of the other type. This suggests that some mechanisms of gene regulation function to compensate for specific deficiencies in the storage protein complement of maize embryos. The timing of embryo and endosperm storage protein degradation in germinating seeds of normal and mutant genotypes are also described here. (Supported in part by a grant from the Standard Oil Company)

L 318 DEVELOPMENTAL REGULATION OF PROTEIN SYNTHESIS DURING FUCUS EMBRYOGENESIS, Darryl L. Kropf, Roswitha Hopkins and Ralph S. Quatrano, Oregon State University, Corvallis, OR 97331.

Zygotes of the brown alga Fucus provide an excellent system for studying cell development in plants. The spherical eggs, both before and soon after fertilization, are apolar. Yet, 1 day later highly localized growth has produced a rhizoid protuberance that gives the embryo a pear-shaped appearance. Our goal is to understand the molecular and cellular basis for the establishment of this polarity. Toward this goal, we have used two-dimensional gel electrophoresis to investigate the changes in protein synthesis that accompany the earliest developmental events. Proteins from radioactively-labeled embryos at 8 distinct developmental stages were analyzed by silver staining, fluorography, and immunoblotting. Ten of the predominant zygotic proteins, including actin and -tubulin, were clearly regulated. The sensitivity of zygotic protein synthesis to external factors, specifically light and developmental inhibitors, was also assessed. Studies employing the transcriptional inhibitor actinomycin D suggested that most of the synthesis during the first day of development was supported by maternal mRNA stored in the egg.

L 319 MOLECULAR MARKERS FOR THE STUDY OF EARLY EMBRYOGENESIS IN COTTON, Sandra E. Meyer and David W. Galbraith, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

The earliest stages of embryogenesis in cotton, Gossypium hirsutum, have been studied using a novel immunological approach. An antibody library was created against globular and early heart-staged zygotic embryos of the cultivar Coker 201. After extensive cross-absorption to extracts of mature seed which had been immobilized on nitrocellulose paper, the antiserum showed strong reactivity to several proteins unique to the early-stage extract, and no reactivity to the mature seed extract. Two major proteins appear to be strongly immunogenic in the early-stage extract, as evidenced by the strong reactivity of their antibodies at high dilutions of antiserum. The presence of these two proteins has been assayed by Western blotting in a variety of other developmental stages and tissues of the zygotic seed and plant, as well as various stages of somatic embryos and callus. Proteins identified in this way represent possible developmental markers for the earliest stages of embryogenesis in cotton.

The storage proteins of mature zygotic seed are present in all stages of somatic embryos, as well as in embryogenic callus. They are not present at significant levels in the early zygotic seed extract, thus indicating that there are differences in gene expression throughout development in the zygotic and somatic systems.

L 320 MOLECULAR STUDIES OF THE HIGH LYSINE GENES OPAQUE-2 AND OPAQUE-6 IN MAIZE, Natale Di Fonzo, Monica Brembilla, Hans Hartings, Massimo Maddaloni, Jaume Palau, Gioacchino Ponziani, Francesco Salamini, Carlo Soave, Richard Thompson and Mario Motto, Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, 24100 Bergamo (Italy).

The high lysine mutants of maize change the endosperm protein pattern in a nutritionally favorable manner. The mutants reduce the level of zeins, the major and nutritional poor endosperm storage proteins of maize. At the molecular and biochemical level, the mechanism of action of the high lysine genes regulating the rate of zeins deposition are largely unknown. However, from the interactions between mutant alleles of these genes, it appears that at least two independent pathways are active in controlling zeins deposition. One pathway involves the Opaque-2 (O2) and the Opaque-6 (O6) genes. Recessive o2 and o6 endosperms not only have a reduced zeins content, but are also devoid of a monomeric cytoplasmic protein with an apparent M.W. of 32 kD (b-32); they are also altered in the level of the enzyme lysine-ketoglutarate reductase involved in lysine catabolism. Taken together, the available evidence suggests that O2 and O6 may influence zein deposition acting on the availability of some amino acids important for zein synthesis.

In order to study at the molecular level the b-32 gene, which appears to play a key role in mediating the action of the O2 locus, cDNA clones, containing sequences related to b-32 were isolated by screening a cDNA library in the expression vector gt11. The nucleotide sequence of this putative full-lenght mRNA clone contains an open reading frame of 909 nucleotides which codes for a polypeptide of 34 kD. The deduced amino acid composition compares favorable with the amino acid composition of active b-32 protein determined by amino acid analysis.

As far as the O2 locus is concerned, genetic analyses suggested that this locus codes for a positive, trans-acting, transcriptional activactor of the zein protein genes. Because the molecular cloning of the O2 locus and the analysis of its structure and expression, could contribute to an understanding of the nature of its regulatory effects, transposon tagging with the transposable element Ac (Activactor) was carried out and 14 mutable o2 alleles were obtained. One of these alleles (o2-m5) contains autonomous Ac element. A DNA sequence flanking the autonomous Ac insertion was found to be o2 specific and provided a probe for the molecular analysis of the O2 locus.

L 321 REGULATION BY ABA OF A HIGLY PHOSPHORYLATED 23-25 kDa OF PROTEINS IN ZEA MAYS EMBRYOS. M. Pagés, A. Goday, D. Sánchez-Martínez, J. Gómez, P. Puigdomènech. Dept. Genética Molecular, C.I.D. (CSIC). 08034 Barcelona (SPAIN)

We have identified a set of proteins of 23-25 kDa covering a pI range of 6.2-8.2, which accumulate gradually during normal embryogenesis of Zea mays and disappear in early germination. These polypeptides can be induced prematurely in immature embryos by abscisic acid (ABA) treatment. Now we report that the most acidic protein forms are due to post-translational modifications of the 23 kDa, pI 8.2 and 25 kDa, pI 8.0 polypeptides, which become highly phosphorylated. A polyclonal antiserum was raised against the 23 kDa protein band which accumulates in mature embryos, and its specificity analyzed by immunobloting of total protein extracts and immunoprecipitation of "in vitro" poly (A) RNA translation products. It was found that this polyclonal antibody recognizes both the 23 kDa and 25 kDa bands and their phosphorylated forms. Primary characterization of cDNA clones corresponding to these ABA induced proteins was done by size selection of the poly (A) RNA from mature embryos by means of high resolution messenger affinity paper (mAP). Labeled single stranded cDNA from this selected mRNA hybridizes specifically with several cDNA clones from a library constructed with poly (A) RNA from mature embryos. Clone MA2F12 was chosen for further studies. By RNA northern hybridization analysis, the RNA encoded by clone MA2F12 is shown to be induced in young embryos upon ABA incubation and to accumulate in mature embryos. Hybrid selection experiments demonstrate that clon MA2F12 is able to identify specifically a discrete closely related group of polypeptides in the 23 kDa and 25 kDa protein set, which are also recognized by the antiserum.

L 322 NUCLEOTIDE SEQUENCE OF A BARLEY GENOMIC CLONE FOR CHYMOTRYPSIN INHIBITOR-2, D.M. Peterson*, J. Forde, M.S. Williamson, W. Rohde+ and M. Kreis, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Biochemistry Department, Harpenden, Herts, AL5 2JQ, U.K. +Max-Planck Institut, 5000 Köln-30, F.R.G.

The chymotrypsin inhibitors 1 and 2 (CI-1 and CI-2) are present in increased amounts in the grain of the high-lysine barley, Hiproly, which carries a "regulatory" mutant gene (*lys1*). Both CI-1 and CI-2 are encoded by small multigene families whose members are expressed in a strict developmental and organ-specific manner. Whereas mRNAs for CI-1 are found only in the developing seed, transcripts for CI-2 have also been detected in leaf tissue. Unlike the endosperm genes, the leaf gene(s) are not under control of the regulatory *lys1* gene. As a step towards understanding the processes which result in these different patterns of expression, we have isolated and characterized an endosperm gene for CI-2 from barley (cv. Vogel-Sanger Gold). The gene is contained on a 5.1 kb EcoRI fragment, and the coding region has been located near the 3' end by restriction mapping. The gene has been sequenced and contains one small intron of 90 bp within the 5' unstranslated region of the mRNA. We will report on our results comparing the 5' and 3' flanking regions with those of other seed protein genes for putative cis-acting regulatory sequences.

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L 323 CONTROL OF CEREAL EMBRYOGENESIS AND THE REGULATION OF GENE EXPRESSION BY ABSCISIC ACID (ABA)*, Ralph S. Quatrano, William R. Marcotte, James C. Litts, Christopher C. Bayley and Sonja A. Schmitz, Central Research & Development Department, Experimental Station, E402, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

Our major goal is to understand the controls operative in the expression of genes and gene blocks during cereal embryogenesis. A set of defined culture conditions allow isolated 10-15 day old cereal embryos to either precociously germinate, or to undergo normal maturation and accumulation of stored reserves. The growth regulator ABA controls this developmental switch *in vitro* and there is strong evidence for a similar role of ABA *in vivo*. Over a 3-5 day culture period in the presence of ABA (1-100μM), immature wheat embryos increase in fresh and dry weight and undergo an accelerated but normal morphogenesis and accumulation of mature embryo proteins. In the absence of ABA, this maturation program is not observed and the embryo germinates into a normal seedling. Some proteins that accumulate in the presence of ABA have been identified and include the lectin wheat germ agglutinin, an abundant protein found in the mature embryo (Em protein) and the globulin storage proteins. Characterization of these genes and the pattern of accumulation of their respective proteins and mRNA's during grain development, and in culture with ABA, revealed that regulation of these genes by ABA occurs at the level of transcription and mRNA stability. Comparison of genomic sequences of these genes revealed that the globulin sequence from wheat shared limited but distinct amino acid sequence homology with the 7S globulins from bean (phaseolin), soybean (conglycinin) and pea (vicilin). However, a strong similarity was noted throughout the protein when hydropathy plots were compared. Sequences 5' to the transcription start site for these three genes that are regulated by ABA did not reveal any region of homology. Constructs of the intact and deleted 5' as well as the 3' regions with the glucuronidase (GUS) reporter gene have been tested in transient assays using protoplasts from monocots and dicots. The same constructs, as well as others containing the entire gene were used in Agrobacterium-mediated gene transfer to selected dicots. Results from both the transient and stable transformation assays will be discussed in relation to the cis-acting sequences involved in the ABA regulation.

* A portion of this research was completed by Ralph S. Quatrano and James C. Litts at Oregon State University (Department of Botany) with support from the USDA Competitive Grants Program (84-CRCR-1-1380).

L 324 DEVELOPMENTAL AND HORMONAL REGULATION OF WHEAT LECTIN mRNA IN EMBRYOS AND ADULT PLANTS, Natasha V. Raikhel, Sebastian Bendarek and Thea A. Wilkins, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

We have investigated the developmental and hormonal regulation of wheat germ agglutinin (WGA) mRNA in wheat embryos and root tips of adult plants. During embryogenesis, synthesis and localization of WGA are temporally regulated and appear to be under the control of abscisic acid (ABA). In hexaploid wheat, WGA consists of 3 isolectins derived from three genomes (A,B and D). We isolated and sequenced a cDNA clone encoding isolectin B of WGA. Southern blot analysis of genomic DNA derived from hexaploid and diploid wheats indicated that (under stringent conditions) this clone could be used as a gene-specific probe for isolectin B. This clone was used as a probe in Northern blot analysis to ascertain the levels of lectin mRNA: 1) during development, and 2) in the presence of exogenous ABA. The results showed that the mRNAs accumulated during embryogenesis and decreased prior to seed dehydration. However, level of the WGA mRNA increased again during germination and was detected in the roots and shoot-base region of adult plants. WGA mRNA was not found in leaf tissue. The level of WGA mRNA increased in immature embryos and during germination in the presence of exogenous ABA.

To analyze the cellular location of isolectin B mRNA, sense and anti-sense transcripts were used in in situ hybridization experiments. The expression of specific mRNA to an anti-sense transcript in the various embryonic tissues and in the adult plants was compared with the localization of the encoded protein.

L 325 MOLECULAR BASIS OF ORGAN DEDIFFERENTIATION IN MONOCOTS, S. Ramagopal, USDA-ARS, Hawaiian Sugar Planters' Association Experiment Station, Aiea, HI 96701.

Many of the developmental processes in plants are plastic in nature. One prime example of such a process is somatic embryogenesis. An essential prerequisite for successful somatic embryogenesis is the ability of a target or responsive cell to undergo dedifferentiation and become meristematic. Elucidating the nature of such target cells appears paramount in understanding somatic embryogenesis and cell lineage in plant development. While we know that such cells are apparently distributed among almost all developed organs in a mature plant and their presumed anatomical location, currently we have practically no knowledge of their biochemical characteristics. Further, although the target cells can be functionally induced by hormones such as auxin, the intracellular molecular responses are unknown. To understand the biochemistry and genetics of dedifferentiation, gene expression was analyzed during the initial phases of dedifferentiation as well as in the steady state cultures of dedifferentiated cells from immature embryos and young roots of barley. The preliminary findings suggest that at least ten unique proteins are induced during dedifferentiation and their expression was independent of the primary donor organ or genotype. The implication of these results will be discussed.

L 326 INDEPENDENT REGULATION OF ABA INDUCED EVENTS IN MAIZE EMBRYOGENESIS, Carol Rivin, Oregon State University, Corvallis, OR 97331

Abscisic acid plays a major role in embryo development, including the inhibition of precocious germination and the induction of embryo maturation proteins. To understand how ABA coordinates different developmental events in maize, we examined the behavior of a set of stage specific embryo polypeptides when normal embryogenesis is disrupted genetically or by experimental manipulation. Changes in polypeptide profiles on 2D gels were followed over the course of normal maize embryo development. The accumulation of many of these proteins appears to require ABA: 1. They are absent in developing viviparous mutant embryos which lack embryo ABA, but they can be restored when these embryos are grown in the presence of ABA in culture, 2. This set of polypeptides accumulates precociously in wildtype embryos cultured in the presence of ABA. Our experiments show that precociously induced polypeptides appear at earlier stages, but in the same order as seen during normal development, suggesting that acquisition of a developmental competence (which may or may not be related to ABA) is required for their accumulation. Culturing staged embryos on high osmoticum leads to the accumulation of a small subset of the ABA regulated polypeptides. Late in seed development, exogenous ABA no longer prevents precocious germination in tissue culture, but it is still effective maintaining the accumulation of the major storage polypeptides.

L 327 **CHARACTERIZATION OF THE 12S GLOBULIN IN B.NAPUS: SEQUENCE OF A CRUCIFERIN PRECURSOR**, J. Rödin, M. Ericson, M. Lenman L-G. Josefsson and L. Rask, Dep. of Cell Research, BMC, Swedish University of Agricultural Sciences, Uppsala, Sweden

The complete protein sequence of a subunit pair in the 12S globulin (cruciferin) of rapeseed has been deduced by sequencing a cDNA clone. By comparing the cDNA sequence with the amino acid sequence from purified polypeptides, it has been possible for us to establish the processing of the cruciferin precursor. From a rape genomic library in the phage Lambda vector EMBL3 several cruciferin clones were isolated using the cDNA as a probe. The genomic clones were characterized by restriction enzyme mapping. These data together with data obtained from Southern blotting analyses of rapeseed DNA, indicate that this subunit pair of cruciferin is encoded by a gene family at most 3-5 genes. We are presently characterizing the cruciferin gene organization with special interest of potential regulatory elements to make possible further studies of these genes and their interaction with regulatory DNA binding proteins.

L 328 PRIMARY EFFECTS OF ALLELOCHEMICALS: DETOXIFICATION MECHANISM DURING ABNORMAL SECONDARY GROWTH OF THE CELL WALL, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.
Some evidence indicates that cellular metabolism in sunflower seedlings is disrupted by thornapple alkaloids (Levitt et al., 1984. New Phytol. 97:213), and as well, may include structural damage to organelles in root tip cells. The concentrations 0.05% alkaloid applied being sufficient to reduce radicle elongation. The nucleus may be much reduced in size. The allelochemical- treated cells indicate a probable slowing down of the metabolism of food reserves (during the early phases of germination). Microbodies and mitochondria have been the subject of recent research. They are known to participate in the uptake of oxygen and the respiratory gas exchange of cells (Hall et al., 1982. Plant Structure and Metabolism, Longman, London). It is possible that the increased size amyloplasts and number of microbodies in root tip cells of sunflower treated with allelochemicals tends to confirm the hypothesis that the detoxification effect of thornapple alkaloids is to interfere with metabolism of energe sources in the cells of germinating seedlings.

L 329 TEMPERATURE-SENSITIVE VARIANTS IN CARROT SOMATIC EMBRYOGENESIS, Jennifer A. Schnall[1], Todd J. Cooke[1], Cheol Ho Hwang[2], and J. Lynn Zimmerman[2], University of Maryland, [1]Department of Botany, College Park, MD 20742, and [2]Department of Biological Sciences, Catonsville, MD 21228. Using a filtration-enrichment selection procedure, we have isolated 21 temperature-sensitive variants in carrot somatic embryogenesis, which develop mature plantlets at the permissive temperature (24 C) and altered phenotypes at the restrictive temperature (33 C). According to their 33 C phenotypes, these ts variants can be placed into six general classes. The No Growth variants fail to grow in embryogenic medium without 2,4-D at 33 C. The Callus Proliferation variants produce abundant callus cells at 33 C. Some Globular-stage Block variants display enlarged globular embryos at 33 C, while others produce normal-sized, arrested globular embryos which develop secondary globular embryos in temperature-shift experiments. The Oblong-stage Block class forms enlarged elongate embryos that lack cotyledons. The Lateral Growth variants consist of modified heart embryos with several outgrowths on their hypocotyl surfaces. Finally, the Root Formation variant produces numerous roots in temperature shifts. The temperature-sensitive periods were determined for all ts variants in order to disclose the timing of gene action. We are currently evaluating mRNA accumulation (by differential hybridization) and protein synthesis (by 2-D PAGE) at different embryonic stages in several variants.

L 330 DIFFERENTIAL INDUCTION OF CYTOCHROME P-450 MONOXYGENASES, Cassie B. Stewart, David H. Brandwein, Wolfgang H. Baur and Mary A. Schuler, University of Illinois, Urbana, IL 61801.

Cytochrome P-450 monoxygenases are enzymes that play key roles in the detoxification of plant xenobiotics, in the biosynthesis of plant hormones and, most importantly, in the production of key intermediates in the synthesis of lignins and flavenoids. Like mammalian P-450 monoxygenases, the plant monoxygenases are localized in microsomal membranes, require O2 and NADPH for catalytic activity and are induced by a multitude of exogenous stimuli. The most prominant plant P-450, trans-cinnamic acid hydroxylase (t-CAH), is a core enzyme required for the biosynthesis of phenylpropanoid products. As such, it is regulated by a wide variety of stimuli including development, wounding, and the application of auxins and herbicides. We have demonstrated that endogenous levels of t-cinnamic acid hydroxylase are differentially regulated in a manner which reflects the lignin biosynthetic profile of seedling tissue. Using CO-difference spectra to evaluate total P-450 content, we have demonstrated that the t-CAH monoxygenase is selectively activated by wounding. In seedlings, which contain a large number of cell types, this activation is highest in the basal sections of pea epicotyls. Thus, induction is influenced by the developmental stage of the tissue which suggests that some plant cells more competently induce the phenylpropanoid pathway. Using a P-450 antibody we have demonstrated that the plant microsomal P-450s are as heterogeneous in size as the mammalian P-450 monoxygenases. Using this information, we have screened an induced cDNA library from basal epicotyl cells for clones encoding t-CAH.

L 331 MOLECULAR CHARACTERIZATION OF OAT SEED GLOBULINS, Mark A. Shotwell, Ruth S. Chesnut and Brian A. Larkins, Purdue University, West Lafayette, IN 47907.

The major seed storage protein of oats is a 12S globulin that has many structural characteristics in common with the 11S storage globulins of legumes. It is deposited in seeds as 320-kD hexamers, each consisting of six 53-kD subunits. Each globulin subunit is composed of a 35-kD acidic polypeptide disulfide-bonded to a 23-kD basic polypeptide. We have isolated full-length cDNA clones corresponding to oat 12S globulin mRNAs from a λgt11 cDNA library. The longest of these clones, pOG2, encodes a complete precursor subunit with a signal peptide of 24 amino acids followed by an acidic polypeptide of 293 amino acids and a basic polypeptide of 201 amino acids. Amino acid comparisons show that the oat 12S globulin is 30-40% homologous with storage globulins of legumes, but 70% homologous with the rice storage glutelin. We estimate there to be 15-20 copies of the oat globulin gene per haploid genome. We have isolated oat globulin sequences from a lambda genomic library and found that the oat globulin gene consists of four exons separated by three short (117-126 bp) introns, similar to most other 11-12S storage globulin genes. We are analyzing the 5′ non-coding regions of the oat globulin gene for their ability to direct the seed-specific expression of heterologous genes in transgenic plants.

L 332 A NUCLEAR PROTEIN AMPLIFIED DURING SOMATIC EMBRYOGENESIS OF DAUCUS CAROTA L. J. Smith*, C. Borkird, M. Krauss, R, Sung, *Eni-Chem Americas, Monmouth Junction, N.J. 08852.

The monoclonal antibody, MAb21D7 has enabled us to study a protein found in nuclei, especially the nucleoli, of rapidly dividing plant cells. The nuclear protein is amplified during the early phases of somatic embryogenesis. This same protein is also found in meristematic regions of the plant such as apical and root meristems, zygotic embryos and early stages of floral bud development. Extracts of small spherical cells collected from suspension cultures contain 4-10X the amount of protein found in more elongated cells from the same culture. Micro-injections of MAb21D7 into single carrot cells stop cell division while serum injected cells resume divisions after a brief lag. Current studies utilizing the methods of electron microscopy and immunogold-conjugated antibodies will further elucidate subcellular localization of the protein during embryo formation. Ultimately we may be able to predict which cells of heterogeneous cell cultures will give rise to somatic embryos and what conditions are most favorable for their growth.

