EVOLUTION AND PLANT DEVELOPMENT

Organizers: Susan R. Wessler, Elliot Meyerowitz and Michael Freeling

January 26 - February 1, 1993; Taos, New Mexico

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Floral Meristems and Flowers-I

D 001 *Abstract Withdrawn*

D 002 DIOECIOUS PLANTS: DISTINGUISHING THE BOYS FROM THE GIRLS, Sarah R. Grant[1], De Ye[1], Sabine Hardenack[1], Fabio Raffaldi[1], Beate Hunkirchen[1], Andreas Houben[2], Timothy E. Huller[1] and Heinz Saedler[1], [1]Max-Planck-Institut fuer Zuechtungsforschung, Cologne Germany, [2]Institut fuer Genetik und Kulturpflanzenforschung, Gatersleben Germany.

The evolution of land plants has culminated in the production of 'perfect' angiosperm flowers in which both male and female sex organs are collected in a floral package attractive to pollenators. However, the evolution of flowers has not stopped at this point. Several mechanisms exist to prevent self-fertilization. Perhaps the most extreme examples are dioecious species in which individuals produce flowers bearing only one kind of sex organ, either male or female. Most species of the genus Silene bear only perfect flowers but a few species are dioecious. Perfect and dioecious species have apparently evolved from a common progenitor that had perfect flowers. Silene latifolia is one of the best characterized dioecious plants. Classical cytogenetic studies have shown that this species has dimorphic sex chromosomes. Males have a single X and a larger Y chromosome, while females have two X chromosomes. Mutant analysis has shown that the Y chromosome bears at least two loci where dominant alleles confer male characteristics on flowers. We are attempting to clone these Y-linked genes in order to obtain the necessary tools to investigate the evolution of sexual differentiation in the genus Silene. Our first goal is to assemble a collection of cDNAs expressed specifically in males that are encoded from the male sex chromosome. We are combining a number of molecular approaches including subtractive cDNA cloning and cloning of Y chromosome DNA obtained by microdissection of metaphase chromosomes. To further identify the sex-determining genes from this collection, we have generated a number of mutants that have lost certain male characteristics. One class of mutants produce hermaphrodite flowers having lost the factors that repress gynoecium development. The second class consists of sterile plants. Gynoecium development is still suppressed but anthers also do not develop. These mutants have lost factors promoting anther development. Tests are in progress to determine if the mutants are Y-linked. Comparison of expression and genomic DNA structure of the Y-linked cDNAs in mutant and wild type plants will suggest which cDNAs have the potential to be sex-determining genes and warrant further analysis.

D 003 GENETIC CONTROL OF PATTERN FORMATION IN FLOWER DEVELOPMENT

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We study a group of *Arabidopsis* genes whose products are necessary for the proper specification of the identity of floral organs, and of floral meristems. Mutations in the floral organ identity genes fall into three predominant phenotypic classes, each class affecting the organ types that appear in two adjacent whorls of the flower. By a series of genetic experiments, we have demonstrated that these three classes of genes specify the four types of floral organ found in *Arabidopsis* in a combinatorial fashion. Molecular cloning of three organ identity genes (*APETALA3*, *PISTILLATA* and *AGAMOUS*) from two of the three classes has been accomplished. The DNA sequence of the three cloned organ identity genes indicates that they are DNA-binding transcription factors of the MADS-box family, which includes human and fungal genes, as well as many additional plant genes. Among the other plant MADS box genes are a homologous series of organ identity genes from *Antirrhinum*, which have similar mutant phenotypes to the *Arabidopsis* genes. An additional indication that the molecular mechanism of organ specification in flowers is similar in distant dicot species comes from experiments in which *Arabidopsis* organ identity genes such as *AGAMOUS* are introduced with constitutive promoters to the genomes of other species (e.g. tobacco), where they act to regulate organ identity as predicted from results of *Arabidopsis* experiments.

Organ identity in flowers is regulated by the spatial pattern of expression of the organ identity genes. This implies that these genes are themselves regulated by prepattern (cadastral) genes, whose function is to regulate the spatial expression pattern of the organ identity genes. An example of such a gene is *SUPERMAN*, whose function is to regulate the initial pattern of expression of the *APETALA3* and *PISTILLATA* genes. An even earlier-acting set of genes are the meristem identity genes, whose mutant phenotype (either alone, or in some cases only in combination with other mutations) is the partial replacement of flowers with inflorescence shoots. There are a number of these genes, including *LEAFY*, *APETALA1*, *CAULIFLOWER*, and several others. *LEAFY* has been cloned, and its expression is in the cells that will later make a flower, at a stage just after they appear on the flank of the inflorescence meristem. Such a flower-specific pattern of gene expression raises the question of the expression and activity of the evolutionary precursor of such a gene in non-flowering plants. Since *LEAFY* is single-copy, it is possible to find its homologue in other plants by direct hybridization. By such methods the homologue of this gene has now been detected in a number of genera, including *Gingko*. Its expression pattern in this non-flowering plant is not yet known. It seems at least possible that information on its site of expression, and similar evolutionary studies of the floral organ identity genes, may indicate the origin or ancestral function of these genes, and thus clarify some of the steps in the evolution of flowers, allowing us to understand for the first time the nature of the genetic changes that lead to morphological innovations.

Floral Meristems and Flowers-II

D 004 DEVELOPMENT AND EVOLUTION OF COMPLEX FLOWERS, Peter K. Endress, University of Zurich, Zurich, Switzerland.

A requirement for the selection of model plants in the study of floral development is relative simplicity in structure, stability of organ number and predictable organ position. This holds for Arabidopsis and Antirrhinum. It is therefore not surprising that both Arabidopsis and Antirrhinum have a similar floral development, although they are not so closely related. A general concept of flower development should, however, encompass all the variant forms that occur in the enormous diversity of the flowering plants. I will show the comparative development of some more complicated flowers: (1) Lecythidaceae with a variable position of numerous stamens, with centrifugal stamen initiation, and differentiation of a polysymmetric and a monosymmetric androecial compartment in the same flower; (2) Asclepiadaceae and Orchidaceae with postgenital and congenital synorganization of structural units that is superimposed on the original pattern and camouflages it. These divergent examples, although not suitable as model organisms, may stimulate ideas about the coverage and generalizations of present concepts of flower development and evolution.

D 005 MODES OF ONTOGENETIC SEQUENCE DIVERSIFICATION IN FLORAL EVOLUTION, **Larry Hufford, Department of Biology, University of Minnesota, Duluth, MN 55812.**

Morphological diversity arises through evolutionary changes in ontogenies. Despite considerable recent interest in ontogenetic evolution, the modes of ontogenetic transformation and their implications for diversification of monophyletic groups remain unclear. The ontogenetic evolution of flowers was explored in the context of phylogenetic hypotheses derived from cladistic analyses of Loasaceae, *Eucnide*, and *Besseya*. Modes of ontogenetic sequence diversification in monophyletic groups were traced by mapping shared ontogenetic sequences and derived transformations on the branches of cladograms. Ontogenetic sequences were treated as hierarchies of developmental events that alter morphology. Using this means of description, four primary modes of ontogenetic sequence transformation have been identified, including deletions, additions, substitutions, and inversions. Deletions and additions are common modes of ontogenetic sequence transformation. The effects of deletions, for example, may range from relatively minor meristic simplifications to extensive paedomorphosis with broad consequences for the evolution of floral form and reproductive biology. Similarly, additions may be involved in simple meristic changes but may have greater consequences when they are involved in reversals and in elaborations following paedomorphosis. Inversions were observed infrequently and are not commonly described in the literature on the diversification of angiosperm flowers. Modifications generally described as paedomorphic (evolutionary juvenilization) were found to arise in some instances from deletions ("strict paedomorphosis") but also from substitutions. In contrast to the widely held assumption that early ontogenetic stages have extensive evolutionary stability, numerous, successful ontogenetic transformations were found to be instituted during this period in the investigated groups. These included some transformations affecting more than one appendage series (e.g., involving corolla/stamen confluence) that greatly modified floral display.

D 006 MODEL SYSTEMS FOR EVOLUTIONARY STUDIES, Elizabeth A. Kellogg, Arnold Arboretum of Harvard University, 22 Divinity Ave., Cambridge, MA 02138.

The major model species for studies of plant biology are maize, tomato, and *Arabidopsis*. All have a detailed genetic map, a sizable collection of mutants, and a large number of workers exploring diverse aspects of their genetics and development. To extend studies of a particular model organism for evolutionary purposes requires a phylogeny as well. Of the three families (Gramineae, Solanaceae, Brassicaceae), the grass family is the best understood phylogenetically, and thus is the best placed at the moment to be a model system for evolutionary studies.

The grass flower is unusual in that it has no clear petals and sepals, although it does have small flaps of tissue that are believed to be vestigial perianth. The flower is subtended by two bracts, and the bracts plus flower make up the floret. In well over half of the genera of grasses, species are distinguished by differences in the size and pubscence of the bracts. Developmental studies on several members of the genus *Poa* (bluegrass)[1] showed that variation in the pubescence pattern on the bracts could be explained by heterochronic shifts in a single developmental program. Growth of the floret occurred in one of two ways: In some species, the bract and the anthers elongated at the same rate from primordium formation through anthesis (the *simultaneous* pattern), whereas in other species the bract attained its full length well before the anthers ceased growing (the *biphasic* pattern). Species with the simultaneous pattern have been shown to be cladistically basal to those with the biphasic pattern[2]. Such allometric and heterochronic change may well have been involved in speciation in many members of the grass family; however, more detailed analysis is difficult because of the genetic and phylogenetic complexity of *Poa*, a genus that exhibits a highly reticulate evolutionary history.

Zea is much more tractable, both genetically and phylogenetically. The phylogeny of the genus is well-understood[3]. Morphological cladograms place *Zea* among a large group of predominantly Asian genera. A number of the phylogenetically informative characters are similar to mutant phenotypes characterized in maize. Branched stems, which are not common among grasses, arise twice among the relatives of maize; three heterochronic mutants, *Teopod 1*, *Teopod 2* and *Corngrass*, also produce branched stems. The close relatives of *Zea* have incomplete flowers; several genes are known that influence sex expression. *Echinochloa*, a genus slightly more distantly related to *Zea*, is characterized by lack of a ligule; *Liguleless-3* is a gene thought to contain a homeobox domain. It is thus possible to ask whether particular cladogenetic events involve changes in these known genes.