L 333 A CHIMERIC GENE CODING FOR A CUCUMBER MOSAIC VIRUS (CMV) SATELLITE RNA MONOMER CONFERS TOLERANCE TO CMV INFECTION

Mark Tepfer, Joëlle Amselem, Laboratoire de Biologie Cellulaire, INRA-Versailles, 78000 Versailles, FRANCE,
Mireille Jacquemond, Station de Pathologie Végétale, INRA-Montfavet, 84140 Montfavet, FRANCE

Cucumber Mosaic Virus (CMV) has a wide host range, and is a significant disease of many crop plants including cucumber, melon, tomato, tobacco, green pepper, lettuce and banana. Certain strains of CMV harbor satellite RNAs, which when present attenuate symptom intensity on most host plants. Using the Ri plasmid of Agrobacterium rhizogenes as a vector, we have introduced a chimeric gene coding for a satellite RNA monomer into tobacco plants. Upon infection with a CMV strain lacking satellite RNA, the pre-satellite RNA transcript is matured to appropriate size and replicated to high levels. Replication of genomic viral RNA is inhibited to levels similar to those observed when control tobacco plants are infected with CMV bearing satellite RNA. Transgenic plants expressing the satellite RNA gene displayed essentially no symptoms on infection with CMV.

L 334 TEMPORAL EXPRESSION AND SPATIAL DISTRIBUTION OF SPECIFIC POLY(A) RNAS DURING THE DEVELOPMENT OF CARROT SOMATIC EMBRYOS, Thomas H. Ulrich, Basil J. Nikolau, Russel H. Bell, Robert A. Millman, and Eve Syrkin Wurtele, NPI, 417 Wakara Way, Salt Lake City, UT 84108.

We have developed a methodology for the physical separation of embryogenic and non-embryogenic cells of carrot growing in the presence of the auxin, 2,4-D. These two populations of cells isolated from the same environment respond differentially when placed in media lacking 2,4-D: the embryogenic cells develop into embryos via the developmental pathway globular, heart, torpedo and germinating embryos at a rate over 1000-fold higher than that of the non-embryogenic cells. To investigate the molecular basis for this differential behavior of these cells, we have cloned seven cDNAs representing specific poly(A) RNAs whose accumulation is altered during this developmental process. Characterization of these poly(A) RNAs by northern blot analysis indicates that their accumulation is temporally manifested in response to the developmental program of the cells and not just in response to auxin treatment. In addition, in situ hybridization techniques have been utilized to determine localization of the poly(A) RNAs in developing embryos. The temporal expression and spatial distribution of representative clones will be presented.

L 335 HEAT SHOCK mRNAs ARE EXPRESSED DURING SEED DEVELOPMENT IN PEA, Elizabeth Vierling and Aiqun Sun, Biochemistry Department, University of Arizona, Tucson, AZ 85721.

We are interested in determining the biological roles of heat shock proteins (HSPs) in higher plants, and, in the long term, understanding the molecular mechanisms of HSP function. We have begun to investigate the expression of HSPs during seed development in pea. cDNA clones encoding HSP70 and two low molecular weight cytoplasmic HSPs, HSP20 and HSP19, were isolated from a library constructed from leaf heat shock mRNA. The cDNAs were used to probe Northern blots of total RNA isolated from developing pea seeds (17 and 23 DAF), dry seed tissues, or seeds imbibed for 7 hours. Seed samples were either obtained from commercial growers (field grown), or grown under controlled greenhouse or growth chamber conditions. The results show that all three mRNAs are abundant in both embryo axes and cotyledons of dry seeds grown under field or controlled conditions. From preliminary quantitative analysis we estimate that the HSP mRNA level in dry seeds is 10-20% of that found in leaves following a two hour heat shock (compared to <0.5% in control leaves). The mRNAs disappear rapidly upon imbibition; after 7 hours the mRNA levels are less than 50% of the amount seen in the dry seed. Analysis of developing seeds indicates HSP mRNA may begin to accumulate as early as 17 DAF. Whether these mRNAs are translated, or only stored in the seed, has not been determined.

L 336 EXPRESSION ANALYSIS OF NORMAL AND MUTANT PHYTOHEMAGGLUTININ GENES, Toni A. Voelker, Arnd J. Sturm, Daniel Riggs, Corinne Dorel and Maarten J. Chrispeels, Department of Biology C-016, University of California, San Diego, La Jolla, CA 92093.
The lectin phytohemagglutinin (PHA) is encoded by two tandemly-linked genes, dlec1 and dlec2, encoding the polypeptides, PHA-E and PHA-L, respectively. In most cultivars of *Phaseolus vulgaris*, PHA is an abundant seed protein (2-3% of total protein), while in the cultivar Pinto, there is no PHA-E and very small amounts of PHA-L. We sequenced the respective genes of the Pinto cultivar (Pdlec1 and Pdlec2) and monitored the accumulation of their transcripts (Voelker et al., EMBO J., 5:3075-3082, 1986). We used four approaches to analyze the regulatory sequences of the PHA genes: sequence comparison of the 5'regions, transformation of tobacco with dlec2 and Pdlec2 and analysis of the gene products, Bal31 promoter resection of dlec2 coupled to an analysis of the gene products, and use of the 5' upstream sequence with a reporter gene. These experiments narrow down the regulatory region to a portion of the upstream 5' end of the gene. Of particular interest is the finding that the difference in expression of dlec2 and Pdlec2 observed in the bean is maintained in tobacco. In addition, we have found that the PHA genes in Pinto are highly methylated relative to their homologues in a normal cultivar. Promoter swaps of the various genes are now in progress.

L 337 TRANSFORMATION OF RICE, WHEAT AND SOYBEAN BY THE MICROPROJECTILE METHOD, Yi-Chang Wang, Jun Cao, John C. Sanford and Ray Wu, Cornell University, Ithaca, NY 14853.
Particle gun can accelerate small tungsten particles (microprojectiles) carrying foreign DNA to high velocities, thus penetrating into and subsequently transforming cells. Enzyme assay two days after bombardment of tungsten particles coated with plasmid containing β-glucuronidase gene on *Oryza sativa* and *Triticum monococcum* cell suspension showed a number of blue cells for each bombardment. Control samples bombarded with particles but without DNA did not give blue cells. The number of blue cells was used as a convenient criterion of transformation efficiency to study the effect of various factors in the bombardment. Using the same method, we also transformed cell suspension of *Glycine max* using chloramphenicol acetyltransferase (CAT) gene as the reporter.

Gene Activation after Germination

L 400 ISOCITRATE LYASE GENE EXPRESSION IN SUNFLOWER, Randy D. Allen, Richard N. Trelease, Terry L. Thomas, Washington University, St. Louis, MO 63130.
A cDNA sequence that encodes sunflower seedling isocitrate lyase (ICL) was isolated from a λ gt11 cDNA library prepared from sunflower seedling mRNAs. The library was screened for recombinants that expressed ICL specific fusion polypeptides using antisera directed against cotton seedling ICL. This cDNA (ICL13) hybridized with a 2 kb transcript in sunflower seedling RNA. ICL mRNA was first detectable in maturing sunflower seeds 19 days after pollination and it remained at a constant low level through seed desiccation. The prevalence of ICL transcripts increased by about 10 fold during the first 2 days after inbibition (DAI) in darkness and began to decline by day 5 after imbibition. Germination of sunflower seedlings in light promoted the accumulaton of ICL transcripts to levels approximately 2 fold over those observed in corresponding dark grown seedlings. After reaching peak levels, prevalence of ICL transcripts in light grown seedlings declined very rapidly, reaching dry seed levels by 5 DAI. Isocitrate lyase polypeptides followed a similar pattern of accumulation and decay in light and dark grown seedlings. Expression of ICL in sunflower is developmentally regulated and is modulated by exposure to light. Mechanisms that control these processes appear to function at the level of mRNA accumulation.

L 401 DEVELOPMENTAL REGULATION OF *MUTATOR* ACTIVITY IN MAIZE, W. E. Brown, P. S. Springer, A. D. Cresse, D. S. Robertson, and J. L. Bennetzen, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.
Transposable element *Mu1* is present in 10-70 copies in *Mutator* lines but is present in 0-4 copies in other maize lines.[1] Elements with the same ends as *Mu1* but different internal sequences are present in all maize lines investigated at copy numbers of 20-60. Several *Mutator*-induced *Bronze* mutations (*bzMum*) were structurally analyzed, and all show insertions of a *Mu1*-like element at various positions within the *Bronze* structural gene. Loss of *Mutator* activity can be accompanied by covalent modification of the *Mu1*-like elements.[2,3] Crossing a plant containing modified *Mu1* elements to a plant containing unmodified elements often leads to modification of all the elements in the progeny.[4] While early leaves have a mixture of modified and unmodified *Mu* elements, later leaves and gametic tissues often contain only modified elements. Modification of some sites is more closely associated with inactivation of *Mutator* activity than is modification at other sites. The modification is specific for the *Mu* elements and does not extend into flanking DNA. Germinal and somatic reversion of *bzMum4* occurs late in development. These revertants are due to a relatively precise excision of the *Mu1*-like element from the *Bronze* locus.

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[2] Walbot, V *et al* (1985) UCLA Symp Mol Cell Biol 35, 333.
[3] Bennetzen, JL (1985) UCLA Symp Mol Cell Biol 35, 343.
[4] Bennetzen, JL *et al* (1987) Mol Gen Genet 208, 45.

L 402 CLONING OF THE PHYTASE GENE FROM GERMINATING SOYBEANS. Alice A. Christen, Beverly Montalbano, and Donna M. Gibson, USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179.
The acid phosphatase, phytase, degrades phytic acid in germinating soybean seed releasing stored phosphate and other minerals e.g., calcium and magnesium. Engineering of the phytase gene might therefore increase the nutritive value of soybeans. Phytase is present in soybean cotyledons 8-12 days after germination. RNA was isolated from germinating cotyledons at five days, prior to the period of maximum phytase activity. Chromatography of total RNA on oligo-dT cellulose resulted in a very low proportion (0.16%) of mRNA. Double-stranded cDNA was synthesized from mRNA and was used in the construction of a cDNA library in the lambda ZAP vector. Polyclonal antibodies to soybean phytase were used to screen for characterization. Work is in progress to characterize these clones by restriction mapping, hybrid select translation, and Northern blotting.

L 403 STAGE-SPECIFIC EXPRESSION IN THE SHOOT APEX DURING EARLY FLORAL DEVELOPMENT, Nathan M. Chu and Ian M. Sussex, Yale University, New Haven, CT 06511
Biennial Hyoscyamus niger (henbane) has an obligate requirement for low temperature followed by long days for flowering. We have described eight developmental stages in the reproductive meristem which are observed when a cold-induced plant is grown under long day illumination (>16 h). Four of the stages (stages I-IV) describe the early phases of development in the meristem during its conversion to reproductive growth. The latter four stages (stages V-VIII) describe the initiation of the four types of floral organs (sepal, petal, stamen and carpel) in the developing floral bud. Biochemical analyses, employing two-dimensional gel electrophoresis (2-D Page), of in vivo labeled polypeptides found in the meristem demonstrate changing patterns of expression which are shown to occur in a stage-specific manner during early floral development. In addition, we have also examined the patterns of newly synthesized polypeptides found in the meristem during vegetative growth under different day length conditions (either long or short days), and during and after cold-induction. These changes in the 2-D page patterns of newly synthesized polypeptides are taken to be representative of changing patterns of gene expression which are responsible for and underlie the morphological changes of the meristem during early floral development. This study serves as a foundation for continuing investigations of the molecular aspects of floral induction and early floral development.

L 404 STRUCTURE AND EXPRESSION OF BRASSICA NAPUS MALATE SYNTHASE GENES, Lucio Comai and John J. Harada, Botany Dept. University of California, Davis, CA 95616.
The glyoxylate cycle has a very specialized role in seedling development; it functions in the conversion of lipid reserves into carbohydrate needed for growth. Malate synthase, a key enzyme in this pathway, is only detected in developing and germinating seeds. Therefore, accumulation of the enzyme is highly developmentally regulated. To begin study of mechanisms involved in regulating malate synthase genes, we isolated B.napus cDNA clones. Each clone reacted with a single size class 2.1 kb mRNA. The nucleotide sequence of the longest cDNA clone revealed an open reading frame which specifies a 558 amino acid polypeptide that is highly homologous to cucumber malate synthase. To analyze malate synthase gene expression, RNA dot blot experiments were done. The studies showed that malate synthase mRNA begins to accumulate during late embryogenesis, is highly abundant in postgerminative cotyledons, and is at very low level in leaves. Genomic DNA gel blot studies and restriction mapping of genomic clones suggest that the enzyme is encoded by a gene family. (Supported by NSF Grant No. DCB-85 18182).

L 405 SPATIAL AND TEMPORAL EXPRESSION OF GERMINATION INDUCED GENES IN *BRASSICA NAPUS*, Robert A. Dietrich and John J. Harada, Botany Dept. University of California, Davis, CA 95616.
The regulation of germination induced genes in *Brassica napus* is being studied as a model for the developmental regulation of gene expression in plants. Developmentally regulated genes that are coordinately expressed may share regulatory elements that are important for their correct developmental expression. To help identify coordinately expressed germination induced genes, we have used *in situ* hybridization experiments to study mRNA accumulation at the tissue level. We found that mRNAs which are prevelent in seedlings can have a complex spatial distribution pattern in embryos and germinating seeds. For example, the amount of malate synthase mRNA increases in the cotyledon from 0 to 48 hours after imbibition, and is evenly distributed in the tissue. In contrast, this message becomes less abundant in the axis over the same time period. Also, while it is evenly distributed in the seed axis, it becomes localized in the cotyledon end of the seedling axis as germination progresses. This detailed information on where and when these genes are expressed should be useful in identifying coordinately induced genes and facilitate the identification of regulatory elements.

L 406 FACTORS CONTROLLING DIFFERENTIAL EXPRESSION OF GLUTAMINE SYNTHETASE GENES IN DEVELOPING ROOTS AND NODULES OF *PHASEOLUS VULGARIS*, Brian G. Forde, Hazel M. Day & Alison P. Hopley, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, U.K.
The glutamine synthetase (GS) gene family in *Phaseolus vulgaris* consists of five known members, which we now refer to as *gsα*, *gsβ*, *gsγ*, *gsδ* and *gsε*. The first three genes code for the α, β and γ subunits that, in various combinations, constitute the cytosolic isoenzymes in different organs. *gsδ* is the gene for the subunit of the chloroplast isoenzyme and *gsε* is a fourth cytosolic-type gene located 3-4 kb downstream of *gsγ*. Previous work has shown that *gsα*, *gsβ* aand *gsγ* are differentially expressed at the mRNA level during plant development (Gebhardt *et al.* 1986, EMBO J. 5, 1429-35). Most notably, *gsβ* is expressed at 20 fold higher levels in roots than in leaves, while expression of *gsγ* is nodule-specific. We have cloned *gsβ* and *gsγ* (with part of *gsε*) and are investigating the *cis* and *trans* factors that regulate their expression.
In one approach we employ a functional assay for *cis*-regulatory sequences that involves fusing the GS promoters to a bacterial reporter gene (GUS) and introducing the constructs into *Lotus corniculatus* via *Agrobacterium rhizogenes*. The transformed roots are nodulated with *Rhizobium loti* and GUS activity is assayed in tissue extracts and by histochemical staining. First experiments indicate that a 2 kb fragment from the 5'-flanking region of *gsγ* is able to direct nodule-specific expression in transgenic plants. A 'gel retardation' assay is being used to detect specific DNA-protein interactions that may be involved in eliciting the host reponse to Rhizobial infection.

L 407 PHYTASE EXPRESSION DURING SEED GERMINATION, Donna M. Gibson[1], Kevin C. Vaughn[2] and Alice A. Christen[1], [1]USDA, ARS, SRRC, New Orleans, LA 70179 and [2]USDA, ARS, Southern Weed Science Lab, Stoneville, MS 38776.
The enzyme phytase may be a model system for examining gene activation after germination since it is active during seed germination as it catalyzes the hydrolysis of phytic acid, the principal storage form of phosphorus in seeds. Soybean phytase reached a peak of activity from day 8 to day 12 in cotyledons of germinating seedlings. The increase in phytase, as indicated by activity or Western blotting, could be partially inhibited by the addition of α-amanitin or cordycepin during the first day of germination, while cycloheximide showed the greatest loss by the third day of germination. Addition of 100 mM phosphate during seed germination also inhibited the increase in phytase. Immunocytochemical staining with purified antisera followed by immunogold labelling of cross-sections of cotyledons of germinating seedlings showed little phytase accumulation by 3 days post-germination, but the staining in protein bodies greatly increased from day 5 to day 10. A cDNA library from five day old cotyledons of germinating seedlings is under evaluation to identify phytase-specific clones.

L 408 HEAT SHOCK RESPONSES DURING EARLY GERMINATION OF ISOLATED EMBRYOS OF WHEAT (*Triticum aestivum*, L.). Kenneth Helm, N. Petersen, R. Abernethy. University of Wyoming, Laramie, Wy. 82071.

We are testing the hypothesis that the ability to synthesize heat shock proteins is an accurate indication of seed vigor, and that this response may promote survival of environmental stresses during early germination. We have compared the relative production of seven hsp's produced in embryos isolated from high and low vigor lots from Nugaines (N83 and N80) and Wanser (W83 and W80) Wheat. Seed vigor was determined by the accelerated aging (high temperature and humidity) test. The relative vigor results were: N83 = 100, N80 = 49, W83 = 100, W80 = 76. Following 1.5 or 12 hrs of imbibition, embryos from each lot were simultaneously labeled with ^{35}S Methionine and subjected to a 42° C heat shock for 90 min. Relative heat shock responses (rhsr), defined as the total area of hsp's 97, 94, 83, 70, 68, and a 17-18 Kd doublet divided by the total area detected on a given lane, were compared between high and low vigor lots from each variety, for both imbibition times. At 1.5 hr. of imbibition, N80 showed a 22.8% decline in rhsr compared to N83, and W80 showed a decline of 22.9% of rhsr compared to W83. At 12 hrs of imbibition, there were no detectable differences in rhsr between the high and low vigor lots of either variety. Uptake and incorporation analyses of protein synthesis in the different lots demonstrate that protein synthesis rates of low vigor embryos are adversely affected to a much greater degree under heat shock conditions than those of high vigor embryos, particularly at very early times of imbibition. Two dimensional gel analysis of the high and low vigor lots show both quantitative and qualitative reductions of the heat shock response in the low vigor embryos. These results support the hypothesis that hsp's are an accurate indicator of seed vigor. The results of two dimensional gel analyses also demonstrate several developmentally regulated, qualitative changes in the heat shock response as germination of the wheat embryos progresses.

L 409 POSTGERMINATIVE REGULATION OF GENES ENCODING PEROXISOMAL PROTEINS IN CUCUMBER COTYLEDONS, John McC. Hunter, B. W. Schwartz, J. S. Sloan and W. M. Becker, University of Wisconsin, Madison, Wisconsin 53706.
The development of peroxisome-specific proteins in cucumber cotyledons is regulated by light during germination. The relative levels of translatable mRNAs encoding the peroxisomal proteins hydroxypyruvate reductase (HPR) and serine:glyoxylate aminotransferase (SGAT) are also regulated by light with a developmental pattern similar to that found for the corresponding proteins. To study further the regulation of the synthesis of these peroxisome-specific proteins, we have synthesized a cDNA library, and isolated HPR-specific and SGAT-specific cDNA clones. The cDNA was produced from poly $(A)^+$ RNA isolated from cotyledons of seven-day light-grown seedlings. This cDNA was then cloned into lambda gt11, and HPR and SGAT clones were isolated using antibodies against the corresponding proteins. Confirmation of the clone identities is in progress using affinity-purified antibodies, antibodies raised against the corresponding fusion proteins, and sequencing of the clones and the corresponding polypeptides. Preliminary Northern blot analysis shows that the SGAT and HPR transcript levels are regulated in the same pattern as the corresponding protein levels. A detailed analysis is underway of these transcript levels in light-grown versus dark-grown seedlings, and in seedlings shifted from the dark to the light after five days.

L 410 Development of Plants from Cultured Shoot Apices of Maize. Erin Irish and Timothy Nelson. Dept. of Biology, Yale University, New Haven, CT 06511

Mature maize plants can develop from isolated and cultured shoot apices, which consist of the the exposed meristem plus one or two leaf primordia. Most culture-derived maize plants have fertile male and female inflorescences and are morphologically normal, with respect to number of leaves at flowering, location of the uppermost ear, and the progression of leaf lengths along the stem. In addition, characteristics considered juvenile, such as the waxy bloom and the leaf striping mutation zb4, are expressed on the lower leaves of the culture-derived plants. Plants derived from shoot apices isolated and cultured at different stages all develop 18-20 leaves, the number produced in seed-grown plants. Determination of the shoot apical meristem in maize occurs very late in development and may be coincident with the switch to floral development.

L 411 GERMIN, A BIOGENETIC SIGNAL OF GROWTH IN GERMINATING WHEAT, IS A GLYCOPROTEIN, Anna Jaikaran and Byron Lane, Biochemistry Dept., University of Toronto, Toronto, Canada (M5S 1A8).

Germin is a water-soluble and pepsin-resistant homopentameric protein (Mr 125 kDa) whose synthesis signals the onset of growth in germinating wheat. The glycoprotein nature of germin was indicated by its reactivity with Schiff reagent, after periodate oxidation, and by its selective labeling when germinated embryos were cultured in media containing [^{3}H]mannose, [^{3}H]fucose or [^{3}H]glucosamine. Because it is refractory to dissociation in SDS-containing media at room temperatures, germin can be seen as an oligomeric doublet in SDS-polyacrylamide electrophoretograms. Double-labeling experiments with [^{3}H]glucosamine and [^{35}S]methionine reveal that the slower-migrating form of germin contains twice as much glucosamine as the faster-migrating one. Apparent resistance to Beta-endo-*N*-acetylglucosaminidase H, glycopeptidase F and alkaline borohydride treatment has prevented quick characterization of glycoprotein linkages. The presence of *N*-linked oligosaccharides in germin is suggested by the accumulation of a series of partially glycosylated germin pentamers when embryos are germinated and cultured in the presence of tunicamycin. These partially glycosylated oligomers are seen as a cluster of 8 bands in SDS-polyacrylamide electrophoretograms: all are pepsin-resistant, all react with antibodies raised against the germin doublet from untreated embryos, and most yield (differing proportions of) 2 monomers, one believed to be fully glycosylated, the other believed to be unglycosylated. The slowest of the 8 oligomers made in the presence of tunicamycin co-migrates with the slower component of the germin doublet (made in untreated embryos), and yields only glycosylated monomer, whereas the fastest-migrating of the 8 ('tunicamycin') oligomers yields only unglycosylated monomer. Concanavalin-A affinity columns selectively adsorb 5 of the 8 pentamers from tunicamycin-treated embryos and partially resolve the two forms of germin present in extracts made from untreated embryos.

L 412 GENE EXPRESSION DURING MERISTEM INITIATION AND ORGANIZATION, Nancy M. Kerk and Ian M. Sussex, Yale University, New Haven, CT 06511.

The initiation and organization of meristem function in localized groups of cells at specific sites is a major event in establishing the patterns of plant development. Yet, little is known of the major molecular genetic events that underlie the developmental pathways of meristem formation, or how these events are related to observable changes in cellular and tissue structure. We have begun to investigate these questions by analysing the initiation and organization of lateral root meristems in radish (Raphanus sativus) where large numbers of meristems can be induced to develop synchronously and rapidly. By analyzing patterns of protein profiles generated by two dimensional gel electrophoresis of in vitro translation products, we have determined times at which to produce cDNA libraries. Clones have been isolated from subtracted cDNA libraries and are being used as probes for genes which are expressed uniquely or preferentially in a stage specific manner in the organizing meristems. These probes are being used to examine the tissue and cellular location of gene products, and to determine whether homologous genes are expressed in other types of meristems.

L 413 CLONAL ANALYSIS OF PHOTOSYNTHETIC CELL-TYPES IN MAIZE LEAVES. Jane A. Langdale and Timothy Nelson, Biology Department, Yale University, New Haven, CT 06511.
Photosynthesis in C4 plants requires Kranz type leaf anatomy in which the veins running the length of the leaf are surrounded by two layers of photosynthetic cells. The inner bundle sheath (BS) cells form a single layer around each vein and the outer mesophyll (M) cells form a layer which is in contact with the epidermis. When fully differentiated these two cell-types must interact to fix CO_2. The C4 pathway requires malate dehydrogenase (MDH), phosphoenolpyruvate carboxylase (PEPCase) and pyruvate phosphate dikinase (PPdK) activities in the M cells and malic enzyme (ME) and ribulose bisphosphate carboxylase (RuBPCase) activities in the BS cells.

We have studied the development of these two cell-types utilizing several striping mutants of maize (sr1, sr2, sr3, j2 and v5). These mutants exhibit varying degrees of striping and as such present a range of clonal sector sizes. An examination of a number of sector boundaries has revealed that BS and M cells arise from separate cell lineages. BS cells are clonally related to the veins they surround. Further analysis of these clonal boundaries, using antibodies prepared against the C4 enzymes as cell-specific markers, has implicated that neighbouring BS and M cells can be activated independently. A study of the mutant ar has indicated that interactions occur between the two cell-types during development.

L 414 EFFECTS OF DEVELOPMENT AND WATER DEFICIT ON GENE EXPRESSION IN SOYBEAN STEMS, Hugh S. Mason, John E. Mullet and John S. Boyer, Texas A & M University, College Station, TX 77843.
When etiolated soybean seedlings are grown at low water potential, stem growth is slowed. Developmentally different regions of the stem exhibit distinct stress responses; e.g. elongating cells maintain turgor pressure by osmotically adjusting, but non-growing mature cells do not. The pattern of salt-extractable cell wall proteins changes with cell maturation. Water deficit alters the pattern of cell wall proteins by increasing the amount of a 28kD protein in the walls of dividing and elongating cells and decreasing the amount of a 70kD protein in the walls of mature cells. Studies of polysome populations in the different stem regions reveal a gradient of polysome content in which the dividing cells have the highest levels and the mature cells the lowest. Transfer of seedlings to vermiculite having low water potential (-0.3 MPa) greatly reduces polysome size and content in dividing and elongating cells, but has little effect on polysomes in mature cells. Fractionation of translation products of polysomal mRNA on two-dimensional gels indicates that gene expression in stems is influenced by both cell maturation and water deficit. Each tissue type is enriched in a specific subset of translation products. Low water potential increases several translation products in elongating cells which are normally most abundant in mature cells and induces several translation products in mature cells. Using antibodies against the 28kD cell wall protein, we have isolated cDNA clones for the 28kD protein and an antigenically related 31kD protein. Translation of mRNA selected by hybridization to these clones yields apparent precursor molecules which are about 2kD larger than the extractable proteins. Sequence analysis of the cDNA clones reveals open reading frames which contain putative N-terminal signal sequences upstream from regions which code for peptides which match the amino acid sequences obtained from N-terminal microsequencing of the extractable proteins. Further studies will examine the regulation of expression of these genes by water stress and normal developmental controls.

L 415 RELATIVE FREQUENCIES OF DIFFERENTIAL REGULATED MESSENGERS DURING THE LIFE-CYCLE AND SALT STRESS OF MESEMBRYANTHEMUM CRYSTALLINUM. Gabriele Meyer and Hans J. Bohnert, University of Arizona, Tucson, AZ 85721.
200 individual clones of a M. crystallinum genomic library were randomly selected. With an average insert length of 14kb, these clones represent approximately 0.5% of the complete M. crystallinum genome. The cloned DNAs were analyzed by hybridization against total DNA and poly A^+ RNAs from plants harvested at different timepoints during the life-cycle and after stress induction by salt treatment.

L 416 A TISSUE-SPECIFIC FACTOR THAT BINDS TO THE UPSTREAM REGION OF A RICE α-AMYLASE GENE IN VITRO, T.-M. Ou-Lee, Robert Turgeon and Ray Wu, Cornell University, Ithaca, NY 14853.

Alpha-amylase is expressed in the aleurone tissue of cereal endosperm in response to gibberellin which is synthesized by the embryo during germination. Extract from rice aleurone layers contained a tissue-specific factor which binds to the upstream region of the α-amylase gene under hormone induction as shown by mobility-shift experiments during agarose gel electrophoresis. The α-amylase DNA binding activity increased proportionally to increasing amounts of aleurone extract and decreased with the addition of unlabelled α-amylase DNA. The binding activity of α-amylase gene was decreased somewhat after incubation at 75°C for 10 min and was inactivated at 100°C. Tissue extract from leaves, roots or de-embryo seeds did not bind to the α-amylase upstream sequences. However, the binding factor to the α-amylase gene was induced in de-embryo seeds by adding GA_3 exogenously. This suggests that the activation of α-amylase synthesis by gibberellin, which is synthesized in embryos of whole seeds, may be mediated by the formation of a binding factor to the α-amylase gene.

L 417 TRANSCRIPTIONAL CONTROL OF NITRATE REDUCTASE SYNTHESIS IN SQUASH AND SOYBEANS, John Smarrelli, Jr., John J. Callaci, and Susan J. Martino, Department of Biology, Loyola University of Chicago, Chicago, IL 60626

Nitrate reductase(EC 1.6.6.1) catalyzes the pyridine nucleotide-linked reduction of nitrate to nitrite in higher plants. In squash cotyledons, nitrate is capable of enhancing in vitro nitrate reductase activity while glutamine represses this activity. Two dimensional polyacrylamide gel electrophoresis, however, revealed no significant changes in the synthesis of other major cellular proteins from plants supplied nitrate or reduced nitrogen sources. In vivo protein labelling and immunoprecipitation studies using anti-nitrate reductase antibodies have demonstrated that changes in nitrate reductase activity are the direct result of de novo protein synthesis. Dot blots of poly A^+ mRNA screened with a 1.2 kb cDNA insert of the nitrate reductase gene have shown nitrate reductase specific mRNA to be most abundant in squash plants given 50 mM nitrate. Little detectable hybridization was seen with mRNA isolated from plants given either no nitrogen or 10 mM glutamine. The combination of 50 mM nitrate/10 mM glutamine resulted in intermediate levels of hybridization. In soybean leaves, the activity of the pH 7.5 NADH-linked nitrate reductase isoform is termed inducible. Activity is present only in the leaves of seedlings which have been supplied nitrate. A cDNA clone that encoded part of the mRNA for squash nitrate reductase hybridized specifically with mRNA for this inducible nitrate reductase isoform. Nitrate induction resulted in an increase in the steady-state levels of mRNA for this isoform after 24 hours, while the addition of glutamine to the nitrate diminished steady-steady state levels of this mRNA.

L 418 DEVELOPMENTAL REGULATION OF A p33-RELATED CELL WALL PROTEIN IN SOYBEAN SEEDLINGS, Mary L. Tierney, Biotechnology Center, Ohio State University, Columbus, OH 43210.

p33 was first identified as an RNA transcript which accumulates in carrot roots after wounding. Using antibodies raised against a synthetic peptide derived from the p33 cDNA sequence p33 was shown to be a cell wall protein. p33 RNA increases markedly within 1 h after wounding, indicating that it may be involved in one of the early cellular responses to physical damage. However, in contrast with several other defense-related proteins, p33 RNAs do not accumulate in carrot roots in response to ethylene or in carrot suspension culture cells in response to a crude endogenous elicitor fraction. A protein immunologically related to p33 has now been detected in soybean seedlings. Cell wall protein isolated from sections of the soybean seedling corresponding to the apical hook, elongating hypocotyl and mature hypocotyl was analyzed by SDS-PAGE and western blot analysis. The anti-p33 sera recognized a single cell wall protein in the soybean apical hook with an apparent molecular weight of 30 kDa. No p33-related proteins were detected in cell wall extracts of the elongating or mature hypocotyl. The localization of a p33-related protein to the apical hook of the soybean seedling leads to the suggestion that p33 may be most abundant in actively dividing tissue. Analysis of RNA preparations from the soybean apical hook indicated that a transcript homologous to carrot p33 cDNAs is synthesized which is of appropriate size to encode the 30 kDa cell wall protein detected with antibodies raised against the p33 synthetic peptide. That this p33-related protein was detected in unwounded tissue indicates that p33 my be an important constituent of the cell wall during plant development.

L 419 **Abscisic Acid-Induced cDNAs in Barley Aleurone Layers**, Bi-Mei Hong, Scott J. Uknes and Tuan-Hua David Ho, Washington University, St. Louis, MO 63130.

The addition of abscisic acid (AbA) to barley aleurone layers induces the expression of several proteins. AbA-inducible cDNA clones have been isolated by differential screening of a lambda gt10 cDNA library with mRNA isolated from tissue treated with or without the hormone. Northern analysis showed that one of the cDNAs hybridizes to a 1.2kb mRNA in AbA-treated layers. The induction of this mRNA appeared as early as 40 minutes after the addition of AbA, reached a maximum at 4-8 hours and was present for greater than 48 hours. An AbA concentration as low as 10^{-9} M induced this mRNA. Higher concentrations(up to 10^{-4} M) induced higher levels of the mRNA. Hybrid-select translation followed by 2-dimensional electrophoresis indicated that the mRNA encoded a very basic 27kD polypeptide. The partial cDNA clone has been sequenced and a putative peptide sequence derived. The regulation of this mRNA in aleurone layers during seed development is currently under investigation.The physiological significance of this and other AbA induced mRNAs will be discussed.

L 420 EFFECTS OF ABA ON GENE EXPRESSION IN ISOLATED WHEAT EMBRYOS FROM DORMANT GRAIN, M. Walker–Simmons and K.E. Crane, USDA–ARS, Washington State University, Pullman, WA 99164–6420.

The regulation of gene expression by abscisic acid (ABA) in mature embryos from dormant and non–dormant grain is being studied. Our intent is to identify genes that are expressed in response to this hormone under the conditions of grain wetting which can restrict germination rates or cause sprouting damage in grain. Previously we have found that ABA is considerably more effective in blocking germination of embryos isolated from mature dormant (cv. Brevor) grain than from non–dormant grain (cv. Greer). In this report polypeptide biosynthesis in response to ABA is examined. Isolated embryos from the dormant or non–dormant grain were imbibed in water with or without ABA for 2, 4, 6 or 8 hours and pulse–labelled with [^{35}S]methionine for the last hour of incubation. Proteins were extracted, separated by SDS–PAGE, and visualized by fluorography. Results demonstrate that there are significant differences in the pattern of polypeptides synthesized in response to ABA in wheat embryos from dormant or non–dormant grain.

Gene Expression in the Mature Sporophyte and During Fruiting

L 500 ISOLATION OF TELOMERIC DNA SEQUENCES FROM ARABIDOPSIS THALIANA
Eric J. Richards and Frederick M. Ausubel, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Telomeres are the structures that form the termini of linear eucaryotic chromosomes. Telomeres stabilize the ends of the chromosomal DNA molecules and facilitate the complete replication of the DNA strands at the extreme termini. Detailed study of telomere structure and function is limited to lower eucaryotic organisms from which telomeric DNA sequences have been isolated. Cloning of telomeres from higher eucaryotic organisms is hampered by the large size of the chromosomes and the attendant dilution of telomeric sequences relative to non-telomeric sequence in the genome.

We have developed a method for constructing genomic libraries highly enriched for telomeric sequences enabling the isolation of telomeres from higher eucaryotic organisms with large chromosomes. The method was used to clone telomeric DNA sequences from A. thaliana. The structure of A. thaliana telomeres is similar to that seen in lower eukaryotes; the cloned A. thaliana telomere is composed of tandemly repeated, C-rich, simple sequence blocks. In addition, the telomeres of A. thaliana are heterogeneous in size as are most lower eucaryotic telomeres. Genomic sequences homologous to the A. thaliana telomeric repeats are present in the genome of other higher plants and some animal species. In the case of corn and humans, these genomic sequences are located at the telomere.

L 501 CHARACTERIZATION OF ARGININE DECARBOXYLASE REGULATION, Erin Bell and Russell Malmberg, University of Georgia, Athens, GA, 30602.
In plants, changes in polyamine concentrations have been correlated with stress responses, hormone responses, cell division, and floral development, but the actual role of polyamines in these responses is still not known. Studies of one enzyme involved in polyamine metabolism have shown that the activity of this enzyme, arginine decarboxylase (ArgDC), can be induced or repressed in response to both developmental and environmental signals. In order to more fully characterize this regulation, we have partially purified ArgDC and used this preparation to raise monoclonal antibodies against the protein. There is circumstantial evidence to suggest that a cDNA we have isolated encodes ArgDC, and with the antibodies in hand a definitive answer will soon be available. If the cDNA proves not to encode ArgDC the antibodies should make it possible to isolate a clone which does. These tools will allow us to examine the regulation of ArgDC and, through the study of both wild type and mutant plants, address the question of what the physiological purposes of this regulation might be.

L 502 Haemoglobin genes in non-nodulating plants, and their expression in non-symbiotic tissue.+* Didier Bogusz, + Cyril A. Appleby, + Elizabeth S. Dennis and + W. James Peacock, *ORSTOM, 213 rue LaFayette 75010, PARIS, FRANCE, + CSIRO, Division of Plant Industry, Canberra, 2601, Australia.
In the plant kingdom haemoglobin genes have been described only within nitrogen fixing symbiotic plants and their expression has been reported only in nodules. We have now detected haemoglobin-related sequences in two non-nodulating species, Trema tomentosa and Celtis australis, by Southern hybridization using Parasponia haemoglobin cDNA as a probe. In Trema, the single band of hybridization suggests that one haemoglobin-related segment occurs in the genome. To study this sequence in Trema we constructed a genomic library, which was screened with the Parasponia haemoglobin cDNA clone. One positive clone was analysed and shown to contain a complete haemoglobin gene. This Trema gene has intron positions identical to those both in Parasponia and legumes. The alignment of its sequence with the Parasponia gene indicates 93% nucleotide homology in the exons and 80% homology in the introns, non-translated leader and 3' untranslated sequences. The putative polypeptide is 161 amino acids long, identical to that of Parasponia haemoglobin and has the 33 amino acid residues common to all plant haemoglobins. Thus, Trema haemoglobin could well be a functional plant haemoglobin. To ascertain whether the Trema gene is functional we searched for its corresponding mRNA and protein in vivo. Northern blot hybridization of Parasponia haemoglobin cDNA to poly $(A)^+$ RNA isolated from Trema indicates transcription of the Trema gene in roots but not leaves. Furthermore, we have detected a native monomeric haemoglobin in Trema roots by Western blots using anti-Parasponia haemoglobin serum. We have also found a low level of transcription of the Parasponia haemoglobin gene in Parasponia non-nodulated roots. We propose that haemoglobin is involved in the respiratory metabolism of root cells of all plants and that its high expression in nodules of nitrogen-fixing plants has required adaptation of the gene regulation pathway.

L 503 METABOLIC, DEVELOPMENTAL, AND ENVIRONMENTAL REGULATION OF β-AMYLASE IN LEAVES OF WILD TYPE AND STARCHLESS MUTANTS OF ARABIDOPSIS THALIANA, Timothy Caspar, Tsan-Piao Lin, Steve Spilatro, Jack Preiss, and Chris Somerville, Michigan State University, East Lansing, MI 48824.
The regulation of leaf amylases was investigated using starchless mutants of Arabidopsis thaliana which are deficient in the activity of either phosphoglucomutase or ADPglucose pyrophosphorylase. Starchless mutants of both types had elevated total amylase activity relative to the wild type. Not all of the amylase isozymes, only the extrachloroplastic, exolytic ones had elevated activities. Immunoblots, using β-amylase antibodies indicated that the increased exoamylase activity was due to an increased level of β-amylase protein. The increased activity in the starchless mutants varied with the photoperiod in which the plants were grown, ranging from 1-3 times the wild type in continuous light to 5-10 times that of the wild type in a 12 h photoperiod. In addition, amylase activity was also regulated, in both the wild type and the starchless mutants, by the developmental stage of the plants and was rapidly affected by shifting plants from a continuous light photoperiod to a 12 h photoperiod. These results suggest that the expression of an extrachloroplastic β-amylase is regualted both developmentally and in response to the carbohydrate status of the leaf.

L 504 CHARACTERIZATION OF CELLULASE GENES FROM PERSEA AMERICANA, Laura G. Cass, Kathleen A. Kirven, and Rolf E. Christoffersen, Department of Biological Science, University of California, Santa Barbara, CA 93106

The ripening of avocado fruit is characterized by softening of the mesocarp tissue. Cellulase is thought to play a major role in softening by participating in degradation of the cell wall. Previous studies have shown that the expression of cellulase activity during ripening is regulated by the appearance of RNA transcripts for the cellulase. In order to study regulatory elements of the cellulase gene, we have cloned and characterized genomic DNA from avocado which hybridizes to a cellulase cDNA. Southern restriction analysis of both genomic DNA and lambda genomic clones indicate that the avocado genome contains two cellulase genes which hybridize to the cellulase cDNA under moderately stringent conditions. One of these genomic fragments either codes for a cellulase which is not expressed in ripening fruit, or represents a pseudogene. Thus we suggest that the ripening-specific cellulase is encoded by a single gene. Furthermore, these two cellulase gene family members are unlinked within the 30 Kb of the genome that has been cloned and mapped.

L 505 WOUND MODULATION OF GENE EXPRESSION IN VARIOUS PLANT TISSUES, Eric Davies, Baoren Liu and Ed Pfeffer, University of Nebraska, Lincoln, NE 68588-0118.

Wound-induced changes in polysome distribution, the rate of protein synthesis in vivo and in vitro and the kinds of protein synthesized in vivo were studied in various plant tissues. In aged pea stems, wounding caused both the formation of polysomes and increased the rate of protein synthesis in vitro, yet decreased the rate of protein synthesis in vivo. In pea roots, different regions responded differently, with the tips showing a marked decline, the mid-region little change and the basal (aged) region showing an increase in polysomes upon wounding, while protein synthesis in vivo declioned in all cases. In leaves of the aquatic plants, Elodea and Vallisneria, as with aged pea stems and basal root tissue, wounding evoked an increase in polysomes and a decrease in protein synthesis in vivo. The effects of wounding, other stresses, and calcium on the rates and kinds of protein synthesized in vivo will be elaborated.

L 506 Tomato Hsp 70 expression during flower development and early germination, Nicklos Duck , Rhonda Wright and Jill Winter, University of Missouri, Columbia, MO. 65211

Hsp 70 multigene families encode both stress inducible and 'cognate' family members. There is homology between family members as well as among hsp 70's of evolutionarily divergent species. The stress and cognate hsp 70 functions remain unknown. Using a tomato hsp 70 cDNA, we have observed the expression of hsp 70 mRNAs in the absence of stress induction in floral tissues of tomato. We have also noticed an unusual hsp 70 stress induction pattern during germination of tomato plants. We will present data characterizing both of these phenomena. We are currently putting an hsp 70 gene, engineered for constitutive expression, into plants in order to determine whether the continuous presence of hsp 70 will alter the development or stress tolerance of the plant.