[1]Kellogg, E. A. 1990. Evolution 44: 1978-1989.
[2]Soreng, R. J. 1990. Amer. J. Bot. 77: 1383-1400.
[3]Doebley, J. 1990. Econ. Bot. 44(3 supplement): 6-27.

D 007 A MODEL OF FLORAL DEVELOPMENT AND EVOLUTION IN THE ZINGIBERALES, Bruce K. Kirchoff, Department of Biology, University of North Carolina at Greensboro, Greensboro.

The flowers of the monocotyledon plant order Zingiberales are remarkable for their structural complexity. This complexity is the result of variation in organ identity, size and shape. The flowers of the banana group (Musaceae, Heliconiaceae, Lowiaceae, Strelitziaceae) are simpler and closer to a typical monocotyledon type (Fig. 1, *Ravenala*, Strelitziaceae). In the ginger group (Zingiberaceae, Costaceae, Cannaceae, Marantaceae), not only are most of the stamen replaced by petaloid staminodes (Fig. 1, *Hedychium*, Zingiberaceae), but the form of the staminodes varies between taxa.

The replacement of stamens with petaloid staminodes in the ginger group is an instance of homeosis. Meyerowitz et al. (1991) proposed a model of flower development based on their work on homeotic genes in *Arabidopsis* (Fig. 1, *Arabidopsis* model). The model is based on three regulatory genes: apetala-3 (AP3), apetala-2 (AP2), agamous (AG). The expression of AP3 alone leads to the production of sepals (SE); AP3 with AP2 yields petals (PE); AP2 with AG yields stamen (ST); and AG alone yields the gynoecium (GY). The model also explains organ identity in the members of the banana group with six stamens (Fig. 1, *Ravenala* model). In the following I discuss only the spacial expression of agamous. In *Ravenala* the model suggests that agamous is expressed over the same spacial domain as it is in *Arabidopsis*. In the ginger group, the existence of the petaloid staminodes can be explained by a reduction in the spacial domain of AG (Fig. 1, *Hedychium* model). Since there is a polliniferous stamen in this group, the spacial domain of AG must be asymmetric (Fig. 1, *Hedychium* model). Stamens are only formed in regions where both AG and AP3 are expressed (Fig. 1, *Arabidopsis* model).

Organ identity is established early in floral development. Only in the later stages does the floral form becomes fully apparent. For instance, allometric growth begins soon after organ initiation, but its effects are most apparent in the final stages of development.

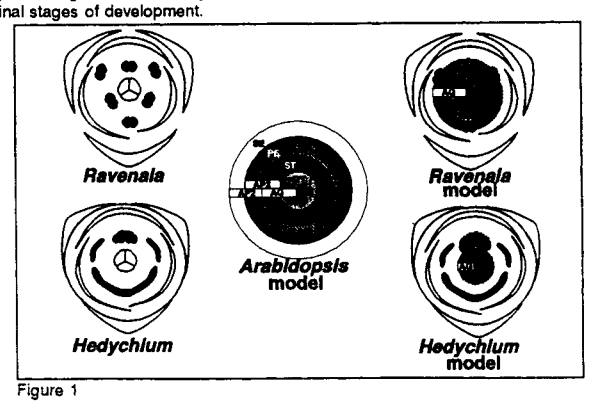

Figure 1

Meyerowitz, E., Bowman, J. L., L. L. Brockman, G. N. Drews, T. Jack, L. Sieburth and D. Weigel. 1991. A genetic and molecular model for flower development in *Arabidopsis thaliana*. Development Supplement 1: 157-167.

Floral Organ Development, Evolution and Function

D 008 EVOLUTION OF FLORAL FORM IN ARABIDOPSIS AND RELATED PLANTS, Peter R. Crane, Department of Geology, Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, IL 60605.

Recent studies of the molecular-genetic control of floral development in Arabidopsis thaliana (Cruciferae) have focused on homeotic mutants in order to understand how the fate of primordia initiated on the floral apex is controlled, and thus how sepal/petal, stamen and carpel differentiation is specified. "Petals" in different groups of angiosperms may have different evolutionary origins (e.g., staminodal, stipular/foliar) but the fundamental regularity of angiosperm floral construction (sepals/petals surrounding stamens surrounding carpels) suggests that results from these studies probably have broad applicability. Another aspect of floral development, which has received less attention, concerns how the number and relative position of primordia on the floral apex is controlled. Because much of the diversity of angiosperm flowers is manifested in terms of these parameters (especially among relatively basal groups) study of the molecular-genetic mechanisms involved is crucial to understanding the evolution of floral form.

The family Cruciferae is notable for extreme uniformity in the number and disposition of floral parts: K2+2 C4 A2+4 G(2). According to classical interpretations (e.g., Eichler) the arrangement of floral organs is fundamentally opposite and decussate, involving six pairs of organs (rather than four whorls) with the apparent whorl of four petals and four (inner) stamens formed by "doubling" of organs in the median (anterior-posterior) plane. In the Cruciferae there is little deviation from this basic pattern. However, in many species of Lepidium there is no "doubling" of the anterior-posterior (inner) stamens (K2+2 C4 A2+2 G(2)), while in Megacarpea (A8-12) additional stamen duplication occurs (although the precise pattern of stamen induction has not yet been studied in detail). In those Capparidaceae that are closely related to the Cruciferae, similar variations on the basic opposite and decussate floral plan occur. Cleome spinosa has an identical number and arrangement of floral organs to that in Cruciferae, while Cleome tetrandra is identical to some species of Lepidium. In more distantly related Capparidaceae (e.g., Boscia) "doubling" of each of two pairs of stamens results in an androecium of eight stamens, while in Capparis less ordered and more extreme duplication occurs to produce many stamens and a pistil with several locules. Recent morphological and molecular (rbcL) analyses of the phylogenetic relationships of Cruciferae-Capparidaceae indicate that they are part of a large natural group in which all floral variants are elaborations of a basically tetramerous (opposite and decussate) plan.

Parallel modifications of floral structure are seen in Papaverales (Papaveraceae-Fumariaceae). Hypecoum may represent the basic condition in the group (K2 C2+2 A2+2 G(2)), while in Chelidonium and Eschscholzia regular duplication (mainly in the median plane) results in around 16-22 stamens. Papaver and related plants are comparable to Capparis, in that more irregular duplication results in both numerous stamens and a gynoecium of many carpel units. Parallel morphological patterns involving stamen-carpel duplication in the Capparales and Papaverales (as well as many other familes) imply straightforward modification of similar developmental processes. What remains to be clarified is whether such patterns are under direct genetic control or are partially mediated by positional and other effects resulting from changes in the size, shape or growth rate of the floral apex relative to changes in the size, shape or growth rate of organ primordia.

D 009 MOLECULAR AND GENETIC ANALYSIS OF GYNOECIUM DIFFERENTIATION, Charles S. Gasser, Kay Robinson-Beers, Stephen B. Milligan, Rachanee Jampates-Beale and Cathrine M. Geil, Section of Biochemistry and Biophysics, University of California, Davis, CA 95616.

Studies on floral homeotic mutants have recently led to significant advances in understanding the molecular events responsible for initiation and maintenance of carpel development. In contrast, the mechanisms controlling development and differentiation of internal components of carpels remain largely obscure. As a model for carpel development, we are studying the regulation of formation of structures within the fused carpels (pistils) of *Arabidopsis thaliana*. We have isolated mutants defective in formation of pistil substructures essential for reproductive competence by screening a mutagenized population for plants which are female-sterile but have pistils with otherwise normal gross morphology. We find that such mutants are most commonly defective in ovule development. The first ovule mutants analyzed define two genetic loci which are essential for normal ovule development. In one mutant, *bell* (*bel1*), only a single integument-like structure is initiated which develops into a thick collar of tissue below the nucellus. The other mutant initiates two integuments which appear to undergo a normal pattern of cell division without the accompanying directional cell expansion necessary for formation of normal integuments. This produces the *short integument* (*sin1*) phenotype after which the mutant is named. Neither mutant is able to form a normal embryo sac. The phenotypes of these mutants indicate connections between normal development of the two integuments, and between integument development and embryo sac formation. The nature and importance of interactions between these mutations, other newly isolated ovule mutations, and mutations affecting the entire gynoecium will be discussed.

Inflorescence Meristems and Cell Fate Choice

D 010 NATURAL GENETIC VARIATION FOR CAPITULUM DIFFERENTIATION IN *MICROSERIS* (ASTERACEAE) , Konrad Bachmann, Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, NL-1098 Amsterdam, The Netherlands

Floret primordia on a composite capitulum arise in a tight spiral phyllotactic pattern from the rim to the center. In addition, there is a radial gradient of organ differentiation from the rim to the center of the capitulum: peripheral florets and fruit often differ in shape, color, hairiness, pappus structure or other characters from central ones. The characters usually change abruptly from one organ to the next one in phyllotactic order. Occasionally, fruits are peripheral-central mosaics for characters with cell-specific expression. If there are changes in several characters along the gradient of one capitulum, these are not simultaneous. The (genes for the) individual characters seem to respond individually to the determining gradient. The differentiation gradient interacts with the geometry of the organ arrangement to produce fixed numbers or proportions of the various floret and fruit morphs in rings around the center ("numerical canalization"). Genetic variation for all quantitative and qualitative aspects of capitulum differentiation can be found in natural populations of the four diploid (2n=18) annual species of *Microseris* in California and Chile. We have isolated many natural variants as inbred lines and characterized their heritable differences and plastic (environmental and age-related) variability under greenhouse conditions. We can show that genetic variation for capitulum differentiation (e.g. for the proportion of peripheral achenes) has arisen even within local, reproductively isolated populations. The genetic basis of this variation has been studied in offspring of artificial interpopulation or interspecies hybrids. Using co-segregating molecular markers (RFLPs, RAPDs), single genes involved in capitulum differentiation have been identified.

D 011 FLOWERING, REVERSION AND THE CONTROL OF ORGAN FATE IN *IMPATIENS*, Nicholas H. Battey, Department of Horticulture, Reading University, Reading, U.K.