L 507 cDNA LIBRARY OF SEQUENCES SPECIFIC FOR MALE STERILITY IN MERCURIALIS ANNUA L. Bernard Durand, Raymonde Durand, Jean Paul-Louis, Elisabeth Cabré, Michel Delaigue, Yu Long Xi, Université d'Orléans, 45067 Orléans Cédex 2, France

In Mercurialis annua, comparative analyses of poly(A^+) RNAs populations from several strains such as fertile ♂ (A B + N cytoplasm), sterile ♂ (A B + S + I r_1R_2), restored fertile ♂ (A B + S + I R_1R_2), normal ♀ (A or B + N cytoplasm), constructed ♀ with sterility determinants (A or B + S + I R_1R_2) were performed by in vitro translation and separation of synthesized peptides by two-dimensional electrophoreses. In each experiment a group of peptides appeared to be specific or each strain. It was possible to correlate each cluster of specific peptides to the presence or absence of each male sterility or sex genes, especially I gene (sterility inducer), R_1 R_2 genes (fertility restorers) and to N and S cytoplasm. The constructed female differed from the normal one by some unexpected specific peptides. Homologous (for instance fertile ♂ cDNAs/fertile ♂ RNAs) and heterologous (for inst. Sterile ♂ cDNAs/fertile ♂ RNAs and reciprocally) hybridization Kinetics studies confirm the specificity of poly A^+ RNAs populations of each strain. I appeared that : 1) two identical phenotypes (restored and normal males ; constructed and normal female) can differ in their program of genetic expression of reproductive organogenesis 2) each cluster of specific peptides is linked to a specific endogenous hormonal balance. 3) the sterility genes behave as regulator ones. 4) some male fertile peptides appeared unnecessary for male fertility (present in normal fertile males absent in restored ones). A cDNA library of the sequences specific for male sterility was performed in λgt 10. The screening of the specific clones was obtained by sterile/fertile ♂ cDNAs hybridization followed by sterile/Sterile one. Northern blotting confirmed the high specificity of these clones issued from screening between cDNAs extracted from strictly homologous organs. Parallel screening was also performed for cDNAs of fertile males.

L 508 GENE EXPRESSION DURING CARNATION FLOWER SENESCENCE, K. A. Lawton, B. Huang, P. B. Goldsbrough and W. R. Woodson, Department of Horticulture, Purdue University, West Lafayette, IN 47907. Senescence of carnation flowers is a highly regulated developmental process that is regulated by ethylene and requires active gene expression. *in vitro* translation of mRNAs isolated from young and senescing petals indicated that many new mRNAs were present in the senescing tissue. Accumulation of these new mRNAs was prevented by treatment with the ethylene action inhibitors, silver thiosulphate and norbornadiene, which also delay senescence. However, exposure of young flowers to exogenous ethylene induced both premature senescence and the accumulation of senescence-specific mRNAs. cDNA clones that correspond to some of the mRNAs expressed during carnation petal senescence have been isolated. These cDNAs have been used to study the levels of specific mRNAs during petal development and senescence, and to determine the role of ethylene in regulating the expression of these mRNAs. One class of cDNAs represents genes whose expression is highly regulated by ethylene. These mRNAs are not present in significant amounts before the ethylene climacteric associated with petal senescence, but accumulate rapidly during the period of autocatalytic ethylene synthesis. This group of mRNAs can be induced by ethylene in young flowers and inhibited by norbornadiene. The second class of cDNAs corresponds to mRNAs that increase in abundance during the course of petal maturation and prior to the ethylene climacteric. Ethylene can increase, and norbornadiene reduce, the levels of mRNAs in this second class. However, the expression of these genes is more temporally regulated and less responsive to ethylene. Our results indicate that carnation petals provide an interesting tissue for studying both hormonal and developmental gene expression.

L 509 EXPRESSION OF FOREIGN GENES IN SOLANUM TUBEROSUM CVS BINTJE AND DESIREE, Freek Heidekamp, Willem J. Stiekema, Wim G. Dirkse, Jeanine D. Louwerse, Yolente Verbeek and *Cees M.P. van Dun; Plant Cell Transformation Group, Research Institute Ital, P.O. Box 48, 6700 AA Wageningen, The Netherlands and *Dept. of Biochemistry, State Univ. of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The research in our group is focussed on the introduction and cell-specific expression of crop-improving genes in potato. To achieve this the following work has been performed.

- The β-Glucuronidase reporter gene, driven by the 35S-CaMV promoter and the NPT II reporter gene, driven by the nos-promoter have been expressed both in potato cvs Bintje and Désirée using a tuber disc transformation procedure.
- The β-Glucuronidase gene was placed under control of a 3.0 kb promoter fragment isolated from a copy of the tuber-specific patatin gene family. The PATGUS construct in BIN19 was introduced into the Agrobacterium strain LBA4404 and used to transform potato cv Bintje. Bintje shoots, rooting on kanamycin containing medium have been obtained.
- The coat protein gene of Tobacco Rattle Virus driven by the 35S CaMV promoter was cloned in pAGS129 and introduced in LBA4404. The resulting strain was used to transform potato cv Désirée. Plants growing on kanamycin containing medium have been obtained.

Characteristics of the different transgenic potato plants will be presented.

L 510 FORMATION OF ADVENTITIOUS INFLORESCENCES ON INVOLUCRAL BRACTS OF SUNFLOWERS IRRADIATED AT 10 OR 16 DAYS AFTER SOWING, Dorothy E. Jegla, Kenyon College, Gambier, OH 43022.

Six plants in a population of 2000 sunflower plants (Helianthus annuus cv. Peredovic) exposed to 500r x-rays at 10 or 16 days after sowing developed miniature inflorescences at the tip of several involucral bracts. Both 10- and 16-day old plants were still initiating vegetative leaves at the time of irradiation - 10-day plants with 2/3 of the in situ leaf number yet to initiate and 16-day plants with approximately 1/3 yet to initiate. Most adventitious inflorescences were surrounded by structurally normal miniature involucral bracts and bore a few to many disc florets. A few inflorescences consisted only of disc florets on the surface of a somewhat swollen area near the tip of the affected involucral bract. These adventitious inflorescences probably were being formed on the involucral bracts at or close to the same time that the disc florets were being initiated in the terminal inflorescence of the shoot; genes which normally would not be expressed in the involucral bracts became active in the bracts at approximately the same time those genes were being expressed normally in the development of the terminal shoot meristem.

L 511 STRUCTURE, DEVELOPMENTAL REGULATION AND STRESS INDUCTION OF TWO GENES ENCODING GLYCINE-RICH PROTEINS IN BEAN, Beat Keller, Norbert Sauer and Chris J. Lamb, The Salk Institute, P.O.Box 85800, San Diego, CA 92138

Two genes coding for glycine-rich proteins (GRP) have been isolated from French bean. They encode proteins of 335 (GRP 1.8) and 272 (GRP 1.0) amino acids, of which 61% are glycine. Both proteins contain an aminoterminal hydrophobic signal sequence, suggesting that they are exported to the cell wall. GRP 1.8 contains five repeats of 22 amino acids, starting with the sequence Glu-His. Both proteins are relatively rich in tyrosine.

On Northern blots, mRNAs have been detected for both genes (GRP 1.8 codes for a 1.8 kb message, GRP 1.0 for one of 1.0 kb). GRP 1.8 is strongly expressed in young hypocotyls and is almost completely switched off during further development. In older hypocotyls, GRP 1.8 is rapidly and transiently induced after wounding, reaching a peak of induction between 4 and 12 hours. In young hypocotyls no increase of expression can be seen after wounding. However, after 24 hours the gene is also down-regulated. GRP 1.0 is only weakly expressed in young hypocotyls and is not wound-induced. The differences in the regulation of these two genes are reflected in different promoter sequences. The promoter of GRP 1.8 shows homologies with other stress-induced genes.

L 512 EXPRESSION OF A MAIZE RIBOSOMAL PROTEIN GENE FAMILY. J. Larkin, J. Hunsperger, D. Culley, I. Rubenstein, and C. Silflow, University of Minnesota, Dept. of Genetics and Cell Biology, St. Paul, MN 55108. During development, protein synthesis requirements vary greatly, and developmental modulation of ribosomal protein (rp) gene expression has been observed in a number of instances. Little is known about the organization or expression of plant rp genes. As an initial approach to understanding the role of ribosome biosynthesis in plant growth and development, we have isolated several maize cDNA clones homologous to the Saccharomyces cerevisiae CRY1 gene, which encodes the 40S rp S14. In maize, this rp is encoded by a small multigene family consisting of 4-8 members. At least three, and probably four, different copies of the gene are expressed. In no other organism have more than two copies of a rp gene been shown to be expressed. We are currently examining the possibility that some members of this gene family may be expressed only in specific tissues. During endosperm development, the highest levels of this rp mRNA are present at ten days after pollination, and mRNA levels for this protein decline continuously from this point on. Thus, expression of this rp gene in endosperm correlates well with the period of maximal cell division rate, and does not show a correlation with maximal nucleolar volume or rDNA amplification. We also find that very little of this rp mRNA is present in pollen, while relatively large amounts of tubulin mRNA are present, suggesting that translation of pollen mRNAs during pollen germination uses preformed ribosomes.

L 513 A NEW SELECTABLE MARKER TO STUDY GENE EXPRESSION IN PLANTS, Bernard Leroux, Bernard Pélissier, Alain Sailland, Michel Lebrun and Georges Freyssinet, Rhône-Poulenc Agrochimie, BP 9163, 69263, Lyon Cedex 09, France.
A gene coding for a nitrilase which detoxifies the herbicide Bromoxynil has been previously isolated (Stalker D.M. and Mc Bride K.E. 1987. J. Bact. 169, 955-960). We wanted to know whether this gene can be utilized as a selectable marker in transgenic plants. To answer this question, we linked various promotors from dicotyledonous and monocotyledonous plants to it and analyzed the expression of the chimeric genes thus made either in vitro on callus or at the mature plant level. By using two types of transit peptide we further tested the effect of the cellular enzyme localization, chloroplastic or cytoplasmic, on the resistance of these plants to Bromoxynil. Results indicate clear differences in the expression of these chimeric genes in transgenic plants and that Bromoxynil resistance can be used as a good selectable marker at the mature plant level.

L 514 EXPRESSION OF A C_3 PLANT RuBisCO SSU GENE IN REGENERATED C_4 PLANTS, Belinda Martineau, H. Jane Smith and Laurens J. Mets, University of Chicago, Chicago, IL 60637
Due to the intercellular localization of enzymes involved in the C_4 pathway, C_4 plants serve as excellent systems for the study of the regulation of cell-specific gene expression. In situ hybridization has been utilized to demonstrate that mRNA transcripts encoding RuBisCO and phosphoenolpyruvate carboxylase are compartmentalized in the same C_4 plant cell types as their respective proteins.[1] Transformation studies with C_4 plants should determine whether the molecular mechanisms regulating cell-specific expression of photosynthetic genes in C_4 plants involve cis-acting genetic elements. We have successfully transformed, via A. tumefaciens infection with a plasmid containing an abundantly expressed RuBisCO SSU gene from petunia and a gene encoding NPTII,[2] and regenerated individuals from the dicotyledonous C_4 plant species, Flaveria palmeri. Of 10 kanamycin resistant plants, at least three are independent transformants. We have demonstrated expression of the C_3 plant RuBisCO SSU gene in the three transformants we have examined thus far. We expect to find correct organ-specific expression of the transferred gene in these plants based on our results with another C_4 Flaveria species. In situ hybridization experiments are underway to determine whether the transferred C_3 plant gene has been induced to express with the same leaf cell-specific pattern as its C_4 Flaveria plant relatives.

[1]Martineau, B. and W.C. Taylor (1986) Plant Physiology 82:613-618.
[2]Dean, C., P. van den Elzen, S. Tamaki, P. Dunsmuir and J. Bedbrook (1985) EMBO J. 4:3055-3066.

L 515 PEROXIDASE EXPRESSION IN COTTON OVULE CULTURES, Jay E. Mellon and Barbara A. Triplett, U.S.D.A., A.R.S., S.R.R.C., P.O. Box 19687, New Orleans,LA 70179.

Peroxidase has been proposed to serve several possible functions in higher plants. The enzyme has been implicated in processes such as host-defense mechanisms, cell wall polymer cross-linking, and lignification. In cotton, peroxidase is localized in ovary walls that subsequently become carpel tissue of the fruit (boll). Peroxidase activity is also associated with developing ovules and fibers (10% of total) in the plant. Cultures initiated from 2-day postanthesis ovules secrete the enzyme into the surrounding medium in several isozymic forms. Secretion of peroxidase activity is detectable at 5 days after culture initiation, but the highest rates of enzyme secretion occur after 15 days in culture. De novo synthesis of secreted peroxidase was indicated by in vivo labeling of ovule cultures with 35S-methionine. Analysis of labeled culture medium by SDS-PAGE and isoelectric focusing gels followed by either fluorography or Western blotting showed incorporation of radiolabel into peroxidase. Biological stress such as fungal infection induces significantly higher levels of an isoperoxidase present at very low levels in non-stressed cultures. The stress-related isoperoxidase has been purified to apparent electrophoretic homogeneity. The regulation of peroxidase expression in cotton ovule cultures is under current investigation.

L 516 CLONING AND EXPRESSION OF THE NADP-MALATE DEHDROGENASE INVOLVED IN THE C4 PATHWAY OF MAIZE, Mary Metzler, Jane Langdale, Beverly Rothermel, Timothy Nelson, Yale University, New Haven, Ct. 06511

We have isolated a cDNA clone for the NADP-dependent malate dehydrogenase (MDH) involved in the C4 pathway of photosynthesis. The protein was purified from maize leaves and antiserum was produced from rabbits that recognized the protein. This antiserum was used to screen a lambda gt-11 cDNA library, and a recombinant clone was identified which produced a fusion protein recognized by the antiserum. We have sequenced this clone and find that it has a high degree of homology to the equivalent protein from spinach. We have determined that the gene is present in low copy number in the genome, that mRNA and protein levels are regulated by light, and that mRNA levels are essentially equal in both mesophyll and bundle sheath cells.

L 517 IDENTIFICATION OF A MONOCLONAL ANTIBODY DIRECTED AGAINST THE DEVELOPMENTALLY-REGULATED CELL WALL PROTEIN EXTENSIN AND ITS USE IN STUDY OF CELL WALL COMPOSITION AND BIOSYNTHESIS, David J. Meyer and David W. Galbraith, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

We have identified the epitope of a monoclonal antibody raised against plant membrane proteins as an epitope which is carried on the extracellular matrix protein extensin. The antigen as found in Nicotiana tabacum cells is rich in proline and poor in leucine, has a very basic pI, and comigrates on two-dimensional gel electrophoresis with authentic tomato extensin. The antigenicity of proteins synthesized in the presence of dehydroproline, a specific inhibitor of prolyl hydroxylase does not appear to be diminished, suggesting that prolyl hydroxylation and glycosylation are not associated with antigenicity. The monoclonal antibody does not cross-react with other hydroxyproline-rich glycoprotein types, and so appears to be a useful new tool for the study of extensin biosynthesis and structure, and possibly also its function. Using this antibody as a probe we have shown the antigen to be localized in subcellular membranes of the ER, golgi, and plasma membrane. We have examined the biosynthesis of extensin as it is associated with plant cell development and cell wall differentiation. The extensin is found to be localized in specific cell and tissue types in the plant as seen in tissue blots probed with the antibody. It is expressed at very low levels in leaves, but is induced to detectible levels after 48 hours of culture in protoplasts derived from this tissue.

L 518 INTEGRATED R2 SEQUENCE IN MITOCHONDRIA OF MALE FERTILE B37N MAIZE ENCODES AND EXPRESSES A 130 KD POLYPEPTIDE SIMILAR TO THE ORF1 OF THE S2 PLASMID. Colleen O'Brien, Gracia Zabala and Virginia Walbot, Stanford University, Stanford, CA 94305. Expression of a 130 kD protein from ORF1 of the integrated form of the R2 plasmid in mitochondria of a male-fertile B37N inbred line is described. By electrophoretic mobility and immuno-crossreactivity the protein appears identical to that synthesized by the closely related S2 episome found in cytoplasmic male sterile maize of the S-type. Protein was detected using antisera raised against a beta-galactosidase:ORF1 fusion product representing the mid region of the ORF1 product. Detection of this protein is suprising since earlier reports indicated that the male-fertile B73N inbred line lacked this protein and that within the first 70 codons of the open reading frame of the integrated R2 of Wf9N there are 11 in-frame stop codons. The integrated R2 of B37N was cloned and this region sequenced, confirming that a continuous open reading frame existed. Normal inbred lines Mo20 and Va26 were also tested for immuoreactivity and showed positive for the 130 kD protein.

L 519 PROTEIN ANALYSIS OF EARLY INFLORESCENCE DEVELOPMENT IN CORN, Alan R. Orr, Brett A. Wagner and Cathy T. Howard, University of Northern Iowa, Cedar Falls, IA 50614. In the genesis of a Zea mays L. ear and tassel there is a sequence of developmental stages beginning with the vegetative meristem and ending with the initiation of the ovule. In the present study, we examine the protein complement associated with the first two stages utilizing high resolution two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) coupled to Gelcode color-based silver stain; and we also use a new method developed by us to quantify the concentration of protein in the same sample solubilized for 2-D PAGE. Inflorescence axes were cut into segments containing either the apical meristem, branch primordia or spikelet primordia and the proteins solubilized with 9 M urea and NP-40. Of the approximately 600 to 900 polypeptides detected, several (27-ear; 23-tassel) showed qualitative changes associated with the apical meristem, branch primordia and spikelet primordia. 65% of these stage-associated polypeptides (SAPS) were clearly visible on the spikelet primordia segment gels, but not on the gels of branch primordia or apical meristems segments. 15% (tassel) and 25% (ear) of the SAPS were observed only on the apical meristem segment gels. Most of the SAPS detected in branch primordia segments also appeared in the spikelet primordia segments. We do not know the function of these SAPS but they are the first reported molecular markers showing modulation of the protein complement during corn inflorescence differentiation. The appearance and disappearance of these markers provide suggestive evidence that corn ear and tassel morphogenesis arises, at least in part, from a modulation in gene expression and/or post-expression activity at specific switch points of inflorescence development.

L 520 DIFFERENTIAL GENE EXPRESSION DURING TOMATO FRUIT FORMATION: EFFECT OF DEVELOPMENTAL PROGRAM AND DIURNAL RHYTHMICITY

Birgit Piechulla, Institut für Biochemie der Pflanze, Untere Karspüle 2, 3400 Göttingen, West-Germany

Differential gene expression during tomato fruit development and ripening was demonstrated for a spectrum of different genes. Genes coding for photosynthesis-specific stromal and thylakoid membrane proteins reach highest steady-state 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. Transcript levels of the chloroplast and mitochondrial ATPase subunits are high in 2-3 week old tomato fruits. Later during fruit development and ripening, the chloroplast ATPase subunits III and A decrease to non-detectable levels, while the mitochondrial ATPase A and B subunits remain relative high, indicating that ATP synthesis during the ripening process is due to the mitochondrial ATPase. During ripening, when high levels of reducing sugars are present, the cytoplasmic fructose-1.6-bisphosphate aldolase mRNAs increase dramatically. These results demonstrate that in addition to specific control mechanisms which are induced by light (known for cab and rbcS), gene activation and inactivation is regulated by a developmental program.

Additionally, significant changes of mRNA levels of cab, rbcS and other genes are measured in fruits harvested at night- and day-time, indicating diurnal mRNA fluctuations. The mRNA fluctuations of the chlorophyll a/b binding protein genes appear with a regular 24 hour periodicity. Under continuous light or dark conditions these diurnal rhythms continue for several days, although the amplitude of the expression level decreases. These results together suggest that circadian rhythms are involved in controlling gene expression in tomato fruits.

L 521 SEGREGATION FOR ENDOSPERM LYSINE AND PROTEIN AS WELL AS INFERTILITY FROM CROSSES OF IN VITRO SELECTED RICE, Gideon W. Schaeffer, USDA, ARS, Plant Molecular Genetics Lab, Beltsville, MD. 20705.

Mutants from anther-derived calli were recovered from inhibitory levels of lysine plus threonine (L+T) and L+T plus S-(2-aminoethyl)cysteine (AEC). Endosperm proteins from L+T-selected mutants had higher lysine as well as protein levels than the starting cultivar. Seeds from mutants had unique soft and crumbly endosperm. A broad spectrum of variation in plant chlorophyll/color was recovered from 2nd generation plants with both normal and elevated lysine from all L+T selections both in the presence and absence of AEC. The improved lysine was transmitted to offspring but some lines became increasingly infertile on selfing and many were totally infertile after 4 selfings. A cross of an L+T-selected mutant(4C) x wild type parent (M101) produced F_2 segregation data consistent with the hypothesis that endosperm lysine is inherited as a recessive character whose expression may be modified by inhibitors. There was a significant negative correlation among progeny from the 4CxM101 cross between seed weight and lysine content with R-squared 0.54. Work is in progress to isolate individual proteins and gene(s) associated with the lysine mutants and to recover segregants from crosses with normal seed set.

L 522 **Systems of communication between nucleus and chloroplast studied with the tomato *ghost* mutant** Pablo A. Scolnik, Dolores Bartholomew and Glenn Bartley. DuPont Plant Science Group, E402-4252, Wilmington, DE 19898.
Leaves of the tomato *ghost* plant show a variegated green/white phenotype due to a somatically unstable mutation in carotenoid biosynthesis. White leaves contain no colored carotenoids, and thus plastids do not differentiate into chloroplasts. Green leaves contain wild-type levels of colored carotenoids, thus chloroplast differentiation is not affected. We measured steady-state mRNA levels for two families of nuclear genes that code for chloroplast proteins: *rbcS* and *cab*, which code respectively for the small subunit of ribulose-1-5-biphosphate carboxylase and chlorophyll a/b binding proteins. mRNA levels for both gene families are low in white leaves but are similar to wild-type in green leaves. Analysis of the rates of transcription in isolated nuclei indicates that the differences in cytoplasmic mRNA levels are due mainly to reduced transcription of the two gene families in white leaves. We are currently focusing on the effects of chloroplast differentiation on hormone biosynthesis in leaves. We find that ABA biosynthesis is blocked in white leaves, but it is normal in green leaves. Many of the secondary phenotypic traits of *ghost* can be attributed to this hormone defficiency. We conclude that chloroplasts play an essential role in leaf development by controlling transcription of nuclear genes that code for photosynthesis-related proteins and by mediating the synthesis of the phytohormone ABA.

L 523 PARTIAL PURIFICATION FROM *ARABIDOPSIS* OF AN ACTIVITY THAT CATALYZES DNA STRAND EXCHANGE, Mary Lopez, Shoshana Klein and Ethan Signer, MIT, Cambridge MA 02139.
Enzymes involved in homologous genetic recombination have been isolated from *Escherichia coli* (RecA) and *Ustilago maydis* (Recl), and similar activities have been found in *Saccharomyces cerevisiae*, *Lilium*, mouse and human. Assays for these activities include binding to single- and double-stranded DNA, reannealing of homologous single DNA strands, ATPase (in some cases DNA-dependent), and particularly exchange of strands between homologous DNA duplexes, which is exclusive to Rec-like enzymes. We have identified and partially purified a Rec-like activity from the crucifer *Arabidopsis thaliana*. Fractions recovered from chromatography of crude extracts on Trisacryl M DEAE and FPLC mono-Q displayed strand exchange activity, as determined by the appearance of a heteroduplex DNA band upon agarose gel electrophoresis. The same fractions also exhibited ATPase activity, dependent on single- or double-standed DNA, in a luciferin-luciferase assay. Further purification of the activity will be described.

L 524 AQUISITION OF COMPETENCE FOR FLORAL DETERMINATION, Susan R. Singer, Cole H. Hannon and Sarah C. Huber, Department of Biology, Carleton College, Northfield, MN 55057
Floral determination in dayneutral Nicotiana tabacum cv. Wisconsin 38 is precisely regulated and the terminal bud produces four nodes after it acquires this new developmental state (Singer, SR and McDaniel, CN 1986 Devel. Biol. 118:587). Floral determination occurs in response to a pervasive signal (Singer, SR and McDaniel, CN 1986 Proc. Natl. Acad. Sci., USA 84:2790). We are investigating whether competent cells respond to a developmental signal that reaches a critical concentration at the time of floral determination or whether cells gain competence to respond to an existing signal at that time. Young seedlings produce fewer nodes when grafted into an apical internode of a flowering plant than when left in situ. When the terminal buds of seedlings having initiated either twelve or twenty-one nodes are grafted into the same apical position on flowering stocks, scions from both groups produce an equivalent number of nodes and a terminal flower. Thus seedlings of both ages appear competent to respond to a signal for floral determination. Grafting N. tabacum scions to long-day N. silvestris stocks results in an even greater reduction in the number of nodes produced before the terminal flower. Thus signal strength appears critical in floral determination. (Supported by USDA (87-CRCR-1-2554) and Research Corporation.)

L 525 GROWTH ABNORMALITIES OF TOBACCO PLANTS TRANSGENIC FOR THE ROL LOCI OF AGROBACTERIUM RHIZOGENES RI PLASMID, A. Spena, Th. Schmülling, and J. Schell, MPI für Züchtungsforschung, 5000 Köln 30, FRG

The hairy root syndrome is a pathological state display by plants regenerated from Ri transformed roots, consisting of high growth rate of roots in culture, adventitious root formation, reduced apical dominance both in stems and roots, altered leaf morphology and plagiotropic roots. Tobacco plants transgenic for the rol A, B, and C loci of the Ri T_L-DNA exhibit all the characteristic symptoms of the hairy root syndrome. Establishment of the full syndrome correlates with expression of the rol loci, and results from the synergistic activities of their products. The biological effects elicited by the rol genes, either by themselves or in different combinations, show that drastic morphological alterations result by manipulating the expression and/or the type of interaction between the rol genes. These results are compounded with the evaluation of the organ specific expression of the genes in a model for the pathogenesis of the hairy root syndrome.

L 526 Patterns of Protein Synthesis in Growing and Quiescent Buds of Pea. Joel Stafstrom and Ian Sussex, Yale University, New Haven, CT 06511.

Lateral buds on intact pea plants (Pisum sativum cv. Alaska) are normally quiescent but can be induced to develop as independent shoots by decapitation of the shoot apex. Most work on this phenomenon ("apical dominance") has focused on the role of hormones and other factors in effecting the transition from the quiescent to the growing state. We are interested in the biochemical events occurring within buds that are correlated with, or perhaps essential for, determining each stable developmental state. Our assay is two-dimensional gel electrophoresis of silver-stained and in vivo-labeled proteins.

18 stained and 25 labeled bud protein were identified whose expression is altered after induction of growth (increase/decrease, qualitative/quantitative). The altered proteins represent 3.4% and 9.1% of the total proteins detected by the two methods, respectively. Proteins from lateral buds are indistinguishable from those of the shoot apex within 24 hr of decapitation. After 6 hr, before the onset of detectable growth, the overall protein profile is more like that of growing than quiescent buds. Induction of bud growth on intact plants by direct application of kinetin results in the same bud proteins as does induction by decapitation. Similarly, inhibition of bud growth by addition of IAA to the stump of decapitated plants results in the quiescent pattern of protein synthesis. Calculation of a "correlation index" (a numerical measure of the overall similarity of protein patterns) for each pair of bud stages reinforces our conclusions based on individual proteins. We are applying what we have learned about bud proteins to construct stage-specific cDNA libraries.

L 527 TISSUE SPECIFIC GENE EXPRESSION DURING CEREAL GRAIN DEVELOPMENT, Jennifer Topping, Claire Marris, Patrick Gallois, Bruce Lee, Kate Murdoch, Mike Jones and Martin Kreis. AFRC Institute of Arable Crops Research, Rothamsted Experimental Station. Harpenden, HERTS AL5 2JQ. U.K.

The development of cereal grains is dependent upon the spatially and temporally regulated expression of a number of genes. We are interested in understanding how the expression of these genes is controlled at the molecular level in the starchy endosperm and the aleurone layer, two tissues which develop and differentiate from the same primary endosperm cell. Sequence analysis of the barley storage protein genes the hordeins has resulted in the identification of a 33bp sequence, approximately 300bp 5' of the start of transcription, termed the '-300 element', which is also found 5' of zeins and gliadins of maize and wheat respectively. The function of this region was studied in transgeneic plants by linking it to a reporter gene (CAT). Analysis of the regenerated transformed plants revealed that CAT activity was present exclusively in the tobacco seed endosperm after 15 days post anthesis. This is the same developmental stage at which the B Hordein genes are expressed in barley. We have further developed a cereal protoplast transient expression system, which allows the rapid assay of gene constructs, and this is being used to study regulatory sequences of other genes including those encoding β amylase and the chymotypspin inhibitors.

The financial support of Ciba Geigy and the EEC (BAP-0099-UK) is acknowledged.

L 528 COTTON FIBER CELL DEVELOPMENT, Barbara A. Triplett (1) and Norma L. Trolinder (2), (1) USDA, ARS, Southern Regional Research Center, New Orleans, LA 70126 and (2) USDA, ARS, Cropping Research Laboratory, Lubbock, TX 97401.
Cotton fibers are greatly elongated (>2.5 cm) single cells which arise from the ovule epidermis. Elongation of fiber cells occurs fairly synchronously over a 15 to 20 day period. Development is completed by the synthesis of a thick secondary cell wall composed principally of cellulose I. The nutritional and hormonal conditions for development of fibers on cultured ovules have been known for some time. More recently, it has been shown that cell suspension cultures from ovule callus will produce elongated cells capable of synthesizing some secondary wall material. The cells elongate for a period of 12 to 14 days and reach lengths of 1-2 mm. Both suspension and ovule cultures initiated from a cotton mutant deficient in the elongation phase of fiber development attain shorter final lengths than cultured cells from isogenic control ovules. X-ray diffraction of purified cell walls from suspension cultures indicates that cellulose is arranged in a Cellulose IV conformation identical to that reported for immature cotton fibers. Western blot analysis of proteins synthesized by fiber suspension cultures shows several proteins immunologically related to proteins synthesized by immature fibers produced in planta.

L 529 MOLECULAR ANALYSIS OF THE PATATIN GENE FAMILY OF POTATO (*SOLANUM TUBEROSUM* L.), David Twell and Gert Ooms, Rothamsted Experimental Station Harpenden, Herts. AL5 2JQ, UK.
The structural diversity and developmentally regulated expression of the patatin multigene family which encodes the major tuber protein of potato was investigated with particular reference to cultivar Desirée. It was estimated that there are 16-18 patatin DNA copies per haploid genome. Most of these showed little allelic variation and contained a characteristic 22bp insert in their untranslated leader DNA (Class II patatin genes), identfified using class-specific oligonucleotide probes. Ten genomic clones corresponding to individual patatin genes were isolated from Desirée. Four clones contained apparent pseudogenes truncated at their 5' ends, whilst the remaining clones contained Class II patatin genes with varying degrees of homology for 400bp of conserved 5' flanking DNA (from 77% to 95%). The role of 5' flanking DNA sequences in controlling organ-specific expression of individual patatin genes was investigated by constructing chimaeric genes including patatin 5' flanking DNA linked to reporter genes encoding bacterial CAT and GUS enzymes, followed by their reintroduction into potato plants using *Agrobacterium* mediated gene transfer. A 3.8 kp 5' flanking DNA fragment of patatin gene LPOT1 linked to the CAT gene was shown to be sufficient to direct tuber specific expression of CAT activity. A different patatin gene (LPOT2) construct involving translational fusion of the 5' flanking DNA and N-terminal coding DNA to the GUS gene was shown to be expressed in stolons tubers and roots of transgenic potato plants *in vitro*. Factors which influence the expression of the constructs such as GA_3, ethylene and photoperiod are currently being investigated.

L 530 EXPRESSION OF "TWIN" GENES FOR GLUTAMINE SYNTHETASE IN NODULES OF PEA. Elsbeth L. Walker and Gloria Coruzzi, The Rockefeller University, New York, NY 10021.
There are at least three isozymes of glutamine synthetase (GS) in peas (GS2, GS1 and GSn), each of which has a unique pattern of expression. The GSn polypeptides are highly expressed in nitrogen-fixing root nodules. There are two nearly identical, but non-allelic genes for GSn in the pea nuclear genome. We have isolated and characterized genomic clones corresponding to these two GSn genes (GS341 & GS132). Nucleotide sequence analysis of the 3' non-coding regions reveals that GS341 and GS132 differ by a 25 bp duplication in the 3' non-coding region of GS132. We have exploited this difference to design 3' S1 probes which distinguish the transcripts corresponding to each GSn gene. Transcripts for GS341 are 3-fold higher than those for GS132 under all conditions examined. The transcript level for each GSn gene is induced 5-10 fold in nodules compared to uninoculated roots. The induction of both GSn transcripts in nodules occurs 18 days post-infection (dpi), and high transcript levels persist until at least 37 dpi. The transcript level for each GSn gene was examined in Fix- nodules produced by inoculation of roots with nifA or nifD mutant Rhizobia. In the Fix- nodules, transcript levels for each GSn gene were equal to those found in wild type Fix+ nodules. These results demonstrate that newly fixed ammonia is not required for the induction of GSn mRNA observed in nitrogen-fixing nodules. Nucleotide sequence analysis of the promoters for GS341 and GS132 indicate that the GSn genes are virtually identical from the start site of transcription (-1) to -550, but show no sequence homology from -550 to -1500. The difference in promoter sequences for GS341 and GS132 may explain the observed difference in the level of expression of these "twin" GSn genes seen in vivo.

L 531 EXPRESSION OF RUBISCO ACTIVASE mRNAs AND POLYPEPTIDES DURING LEAF CELL DEVELOPMENT IN BARLEY, Jeffrey M. Werneke, Raymond E. Zielinski, and Michael E. Jenkins, Dept. of Plant Biology, University of Illinois, Urbana, IL 61801.
Rubisco activase is a stromal polypeptide that appears to be required by higher plants and algae to fully activate rubisco in vivo (Plant Phys 84, 930, 1987) We have used specific spinach rubisco activase polyclonal antibodies and barley activase cDNA clones to assess the relative steady-state levels of rubisco activase mRNAs and protein in the naturally occurring developmental gradient that exists in the leaves of 7-day-old barley seedlings. We have found that rubisco activase protein and mRNA increases with cell age, and that these increases parallel the changes observed in rubisco holoenzyme accumulation, and rubisco small subunit (rbcS) mRNA accumulation. In contrast, when etiolated, seven day old barley seedlings are exposed to light, rubisco activase mRNA levels increase approximately 50 fold after 6 hours, whereas rbcS mRNA levels increase by only about 1.5 fold during the same time period.

Hormone and Light Effects

L 600 GA REGULATED EXPRESSION FROM A WHEAT α-AMYLASE PROMOTER IN OAT ALEURONE PROTOPLASTS, A.K. Huttly and D.C. Baulcombe, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2LQ.
α-amylase is encoded in wheat by a large multigene family of approximately 30 members. These can be divided into 3 different classes based on gene structure, their expression and pI of their products. Those genes active in the aleurone layer of germinating wheat are subject to control by gibberellin. In order to investigate the nature of this control oat aleurone protoplasts have been isolated and are being used to assay α-amylase promoter constructs in transient expression experiments. Initial experiments have involved α-amylase and CaMV 35S promoters fused to the reporter gene GUS. Expression of the CaMV 35S construct was found to be independent of the presence of GA in the protoplast incubation media and could be detected at high levels 3 and 5 days after transformation. In contrast, expression of the α-Amy2 gene promoter was dependent on the presence of GA and could only be detected after a lag phase of 2-3 days incubation in parallel with the expression of oat α-amylase from the endogenous α-amylase genes. A patatin-GUS fusion was not expressed in these protoplasts. These experiments show that expression of the wheat α-amylase gene introduced into oat aleurone protoplasts was specifically regulated by a GA stimulated process.

L 601 CONTROL OF MATURATION GENES IN WHEAT BY ABA, Sonja K. Berge and Ralph S. Quatrano, Oregon State University, Corvallis, OR, 97331.
Mature wheat seeds provide a useful system to study embryo development as they pass from the quiescent state of the mature dry seed to the active state of germination and seedling formation. The plant growth regulator abscisic acid (ABA) appears to play a role in the regulation of gene expression during embryogenesis. Previous studies suggest that during embryogenesis transcription of genes constituting an embryo maturation set is promoted by ABA while transcription of germination specific genes is inhibited. Is the control of the maturation gene set by ABA limited to early embryogenesis? Does desiccation of the seed in the later stages of development prevent the gene set from responding to ABA in seedlings? A number of gene sequences and products of the maturation set were used to study the response of mature wheat embryos, germinating seedlings, and mature tissue to altered levels of ABA. Preliminary results indicate that certain genes of the maturation gene set in wheat, which respond to ABA, remain competent to respond to ABA in vegetative tissue. Such results suggest a more general function for these genes, that their expression may not be limited to embryogenesis, and that desiccation does not prevent their activation by ABA at later stages.

L 602 ALTERED POLPYPETIDE BIOSYNTHESIS IN DROUGHT-STRESSED ABA DEFICIENT TOMATO MUTANTS, Elizabeth A. Bray, University of California, Riverside, CA 92521.

Drought stress triggers ABA biosynthesis resulting in ABA accumulation in stressed leaves. ABA serves to reduce transpirational water loss from the plant. The ABA-deficient mutant of tomato, *flacca*, is drought sensitive because ABA biosynthesis is not induced in response to stress. This mutant has been used to distinguish polypeptides that are synthesized during drought stress in response to elevated ABA levels from those that are induced by altered water relations. Detached leaves from wild-type tomato, cv. Ailsa craig, and *flacca* were stressed, kept turgid or treated with ABA. Leaflets were labeled with ^{35}S-methionine for 2 h after varying periods of stress. 2-D gels demonstrated that a set of polypeptides are synthesized during stress in the wild type that are not synthesized in response to stress by the ABA-deficient mutant. Many of polypeptides produced in response to stress have molecular weights in the range of 15 to 40 kd. Polypeptides could be detected after a 1-h stress period. ABA treatments of *flacca* induced the synthesis of many of the stress polypeptides that were absent in the stressed mutant. These results support the hypothesis that a group of polypeptides are synthesized in response to drought-induced ABA.

L 603 MASS SPECTRAL IDENTIFICATION OF A HISTIDINE⟶TYROSINE SUBSTITUTION IN NON-BINDING SUBUNITS OF A CYTOKININ-BINDING PROTEIN, Chris Brinegar, San Jose State University, San Jose, CA 95192; Charles Hauer and Don Hunt, University of Virginia, Charlottesville, VA 22901; J. E. Fox, Miles Laboratories, Elkhart, IN 46514.

In a previous study using a radio-labeled azido derivative of the synthetic cytokinin benzyladenine (BA), a single histidine residue was shown to be the site of photoaffinity labeling within a 12-residue peptide of the wheat embryo cytokinin-binding protein. Binding studies, however, have indicated that only one BA binding site exists in the holoprotein, a trimer of 54 kDa subunits. In our attempts to completely sequence the protein by tandem quadrupole mass spectrometry, heterogeneity has been discovered in that same 12-residue sequence. In addition to obtaining a histidine-containing sequence identical to the photoaffinity labeled site, a sequence with tyrosine at that position has also been found. This is evidence that some subunits may be incapable of binding BA because of a His⟶Tyr substitution. A model of how this substitution may affect the structure of the binding site will be presented.

L 604 LIGHT-REGULATED EXPRESSION OF A PHYTOCHROME GENE FROM OATS IN TRANSFORMED TOBACCO. Wesley B. Bruce and Peter H. Quail, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

The expression of phytochrome genes from *Avena sativa* (oats) is strongly light regulated at the protein and mRNA levels. Steady-state levels of phytochrome transcripts in oats decline >90% within 3 h of a saturating pulse of red light or in continuous white light. This reduction in transcript levels can be abrogated by immediate irradiation with far-red light after the red pulse thus demonstrating a classical phytochrome response. We have now examined light regulated expression of an oat phytochrome gene and various translational fusion constructs with the CAT reporter in transformed tobacco plants. Transformed plants were placed in the dark for 4 days following a 15 min far-red pulse. Significant levels of CAT activity were seen in the extracts from the re-etiolated tobacco plants, whereas no activity was detected in plants remaining in the normal 16 h light/8 h dark cycle. Transcript levels of the phytochrome/CAT constructs were directly compared to those of endogenous tobacco phytochrome. The effects of various phytochrome promoter constructions on light regulation were also examined.

L 605 $tRNA^{Glu}$ IN THE SYNTHESIS OF Δ-AMINOLEVULINATE AND CHLOROPHYLL. Ph. Bruyant, S. Berry-Lowe, S. Gough, G. C. Kannangara and D. von Wettstein; Carlsberg Laboratory, Dept. of Physiology, Gamle Carlsberg Vej 10, 2500 Copenhagen Valby, Denmark.
The tetrapyrrole ring of chlorophyll is made from eight molecules of δ-aminolevulinate (ALA). ALA formation is a regulated step in chlorophyll synthesis. In chloroplasts ALA is synthesised from glutamate, which is first activated by ligation to a tRNA (δ-ALA-RNA). The activated glutamate is reduced by a dehydrogenase to glutamate 1-semialdehyde and then isomerised by an aminotransferase into ALA. The nucleotide sequence of δ-ALA-RNA fits the clover leaf structure of tRNA and the anticodon UUC identifies it as a glutamate tRNA. The δ-ALA-RNA gene is located in the chloroplast DNA and has been sequenced. The barley chloroplast glutamate-tRNA ligase, with an apparent molecular weight of 54,000, has been purified and shown to activate glutamate for both chlorophyll and protein synthesis. It also charges glutamate onto the two glutamine tRNAs identified in chloroplasts; the glutamyl-$tRNA^{Gln}$s are subsequently converted into glutaminyl-$tRNA^{Gln}$s. The chloroplast enzyme can charge *E. coli* $tRNA^{Glu}$ and is immunologically related to *B. subtilis* glutamate-tRNA ligase. The barley ligase can be easily purified by immunoaffinity chromatography, which is the only technique found so far that achieves the separation of an active dehydrogenase fraction from the ligase. The light dependent regulation of ALA synthesis is being studied in barley mutants which either overproduce protochlorophyllide or are blocked in chlorophyll synthesis. The activity and content of δ-ALA-RNA in mutants are higher in the former and lower in the latter compared with those in wild type. We are also analysing the formation of the nuclear encoded components performing the conversion of glutamate into ALA.

L 606 LIGHT INDUCED DECREASE IN β-TUBULIN MRNA ABUNDANCE, Stephen A. Costigan, Zhifan Zhao and James T. Colbert, Department of Biology, Colorado State University, Ft. Collins, CO 80523.
The oat β-tubulin cDNA clone pβ1 was used to measure β-tubulin mRNA abundance in etiolated seedlings of both *Avena sativa* and *Hordeum vulgare*. Irradiation of 4-day-old etiolated oat seedlings with ten seconds of red light or by transfer to continuous white light resulted in a 50% decrease, relative to unirradiated controls, in β-tubulin mRNA abundance by 3 to 6 hours post-irradiation. A similar decrease in β-tubulin mRNA abundance was observed in 4-day-old etiolated barley seedlings given five seconds of red light. These data add an additional gene to the relatively short list of genes known to be negatively regulated by light (1). We are currently investigating whether phytochrome mediates this response to light. This work was supported by the Colorado State University Agricultural Experiment Station.
1. Tobin, E.M. and J. Silverthorne (1985) Ann. Rev. Plant Physiol. 36:569-593.

L 607 CYTOKININ INDUCED mRNA CHANGES IN *N. PLUMBAGINIFOLIA* CELLS, Janice A. Dominov and Stephen H. Howell, University of California, San Diego, La Jolla,CA 92093.
The importance of cytokinins for the growth and development of plant cells has been observed for many years, yet the mechanisms by which this class of plant hormone acts are largely unknown. Analysis of such mechanisms has been hindered by the lack of adequate means to monitor the initial changes in gene expression or cell physiology in response to altered hormone concentration. We have used *Nicotiana plumbaginifolia* cell cultures capable of growth in the absence of exogenous cytokinin as a system in which to identify early, specific changes in gene expression in response to cytokinin application. Total mRNA from cells exposed to the cytokinin N^6-benzyladenine (BA) for various lengths of time was translated *in vitro* and the protein products analyzed by 2-dimensional electrophoresis and compared with untreated controls. The level of several proteins increased within 24 hours of BA exposure, some changes evident as early as 6 hours of treatment. Such changes in translatable mRNA populations demonstrate the utility of this system for detecting fairly rapid changes in gene expression in response to cytokinin. A cDNA library from 6 hour hormone-treated cells is currently being screened in order to identify specific transcripts affected by altered cytokinin concentrations.