Impatiens balsamina L. (cv. Dwarf Bush Flowered) initiates terminal flowers in short days (inductive conditions). However, if transferred to long days (non-inductive conditions) during flower development, the meristem reverts to leaf initiation and already initiated floral organs take on vegetative characteristics. Transfer of plants from inductive to non-inductive conditions after varying lengths of time can therefore be used experimentally to create a series of 'partial flowers'. For example, after minimum induction, plants typically show only the first step in flower development (leaves without axillary buds are inserted close together on the stem due to a lack of internode development); a larger number of inductive cycles gives normal petals followed by leaves; inductive treatments between these extremes cause plants to initiate a zone of leaf-like organs which are not separated by internodes and which contain areas of petal pigment, followed by whorls of leaves separated by long internodes (1). This latter type of partial flower therefore has the phyllotaxis of a flower but is made up of leaves, indicating that determinacy and organ identity have been uncoupled from phyllotaxis.

Furthermore, the leaves produced are not simply vegetative, because they are 'primed' to respond to further inductive conditions: when the plants are returned to inductive conditions the seven youngest 'leaf' primordia switch to petal development (2). The ability to respond to inductive conditions decreases as the primordia grow larger, and when they are more than about 750µm long they become irreversibly determined as leaves (3). The meristem itself is also 'primed' because resumption of flowering on return to inductive conditions does not involve the transition stages that occur during normal flowering; instead, petals immediately follow leaves (2).

Impatiens therefore provides the opportunity to study how environmental signals are coupled to the internal developmental programmes controlling flower morphogenesis. Recently initiated work will focus on the effects of environmental signals on the expression of homologues of the homeotic genes *floricaula* and *deficiens* in the *Impatiens* shoot meristem and developing primordia.

1. Battey, N.H. and Lyndon, R.F., 1984. *Ann. Bot.* **54**, 553-67.
2. Battey, N.H. and Lyndon, R.F., 1986. *Ann. Bot.* **58**, 333-41.
3. Battey, N.H. and Lyndon, R.F., 1988. *Ann. Bot.* **61**, 9-16.

D 012 GENETIC ANALYSIS OF THE MORPHOLOGICAL EVOLUTION OF MAIZE, John Doebley and Adrian Stec, Plant Biology Department, University of Minnesota, St. Paul, MN 55108

The combined use of molecular markers and quantitative genetic models provides a powerful means for dissecting the inheritance of complex traits involved in morphological evolution. This approach was used to investigate the genetic basis of the dramatic morphological differences between maize (*Zea mays* ssp. *mays*) and its probable progenitor, teosinte (*Z. mays* ssp. *parviglumis*). Results indicate that the key traits differentiating maize and teosinte are all affected by multiple loci, although for most traits, the data are consistent with a mode of inheritance that would involve one or two major loci plus several minor loci. Our data are not consistent with the model that the traits differentiating maize and teosinte evolved by selection at numerous loci with equal and small effects on the phenotype. Most of the variation for the dramatic differences in inflorescence morphology between maize and teosinte is explained by five restricted regions of the genome. These five regions and their effects on inflorescence development are currently being studied in near-isogenic backgrounds. One of these regions possesses a single major locus that our colleagues and we have designated *TGA1* (*teosinte glume architecture*). This locus controls glume development including its size, orientation and degree of induration. The maize allele at *TGA1* enables the production of staminate-like glumes on a pistillate background. A second region has a large effect on plant architecture, controlling a switch from long lateral branches tipped by tassels (teosinte phenotype) to short branches tipped by ears (maize phenotype). This region encompasses a previously described maize locus, *TB1* (*teosinte branched*), that has similar effects, suggesting that a mutation at this locus was involved in maize evolution. A third region affects development by altering the pattern of internode elongation, phyllotaxy and sex expression in the inflorescence. This region encompasses a known maize locus, *TE1* (*terminal ear*), with similar phenotypic effects. A fourth region appears to possess a single undescribed major locus that controls the switch from distichous to decussate inflorescence phyllotaxy. These results suggest that mutations at a small number (five) of regulatory loci may have been the initial steps in the domestication of the maize, supporting a model for maize evolution proposed by George Beadle in 1939.

D 013 INFLORESCENCE VERSUS FLORAL MERISTEM DEVELOPMENT IN *ARABIDOPSIS*: THE ROLE OF *LEAFY*
Detlef Weigel, and Elliot M. Meyerowitz, California Institute of Technology, Division of Biology 156-29, Pasadena

Development of a flower begins with the formation of a floral meristem, which derives from the inflorescence meristem. Mutations in the *Arabidopsis* gene *LEAFY* cause a partial transformation of flowers into inflorescence shoots, indicating that *LEAFY* promotes development of floral meristems as opposed to inflorescence meristems. Genetic studies show that *LEAFY* interacts with at least one other gene, *APETALA1*, in the determination of floral meristems, since the transformation of floral into inflorescence meristems is more complete in *apetala1; leafy* double mutants than in either single mutant. The combined action of *LEAFY* and *APETALA1* is reflected by the effect of single and double mutants on the expression of homeotic genes. As in wild-type flowers, the homeotic gene *AGAMOUS* is expressed in the center of developing *apetala1* and *leafy* single mutant flowers, but not of *apetala1; leafy* double mutant flowers.
We have cloned the *LEAFY* gene, and, as a first step towards understanding the molecular basis of *LEAFY* function, we have analyzed its expression by in situ hybridization. Consistent with its role in determining floral meristem identity, *LEAFY* RNA is detected in floral, but not in inflorescence meristems. *LEAFY* expression precedes expression of homeotic genes, at least some of which are activated by *LEAFY*. In wild type, *LEAFY* expression is shut off in the floral meristem during stage 3 of flower development, shortly before the floral meristem terminates with the formation of carpel primordia. Interestingly, this negative regulation of *LEAFY* expression appears to be dependent on feedback from homeotic genes, as *LEAFY* continues to be expressed in the (indeterminate) floral meristem of *agamous* mutants. *LEAFY*, however, cannot be the only mediator of floral meristem indeterminacy in *agamous* mutants, since *agamous; leafy* double mutant flowers display the same indeterminacy as *agamous* single mutant flowers.
The molecular analysis revealed furthermore that *LEAFY* is the *Arabidopsis* homolog of the *Antirrhinum* gene *FLORICAULA*. Like *LEAFY*, *FLORICAULA* promotes floral over inflorescence meristem development. However, *Antirrhinum* plants mutant for the *FLORICAULA* gene exhibit a more dramatic phenotype than *leafy* single mutants in *Arabidopsis*, i.e., a phenotype more similar to *leafy; apetala1* double mutants. The functional comparison of *LEAFY* and *FLORICAULA* shows that, although the basic mechanisms controlling flower development in dicotyledonous plants are conserved, distantly related species utilize these conserved factors in different ways. Using a PCR approach, we have detected *LEAFY* related sequences in maize, a monocot, as well as in gingko, a non-flowering seed plant. We hope that these studies will ultimately contribute to our understanding of the evolution of flowering plants.

Vegetative Meristems-I

D 014 NOVEL MORPHOLOGICAL PATTERNS RESULT FROM THE INTERACTION OF MAIZE MUTANTS AND VARIANTS AFFECTING DEVELOPMENTAL TIME, Michael Freeling and laboratory, Department of Plant Biology, University of California Berkeley, CA 94720

From observations of early events in leaf development, and characterizations of mutants of about two dozen genes that remove leaf parts or change leaf patterns, some generalizations are possible (review and analysis: Freeling, 1992, Dev. Biol. 153, 44-58). 1) From the meristem to the primordium -- plastrochon 3-4, ca. 6 mm long -- involves an approximately even distribution of cell-divisions. All of our mutants act before the primordium. 2) The mutants that remove the ligule-auricle are known to involve the sending or receiving, of a "make ligule" signal emanating from the twin midrib-blade foci. Dominant mutants fall into two classes. 3) The first includes the four KNOTTED-like functions: dominant neomorphic mutants of Kn1, Rs1, Lg3 (all express different homeodomain proteins ectopically) and Lg4. 4) Each is developmentally retarded, and 5) move the ligular boundary out toward the tip of the leaf. 6) Kn1 and Rs1 (Becraft, unpublished) are domain-nonautonomous, and 7) mutant alleles of different genes act in specific regions of the primordium as if the leaf were a patchwork of domains that develop coordinately only because cells in the various compartments progress synchronously along developmental schedules; mutants upset synchrony. See poster by J. Fowler and coworkers. Recessive leaf part-deletion mutants support this "multiple maturation schedules" hypothesis.

A second more complicated class of dominant mutants not only affect the leaf in similar fashion to the Kn1-like group, but affect development in meristems and other organs as well. Among the mutants in several different genes analyzed, Hsf1-O has been described in some detail (see Freeling, Bertrand-Garcia and Sinha, 1992, BioEssays 14, 227-236). This retarded stage-transition mutant displays very different phenotypes in different time-to-flowering backgrounds. Lxm1-O is another heterochronic mutant showing novel phenotypes as different time-frames "slip." See poster by D. Porter, this lab. The interaction of mutants and variants in different developmental time-keeping pathways may generate Goldschmidtian hopeful monsters (i.e. evolutionary novelty) without need for special mechanisms of mutagenesis.

D 015 DOMAINS OF GRASS LEAVES ARE IMPRINTED BY THE VEGETATIVE MERISTEM: CELLULAR & GENETIC ANALYSIS OF MAIZE AS A MODEL SYSTEM, Anne W. Sylvester, Dept. of Biological Sciences, University of Idaho, Moscow ID 83843

Grass leaves are modular organs that emerge from the meristem in a relatively conserved manner. The leaf primordium encircles the apex at inception with subsequent development of three primary leaf regions, a blade, a sheath, and a joint-like ligular region. The question of how and when cells in each region acquire their appropriate identity is investigated from a cellular and genetic perspective, using maize as a model system. The guiding principle is that developmental decisions are made in a three-step hierarchy, starting at fertilization when embryo poles are established, continuing at the level of the meristem, and finally at the level of the primordium itself. Choices in cell identity are traced from the leaf down to the level of the meristem by combining mutant analysis with cellular reconstruction of early events. Mutations that alter the normal development pattern at presumably different stages in the hierarchy are investigated. Mutants examined are those that alter the position of leaf parts early and late (*Laxmidrib1-0 and Liguleless3-0*) as well as those that alter the extent of cell differentiation early and late (*leafbladeless1-0* and *liguleless1-0*). Genetic analysis confirms that *Lg3-0* is semi-dominant. The *Lxm1-0* mutation appears to have pleiotropic effects on tassel, ear, and leaf cell identities with increased temperature or decreased time to flowering. Cell behaviors of wild-type and mutant siblings are viewed in a three-dimensional framework: the longitudinal dimension spans leaf tip to base, the lateral from margin to margin and the transverse from adaxial to abaxial side. Patterns of cell aging within the three-dimensional framework are identified by a combination of microscopical and experimental methods, including epifluorescence, scanning electron and UV-sensitive CCD-video microscopy. Two features are used to mark changes in cell age: first, immunocytochemisty, using fluorescently-labelled tubulin antibodies, shows that changes in cytoskeletal arrays correlate with early steps in cell aging. Second, lignification of the secondary wall marks a later aging process as indicated by subtle shifts in the wavelength of UV-light-induced autofluorescence. Rate of cell aging is found to be regional-specific in each of the three dimensions, suggesting the leaf is a complex of independent but interacting cellular domains. Heterozygotes of *Lg3-0* show altered aging patterns in cells through the transverse dimension, i.e., on the adaxial but not abaxial sides, within the midrib domain alone. Similar boundaries are identified in *Lxm1-0* and *lbl1-0*. The blade/midrib junction is viewed as a border that defines distinct leaf domains. CCD-videomicroscopy of whole leaves confirms that changes in vascular spacing occur at the blade/midrib junction of mutant leaves relative to wild-type. The major leaf domains are thus determined at least as early as the time that vascular spacing is established. To test this idea, computer-aided reconstructions of shoot apices are used to trace provascular patterns that serve as markers for information imprinted at the level of the meristem.