L 608 THE EXPRESSION OF INDIVIDUAL MEMBERS OF THE rbcS GENE FAMILY IN POTATO IS DIFFERENTIALLY REGULATED

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We have obtained genomic clones carrying four of the five genes coding for the small subunit of Ribulose-1,5-bisphosphate carboxylase of potato (Solanum tuberosum). One gene (rbcS1) is unlinked to the other genes and is interrupted by three introns. The other three genes (rbcS2a,2b,2c) are clustered in a tandem array and contain two introns each.
We have developed a method which allows to measure the level of transcription of all four rbcS genes simultaneously. The analysis shows, that the rbcS1 transcript is generally present at a higher concentration than the transcripts of the other three genes. The relative levels of the mRNAs however are found to vary between different organs and developemental stages of the plant. The expression of the four genes also reacts differentially to various light induction conditions.

L 609 DIFFERENTIAL REGULATION OF THREE CHLOROPHYLL A/B-PROTEIN COMPLEXES AT THE POST-TRANSCRIPTIONAL LEVEL, Beverley R. Green and Michael J. White, Botany Dept., University of B.C. Vancouver, B.C., Canada V6T 2B1.

The three Chl a/b complexes of higher plants (CP29 and LHCII in PSII; HCI in PSI) have polypeptides of 20-30 kD which share partial sequence homology. The polypeptides of all three complexes are absent in etiolated barley, and are accumulated in parallel with Chl b upon illumination. However, the absence of Chl b in the barley mutant chlorina f2 does not prevent accumulation in the light of the apoprotein of CP29 (the core a/b antenna of PS II) nor a minor 25 kd LHC II polypeptide. Using a sensitive immunoblotting method, at least one of the LHCI polypeptides could also be detected, but the two major LHCII polypeptides were almost totally absent. In contrast, barley plants grown under intermittent light, which also lack Chl b, do not have any detectable LHCI or major LHCII polypeptides, although they accumulate the CP29 and 25 kD polypeptides.

These results show that the availability of Chl b has different effects on the synthesis/accumulation of the different a/b-binding polypeptides. The striking difference between CP29 and the major LHCII polypeptides could be related to the lower amount of Chl b in CP29 (Chla/b=3-4 cf. 1.2 for LHC II). However, other factors must be involved in regulating the LHC I polypeptides. (Supported by NSERC).

L 610 **MONOCLONAL ANTIBODIES TO AN IMMUNODOMINANT CARBOHYDRATE EPITOPE PRESENT ON A VARIETY OF PLANT CELL SURFACE MACROMOLECULES**, Michael G. Hahn, Rolf Craven, Eva Bucheli and Felice Cervone. Univ. of Georgia/U.S. Dept. of Energy Complex Carbohydrate Research Center, USDA Russell Center, P.O. Box 5677, Athens, GA 30613.

Monoclonal antibodies (McAbs) are useful tools to probe the structure of plant cell surface components, and to follow cell surface macromolecules as they are biosynthesized. McAbs were generated to *Nicotiana tabacum* leaf protoplasts and to rhamnogalacturonan I (RG-I), a plant cell wall pectic polysaccharide. The epitope(s) recognized by four anti-protoplast McAbs have been shown by indirect immunofluoresence to occur on cell surfaces of plant protoplasts and walls of intact cells. These epitope(s) were periodate sensitive. Four anti-protoplast McAbs and two anti-RG-I McAbs show similar staining patterns when reacted with Western blots of either total cellular proteins from *N. tabacum* or an arabinogalactan protein purified from those extracts. All six McAbs react with RG-I, but not with rhamnogalacturonan II, another primary cell wall pectic polysaccharide. The six McAbs also bind to a plant cell wall-localized glycoprotein that specifically inhibits fungal endopolygalacturonases. The data suggest that a wide array of plant cell surface macromolecules carry common structural motifs, and that a carbohydrate epitope common to these various macromolecules is immunodominant in mice. The epitope(s) recognized by the McAbs are being characterized. Our observations suggest that many cell surface macromolecules are similarly glycosylated. Thus, alternate strategies are likely to be required to generate McAbs to specific plant cell surface macromolecules. (Supported by grant #DE-FG09-85ER13426 from the Department of Energy)

L 611 MOLECULAR ANALYSIS OF PHENYLPROPANOID & FLAVONOID ENZYMES IN DEVELOPING SEEDLINGS of FAGOPYRUM and BRASSICA. G. Hrazdina, S. Reeves, Y.J. Sun & A.M. Zobel, Institute of Food Science, Cornell Univ., Geneva, NY 14456.

Etiolated buckwheat (Fagopyrum esculentum M.) and red cabbage (Brassica oleracea cv. Red Danish) seedlings respond to illumination inter alia by synthesis of flavonoid compounds. The key enzymes of the flavonoid metabolic pathway(s) are Phenylalanine Ammonia Lyase (PAL, E.C. 4.3.1.5), which channels phenylalanine from protein biosynthesis into the phenylpropanoid pathway; Chalcone Synthase (CHS, E.C. 2.3.1.74), the first enzyme of the specific flavonoid pathway that condenses 3 molecules of malonyl-CoA with one molecule of p-coumaryl-CoA to form naringenin chalcone; and a flavonoid Glucosyltransferase (GT, E.C. 2.4.1.91) that renders the end product soluble and possibly determines the vectorial transport of the metabolic products and their accumulation in the vacuole. In both plants flavonoid compounds were found to accumulate in the epidermal layers. Phenylalanine ammonia lyase and chalcone synthase were isolated from illuminated Fagopyrum, and the flavonoid glucosyltransferase from Brassica seedlings at the developmental stages when enzyme activities were at their peak (Fagopyrum 18 h, Brassica 36 h illumination). During the isolation procedures using standard techniques, all three enzymes showed signs of heterogeneity, although denaturing gel electrophoresis showed homogeneous protein preparations. We have developed specific polyclonal (CHS, GT) and monoclonal (PAL) antibodies against the three enzymes and probed their homogeneity on two dimensional immunoblots. Phenylalanine ammonia lyase from Fagopyrum seedlings showed two different subunit groups consisting of approximately six isoforms each. Chalcone synthase from the same plant was shown to consist of approximately six major isoforms. Both the PAL and CHS isoforms showed differences in charge, but not in M_r. The flavonoid glucosyltransferase from Brassica seedlings showed partial separation into three major components during chromatofocusing on a PBE column. Three major forms of this enzyme that differed in M_r were detected on denaturing gels. The approximate number of flavonoid compounds in illuminated Fagopyrum seedlings seems to coincide with the number of isozymes in the one PAL group and with CHS. In Brassica seedlings, three major glycoside groups of flavonoids were detected, coinciding with the number of glucosyltransferase isoforms. These data suggest the involvement of multiple parallel pathways in the production of the diverse flavonoids in these plants. Members of diverse flavonoid groups were implicated in the nodulation of legumes and in the disease resistance mechanisms of some plants.

L 612 GENE EXPRESSION DURING GROWTH AND MATURATION OF THE CONIFER LARIX LARICIA, Keith W. Hutchison, Patricia B. Singer and Michael S. Greenwood, The University of Maine, Orono, Maine 04469.

Maturation, or phase change, in conifers is associated with a decrease in the rate of growth, a decrease in the ability to root cuttings, a decrease in the ability to propagate cells in culture, the onset of the capacity for sexual reproduction and the increase in the amount of chlorophyll produced by the foliage. To understand this process at the molecular level, and to understand gene expression in conifers, in general, we have constructed cDNA libraries from juvenile and mature plants of *Larix laricina*, or larch. These libraries have been screened for clones which represent sequences which are differentially expressed between juvenile and mature plants. In addition, clones for the small subunit of ribulose-bisphosphate carboxylase/oxygenase (*rbc*S) and for the light harvesting complex (chlorophyll a/b-binding) protein (LHCP)

cDNA clones which encoded for *rbc*S were identified using a probe from corn. Sequences which represent LHCP were identified using a probe from pea. Both genes are expressed in foliage but not in roots, and both are part of multi-gene families. However, though the *rbc*S sequences are expressed at the same levels in both juvenile and mature trees, LHCP sequences showed a strong differential expression, being expressed at much higher levels in newly expanded foliage from juvenile plants than the same foliage from mature plants.

L 613 THE EFFECT OF ENDOGENOUS AND EXOGENOUS PHYTOHORMONES ON THE REGENERATION OF SUGARBEET (BETA VULGARIS L.) PLANTS.Frans A. Krens,D. Jamar,W. v.d. Molen and H. Huizing, Foundation for Agricultural Plant Breeding, P.O.Box 117,6700 AC Wageningen, The Netherlands.

Inoculations of GA3 elongated sugarbeet stems with Agrobacterium tumefaciens strains LBA4001 (wild type) and LBA1501 (shooter mutant) both gave rise to the same response: unorganized tumor growth. This phenomenon and other in vitro properties of sugarbeet plants and cell suspension cultures suggest that the endogenous level of auxins in B.vulgaris is elevated as compared to other plant species. However, direct measurements do not confirm this(1). When these tumors were isolated and cultured on media containing 10 uM BA, shoot regeneration occurred both on 4001 and 1501 derived tumor calluses. This might indicate that the sensitivity of cytokinin receptors is much greater than of auxin receptors in these tissues. Unfortunately, no transgenic shoots were obtained. Transformed callus obtained after leaf-disc transformation did not show this response. Firstly, no direct regeneration of transformed sugarbeet shoots occurred, secondly, placing the octopine positive calli on different media varying in hormone composition and concentrations did not result in any plant regeneration. A difference with the former stem inoculations is that the explants were immediately exposed to 10 uM BA which might influence the endogenous cytokinin levels even in later stages, since the presence of BA has been demonstrated in tissues even 4 weeks after removal of BA from the medium(1). Several Agrobacterium strains carrying only the cytokinin locus of the original T-DNA are used in studies to determine the effect of raising the endogenous cytokinin levels on organogenesis. Preliminary experiments on leaf-discson media with 10 uM BA showed no shoot formation. Experiments in which different plant organs were used as explant sources which were placed on media with different combinations of BA and 2,4-D, showed a marked divergence in shoot regenerating capacity. Sofar, the data suggest organ-specific phytohormone sensitivity in sugarbeet.

1. R. Horseele, Report University of Antwerpen.1986

L 614 SLENDER BARLEY: A CONSTITUTIVE GIBBERELLIN-RESPONSE MUTANT, Michael B. Lanahan and Tuan-Hua David Ho, Washington University, St. Louis, MO 63130

In barley (*Hordeum vulgare* L. cv Herta), *slender* (*sln1*) is a single locus recessive mutation which causes a plant to appear as if it had been grown in saturating concentrations of the phytohormone gibberellin (GA). The phenotype of slender barley suggests that either it is over-producing GA or its tissues are continuously in GA-induced states independent of hormone concentrations. We have investigated two of the GA-mediated processes in slender barley, shoot elongation and the induction of hydrolytic enzymes in aleurone layers. Shoot elongation is severely retarded in normal barley if the biosynthesis of GA is blocked by an inhibitor, ancymidol. However, the slender mutant continues to elongate in the presence of ancymidol. In isolated normal aleurone layers, the synthesis and secretion of a-amylase, protease, and nuclease are induced by exogenously applied GA. However, in the aleurone layers of the slender mutant these enzymes are induced even in the absence of GA. This GA-independent synthesis of hydrolytic enzymes is still susceptible to inhibition by another phytohormone, abscisic acid (ABA). Bioassays of the slender mutant and their normal siblings show no detectable differences in endogenous levels of GA-like substances. We suggest that the slender mutation allows competent tissues to fully express, or over express, appropriate GA-induced processes independent of GA concentrations. We also conclude that shoot elongation, and hydrolytic enzyme secretion in aleurone layers, share a common regulatory element.

L 615 **AUXIN STARVATION, AND TREATMENT WITH GLUCAN ELICITOR ISOLATED FROM PHYTOPHTHORA MEGASPERMA INDUCES SIMILAR RESPONSES IN SOYBEAN CULTURED CELL SUSPENSIONS,** J.J. LEGUAY and J.P. JOUANNEAU, Laboratoire de physiologie Cellulaire Vegetale, C.N.R.S., BP n°1 91190 Gif sur Yvette, France.

Auxin (2,4-Dichlorophenoxyacetic acid) starvation of soybean cell suspension induces accumulation of a well known isoflavonoid-derived phytoalexin (glyceollin) and a highly elevated catalitic activity of two of the enzymes associated with glyceollin biosynthesis, phenylalanine ammonia-lyase and chalcone isomerase, is described. Moreover, stimulation of catalitic activity of enzymes chitinase and ß 1,3-glucanase involved in plant defense against pathogens is observed at the same time. Analysis of patterns of *in vitro* protein synthesis shows that a set of polypeptides are induced by both treatments. Our results provide evidence of a dual control by auxin and fungal elicitor working in an opposite manner on the inducibility of enzymes and proteins playing a role in the induced defense response of soybean.

L 616 MANIPULATION OF AUXIN, CYTOKININ AND GUS LEVELS USING THE MAIZE HSP70 PROMOTER, June I. Medford and Harry J. Klee, Monsanto Company, St. Louis, Missouri 63198.

Development in plants is controlled by both cell autonomous and non-cell autonomous events. Examples of non-cell autonomous control regulators are phytohormones. We have produced transgenic plants containing the Agrobacterium tumefaciens genes for auxin and cytokinin biosynthesis fused to the maize HSP70 (heat shock) promoter. As a control, we have examined expression from the HSP70 promoter with the reporter gene β-glucuronidase (GUS). Histological and fluorometric analyses have shown that even under non-induced conditions, the HSP70 promoter is functional in the pistils and anthers of transgenic flowers. After induction by heat shocking *in vivo* for 2 hours, GUS levels are increased approximately 30-fold. Induction experiments have also been carried out on plants transformed with chimeric auxin and cytokinin biosynthesis genes. Transgenic Arabidopsis and petunia plants show phenotypes characteristic of transient bursts of auxins and cytokinins. We are currently conducting experiments giving transient expression of phytohormones at various times in development.

L 617 TUBULIN GENE EXPRESSION DURING INTERNODE ELONGATION IN OATS,
Nandini Mendu and Carolyn Silflow, University of Minnesota,
Department of Genetics and Cell Biology, St.Paul, MN 55108

Microtubules play key roles in the processes of cell division and cell enlargement during plant growth and developement. We are studying the organization and expression of genes coding for alpha and beta tubulin, the major components of microtubules, in hexaploid oats. Southern blots of oat DNA suggest that the genome contains 8-10 alpha tubulin genes and 12 -15 beta tubulin genes. To examine tubulin gene expression associated with cell elongation, we have used the previously well-characterized system of the excised penultimate internode segments (Kaufman,P.B.,1965, Physiol. Plant., 18: 703). In this system the GA_3 induced in vitro elongation is entirely due to cell elongation and not cell division. Internode segments treated with GA_3 elongate 4-5 cm over control untreated internodes by 48h. Addition of colchicine, which causes the depolymerization of microtubules, inhibits the GA_3 induced elongation by 60% and causes the cells to become laterally distended. In contrast, abcissic acid, does not cause the cells to increase their width even though it inhibits the GA_3 induced elongation by about 50%. These observations implicate a role for microtubules during cell elongation. We find that elongation is coincident with enhanced expression for both alpha and beta tubulin genes. Transcripts for both alpha and beta of tubulin show an increase with time, reaching a maximum (2-4 fold) at 24h when the internodes have elongated to approximately one-third the length they attain in vitro.

L 618 SEQUENCE OF THE GENE FOR PHOSPHORIBULOKINASE (PRK) FROM SPINACH. Sylvia Milanez and Richard J. Mural. The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and the Protein Engineering Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831. We have constructed a λgt11 spinach cDNA library and have isolated from it two clones for PRK, a light-regulated enzyme of the Calvin cycle. DNA sequence analysis of these clones showed that they code for a protein which contains several tryptic fragments previously isolated from the mature spinach enzyme and whose amino acid sequences have been published (Krieger, et al. 1987. Biochem. Biophys. Acta 915:112-119; Porter and Hartman, 1986. Biochemistry 25:7314-7318). These two clones share a common region of about 1.3 kbp which codes for a predicted mature spinach PRK of 351 amino acids with a molecular weight of about 39300. The clones also encode a part (56 amino acids) of a leader peptide which is presumably processed during the maturation of PRK. Using these clones to probe total spinach DNA, we have shown that PRK in spinach is encoded by a single copy gene. (Sponsored by OHER, USDOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. S.M. was supported by the NIH Training Grant GM 07438 through the University of Tennessee.)

L 619 MORPHOGENESIS OF TOBACCO THIN-CELL-LAYERS IN LIQUID MEDIA: BIOASSAY OPTIMIZATION FOR STUDYING OLIGOSACCHARIN ACTIVITY AND GENE EXPRESSION. Debra Mohnen, Stefan Eberhard, Victoria Marfa-Riera, Nancy Doubrava, Teresa Gruber, Maria Elena Mayorga, Patrick Toubart, David Gollin, Alan Darvill and Peter Albersheim. Univ of Georgia/Dept of Energy Complex Carbohydrate Research Center, Dept of Biochem, USDA Russell Ctr, P.O. Box 5677, Athens, GA 30613.

The early experiments that suggested oligosaccharins (cell wall oligosaccharides with regulatory activity) may regulate plant organogenesis were performed by incubating tobacco thin-cell-layers (TCLs) on liquid media in the absence or presence of plant cell wall fragments (Tran Thanh Van et al (1985) Nature 314:615). Due to the paucity of information on TCL organogenesis in liquid media, we have found it necessary to characterize and optimize the TCL bioassay for reproducibility of the morphogenesis programs. We will present the results of these optimization studies, which include the importance of uniform donor plants, use of multi-well dishes for individual TCL culture, and the effects of light, hormone concentration, pH and cell wall fragments on organogenesis. In addition, our approach to using the TCL system to study gene expression during organogenesis will be presented. We will describe our use of the TCL bioassay in the purification of a pectic polysaccharide that can alter organogenesis of TCLs.

Supported by NIH Fellowship 1 F32 GM11857-01 BI-1 and DOE Grant DE-FG09-85ER13425.

L 620 PHOTOREGULATED EXPRESSION OF AL-3 GENE IN *NEUROSPORA CRASSA* G. Morelli*, M.A.Nelson# and G. Macino# *Istituto Nazionale della Nutrizione v.Ardeatina 546 00179 Roma #Dip. Biopatologia Umana Universita' "La Sapienza" 00185 Roma Italy

The fungus *Neurospora crassa* is a very promising system to study the intermediate steps leading from the activation of the photoreceptor to the induction of gene expression. *N.crassa* is an extremely well-developed genetic system and adequate preliminary results concerning photobiology are available. The exposure of dark-grown cultures of *N.crassa* to a brief pulse of blue light generates several effects. One of the short-term effects is the de novo synthesis of carotenogenic enzymes. Albino mutants that are blocked in carotenogenesis (due to defects in the structural genes for pigment formation) have been isolated and characterized (al-1,al-2,al-3). Two mutants in regulatory genes whose products are required for the light-induction process have already been identified (called white collar, wc-1 and wc-2). We recently cloned the al-3 gene by complementation of a mutant strain. The gene probably codes for the geranyl-geranyl pyrophosphate synthetase. The expression of this gene is controlled by blue light. Experiments are under way to define the sequence of the gene al-3 and to isolate the wc-1 and wc-2 genes, whose products regulate the photoinduction of the al-3 gene. The photoinduction of carotenogenesis in *N.crassa* may serve as a model system for the photoinduced processes that occur during development in eucariotic organisms.

L 621 REGULATION OF CHLOROPLAST GENE EXPRESSION BY LIGHT, John E. Mullet, Tineke Berends Sexton and Patricia E. Gamble, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2128.

The chloroplast genes, psbD and psbC, encode two polypeptides of Photosystem II. These genes are adjacent in the barley chloroplast genome and are part of a 5.7 kbp transcription unit. During plastid biogenesis in dark-grown barley, translation of the psbD gene product is readily detected. In contrast, psbC gene product translation is only detected when plants are illuminated. Etioplasts of dark-grown barley contain four transcripts which contain psbD and psbC sequences. Furthermore, the light-induced increase in psbC gene product translation occurs prior to light-induced changes in the psbD-psbC transcript population. Therefore, psbC gene product accumulation is regulated by light at the level of protein translation or protein stability.

Illumination of dark-grown plants for 72 h causes a decline in psbD-psbC transcripts found in etioplasts. However, two different psbD-psbC transcripts accumulate in illuminated plants. The light-induced psbD-psbC transcripts accumulate during a phase of chloroplast maturation when transcript levels and translation of other chloroplast genes such as rbcL, psaA-psaB, psbB and atpB/E decline. This suggests that the light-induced accumulation of psbD-psbC transcripts is required to maintain relatively high levels of psbD and psbC gene product translation in mature chloroplasts. Evidence for this possibility will be presented and discussed in relation to chloroplast biogenesis in barley leaves.

L 622 PRIMARY STRUCTURE AND IN VITRO TRANSLATION OF GENES ENCODING A QUINONE AND A CHLOROPHYLL BINDING PROTEIN OF PHOTOSYSTEM II, Eva Neumann, Carlsberg Laboratory, Copenhagen, Denmark.

The core of photosystem II (PSII) comprises four integral membrane polypeptides: D-1 and D-2 are suggested to bind the reaction center chlorophyll as well as the primary and secondary electron acceptors. Presence of the two chlorophyll binding proteins of 44 and 47 kd in the thylakoid membrane is also essential for the physiological function of PSII, which suggests a coordinated regulation of gene expression. I present the primary structure of the barley genes psbD, encoding D-2, and psbC, encoding the 44 kd protein. The two genes overlap in the chloroplast genome. Analysis of the nucleotide sequence elucidates some features of the observed RNA heterogeneity: a) Putative promoter consensus sequences upstream of the psbD gene were identified. Their significance for recently established light-induced transcription will be discussed. b) A sequence region in the 3' end of psbD allows the prediction of a hairpin structure, which might serve as a processing signal for an RNA exonuclease. Translation experiments of in vitro derived psbD transcripts in a rabbit reticulocyte lysate give three products of 30, 17 and 48 kd, respectively. All three polypeptides precipitate with antibodies directed against D-2. Also presented are in vitro translation experiments performed in the presence of different quinones. The specific effect of quinones on the translation of psbD will be discussed.