Vegetative Meristems-II

D 016 THE SUPPRESSION HYPOTHESIS: THE ROLE OF PLACE-DEPENDENT SUPPRESSION OF GROWTH IN MORPHOGENESIS AND PHYLOGENY, Dominick V. Basile and Margaret R. Basile, Lehman College of CUNY, Bronx, NY 10468.

The suppression hypothesis is based on the premise that once cell proliferation and enlargement starts in a developing plant or plant part, there must be some mechanism to suppress these growth processes at specific times and in specific places, else plants/parts would have no predictable size or shape. The hypothesis, "that many if not most of the differences by which we distinguish the form changes that occur during the stages of ontogeny and the course of phylogeny are due to differences with respect to the time and place cell proliferation and/or enlargement is suppressed," logically follows. Although it cannot be experimentally verified, it is proposed that place-dependent suppression of growth has played critical roles in the genesis of form in land plants (embryophytes) since their origin from presumed algal ancestors. Comparative studies indicate that the genesis of the vegetative bodies of the great majority of non-embryophyte photoautotrophs is by cell proliferation diffuse throughout the developing body. In sharp contrast, the genesis of the vegetative bodies of embryophytes is by cell proliferations restricted to relatively small populations of cells, meristems. For this to have happened, a regulatory system whereby one subpopulation could act to suppress further proliferation in all the remaining cells in a developing individual had to have evolved in preembryophytes. Such considerations bring us to propose that apical growth and apical dominance are a consequence of the evolution of a mechanism for sub-apical suppression. "Subapical suppression," in our judgement, is the earliest and one of the most critical roles for place-dependent suppression of growth. Strict apical growth apparently was the only mode in Rhyniophytes, the original land plants, and continues to be the most characteristic mode of axial development of all extant phyla of embryophytes.

Comparative studies also indicate that evolutionary diversification from the original Rhyniophyte-type organization was primarily toward greater size and more complex morphology. Counter to this primary tendency has been a recurrent, secondary tendency to reverse the trend. The secondary tendency is often referred to as "reductive" or "regressive evolution" by comparative morphologists. We think that place-dependent suppression of growth is largely responsible for "reductive"/regressive evolution, and, as such, constitutes a second major role in plant morphogenesis and phylogeny.

The series of experiments that led us to think that "reductive evolution" was a manifestation of place-dependent suppression also provided evidence as to the system that may regulate place-dependent suppression. The experiments, conducted with liverwort gametophytes, flowering plant seedlings, and stem tissues, implicated the interrelated actions of auxin, ethylene, and specific arabinogalactan proteins (AGPs). Of the three components of this morphoregulatory system, the AGPs are the most interesting; primarily because they are likely to be more immediately and specifically involved than the hormones, and because we have been able to detect pronounced differences in AGPs correlated with suppressed vs desuppressed cell proliferation.

The AGPs in the liverwort and lettuce tissue with which we work exhibit a relatively wide range in buoyant densities when separated on CsCl gradients. When cell proliferation in suppressed organ primordia in liverwort gametophytes and stem pith (sporophyte) tissue of lettuce were experimentally desuppressed, AGPs at the high end of the buoyant density range, present in extracts before desuppression, were absent afterwards. We suspect, therefore, that some component of the high buoyant density AGPs plays a critical role in maintaining those cells still capable of dividing in a suppressed state. A program to develop monospecific antibodies to high buoyant density AGPs present in suppressed but absent in desuppressed/meristematically active cells is in progress. If the project is successful, we will have one or more monoclonal antibodies that will not only discriminate between suppressed vs meristematically active cells, but will also help isolate and characterize AGPs involved in the place-dependent suppression of growth. Perhaps then a mechanism of action will suggest itself

D 017 PATTERN FORMATION IN THE MOSS PHYSCOMITRELLA PATENS, David J. Cove, Department of Genetics, University of Leeds, Leeds, U.K.

The early stages of moss gametophyte development provide excellent material for the study of the control of the timing and plane of cell division. The cell lineages involved can be described in probabilistic terms and the effects of different light regimes and the application of plant hormones can be quantified. A large range of mutants altered in the pattern of development have been isolated[1]. Although most such mutants are sexually sterile, their genetic analysis is possible using somatic hybrids produced by protoplast fusion[2]. Techniques for the genetic transformation of P. patens have been devised[3] and recent improvements have increased transformation frequencies considerably. Attempts to tag developmentally important genes have so far been unsuccessful but the improved transformation procedure now makes gene isolation by mutant complementation feasible. The isolation of moss genes using heterologous probes is being developed. Two myb homologues have been identified and expression of these genes in antisense has been shown to be lethal[4].

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D 018 GENETIC AND MOLECULAR ANALYSIS OF ROOT CELL DEVELOPMENT, John Schiefelbein, Susan Ford, Moira Galway, Mark Kinkema, Jim Masucci, Yonca Ilkbahar, and Carrie Begin, Department of Biology, University of Michigan, Ann Arbor, Michigan 48109.

The proper development of plant cells depends on the precise control of cell division, cell expansion, and cell differentiation. The analysis of root hair development in *Arabidopsis thaliana* provides an opportunity to examine the genetic and molecular basis of these processes. Root hairs are extensions of epidermal cells that develop by tip growth and are not required for the growth of the plant as a whole. Genetic studies show that at least 12 *Arabidopsis* loci are involved in the development of root hair cells. Some of the gene products are required during early stages in root cell differentiation, where they influence the number and arrangement of hair-forming and non-hair-forming epidermal cells. Several loci appear to affect the expansion of root hairs, since the mutant phenotypes display alterations in root hair morphology (*e.g.* bulging, branched, or singed hairs). The recent isolation of T-DNA-induced root hair mutants has provided an opportunity for the molecular isolation of some of these genes. In a related line of investigation, genes encoding myosin isoforms have been characterized from *Arabidopsis*. Myosin is a molecular motor that is believed to drive the transport of various cellular components along actin filaments (*e.g.* cytoplasmic streaming, directed vesicle transport during tip growth). The molecular mechanisms uncovered by these studies are likely to represent fundamental, evolutionarily-conserved processes that are utilized by other cell types and other members of the plant kingdom.

Patterns in Plant and Meristems Evolution

D 019 THE PHYTOMERIC STRUCTURE OF BARLEY. Gerd Bossinger and Francesco Salamini, Max-Planck-Institut für Züchtungsforschung, Köln, Germany.

Based on a histological and morphological description of organ development of *Hordeum vulgare*, a model is proposed for grass development based on the metameric (phytomeric) structure of the plant. During development two distinct types of organs appear which show basic differences in their histogenesis. These organs are of foliar origin when organized according to the histogenesis of a leaf primordium ('founder' cells distributed transversely to the apex in an almost complete ring; first periclinal cell divisions in the tunica layer). Bud-type organs are indicated as being of cauline origin (meristematic activity first in the corpus; activity restricted to a narrow region from which it proceeds in an outward direction). The event common to the initiation of both foliar and cauline types of organs *is periclinal* cell division. To define the type of growth each organ will assume it then is of basic importance where periclinal cell division first happens and in which other layers divisions follow.

The concept of a single repeating unit can be used in grasses to describe their metameric structure. The basic element can be considered a unit of growth. This unit is referred to as the 'phytomer', a structure which, when produced in a series, defines a structure of higher order. We share the view of Weatherwax (1923) and Sharman (1942) who see the phytomer as an internode together with the leaf at its upper end and the bud at its lower end in position opposite to the leaf. These phytomers (type 1) appear along the plant axis in different variations establishing distinct regions depending on the development of the different phytomer parts (leaf, internode, bud and adventitious root). These six regions are vegetative, tiller region (A) and culm (B), and reproductive, rachis (C), first order branch (D), rachilla (E) and floret axis (F).

The formation of a lateral apex is one of the common modules proper to the organization of the phytomer. The first evidence revealing an incoming new apex is the occurrence of one or few periclinal cell divisions localized in tissues histogenetically derived from the corpus. This is followed by a cauline mode of growth. This phase is particularly interesting for the origin of what we introduce as type 2 phytomers. The relevant homologies of organs present at ramifications (all bi-keeled and all products of a phytomer fusion) are based on the common morphogenetic events proper to the initiation of a new lateral apex. In this sense, homology can be used to hypotesize similar basic sets of morphogenetic events used to assemble organs as different as coleoptile, prophyll, subtending glumes, palea, stamens and possibly lodicules. Because of the large developmental flexibility of type 2 phytomers, the formation of a new lateral apex starting with the determination of the first fused phytomer, represents a developmental window open to several morphological solutions, depending on the position of the new apex.

During the formation of the sporophyte, the first instance when such a type of genetic program is activated is the organization of the shoot apex in a lateral position of the proembryo. The first organ formed, the coleoptile, results from the fusion of two leaf primordia. This phase of development represents the essence of embryogenesis. The shoot apical meristem organizes itself in layers and displays morphogenetic activity as seen by the formation of lateral appendages. Whenever new differentiation events are necessary, new non-dormant apices are programmed in lateral positions of type 1 phytomers and develop into specialized organs. Due to its basic similarity to the most relevant part of embryogenesis, we have indicated this repetitious origin of lateral organs as recurrent embryogenesis (i.e., the inception of phytomers type 2). In contrast, the ability of a shoot apex to organize a long linear succession of phytomers type 1 can be seen as continuous embryology. While continuous embryology provides elongation of organs or plant parts by addition of type 1 units, recurrent embryogenesis organizes new lateral points of growth and covers relevant needs in differentiation. Species specific modules of recurrent and linear growth are responsible for the final shape of the plant.

One purpose of our work was to classify morphological mutants of barley according to the disruptive effects mutations induce on the organization of the plant body. Based on the proposed model of the plant structure, the mutants could be classified into four classes. These are transitions, organ modifications, addition of phytomers and suppression of phytomers. Transitions are present if a phytomer variant which is typical for one region of the plant appears in an other region (short internodes in *densinodosum*). Organ modifications show altered parts of one phytomer variant (*e.g. liguleless*). Addition of phytomers occurs if normally dormant bud primordia sprout (e.g. the terminal meristem of the floret axis in *awned palea* or that of the rachilla in *branched*). A suppression of phytomers is present if a lateral bud primordium is not initiated (e.g. no tillers in *uniculm*, no lateral spikelets in *Deficiens*) or if less phytomers are produced along the plant axis (*reduced internode number*).

D 020 CLADISTIC AND PALEOBOTANICAL PERSPECTIVES ON THE ORIGIN OF ANGIOSPERM ORGANS, James A. Doyle, Section of Botany, University of California, Davis, CA 95616.

Cladistic analyses of living and fossil seed plants (Crane 1985; Doyle and Donoghue 1986, 1992) help clarify the evolutionary history of the familiar organs and patterns of development in angiosperms. Even if molecular analyses give the correct relationships among modern taxa, the gaps between surviving seed plant groups are so great that the complex sequence of morphological transformations cannot be reconstructed without morphological analyses that include fossils as well.

Axillary branching apparently arose between Devonian "progymnosperms," which had unequal apical branching and dichotomous simple leaves, and the earliest Late Devonian-Carboniferous seed plants ("seed ferns"). This change coincided with conversion of branch systems into fern-like compound fronds and origin of scale-like cataphylls (arrested frond primordia?). The typical simple leaf of angiosperms was ultimately derived from a seed fern frond, but because angiosperms are more closely related to Bennettitales, *Pentoxylon*, and Gnetales, which show varying degrees of leaf simplification, it is likely that leaf evolution passed through a stage with one order of pinnate venation. Comparison with leaf development in ferns suggests that origin of the "reticulum" of higher vein orders was linked to truncation of marginal growth and expansion of diffuse growth, but better understanding of leaf development in other seed plants is needed.

The reproductive structures of angiosperms have an especially complex history. Ovules of primitive seed ferns had an integument probably derived from outer members of a cluster of sporangia in progymnosperms and were borne in cupules corresponding to the progymnosperm fertile appendage. These two layers might be homologized with the two integuments of the angiosperm ovule. However, cladistic results indicate that the common ancestor of extant seed plants had non-cupulate ovules borne directly on sporophylls, implying that the outer integument of angiosperms is a new structure. Because angiosperms are positioned between Mesozoic seed ferns and Bennettitales, *Pentoxylon*, and Gnetales, the typically anatropous ovules of angiosperms may be derived from the anatropous "cupules" of Mesozoic seed ferns, which were borne in two rows on a sporophyll rachis, suggesting they are ovule-bearing leaflets. These comparisons would imply that the carpel is derived from the sporophyll rachis, which would require a drastic change in proportions and form to enclose the ovules. Since angiosperms, Bennettitales, *Pentoxylon*, and Gnetales (anthophytes) all have sporophylls aggregated into flower-like strobili, the flower presumably originated in their common ancestor, before the closed carpel. This suggests that genes controlling the sequence of floral parts in *Arabidopsis* may have taken on their present functions in the common ancestor of anthophytes; if so, they should also be found in Gnetales.

Different aspects of this scheme vary greatly in robustness. Although a relationship between angiosperms and Gnetales is supported by molecular data, how Mesozoic seed ferns, Bennettitales, and *Pentoxylon* fit in is uncertain because many of their characters are unknown. For example, a direct relation between angiosperms and Bennettitales, which have single orthotropous ovules borne directly on a floral axis, would require a less conventional origin for the standard carpel bearing several anatropous ovules - perhaps as in Meyen's gamoheterotopy hypothesis, by adoption of the developmental program for the laminar microsporophylls. Also, although all available data strongly support the monophyly of angiosperms, they conflict on which groups are basal, and the original floral morphology could be either *Magnolia*-like or much simpler, as in many "paleoherb" groups (herbaceous magnoliids, monocots).

D 021 ANALYSES OF DEVELOPMENTAL PATTERNS: BRIDGING THE GAP BETWEEN GENETICS AND MORPHOGENESIS. G. Ledyard Stebbins, Department of Genetics, University of California, Davis, CA 95616

Based upon morphogenetic investigations, two types of developmental processes are recognized in plants: (1) in a single organ, passage from a meristematic to submeristematic or secondary differentiation, then a postmeristematic maturation state, (2) the succession of different organs produced from seed germination to adulthood. Molecular geneticists have identified regulator genes that govern onset of successive stages of (2), but little is known about the genetic bases of progression from meristematic to postmeristematic stages, and the termination of growth. Evidence is presented to indicate that developmental succession is under the influence of growth substances: cytokinins promote the meristematic condition, IAA, NAA and others the postmeristematic condition, GA^3 submeristematic condition. Hence mutations of genes that affect production and movement of growth substances should affect meristem-adult progression, as would inhibitors of mitosis and promoters of wall growth. With respect to organ primordia succession, the number of organs of a particular kind found in an adult flower is correlated with the length of developmental time occupied with the differentiation of its primordia. Comparative diagrams based upon this principle show how altering timing of processes can alter adult morphology.

D 022 PATTERNS OF PLANT EVOLUTION INFERRED FROM NUCLEAR GENES, Elizabeth A. Zimmer, Smithsonian NMNH Laboratory of Molecular Systematics, Washington, DC 20560.

Ribosomal genes (rDNAs) have been the nuclear genes characterized from the largest number of plant populations and species. These genes are found as highly-reiterated, highly-homogeneous repeat units in tandem arrays at one or several locations in the plant genome. Initial comparative restriction endonuclease ' vses of ribosomal gene families revealed base substitution and length var n concentrated in the nontranscribed spacer regions of the repeats. Such ' iation has been most useful in delineating intraspecific and intergeneric relationships and in identifying natural occurrences of hybridization among plant populations and species. With the use of primers complementary to conserved nucleotides and direct sequencing methods, the coding regions of rDNA repeats can be compared across the entire range of green plant lineages. The different transcribed regions of the repeat unit denoted as "18S," "26S," and "ITS," generally provide increasing degrees of resolution for interspecific comparisons; however, the rate of change of each of these regions may vary among different taxonomic groups at the same hierarchal level. For example, ITS (internal transcribed spacer) regions of the rDNA repeat, which tend to be unalignable among different genera and tribes of the recent sunflower family *Asteraceae*, can be compared readily among the much older family,*Winteraceae*. While the ITS region recently has become the intense focus of plant molecular systematists interested in biogeography and speciation, over the past five years the more conservative coding regions have been sequenced in efforts to infer evolutionary trees relating the major groups of land plants and green algae. These ribosomal gene-based trees yield relationships which, in part, have generated renewed interest by biologists studying the origin of the flower, in the gymnosperm order *Gnetales* and in the paleoherbaceous lineages, *Aristolochiales*, *Nymphaeales*, *Piperales* and monocots. Comparisons of rDNA-based trees with those derived from morphological characters show some degree of complementarity of the data sets, although the exact nature of the angiosperm radiation remains unresolved. In fact, it is clear from these initial investigations that additional gene comparisons (e.g. of conservative organellar sequences, of protein-coding "housekeeping genes" and of genes producing morphological variation in plants) are needed to resolve the mystery of flowering plant origins. Parallel gene studies, which should include extensive sampling of paleoherb and woody magnoliid lineages and of land plant groups with good fossil records, will enhance our understanding of molecular character reliability and rates of evolution. Among other nuclear genes, a large number of sequence types have been isolated and compared from a few "model genetic organisms" such as maize, tomato, pea, *Arabidopsis* and *Chlamydomonas* or agronomically-important plants such as wheat, rice and soybean. These initial comparisons provide the basis for developing PCR-mediated gene isolation strategies for determination of the patterns of plant sequence evolution with respect to both structure and expression. Such strategies are essential to the inclusion of field-collected materials from key ancestral lineages such as the *Gnetales* and paleoherbs. These more limited comparative studies also indicate that most plant genes are encoded by small gene families; therefore, careful assessment of homology is critical for the production of robust hypotheses of plant molecular evolution.

Embryos and Embryogenesis

D 023 EARLY EVENTS IN APICAL-BASAL PATTERN FORMATION IN THE ARABIDOPSIS EMBRYO, Gerd Jürgens, Ulrike Mayer, Thomas Berleth, Ramón A. Torres Ruiz, Simon Miséra, Gabriele Büttner, and Max Busch, Institut für Genetik und Mikrobiologie, Universität München, Munich, Federal Republic of Germany.

The seedling which represents the primary plant body organization shows two patterns, one along the apical-basal axis of polarity and the other perpendicular to it. The latter (radial) pattern comprises the main tissue types such as epidermis, ground tissue and vascular tissue, while the apical-basal pattern includes the following elements: shoot pole (shoot meristem and epicotyl), cotyledons, hypocotyl, embryonic root (radicle), root meristem, and root cap. We are investigating the mechanisms that generate the body organization in the *Arabidopsis* embryo, using a genetic approach. As a first step, we have isolated and characterized a fairly large number of EMS-induced putative pattern mutants which affect different aspects of the body organization: apical-basal pattern, radial pattern, and shape. Four genes involved in apical-basal pattern formation have been studied in detail. The analysis of their mutant phenotypes suggests that these genes are involved in the early partitioning of the apical-basal axis which generates three large subregions: apical, central and basal. In order to learn more about the functions of these genes, we have initiated their molecular cloning. At present, we are focusing on the *gnom* gene which seems to act earlier than the other three genes and might play a pivotal role in organizing the apical-basal axis, as suggested by its mutant phenotype.