L 623 ORGANIZATION OF PHOTOSYSTEM II PIGMENT-PROTEINS, Gary F. Peter and J.P Thornber, Dept of Biology, University of California, Los Angeles,90024

A photosystem (PS) is composed of multiple pigment binding proteins. These proteins form two distinct complexes: a core complex (CC) which contains those polypeptides and cofactors essential for a stable and photochemically active complex in vivo and a light harvesting complex (LHC) where most of the antenna pigments are organized but which is not essential for a stable CC in vivo. We have used nondenaturing Deriphat-PAGE to separate PS II into seven pigment-proteins. We show that LHC II is composed of four distinct pigment-proteins a, b, c, and d. We have studied the requirements of these LHC II pigment-proteins for stable complex formation and their organization with each other and CC II by 1) purifying CC II depleted of various LHC II pigment-proteins, 2) reconstituting LHC II subunits with these complexes, 3) analyzing mutants that lack various LHC II subunits and 4) determining the presence of LHC II pigment-proteins in plants grown under various light regimes. We will present these data and a model that describes the organization of PS II with an emphasis on the dynamic aspects of PS II structure and function.

L 624 GIBBERELLINS (GAs) IN RELATION TO FLOWERING IN SHORT- (SDP) & LONG-DAY (LDP) PLANTS. Richard P. Pharis, David Pearce, Loeke Janzen, Lloyd T. Evans, Rod W. King and Lewis N. Mander, Univ. Calgary, Calgary, AB, Canada (RPP, DP, LJ), Plant Industry, CSIRO, Canberra ACT 2601, Australia (LTE & RWK), Res. Sch. Chem., ANU, Canberra.

GA metabol. and conc. in leaves, or exported from them during & after the 1 day required for floral induction, was examined in LDP Lolium temulentum and SDP Pharbitis nil.

In Lolium changes (increases) in leaf GAs after 1 LD are complex, & some are reflected in the GA spectrum of phloem exudate & shoot apices. Monohydroxylated GA_4 and GA_7 are prominent in phloem exudate, & putative 11β-OH GA_7 is found mainly in leaves. Putative polyhydroxylated GA-like substances and/or their glucosyl conjugates increase in leaves, their phloem exudate, & in shoot apices 24-72 hr after the 1 LD. This is consist. with the particular effectiveness of the polyhydroxylated GA_{32} in promoting floral initiation, with minimal effect on stem elongation. GA_1, in contrast, has little effect on flowering, but causes sig. increase in stem elongation. Thus, there is some specificity in flowering resp. to GAs, & this is influenced by the double bond in ring A, as well as no. & location of hydroxyls.

In Pharbitis the metabolism of native GA_5 in the cotyledons during & after 1 SD was examined with [^{3}H] GA_5. More free GA_1 and GA_3, and less of their conjugates were formed from GA_5, both rel. to non-inductive LD. This increase of free GAs & their decreased conjugation is consistent with the floral promotion that occurs when GAs are applied just before inductive dark to a GA-deficient dwarf Pharbitis strain.

Thus, both LDP & SDP photoperiod induct. is assoc. with enhanced prod. of specif. GAs.

L 625 EPITOPE MAPPING: A SYSTEMATIC APPROACH DEMONSTRATED WITH pUC18 FUSION PROTEINS DERIVED FROM PHYTOCHROME cDNA, Laura Thompson,* Lee Pratt,* Lyle Crossland,† and Marie-Michèle Cordonnier,† *Botany Department, University of Georgia, Athens, GA 30602, and †CIBA-GEIGY Biotechnology, Research Triangle Park, NC 27709.

Recombinant DNA technology permits elucidating with high resolution the location of epitopes along the primary sequence of a large protein. We demonstrate here a three-step, systematic approach to epitope localization, using phytochrome cDNA and monoclonal antibodies to this chromoprotein. An approximately 2 kb cDNA fragment, which codes for amino acids 464 through the C-terminus of phytochrome, was isolated from λgt11. (1) This cDNA was first isolated and then cut by selected restriction enzymes, removing about 300 bp at a time from both the N- and the C-termini, independently. After ligating these fragments in frame into pUC18, fusion proteins were screened by immunoblotting following SDS PAGE. In this way, epitopes can be defined to within about ±50 amino acids. (2) A pUC18 construct containing one of the previous cDNA fragments, which terminates on the 5' side of the epitope, was opened at its 3' end and then digested in the 5' direction with Bal31. Resultant fragments, each 15-20 bp smaller than the previous one, were re-ligated and fusion proteins immunochemically screened as above. Putative epitopes are thereby identified to within ±3-5 amino acids. (3) An analogous Bal31 digest series was produced, but beginning at the 5' end of an appropriate fragment from the first step, and proceeding in the 3' direction. With this series, putative epitope assignments can be tested. It is therefore possible to identify an epitope precisely and unambiguously. Moreover, this approach does not rely on chance as is the case with most other methods that utilize recombinant DNA technology.

L 626 Isolation and Characterization of Potato Genes Hybridizing to a Bean Chitinase Probe. Daniel Laflamme and Robert Roxby, University of Maine, Orono, Maine 04469.
A bean chitinase cDNA probe [Broglie et al. PNAS 83, 6820 (1986)] has been used to isolate hybridizing genomic clones from potato. One of the genomic clones has been characterized by restriction mapping and found to be 12 kb in length with no internal EcoRI or SmaI sites and 4 internal HindIII sites. The sequences hybridizing to the bean probe are contained entirely within one internal 4.2 kb HindIII fragment, which was subcloned (pCH1H4.2) and used to probe RNA blots. Dot-blots of messenger RNA show about 5-fold induction of sequences hybridizing to pCH1H4.2 in response to ethylene treatment. Northern blots show a single hybridizing band band of about 1kb. A cDNA library was screened with pCH1H4.2 and one of the selected cDNA clones has been partially sequenced. It is 900 bases long and contains a central SmaI site and therefore is not a product of the gene represented by the cloned probe. The potato clone is not of full length, including neither transcription start or stop signals, but contains sequences corresponding to the middle 90% of the bean gene. The amino acid sequence deduced from the single open reading frame of the cDNA clone is 61% homologous to the bean sequence.

L 627 PHYSCOMITRELLA PATENS AS A POTENTIAL SYSTEM TO STUDY PHOTOREGULATION OF GENE EXPRESSION, Didier G. Schaefer and J.-P. Zrÿd, LPC, University of Lausanne, 1015 Lausanne, Switzerland.

The moss Physcomitrella Patens is a particularly suitable system to study photomorphogenesis. Growing on a simple mineral medium, its life cycle is completed within three months. The germination of its spores and the development of its protonemata and its gametophores are photoregulated. Phytochrome is implied at least in the last two steps (Cove et al.,1978, Photochemistry and Photobiology 27, 249-254). The first division of P. Patens protoplasts is strictly photodependent with phytochrome involved. Polarotropic orientation of this division has been demonstrated (unpublished data from our lab).

We have built a genomic library and screened it with heterologous cDNA probes for the small subunit of the RUBISCO and the chlorophyll a/b binding protein. Several positive clones were isolated and are now being characterised. We intend to construct an expression vector under the control of the promoter and the polyadenylation sequences of these genes in order to develop a light induced transient assay system.

L 628 HORMONE-SENSITIVE ION CHANNELS IN PLANT PROTOPLASTS, Charles L. Schauf, Kathryn J. Wilson and Marsha E. Stockton, Dept. Biology, IUPUI, Indianapolis, Indiana 46223
Plant hormones regulate growth and a variety of physiological responses, but their cellular mechanism of action is not well-defined. We used patch voltage-clamp to study a K^+ channel in Vicia faba guard cell protoplasts whose activity was enhanced by micromolar levels of abscisic acid (ABA) and a Cl^- channel in Asclepias tuberosa suspension cell protoplasts blocked by ABA. Single channel K^+ conductance in Vicia faba was 65 ± 5 pS and mean open time was 1.25 ± 0.30 msec at 150 mV. These K^+ channels were blocked by TEA^+ and internal Cs^+, but not by 4-aminopyridine. In addition, ABA increased mean open time without changing the single-channel conductance or reversal potential, and induced long-lasting bursts of channel openings. That a similar, Ca^{++}-mediated response exists in gurad cells in vivo is suggested by the effects of ABA and ion channel blockers on stomatal openings in epidermal peels and guard cell protoplast volume. Asclepias tuberosa suspension-cell derived protoplasts had both K^+ and Cl^- channels with single channel conductances of 40 ± 5 pS and 100 ± 17 pS respectively. Mean open time for these TEA^+-sensitive K^+ channels was 4.5 ± 0.4 msec at 100 mV, while Cl^- channels had a mean open time of 8.8 ± 1.2 msec and were blocked by Zn^{++}, ethacrynic acid, and ABA. Since internal and external composition is controlled patch-clamped protoplasts are ideal systems in which to elucidate the role of ion channels in plants and their possible modulation by intracellular second messenger systems.

L 629 RbcS GENES IN SOLANUM TUBEROSUM: CONSERVATION OF TRANSIT PEPTIDE AND EXON SHUFFLING DURING EVOLUTION, F.P. Wolter, C.C. Fritz, J. Schell and P.H. Schreier[1,2]
1. Max-Planck-Institut für Züchtungsforschung, Egelspfad, 5000 Köln 30, FRG
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Five genes of the rbcS gene family of Solanum tuberosum (potato) were studied. One of these is a cDNA clone, the other four are located on two genomic clones representing two different chromosomal loci containing one (locus 1) and three genes (locus 2), respectively. The intron/exon structure of the three genes in locus 2, is highly conserved with respect to size and position. These genes contain two introns whereas the gene from locus 1 contains three introns. Although in most cases the amino acid sequences in the transit peptide part of different rbcS genes from the same species varied considerably more than the corresponding mature amino acid sequences, one exception found both in tomato and potato indicates that the transit peptide of rbcS could have a special function. A comparison of the rbcS genes of higher plants with those of prokaryotes offers suggestive evidence that introns first served as spacer material in the process of exon shuffling and then were removed stepwise during the evolution of higher plants.

L 630 **RESVERATROL SYNTHASE AND CHALCONE SYNTHASE: RELATED GENES AND PROTEINS, BUT DIFFERENT ENZYME FUNCTIONS,** **Joachim Schröder and Gudrun Schröder, Universität, D-7800 Freiburg, FRG.**

Stilbenes are phytoalexins produced in some plants in response to various stresses. Resveratrol synthase (RS), the key enzyme, synthesizes resveratrol from p-coumaroyl-CoA and malonyl-CoA. We isolated RS cDNA clones from libraries with RNA from peanut (Arachis hypogaea) cultures specifically induced for RS, and we identified two overlapping genomic clones in EMBL3 libraries. One contains the 5'-end, the other the 3'-end of RS genes, and the two are nearly identical in an overlap of about 490 bp. A single intron was identified, and the predicted protein subunit size is 42.7 kDa, in close agreement with the size observed in SDS gels (43 kDa). Genomic DNA and cDNA reveal substantial differences, indicating that the peanut cell culture contains more than one RS gene. Chalcone synthase (CHS), a key enzyme of flavonoid biosynthesis, uses the same substrates as RS, but synthesizes a different product (naringenin chalcone). Comparisons show that RS and CHS are related. Homology extends throughout the coding region, and the intron is in RS and CHS at the same position . However, RS and CHS proteins reveal substantial differences in three regions which are highly conserved in all CHS. It is proposed that the two enzymes possess a common scaffold necessary for substrate binding and the basic mechanism of catalysis, and that the differences reflect the specificity for synthesis of different products.

L 631 MOLECULAR MECHANISMS OF ION TRANSPORT INVOLVED IN PLANT CELL FUNCTION J.I. Schroeder & R. Hedrich, Max-Planck-Inst. f. Biophys. Chem. 3400 Göttingen, W.-Germany

Ion transport across the plasma membrane and the tonoplast are essential to processes of higher plant cells during growth and development. By application of the patch-clamp technique mechanisms of ion transport across these membranes have been identified at the molecular level.
Two types of K^+ channels were observed in the plasma membranes of both guard and epidermal cells which allow uptake and release of K^+. Fluxes of K^+ through these channels are strongly regulated by the membrane potential (Schroeder, Raschke & Neher (1987) PNAS, 84: 4108). Thus, blue light activated pumps allow K^+ uptake through K^+ channels by inducing hyperpolarization. Channels activated by stretching of the plasma membrane allow K^+ release by depolarization. Patch-clamp studies on vacuoles revealed electrogenic pumps and two types of ion channels. The direction of ion flux across the tonoplast is determined by the tonoplast potential and the cytoplasmic Ca^{++} concentration which modulate vacuolar channels (Hedrich & Neher (1987) Nature, 329:833). The ion transport mechanisms of both the plasma membrane and the tonoplast and their regulation by different stimuli can be shown to allow plant cells to control a wide variety of processes, when the functional aspects of both membranes are combined in a model.

L 632 EFFECTS OF ABSCISIC ACID, APPLIED DURING THE PERIOD OF ENDOSPERM CELL DIVISION, ON MAIZE CARYOPSIS DEVELOPMENT, Tim L. Setter, Patricia N. Myers, Eric S. Ober, James T. Madison, and John F. Thompson. Cornell University and USDA-ARS, Ithaca, NY 14853.

Maize caryopsis development and growth are sensitive to water stress imposed during the early stages after pollination. To test the effects of elevated abscisic acid (ABA) concentrations, caryopsis explants were cultured in vitro with ABA applied via the medium. ABA (9×10^{-5} M) applied at 6 d after pollination decreased endosperm cell division rate, starch granule number, and final dry weight. Also, DNA endoreduplication, as estimated by average nuclei diameter, was decreased by ABA treatment. Cell division and starch granule multiplication rates were decreased to a lesser extent, or unchanged from controls when ABA applications were delayed until later stages of development. In further work, we are examining the time course and extent of ABA increase and starch-pathway enzyme synthesis during water stress of intact plants. Although leaf water potentials decreased substantially as stress developed, caryopsis water potentials were less affected. Caryopsis starch synthesis was substantially decreased by stress. Initial data indicate that ABA concentrations were higher in both leaves and caryopses of plants stressed for 5 days.

L 633 PHOTOREGULATED AND CELL-SPECIFIC EXPRESSION CHARACTERISTICS OF THE ARABIDOPSIS RBCS GENES IN A HOMOLOGOUS GENETIC BACKGROUND AND IN TRANSGENIC PLANTS. Michael P. Timko, Enno Krebbers*, Lydia Herdies*, and Elenora De Almeida*. Department of Biology, University of Virginia, Charlottesville VA 22901 and * Plant Genetic Systems, Gent Belgium. The four members of the gene family which encodes the small subunit polypeptides of ribulose-1,5-bisphosphate carboxylase in the crucifer Arabidopsis thaliana have been isolated. The structure, genomic organization, and expression characteristics of these genes have been examined. We present here a description of the cell-specific and photoregulated expression characteristics of the individual members of the gene family in their normal genetic background. We show that all four members are expressed but that their individual expression characteristics vary. We discuss the role of the photoreceptor phytochrome in the transcriptional regulation of the individual family members. Through the construction of chimaeric genes based upon the Arabidopsis rbcS gene promoters and their subsequent expression in foreign genetic backgrounds we have begun to elucidate the important transcriptional regulatory sequences present in these genes. These analysis have provided the basis for the identification of the important regulatory factors active in the proper regulation of this gene family.

L 634 INSTABILITY OF Sn, A LIGHT DEPENDENT AND TISSUE SPECIFIC GENE COMPLEX IN Zea mays. Chiara Tonelli*, Gabriella Consonni*, Angelo Viotti** and Giuseppe Gavazzi*. *Dip.di Genetica, Univ. di Milano, **Ist.Biosintesi Vegetali-CNR, Milano, Italy.

Sn is a light dependent gene complex, closely linked to the R locus, responsible of anthocyanin accumulation in specific plant and seed tissues of maize. Previous studies have suggested a transactive regulatory role of Sn on the activity of the structural genes involved in flavonoid biosynthesis as well as a link between Sn and presence of a photoreceptor responding to the blue light. We have isolated several Sn accessions, differing in the level of pigmentation. One of them, referred to as Sn-s, is unstable giving rise to frequent weakly pigmented derivatives, referred to as Sn-w. Data related to the instability of Sn are presented in order to understand the basis of this phenomenon. Sn-s selections keep showing instability in succeeding generations. Heterozygotes Sn-s/sn plants carrying appropriate outside markers yield in their progeny frequent Sn-w derivatives that are recovered on either homologues. Passage from one homologue to the other is not associated to outside markers recombination and is affected in its frequency by the R constitution of the sn marked chromosome. Sn-w derivatives do not spontaneously revert to the original phenotype, however treatments with a DNA methylation inhibitor lead to a phenotypic reversion thus suggesting an involvement of DNA modification in their origin. Analysis at the DNA level seems to support this hypothesis.

L 635 CHARACTERIZATION OF A cDNA CLONE FOR BEAN ABSCISSION CELLULASE AND REGULATION OF CELLULASE GENE EXPRESSION BY ETHYLENE AND AUXIN, Mark L. Tucker, Roy Sexton, Elena del Campillo, and Lowell N. Lewis, Univ. of Calif., Berkeley, CA 94720

We recently reported the sequence of a full-length cDNA clone (pAV363) of an avocado fruit cellulase (Plant Molec. Biol. 9:197-203). This avocado cellulase clone was used to probe a cDNA library prepared from poly(A)$^+$ RNA from leaf abscission zones of ethylene treated bean explants (Phaseolus vulgaris c.v. Red Kidney). We identified and sequenced a 595 nucleotide clone (pBAC1) having extensive sequence homology with the avocado cellulase cDNA. The 2.0 kb bean mRNA complementary to pBAC1 codes for a polypeptide of approximately 51 kD (shown by hybrid-selection followed by in vitro translation). A 51 kD polypeptide from the in vitro translation products of abscission zone poly(A)$^+$ RNA is immunoprecipitated by bean cellulase antiserum. Ethylene at 5 µl/l induces leaf abscission and tissue specific expression of cellulase activity and accumulation of RNA hybridizing to pBAC1. In the presence of ethylene, plants treated with 5 x 10^{-6} M IAA four hours prior to ethylene treatment showed no substantial accumulation of RNA hybridizing to pBAC1 or expression of cellulase activity. 2,5-Norbornadiene, a competitive inhibitor of ethylene action, inhibits the development of abscission and expression of cellulase activity. Treatment of explants with 1,000 µl/1 2,5-norbornadiene after a 31 hour exposure to ethylene, a suitable length of time to detect cellulase message and activity, caused a decline in polysomal mRNA hybridizing to pBAC1 and slowed the increase in cellulase activity. This latter result suggests that ethylene is required to sustain cellulase gene expression in bean leaf abscission.

L 636 PHOTORECEPTORS MEDIATING LIGHT-STIMULATED BEAN LEAF EXPANSION, Elizabeth Van Volkenburgh and Robert E. Cleland, University of Washington, Seattle WA 98195

Expansion of dicotyledonous leaves to full size requires light. Although some cell division occurs in dim red light, maximum leaf and cell size are obtained only after exposure to relatively bright white light. This response has been characterized for primary bean (Phaseolus vulgaris) leaves, grown in low fluence red light (4 umol $m^{-2}s^{-1}$) for 10 days to allow for completion of cell division. Subsequent exposure to higher fluence white light (400 umol $m^{-2}s^{-1}$) stimulates cell expansion and more than doubles leaf growth rate. The response can be assayed either in intact leaves or excised leaf discs floating on minimal nutrient solution (0.01M KCl plus sucrose).

Light-stimulated cell growth is maximal at 100 umol $m^{-2}s^{-1}$ for discs exposed to continuous white light. The reciprocity test fails. An action spectrum for disc expansion has been constructed showing that blue (460 nm) and red (660 nm) light stimulate growth. Simultaneous exposure of discs to red and farred (730 nm) light elimnates the red light response. Normal green discs exposed to the photosynthetic uncoupler DCMU, as well as discs excised from tentoxin-treated leaves containing little chlorophyll, grow normally when supplied with sucrose. On KCl alone, neither grow in response to light.

These results confirm that chlorophyll and the products of photosynthesis are not directly required for leaf cell growth, and that phytochrome (and possibly and additional blue light receptor) mediate this light response. The well described physiology of leaf cell enlargement, including proton efflux, K^+ efflux, and wall loosening may now be linked to phytochrome activity. The nature of this interaction is being pursued further.

L 637 INVOLVEMENT OF AGROBACTERIUM RHIZOGENES TL-DNA GENES IN PLANT MORPHOGENESIS. Frank F. White and Farida Shaheen, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

The expression of TL-DNA genes of Agrobacterium rhizogenes strain A4 (pRiA4) in transformed plants induces a complex modified phenotype. The altered phenotype in tobacco include wrinkled leaf morphology, branched roots, reduced apical dominance, reduced fertility, altered plant size and flowering time. To determine which gene or genes on the TL-DNA are responsible for the modified phenotype, we have regenerated transgenic Nicotiana tabacum var. xanthi plants containing one or more genes. The TL-DNA or segments of TL-DNA were cloned into a binary vector. The T-DNA in binary vector was introduced into plant via Agrobacterium-mediated gene transfer. Some regenerated plants containing only three TL-DNA genes; rolA, rolB, and rolC showed the typical TL-DNA induced plant phenotype (severely wrinkled leaves, small plant size). However, other plants showed a weak phenotype (slightly wrinkled leaves, intermediate plant size, early flowering and reduced apical dominance). These results suggest that the less wrinkled phenotype is due to reduced expression of one or more of the three TL-DNA genes. To further explore the role of individual genes we have introduced the individual genes rolA, rolB, and rolC into tobacco in order to assign each gene to a specific phenotype.