D 024 DO PLANTS EXHIBIT CLASSICAL EMBRYOGENESIS? PERSPECTIVES TOWARD A SYNTHESIS BETWEEN DEVELOPMENTAL MORPHOLOGY AND MOLECULAR GENETICS OF PLANTS. Donald R. Kaplan[1] and Todd J. Cooke[2], [1]Department of Plant Biology, University of California, Berkeley, CA 94720 and [2]Department of Botany, University of Maryland, College Park, MD 20742.

Two distinct approaches have been used to describe the development of angiosperm embryos. The predominant approach, which was pioneered in Hanstein's (1870) studies of Capsella and a few other genera, places primary emphasis on cell lineages that are thought to give rise to specific structures in the developing embryo. This approach has resulted in the classical concept of the angiosperm embryo, i.e. it consists of a series of unique morphological states expressed within the seed, with unique structures such as the cotyledons being produced by almost invariant cell lineages. This concept has several appealing aspects, including its remarkable similarity to current concepts of animal embryogenesis. However, it does not account for the morphological diversity observed in dicot embryos. For example, 1) the embryos in mature dicot seeds have progressed to different stages of embryonic development, ranging from 2-celled proembryos of Monotropa to advanced embryos of Pisum with 4 to 6 leaf primordia, and 2) the embryos of many dicots, such as Gossypium, exhibit highly variable cell lineages. Finally, the cell-lineage approach fails to satisfactorily address the problem of meristem initiation. The other approach, which is epitomized by the earlier work of Hofmeister (1849, 1861), surveys the diversity of angiosperm embryogenesis in the attempt to uncover general principles applicable to all angiosperm embryos. Using this comparative morphological approach to examine dicot embryogenesis, we conclude that the so-called globular stage is better described as consisting of an apical pole and a basal pole, which represent the incipient shoot and root apical meristems, respectively. All subsequent events are then seen as the elaboration of these poles whereby they will gradually acquire the distinctive features associated with vegetative apical meristems. Thus, the cotyledons, radicle, and plumule which are typically viewed as unique structures arising via unique processes are instead seen to represent the first expression of the same processes occurring throughout the development of the vegetative plant body. This morphological perspective will also be used to analyze the fascinating mutants now available in Arabidopsis embryogenesis. Although these mutants have generally been interpreted in terms of cell lineage-based mechanisms, such interpretations must necessarily result in experimental models that are relevant only to a few other dicots. We shall evaluate these mutants in terms of broad morphological principles which we hope will suggest underlying mechanisms that are universally applicable to dicot embryogenesis.

D 025 STRUCTURE AND EVOLUTION OF THE REGULATORY NETWORK CONTROLLING MATURATION OF THE SEED. Donald R. McCarty, Ute Hoecker, Leonard Rosenkrans, Christian B. Carson, Vimla Vasil, Indra K. Vasil and Margaret E. Stoll. Horticultural Sciences Dept. University of Florida, Gainesville, FL 32611.

During the late phase of seed formation the embryo and portions of the endosperm which will remain viable in the dry seed undergo a maturation process in which growth is arrested and dessication tolerance is acquired. The viviparous mutants of maize define genes that are specifically required for seed maturation. Most of the viviparous mutants block synthesis of abscisic acid (ABA), a hormone that has been widely implicated in regulation of maturation related gene expression. In contrast, the *viviparous-1 (vp1) mutant specifically reduces hormone sensitivity in seed tissues.* In addition, *vp1* blocks synthesis of anthocyanin pigments in the aleurone and embryo. *Vp1* controls the anthocyanin pathway by activating transcription of the *C1* regulatory gene. In addition to activating pathways associated with seed maturation, *Vp1* also has a role in repressing genes that are normally activated during seed germination.

Functional analyses based on transient expression in maize cells indicate that the VP1 protein can function as an activator, as well as, a repressor of transcription. At least four domains are resolved within VP1 which differentially affect three distinct in vivo functions: i) activation of the Em LEA gene associated with embryo maturation, ii) activation of the *C1* gene and iii) repression of an α-amylase gene. One of the domains corresponds to exons that are highly conserved in homologous genes from barley and *Arabidopsis* (Giraudet et al. Plant Cell, in press). The other three functional domains reside in the highly diverged large first exon of the *Vp1* gene. The regulatory functions of VP1 that we have analyzed, thus far, are mediated through distinct cis-acting elements in the promoters of the Em, *C1* and amylase genes. Genetic variation in maize which affects the interaction between *Vp1* and *C1* suggests some insight into how at least part of this complex regulatory network evolved.

D 026 ESTABLISHMENT OF CELL POLARITY IN *FUCUS*. Ralph S. Quatrano, Vincent T. Wagner, Brad Goodner and Leigh Brian. Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

Zygotes of the brown alga *Fucus* provide a model system to study the basic mechanisms involved in the generation of zygote asymmetry and directional transport of unique cytoplasmic components to the resulting daughter cells of the two-celled embryo. These polar processes are common to many different organisms (from yeast, algae, amphibians, and nematodes, to vertebrates, mammals and seed plants), but are difficult to study and experimentally manipulate in most of these systems. In contrast, the polar axis of the *Fucus* zygotes is labile and can be oriented and reoriented by a number of gradients, the simplest of which to manipulate is unilateral white light. There is no apparent spatial patterning in the egg, and "polar determinants" are not localized in the egg and rotated by such vectors; the polar axis and localized cytoplasmic components arise epigenetically.

Polarization of the developmental axis by light can be shown to have at least two basic components, axis formation and axis fixation. Fixation occurs between 10 and 12 hours after fertilization after which other orienting vectors are incapable of forming a different axis. Data from earlier experiments indicate that at least two components were essential for fixation; a localized actin network at the fixed site of rhizoid formation and a cell wall. Our working hypothesis at present is that axis fixation involves transmembrane bridges at the presumptive rhizoid pole linking the cell wall to the microfilament cytoskeleton. These structural components anchor membrane proteins (e.g. a localized calcium channel), and possibly provide "tracks" for the directional transport of particles needed at the site of polar growth (e.g. F granules). Our evidence for transmembrane linkages between the cytoskeleton and the cell wall to fix the polar axis in *Fucus*, has led to a structural model of the rhizoid tip, i.e. and AXIS STABILIZING COMPLEX (ASC) (1). Evidence for this model will be presented and discussed, including evidence for the presence of actin-binding proteins, integrins (2) and a vitronectin-like protein in this complex (3). In addition, preliminary data will be presented that describes the presence of two gene products in *Fucus* that correspond to proteins that are necessary for polar bud formation in yeast, i.e. members of ras-like (BUD1 and CDC42) and neck filament (CDC3) family. The expression of these genes and their localization in *Fucus* will be discussed.

Supported by NSF Plant Biology Fellowship to V.T.W. (DIR-9104253) and research grants to R.S.Q. from the NSF (DCB-8917540) and the Office of Naval Research (NOOO14-91-J4128).

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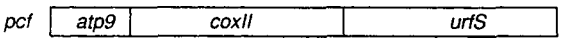
Evolution and Organization of Plant Genomes

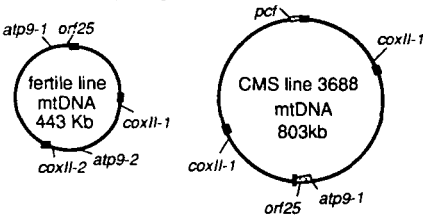
D 027 ALLELIC DIVERSITY AT THE MAIZE *B* GENE: DIFFERENT PROMOTER SEQUENCES DETERMINE DIFFERENT TISSUE-SPECIFICITIES, Vicki L. Chandler, Devon Brown, Pablo Radicella, Matt Beaudet, and Garth Patterson, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The *B* gene encodes a transcription factor of the basic-helix-loop-helix class, which controls the synthesis of the anthocyanin pigments in maize. This gene, as well as the homologous *R* gene family, displays extensive allelic variation in that different alleles (~30) cause distinct distributions of anthocyanin pigments in different tissues and at different developmental times. Our recent experiments have demonstrated that anthocyanin synthesis correlates with tissue-specific *B* mRNA accumulation in the various *B* alleles. We have generated restriction maps of six different *B* alleles. All the *B* alleles are simple, containing a single coding region. Restriction sites within the coding region and nearby 3' flanking sequences are highly conserved, whereas the restriction maps in the 5' flanking regions are all quite distinct. Cloning and sequence comparison of two alleles has demonstrated high sequence identity in the coding and 3' flanking regions (98% and ~90%, respectively). In contrast, the most 5' region of the mRNAs and 5' flanking sequences share no significant sequence identity. This result suggests that the alleles diverged from each other by complex genome rearrangements rather than by simple base pair substitutions. We have used the high velocity microprojectile transformation assay to demonstrate that the differential expression of the two alleles in the seed is determined by their 5' variant sequences. Thus, the variation in tissue-specific anthocyanin synthesis in plants with these different *B* alleles is controlled at the level of *B* gene expression. The extensive allelic diversity at *B* and *R* may have arisen because of the easily scorable phenotype and the nonessential nature of the anthocyanin pigments. The evolutionary implications of the ectopic expression of a regulatory gene that can occur by juxtaposition of novel DNA sequences will be discussed.

D 028 EVOLUTION OF MITOCHONDRIAL GENOMES AND CYTOPLASMIC MALE STERILITY, Maureen R. Hanson[1], Helen T. Nivison[1], Catharine A. Conley[1], Anthony Moore[2], Hsu-Ching Chen[3], Henri Wintz[3], Rainer Köhler[1], Claudia Sutton[1], and Otto Folkerts[4], [1]Genetics and Development, Cornell University, Ithaca, NY 14853-2703, [2]Department of Biochemistry, University of Sussex, Falmer, Brighton, UK, [3]IBMP, Strasbourg, France, [4]Dow Chemical Company, Midland, MI 48674.

Abnormal mitochondrial genes arising from recombination events have been correlated with cytoplasmic male sterility in *Petunia* and other species. Recombination events also serve to reorganize mitochondrial genomes, resulting in diversity between closely related plant species. The CMS-associated recombinant locus in *Petunia* contains three genes: *pcf* (a fusion of portions of *atp9*, *coxII*, and an *urf*), *nad3*, and *rps12*. Light and scanning electron microscopy has revealed aberrant development at the time of meiosis in CMS lines in contrast to normal fertile and CMS-restored lines containing a single dominant nuclear restorer (*Rf*) gene. Respiratory studies with electrodes that measure ubiquinone pool reduction and oxygen uptake indicate a difference in partitioning of electron transport between the cytochrome oxidase and alternative oxidase pathways. A *pcf*-encoded 25 kD protein is found in mitochondria of CMS lines but is much reduced in abundance in *Rf*-containing fertile lines. Immunological and N-terminal analysis indicates that this protein is processed from a larger precursor. The 25 kD protein was found in both the membrane and soluble fractions of mitochondria from a CMS line. Two different promoters (35S and TA29) and two different transit peptides (*Nicotiana* B-ATPase and *Neurospora* ATP9) have been used to target PCF protein to mitochondria of transgenic *Petunia* and tobacco plants. Expression of the chimeric genes and phenotypes of transgenic plants will be described.





Unifying Cellular Mechanisms

D 029 CYTOGENETICS OF MALE MEIOSIS IN MAIZE, W. Zacheus Cande[1], R.Kelly. Dawe[1], John.W. Sedat[2], and David A. Agard[2] [1]Department of Molecular and Cell Biology, 341 LSA, University of California, Berkeley, CA 94720 and [2]Department of Biochemistry and Biophysics., University of California, San Francisco CA 94143 and The Howard Hughes Medical Institute.

Microsporogenesis in maize procedes through a well-defined developmental sequence. The superb cytology of the meiocyte and the ability to generate mutants that affect meiosis and subsequent pollen development makes this an ideal system for studying the changes in chromosomal and cytoskeletal structure and function that occur during meiotic prophase and meiosis I and II. Examination of several existing male sterile mutants suggests that abnormal meiosis can be correlated with disruption of the cytoskeleon. For example, dv contains divergent spindle poles, ms28 has microtubule arrays with altered microtubule dynamics that disrupts the transition from meiosis I to meiosis II, and the ms43 spindle falls apart prematurely in anaphase I. The mutant ameiotic is a recessive gene that replaces meiosis I with a mitotic division. All identifiable cellular features are typical of a somatic division and surprisingly a pre-prophase band of microtubules (PBB) predicts the future division plane. Although meiotic division planes are tightly controlled and predictable, a PPB has never been observed in wild type cells.

We are using three dimensional imaging technology to study chromosome behavior in living and fixed material during meiotic prophase . For studies of fixed cells, nuclei are fixed with 4% paraformaldehyde in a buffer optimized for maintaining chromatin structure and immobilized without squashing. Using a standard light microscope, images of DAPI-stained chromosomes are collected on a computer using a cooled charge-coupled device (CCD). Nuclei are optically sectioned at 0.2 micron intervals with an automated stage. A collection of images representing an entire nucleus are then submitted to mathematical deconvolution to remove the out of focus information. The deconvolved data are viewed on screen as rotating, three dimensional images. Knobs (large heterochromatic regions) are visible in premeiotic interphase, providing the relative positions of individual loci prior to synapsis. Throughout early prophase, chromosome knobs lie at the nuclear envelope. Individual chromosomes have been followed and computationally straightened from both mitotic and meiotic prophase nuclei. Homologous pachytene chromosomes twist about each other in patterns that appear to be consistent from cell to cell. Optical cross sections are accurate enough to resolve single chromosome strands. As a result, two stages of pachytene have been identified: one with the sister chromatids tightly oppressed, and one with all four strands visible. Synaptic mutants and haploid plants have been used to analyze how the sister chromatids are held together during meiosis I.

Late Abstracts

PHENOTYPIC PLASTICITY : ALTERNATIVE FATES OF MERISTEMS, Pamela K. Diggle, Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, CO 80309-0334.

The current resurgence of interest in the relationship between development and evolution has originated among systematists and other biologists studying evolution at the 'macro' scale. Generally, these biologists are working above the species level and are interested in the origin and evolutionary diversification of clades of organisms. One result of this research is the detection of patterns of relationship, and development has been successfully invoked as one of the processes that might explain the observed patterns. Much of our theory of evolutionary process, however, comes from the fields of microevolution such as population biology or population genetics. Development has yet to be incorporated into these fields of evolutionary biology. Is it possible to integrate our understanding of developmental mechanisms into theories generated in the microevolutionary realm? One approach to integration of development and population biology involves the study of variation. Basic to theories of population biology is the study of variation, the amount and pattern of variation and the fate of variants in populations. A basic assumption is that mutation (the ultimate source of genetic variation) is random and that this translates into random phenotypic variation. It is development, however, that translates genetic variation into phenotypic variation. Thus, developmental mechanisms may influence or bias the types of variation possible within populations. Incorporation of developmental models into microevolutionary thought may expand the ability of population biologist to understand variation, which is believed to be the raw material of evolution. Two model systems for the study of development and population level variation are discussed: *Solanum hirtum*, an andromonoecious species producing both hermaphrodite and staminate flowers within each inflorescence, and *Polygonum viviparum* a species that produces both sexual and a sexual propagules within each inflorescence. The two types of structures in each species develop from morphologically indistinguishable primordia, and the ratio of these structures varies among individuals in natural populations. What determines the developmental fate of these reproductive meristems (and thus, what is the explanation for this variation among individuals)? Many theories concerning the evolution of andromonoecy suggest that the ratio of flower types should be plastic and vary with resource availability. This hypothesis has been confirmed for *S. hirtum*, however, the limits to this plasticity are set, in part, by the developmental mechanisms involved in floral sex determination. In *P. viviparum*, in contrast, the limits to phenotypic plasticity are determined by the process of preformation. These two systems will be discussed as examples of the use of developmental systems for the investigation of evolutionary theories, and of the way in which properties of development may set limits on the types of variation possible in natural populations.

PATTERNS OF FLORAL ORGAN INITIATION: NUMBER, SITE, AND SEQUENCE, Shirley C. Tucker, Department of Botany, Louisiana State University, Baton Rouge.

Initiation of organs by a floral apical meristem includes at least five components: vertical sequence among different types of organs, order among like organs, determination of number placement or site determination, and time sequence. This talk will deal with patterns of divergence in the last three of these components.

The number of organs in a flower is usually limited by constraints. By what patterns of ontogenetic divergence can organ number be altered? Changes can either be proliferations or reductions. Proliferation can occur by increasing meristem duration, by increasing meristem surface area, by ring meristems, common primordia, organ subdivision (dedoublement), obdiplostemony, or by homeotic conversion of other organs. Increase in meristem size can increase merosity throughout the flower, or in number of a particular organ type, depending on the timing of the size increase. Increasing meristem surface includes both centripetal and centrifugal amplification. Dedoublement, occurs in floral organogenesis among Malvaceae, and in Anemopsis (Saururaceae). Homeotic conversion can result from delay in commitment of an organ, as in stamens becoming petaloid in Neptunia (a Mimosoid legume), or petals becoming stamens in the caesalpinioid legume Saraca. Stamen proliferation may accompany suppression of gynoecia or heteromorphy in an inflorescence, common in mimosoid legumes.

Reduction in organ number can occur by reduction of meristem surface, by attenuation of meristem duration, by failure of some organs to be initiated, or by suppression of organs already initiated. Such reduction commonly is combined with site changes, or changes in timing. One common consequence of reduction or suppression is unisexuality.

Site changes of organs are uncommon in most flowers. They can occur by reduction of organ number, by dedoublement, or as a consequence of disruptive changes such as the ring meristem found in legumes Ateleia and Swartzia. Some apparent site changes are due to fusion of a pair of organs, as in the shift from five to four sepals in Saraca. A major discontinuity in organ position distinguishes subfamilies of legumes. The median sagittal sepal is adaxial in Mimosoideae, abaxial in Papilionoideae and most Caesalpinioideae. In each pattern, all subsequent organs in the flower are also shifted. A few caesalpinioid taxa show the mimosoid pattern. A site shift in the flower evidently occurred at the time of diversification of subfamilies from an ur-caesalpinioid type.

Changes in organ sequences are also uncommon, co-occurring with disruptions such as missing of organs, disruption of organ number and site changes, or teratologies. Some sequence changes have become established such as unidirectionality in each whorl (in papilionoid legume flowers), and overlapping initiation between adjacent whorls, occurring in Pisum and some other specialized papilionoid legumes.

These distinct components of organ initiation (level, number, site, and sequence) need to be teased apart for separate consideration before attempts to examine the causative bases of organ initiation.

Floral Meristems and Flowers

D 100 THE HOMEOTIC GENE *fbp2* REGULATES FLORAL ORGANOGENESIS IN PETUNIA AND ENCODES A NEW CLASS OF MADS BOX PROTEINS

Gerco C. Angenent, Marco Busscher, John Franken, Lucia Colombo and Arjen J. van Tunen
Centre for Plant Breeding and Reproduction research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands.

We have isolated and characterized two flower specific MADS box genes from petunia, designated *floral binding protein*(*fbp*) genes 1 and 2. The *fbp1* gene is exclusively expressed in petals and stamens and is activated at the moment that the floral organ primordia appear. In later stages of floral development, this gene is initiated again in anther tissues, suggesting an additional role of *fbp1* during anther maturation. The function of the *fbp1* gene was investigated using a reverse genetic method. The *fbp1* expression was reduced by sense co-suppression in transgenic petunia plants. The flowers of these plants show a partial reversion form petals into sepals and the development of stigma and style tissues on top of the anthers. These results demonstrate that *fbp1* represents a class B homeotic gene determining the formation of petals and stamens.

A similar co-suppression approach was used to investigate the function of *fbp2*, a gene which is specifically expressed in petals, stamens, and pistils. Inhibition of *fbp2* expression in transgenic plants resulted in highly aberrant flowers. These plants possessed a green corolla which was reduced in size. Furthermore, the stamen are replaced by green petaloid structures and new floral buds appear in the fourth whorl. These homeotic transformations were accompanied with a down-regulation of a cognate petunia homolog of the *agamous* (*ag*) gene which determines whorl three and four formation in Arabidopsis. In contrast to this, no effect on the steady state mRNA levels of the *fbp1* gene was observed. These results suggest that the *fbp2* gene belongs to a new class of floral homeotic genes and facilitates an important role in the regulation of genes required for the determination and differentiation of the three inner whorls.