L 638 INDUCTION OF THE GH3 PROTEIN IN REPONSE TO AUXINS, Richard M. Wright and Tom Guilfoyle, University of Missouri, Columbia, Mo. 65211

Antisera were raised to a 70 kd protein encoded by a 2,4-D inducible mRNA, GH3. These antisera have been used to probe protein blots to study the specificity and kinetics of the GH3 induction response as well as the species specificity and intracellular location of the protein. Synthesis of the GH3 protein is induced by a variety of auxins. Other plant hormones such as gibberellic acid, cytokinin and ethylene added with or without 2,4-D did not alter the level of GH3 protein induction. Detectable levels of the GH3 protein are induced by 2,4-D within 2hr in elongating hypocotyl sections, root sections, and etiolated plumules, and within 15min in soybean suspension cells. The GH3 protein was found only in the S100 fraction and was not associated with the nucleus or the cell wall in fractionated plumule tissue. This antisera also reacted to a 70kd protein induced in other unrelated dicots after 2,4-D treatment.

Cis- and Trans-acting Factors

L 700 CONSTRUCTION OF A HEAT INDUCIBLE EXPRESSION CASSETTE, W. Michael Ainley and Joe L. Key, University of Georgia, Athens, Ga. 30602.

A heat inducible expression cassette has been constructed to: 1) study the DNA sequence elements required for regulation of the level of expression and thermal control of a heat shock gene and 2) study the expression of sense or antisense orientations of any sequence of interest. The construct includes the promotor and all but 5 bases of the mRNA leader from the soybean 2019E gene, the polylinker from pUC18, and the fragment from the nopaline synthase gene that contains the polyadenylation signals and sites. The promotor of the 2019E gene is potentially the most useful of the soybean heat shock genes described thus far for use in such a vector, having undetectable expression at control temperatures and extremely high expression at heat shock temperatures. Analysis of a construct containing the ß-glucuronidase (GUS) coding sequence cloned in the cassette and introduced into Nicotiana plumbaginafolia protoplasts by electroporation shows that the promotor has high expression at heat shock temperatures but after optimization has roughly 10% of the heat induced level at control temperatures. Preliminary experiments show that within 2 hr at heat shock temperatures protoplasts transformed with the heat inducible GUS expression construct produce GUS at a level that is comparable to the amount accumulated in 24 hr following electroporation with the same concentration of a constitutively expressed 35S-GUS construct. Expression constructs are being made to test the importance of having the entire mRNA leader and 3' nontranslated sequence fused perfectly to an open reading frame of interest. Constructions bearing the polyadenylation sites from the nopaline synthase gene and the 2019E genes will be compared as to how each affects regulation of this gene.

L 701 THE GENETIC REGULATION OF THE S-LOCUS IN *Brassica campestris* BY A TRANS-ACTING FACTOR, Bicka A. Barlow and June B. Nasrallah, Cornell University, Ithaca, NY 14853.

Self-incompatiblity in *Brassica campestris* is determined by the S-locus. Genetic analysis of a naturally occuring self-compatible line indicates that the self-compatible phenotype is determined by a second unlinked locus, called the modifier locus. The F_1 plants from a cross of the compatible line and an incompatible line are self-incompatible. Analysis of the DNA shows a number of restriction fragment length polymorphisms (RFLP) that are specific to each parent and that are present in the heterozygous F_1. Northern analysis shows that the level of the S-locus specific message is greatly reduced in the compatible parent relative to the incompatible parent, and in the incompatible F_1 the message is also significantly reduced. To determine whether the function of the modifier locus is at the level of transcription rather than translation the S-Locus Specific Glycoprotein (SLSG) levels are being determined in each of these plants. The analysis of the RFLP's, RNA levels and SLSG levels is being continued in the F_2 generation. The proposed function of the modifier locus is as a trans-acting, positive activator of transcription of the S-locus.

L 702 ELICITOR REGULATION OF A PLANT DEFENSE GENE PROMOTER IN ELECTROPORATED PROTOPLASTS,Michel Dron, Steve D Clouse, Richard A Dixon, Michael A Lawton, Chris J Lamb, The Salk Institute, San Diego, Ca 92138.
Plants respond to microbial attack by synthesis of phytoalexins,stimulation of lytic enzymes such as chitinase and reinforcement of cell walls by deposition of lignins and hydroxyproline-rich glycoproteins. Elicitors, wounding or infection rapidly stimulate the transcription of a specific set of genes involved in the erection of these defenses. To investigate this early event in the activation of resistance mechanisms we have studied the expressionin electroporated soybean protoplasts of a chimeric gene comprising the 5'-flanking region of a defense gene encoding chalcone synthase fused to a bacterial chloramphenicol acetyl transferase gene. Glutathione and fungal elicitor caused a rapid and transient expression of the chimeric gene. Functional analysis of 5' deletions showed that promoter activity was determined by an elicitor-regulated activator located between the TATA box and -170 and an upstream silencer between -170 and -326. Cinnamic acid, the reaction product of Phenylalanine ammonia lyase activity has a strong positive or negative effect on the expression of this chalcone synthase-CAT chimeric gene depending on the concentration at which it is added. We present a model in which cinnamic acid plays a key regulatory control in the synthesis of phytoalexins.

L 703 THE Tn10 ENCODED TET REPRESSOR-OPERATOR-INTERACTION IS FUNCTIONAL IN PLANT CELLS, Christiane Gatz, Institut für Genbiologische Forschung, Ihnestr 63, 1 Berlin 33 FRG.
The Tn10 encoded tet repressor-operator-system was used to regulate transcription from the 35S cauliflower mosaic virus (CaMV)-promoter in plant protoplasts. Expression was monitored in a transient expression system using electroporated tobacco protoplasts. The tet repressor, being expressed in the plant cells under the control of eucaryotic transcription signals, blocks transcription of a 35S-CaMV-promoter-chloramphenicol acetyltransferase (CAT) fusion gene when one of the operators is located between the CAAT-box and the TATA-box and the second beween the TATA-box and the transcription start site. In the presence of sublethal concentrations of the inducer tetracycline expression is restored to full activity. Location of the operators downstream of the transcription start site does not significantly affect transcription in the presence of the repressor. These experiments show for the first time that a procaryotic regulatory protein can function in plant cells. The tet repressor-operator-system may be useful as an on/off switch for the regulation of gene expression in gene transfer experiments.

L 704 CLONING OF THE MAJOR CAPSID PROTEIN OF ALGAL VIRUS PBCV-1, Michael V. Graves Russell H. Meints, University of Nebraska-Lincoln, Lincoln, Neb. 68588.
PBVCV-1 is a large polyhedral dsDNA (330 kb) virus which infects an unicellular, eukaryotic, exsymbiont Chlorella-like green algae. Large quantities of the virus can be purified from infected algal cultures and a sensitive plaque assay has been developed. Previous studies indicate the infectious cycle can be divided into periods of early and late gene expression. Thus, this system provides a novel means of studying the regulation of gene expression in a plant-virus system. Viral structural proteins were purified via SDS-PAGE and the putative major capsid protein (MW = 54kD) was used to raise a polyclonal antiserum. This antiserum is currently being used to screen a viral genomic expression library in order to identify the gene. This will facilitate the determination of the structure of the cis-acting factors involved in gene expression. The antiserum is also being utilized to study the time course of capsid protein biosynthesis in infected cells.

L 705 **GENE REGULATION OF STORAGE PROTEINS IN BRASSICA NAPUS**
H-O Gustavsson, M. Ellerström, M. Ericsson, S. Höglund, L. Rask and L-G Josefsson. Dep. of Cell Research, Swedish University of Agricultural Sciences, Uppsala Biomedical center, Box 596, S-751 24 Uppsala, Sweden

The Napin gene family of Rapeseed is only expressed in embryonal cells and thus constitutes a strictly regulated gene system. One of several likely factors involved in effecting the regulated expression of these genes are trans acting factors. One class of such factors have in other systems been shown to interact with regulatory cis sequences in a process that ultimately guides RNA polymerase II to its proper initiation site.

Several species of sequence specific DNA-binding proteins are detectable in crude nuclear extracts obtained from developing B. napus seeds. We have shown that some of these are able to interact with different sequence motifs that are present in the promoter of a Napin gene. Our present efforts are biased towards a further characterization of these proteins as well as towards their purification and cloning, the ultimate aim being to establish their role in the regulation of the Napin genes.

L 706 CHARACTERIZATION OF CELLULAR FACTORS GOVERNING GENE EXPRESSION WITHIN SINGLE CELLS OF TRANSGENIC PLANTS. Kristi R. Harkins, David W. Galbraith and Richard A. Jefferson*, University of Nebraska, Lincoln, NE 68588 and Plant Breeding Institure, Maris Lane, Trumpington, Cambridge CB2 2LQ England*.
Studies of the expression of beta-glucuronidase in transgenic Nicotiana plants have led us into the development of flow cytometric procedures for the isolation of homogeneous populations of protoplasts with uniform chlorophyll content and cellular diameter. Analysis of uniform populations of mesophyll protoplasts sorted according to size has revealed linear relationships between protoplast diameter, chlorophyll content and chloroplast number. These relationships provide possible insight into how the organelles may interact via diffusion of molecular signals. In order to determine the nature of these molecular signals, we have developed flow cytometric procedures for the isolation of populations of protoplasts devoid of chlorophyll that originate from the epidermal and perivascular tissues. We have found that expression of beta-glucuronidase under the control of light regulated promoters is suppressed in these protoplasts, and are currently searching for cellular factors that can reconstitute expression. Finally, the ability to separate cells on the basis of photosynthetic capability allows us to study how metabolic changes affect light-regulated chimaeric gene expression. These studies provide further information of the controls involved in cell specific gene expression.

L 707 NUCLEAR PROTEIN BINDING TO A *CIS*-ACTING SILENCER IN A DEFENSE GENE PROMOTER, M.A. Lawton, K. Kragh, S.M. Jenkins, M. Dron, S.D. Clouse, R.A. Dixon and C.J. Lamb, Plant Biology Laboratory, Salk Institute, P.O.Box 85800, San Diego, CA 92138
Plant defense gene activation is an early step in the induction of defenses in response to elicitors, wounding or infection. Functional assay in electroporated protoplasts showed that the promoter of a gene encoding the phytoalexin biosynthetic enzyme chalcone synthase (CHS) contains an upstream silencer between -173 and -326, as well as an elicitor-regulated activator between the TATA box and -173. Binding of nuclear proteins to the silencer *in vitro* was detected by gel retardation and DNase I protection footprints. Footprinting identified binding to three discrete sites in the element. Binding was specifically competed by CHS promoter sequences and by the promoter of a co-ordinately induced defense gene, but not by unrelated sequences eg. CHS cDNA. Electroporation of the silencer element enhances in *trans* the activity of the CHS promoter, presumably by competition for binding of the *trans*-acting repressor(s). This suggests an approach to engineer increased expression of a set of co-ordinately regulated biosynthetic genes involved in a multigenic defense response.

L 708 A BEAN cDNA HOMOLOGOUS TO CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASES, Michael A. Lawton and Christopher J. Lamb, Salk Institute, La Jolla, CA 92037. Protein kinases are important components in the transduction of many intracellular and extracellular signals in mammals, yeast and *Drosophila*. We have initiated a search for plant genes encoding protein kinases in order to examine their role in developmental and environmental signal transduction pathways. A cDNA library from elicitor-treated bean suspension cells was screened sequentially with degenerate oligonucleotide probes encompassing conserved regions of known protein kinase genes. One recombinant, containing a 2.1 kb cDNA, hybridized to both oligonucleotide probes. Partial DNA sequence showed that the regions hybridizing to the oligonucleotide probes could potentially encode the conserved amino-acid sequences found at equivalent positions in known protein kinases. Comparison of the derived amino-acid sequence of adjacent regions revealed additional extensive homology to known protein kinases, with homology tending to diverge in regions that are not highly conserved among other protein kinases. Compared to known sequences, the bean cDNA sequence most closely resembles the mammalian cyclic nucleotide-dependent protein kinases. The availability of this sequence should aid in the isolation and biochemical characterization of this putative protein kinase. The approach used to identify this sequence is being used to isolate other sequences representitive of classes of protein kinases.

L 709 INTERACTION OF NUCLEAR PROTEINS WITH β-CONGLYCININ GENE UPSTREAM SEQUENCES, Randy D. Allen, Philip A. Lessard, Roger N. Beachy, Washington University, St. Louis MO 63130. Crude nuclear extracts of soybean embryos contain proteinaceous factors that interact specifically with DNA sequences located between 140 and 208 bp 5' of the transcription start site of the β-conglycinin α' subunit gene. These sequences are included within a region of the α' gene which has been shown to act as a developmentally regulated, embryo-specific enhancer in transgenic tobacco seeds. Levels of binding activity detected in nuclear extracts correspond with levels of β-conglycinin gene expression in maturing embryos. Peak binding activity was observed in extracts from embryos of developmental stages in which β-conglycinin transcript accumulation is maximal. A major β-conglycinin gene binding factor has been partially purified from nuclear extracts and characterized. The specific nucleotide sequences that are recognized by this factor are being determined, and possible interactions of this or other factors with 5' sequences of other embryo-specific genes is under investigation. Our goal is to understand the role of sequence-specific DNA-binding proteins in the developmental regulation of gene expression in plants.

L 710 RECOGNITION OF E. coli TRANSCRIPTION TERMINATORS BY SPINACH CHLOROPLAST RNA POLYMERASE, Emil M. Orozco, Jr. and Liang-Jwu Chen. USDA Agricultural Research Service and Department of Agronomy, University of Illinois, Urbana, IL 61801. Spinach chloroplast RNA polymersase will efficiently terminate transcription of supercoiled DNA at the E. coli threonine attenuator. Transcription from a plasmid promoter cloned in front of this terminator results in a discretely-sized RNA that can be directly assayed by polyacrylamide gel electrophoresis. Recognition of prokaryotic terminators by the chloroplast enzyme is not a general phenomenon, however. The chloroplast enzyme terminates very inefficiently at the rrnB terminator and hardly at all at the rrnC terminator. We are currently characterizing the parameters of this new method of assaying in vitro transcription. This method should be useful for the characterization of plastid promoters and the purification of chloroplast RNA polymerase and transcription regulatory molecules.

L 711 **ANALYSIS OF PR-1 PROTEIN GENES IN TOBACCO - EVIDENCE FOR A TRANSPOSITION EVENT**, Ursula M. Pfitzner, Howard M. Goodman[1] and Artur J. P. Pfitzner, Botanisches Institut, Menzingerstr. 67, D-8000 München 19, FRG, [1]Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Pathogenesis-related proteins (PR-proteins) are a heterogeneous group of host encoded proteins, which are induced in plants by different external stimuli such as pathogen attack or exposure of the plant to certain chemicals. We have isolated and sequenced 3 tobacco genomic clones encoding PR-1 genes. Two of these correspond most presumably to pseudogenes, whereas the 3rd gene is essentially identical to the PR-1a cDNA clones. The 5' flanking region of this gene contains direct repeat elements and a heat shock element like motif, which might be involved in the transcriptional regulation of PR-1 genes. One of the pseudogenes contains an insertion of heterologous DNA in the 5' upstream region which results in the duplication of a 13 bp target sequence. By Southern blotting, we demonstrated that this insertion is present at other positions in the tobacco genome unrelated to the PR-1 gene family. These data are in favour of a transposition event in tobacco.

L 712 ISOLATION OF TELOMERIC DNA SEQUENCES FROM ARABIDOPSIS THALIANA
Eric J. Richards and Frederick M. Ausubel, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Telomeres are the structures that form the termini of linear eucaryotic chromosomes. Telomeres stabilize the ends of the chromosomal DNA molecules and facilitate the complete replication of the DNA strands at the extreme termini. Detailed study of telomere structure and function is limited to lower eucaryotic organisms from which telomeric DNA sequences have been isolated. Cloning of telomeres from higher eucaryotic organisms is hampered by the large size of the chromosomes and the attendant dilution of telomeric sequences relative to non-telomeric sequence in the genome.

We have developed a method for constructing genomic libraries highly enriched for telomeric sequences enabling the isolation of telomeres from higher eucaryotic organisms with large chromosomes. The method was used to clone telomeric DNA sequences from A. thaliana. The structure of A. thaliana telomeres is similar to that seen in lower eukaryotes; the cloned A. thaliana telomere is composed of tandemly repeated, C-rich, simple sequence blocks. In addition, the telomeres of A. thaliana are heterogeneous in size as are most lower eucaryotic telomeres. Genomic sequences homologous to the A. thaliana telomeric repeats are present in the genome of other higher plants and some animal species. In the case of corn and humans, these genomic sequences are located at the telomere.

L 713 EXPRESSION OF CHORION-Adh PROMOTER FUSIONS IN DROSOPHILA, Charles P. Romano and Fotis C. Kafatos, Harvard University, Cambridge, MA 02138

During Drosophila oogenesis, the follicle cells express and amplify the chorion (eggshell) genes with temporal, tissue, and sex specificity. To identify the cis- and trans- regulators of chorion gene expression, we have fused the "late" s15 chorion gene promoter to the alcohol dehydrogenase (Adh) structural gene and examined fusion gene expression in Adh null flies by P element mediated germline transformation. As chemical selections both for and against ADH activity are well established in Drosophila, the chorion-Adh fusion constructs may enable us to directly select trans-acting mutants affecting chorion promoter function. The s15-Adh fusion genes are expressed with the same temporal, tissue, and sex specificity as the endogenous s15 gene, indicating that the 5' flanking and promoter regions are sufficient to confer such specificity. However, steady state s15-Adh fusion gene mRNA levels are about 25-fold lower than the levels produced by a "marked" s15 gene constructs containing the s15 structural gene and 3'flank. Experiments are currently underway to determine if this quantitative effected is mediated at the transcriptional or post-transcriptional level.

L 714 EXPRESSIONS AND REGULATIONS OF CORN NITRITE REDUCTASE IN HYDROPONICALLY GROWN SEEDLINGS AND CELL SUSPENSIONS. S. Rothstein, K. Lahners, V. Kramer, E. Back, and L.S. Privalle CIBA-GEIGY Biotech. Res., Res. Tri. Park, NC 27709. Nitrite reductase (NiR) expression is controlled by nitrate in many plants and fungal systems. We have isolated the cDNA clone for corn nitrite reductase using the spinach nitrite reductase gene as a heterologous probe. The two proteins were found to be 83% homologous at the amino acid level with key cysteines involved in prosthetic group binding being conserved. NiR mRNA, activity, and protein levels have been monitored in two controlled systems, two week old hydroponically grown corn seedlings and Black Mexican Sweet suspension cell cultures. Our results indicate that there is a nitrate concentration dependent induction of the mRNA which peaks between 1 and 2 hours followed by a sharp decrease. NiR protein and activity is slightly delayed but nonetheless peaks and decreases to the original level within 24 to 48 hours in either system even though the nitrate levels in the medium remain constant. Nitrite itself can induce the expression of NiR mRNA with similar kinetics. Ammonia does not prevent nitrate induction of NiR. These results demonstrate that the nitrate concentration in the medium is not the sole determining factor in the regulation of this gene. One hypothesis that would explain these results is that under relatively high nitrate concentrations a nitrate-inducing regulatory protein is limiting.

L 715 HEAT SHOCK INDUCTION OF A MINIMAL PROMOTER FROM T-DNA BY DROSOPHILA HSP70 HEAT SHOCK ELEMENTS. David Wing and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, West Germany.
Segments of the Drosophila hsp70 promoter were tested for their ability to convey heat shock regulation to a heterologous promoter in tobacco cells using a vector system designed to assay for inducible enhancer domains. The promoter fragments were cloned 5' of a 94 bp sequence containing the "CAAT" and "TATA" boxes of the T-DNA indole-3-acetamide hydrolase gene. These chimeric promoters were ligated to a neomycin phosphotransferase II (NPT II) reporter gene located on a binary vector suitable for A. tumefaciens mediated DNA transfer to plant cells. The 94 bp minimal promoter provided a consistently low baseline of expression over which increased expression due to the inserted inducible enhancer sequence could be assayed. After heat shock for 1 hour at 40°C, tobacco calli containing a chimeric gene with a 216 bp fragment with 3 heat shock elements (HSE) had increased levels of NPT II mRNA. But only after incubation at 22°C for 1 hour did the NPT II activity quickly increase 10-20 fold. A chimeric gene with a 140 bp fragment with 2 HSEs also had heat shock induced NPT II activity in tobacco calli. The chimeric genes thus showed 2 levels of heat shock gene regulation, induced transcription from the chimeric promoter using Drosophila HSEs, and repressed translation of the mRNA not having the sequence of a heat shock mRNA.

L 716 EARLY EVENTS IN CEREAL EMBRYOGENESIS, P. Higgins[1], R.L. Lyne[2] and D.J. Bowles[1] ([1]Department of Biochemistry, University of Leeds, Leeds LS2 9JT; [2]Shell Research Ltd., Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG).
Pollen and anther culture offers a good experimental system to study the mechanisms controlling embryogenic development in plants. Under optimal culture conditions embryos can be formed from immature pollen grains without an obvious intervening callus phase. Evidence will be presented to show that microspore-derived embryos are very similar to their zygotic counterparts. This similarity is expressed in general morphology and polypeptide composition. Patterns of gene expression during early stages of embryo development in vitro and in vivo have been investigated and compared to that of undifferentiated cell proliferation. The implications of these results will be discussed in the context of regulatory events during the embryogenic process.

L 717 OLIGOSACCHARIDES AND CELL-SIGNALLING IN TOMATO PLANTS, H.M. Doherty, D.J. Bowles, Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K.; R.R. Selvendran, AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich, NR4 7UA, U.K.

Wounding of tomato leaves leads to changes in gene expression that include the synthesis of serine proteinase inhibitors (PI's). Induction of PI's is also caused by the application of cell wall fragments to the cut ends of stems and petioles. (1) Evidence will be presented that the response can be inhibited by treatment with aspirin and related hydroxybenzoic acids. Inhibition by aspirin is rapid and reversible, but occurs only when aspirin is applied as a pretreatment; following induction by pectic oligosaccharides the drug is without effect. The effect of inhibitors and the role of oligosaccharides as the mediators of the wound response will be discussed.

(1) Green, T.R., Ryan, C.A. (1972) Science 175 776-777.