D 101 MUTATIONS AFFECTING THE SEXUAL PHENOTYPE OF THE CERATOPTERIS RICHARDII GAMETOPHYTE. Jo Ann Banks, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47906

The homosporous fern *Ceratopteris richardii* has been developed as a model genetic system for studying many aspects of gametophyte development, including the process of sex determination. *Ceratopteris* gametophytes are either hermaphroditic or male. The determinant of sexual phenotype is the pheromone antheridiogen (or A_{CE}) which promotes male development. In the absence of A_{CE}, individual spores develop as hermaphrodites with archegonia, antheridia and a defined marginal meristem. The hermaphrodites produce and secrete A_{CE} into their surroundings yet are insensitive to its effect. In the presence of A_{CE}, individual spores develop as males lacking both archegonia and a defined meristem. Thus, in a population, gametophytes that germinate and develop rapidly become hermaphroditic and secrete A_{CE}, whereas those that germinate or develop more slowly become male under the influence of the pheromone. Abscisic acid (ABA) blocks the A_{CE} response in *Ceratopteris*.

In an effort to understand how A_{CE} governs the sex of the *Ceratopteris* gametophyte, several classes of mutations affecting the normal response to A_{CE} and ABA have been isolated. The first class of mutations, designated *her* (for hermaphroditic) are hermaphroditic even in the presence of A_{CE}. These mutations most likely affect the ability of the gametophyte to recognize or transduce the A_{CE} signal to promote male development and suppress hermaphroditic development. The second class of mutations, designated *fem* (for feminine) produce archegonia, a meristem and no antheridia. The third class of mutations, designated *mal* (for male), produce only antheridia and no meristem or archegonia in the absence of A_{CE}. The fourth class of mutations, designated *jan* (for Janus, the Roman god with two faces) initially develops as a male in the presence of A_{CE}, but quickly switches to the hermaphroditic form in the continued presence of A_{CE}. The final class of mutations, designated *abr* (for ABA resistant) are hermaphroditic at concentrations of ABA that, in the wild type, suppress antheridia formation in the hermaphrodite. By studying the interactions among these genes and suppressors of them, a regulatory pathway of gene control either activated or inactivated by A_{CE} can be deduced.

D 102 ESTABLISHMENT OF POLARITY IN MICROSPORES OF ORCHIDS, Roy C. Brown and Betty E. Lemmon, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504-2451

The first pollen mitosis in angiosperms is highly asymmetrical and results in a small generative cell and a large vegetative cell. Polarization of the microspore in preparation for this critical division includes migration of the nucleus to a position adjacent to the plasma membrane. Movement of the nucleus is microtubule dependent. In the moth orchid *Phalaenopsis*, an extensive generative pole microtubule system (GPMS) forms a polar structure at the distal surface and marks the path of nuclear migration. In *Tradescantia*, nuclear migration is reported to occur in the absence of a specialized microtubule system. A comparative study of three additional orchids representing different subfamilies of Orchidaceae has shown that a GPMS is present in *Habenaria*, but absent in *Spiranthes* and *Cypripedium*. Microspores of both *Habenaria* and *Palaenopsis* remain in exineless tetrads. Pollen mitosis in *Cypripedium*, which produces monads enclosed by exine, is most like *Tradescantia*. These data suggest that the GPMS may have evolved in association with the aggregation of pollen into polyads in specialized massulate and polliniate orchids. Variation in specific structures and processes involved in unequal pollen mitosis suggests that this process might provide a model for study of polarity in development of higher plants.

D 103 IDENTIFICATION OF REGULATORY GENES INVOLVED IN PLANT DEVELOPMENT, Jan Dockx, N. Quaedvlieg, P. Weisbeek and S. Smeekens, Department of Molecular Cell Biology, University of Utrecht, The Netherlands

Light plays a critical role in the plants life cycle. It is essential for normal plant growth and development, not only as a source of energy but also as a stimulus that regulates numerous developmental and metabolic processes. Studies of several light-regulated genes from different species demonstrate that DNA elements responsible for light-responsive expression are usually located within 5' upstream sequences. An increase in the transcript levels of these genes occurs in response to light. The best known photoreceptor is phytochrome and it has been shown that this photoreceptor can mediate gene expression. Such observations suggest that many developmental processes in plants are controlled by interaction of regulatory proteins with specific DNA sequences.
Transcription factors can be divided into classes on the basis of their mode of interaction with the target promoter sequence. Different protein domains responsible for DNA recognition have been identified (e.g. zinc finger, helix-turn-helix, homeobox, leucine zipper etc.). We are interested in genes which control light-dependent plant development. Our hypothesis is that in many cases these genes will be transcription factors. More precisely we want to work out the chain of events by which light, through photoreceptors, gives signals to these developmental processes. The strategy we follow to isolate transcription factor genes involved in these processes is described as follows. A transcription factors library has been generated by screening a genomic lambda zap library with probes that encode DNA-binding motifs identified in plants, yeast, and animals.
We are now isolating transcription factor genes which are differentially expressed during light-dependent development.

D 104 *AGL1* AND *AGL2*, TWO *AGAMOUS*-RELATED MADS-BOX GENES, ARE DIFFERENTIALLY EXPRESSED DURING FLOWER DEVELOPMENT, Catherine A. Flanagan and Hong Ma, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724-2212

Several floral homeotic genes, such as *Agamous* and *AP3* from *Arabidopsis thaliana* and *DefA* from *Antirrhinum majus*, have been shown to be members of the MADS-box gene family, which includes the known transcription factors MCM1, a yeast protein involved in mating-type control, and SRF, the mammalian serum response factor. The MADS-box is a conserved region in *MCM1* and SRF shown to be within the DNA binding and dimerization domain. Thus, the floral homeotic MADS-box genes are proposed to be transcription factors involved in regulating flower development. To determine if other MADS-box genes might also be involved, several MADS-box genes were cloned from *Arabidopsis thaliana* by their homology to *Agamous* and named *AGL* genes, for *Agamous*-Like. We are characterizing two of these genes, *AGL1* and *AGL2*, which are known to be preferentially expressed in flowers. To determine what role these genes might have in flower development, we have analyzed, using gene-specific ribo-probes and RNA *in situ* hybridization, the specific expression patterns of *AGL1* and *AGL2* within the flower. Detailed results will be presented showing that *AGL1* and *AGL2* are differentially expressed, both spatially and temporally, during *Arabidopsis thaliana* flower development.

C.A.F. is an American Cancer Society Postdoctoral Fellow

D 105 EXPRESSION OF GENES FOR CAROTENOID BIOSYNTHESIS DURING TOMATO FLOWER DEVELOPMENT

Giovanni Giuliano[1], Pablo Scolnik[2], Glenn Bartley[2] and Benedetto Aracri[1]

1ENEA, PO BOX 2400, Roma 00100 AD, Italy and DuPont Experimental Station, PO Box 80402, Wilmington, DE 19880-0402, USA

Carotenoids are terpenoid pigments that are accumulated in leaves, flowers and fruits of plants. In many flowers they colour anthers and petals and may serve as insect attractants for pollination. We have cloned and characterized a gene, *pds*, encoding phytoene desaturase, a key enzyme in carotenoid biosynthesis. Using a sensitive RT-PCR assay, we studied expression of *pds* and *psy*, a second gene involved in the same biosynthetic pathway. Both genes are very highly expressed in flowers. For *pds*, flower tissue is the most highly expressing tissue in the plant, while for *psy* expression has been found to be higher in ripening fruits. Expression is higher in fully opened than in unopened flowers and in organs of the second and third whorl (petals, anthers) over sepals and ovaries. Roots contain very low, albeit detectable levels of both transcripts.Thus, a second class of genes controlling pigment biosynthesis, besides those for flavonoid biosynthesis, show high levels of expression in flowers.

D 106 OVEREXPRESSION OF THE HOMEOTIC FLORAL GENE *GREEN PETAL* IN *PETUNIA* RESULTS IN THE HOMEOTIC CONVERSION OF SEPAL INTO PETAL

Ursula Halfter, Nazeem Ali, Ling Ren and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021

Proper flower development in higher plants requires the correct expression of a set of homeotic genes in the developing floral organs. A model based on studies in *Arabidopsis* and *Antirrhinum* has been proposed which suggests that the identity of floral organs is specified by the combinatorial expression of four homeotic genes (Meyerowitz et al., 1991; Carpenter and Coen, 1990). Petal development in *Antirrhinum*, e.g., requires the expression of the homeotic genes *deficiens* and *globosa* and in addition the presence of *ovulata* while stamen identity is determined by the additional expression of *plena* instead of *ovulata*. The identity of sepals however is determined by the sole expression of *ovulata* and by the absence of *deficiens / globosa* expression.

We have analyzed flower development in *Petunia hybridia* by overexpression of the *green petal* gene, the *deficiens* homologue from *Petunia*. The ectopic expression of this homeotic flower gene results in a partial conversion of sepals into petals. Data on the molecular characterization of the *green petal* gene, its expression pattern and interaction with other homeotic *Petunia* genes will be presented. We will discuss the differences of *Petunia* flower development in comparison to the models in *Arabidopsis* and *Antirrhinum*.

Carpenter and Coen (1990) Genes Dev. **4**, 1483 - 1493.

Meyerowitz et al. (1991) Development Suppl. **1**, 157 - 168.

D 107 THE *FRUITLESS* GENE REGULATES OVULE MORPHOGENESIS IN *ARABIDOPSIS THALIANA*, George W. Haughn[1], Zora Modrusan[1], Leonore Reiser[2], Robert Fischer[2] and Kenneth Feldmann[3], [1]Biology Dept, Univ. of Saskatchewan, Saskatoon, SK, S7N OWO, Canada, [2]Dept of Plant Biology, Univ. of California, Berkeley, CA, USA, 94720, [3]Dept. of Plant Sciences, The Univ. of Arizona, Tuscon, AZ 85721, USA.

We are analyzing *Arabidopsis thaliana* mutants defective in ovule morphology in an attempt to determine the mechanisms controlling ovule development. Female sterile mutants were isolated from a population of T-DNA transformed *Arabidopsis* plants. Two of these mutants, Fruitless (Fts) and Female Sterile (Fms), were found to have similar abnormal ovule morphologies. Genetic analysis indicated that both Fts and Fms phenotypes are due to recessive alleles of the same nuclear gene we tentatively designate as *FTS*.

The morphology and ontogeny of Fts and Fms ovules were examined using SEM and light microscopy. Ovule development deviates from that of the wild type at the time when the integuments are initiated. A single irregular integument forms that does not enclose the nucellus. Many of these deformed Fts ovules degenerate as the flower senesces. However the outer integuments of some ovules develop into distinctive carpel-like structures with cell types characteristic of ovary, style and stigma. Thus the *fts* mutant shows homeotic transformation of integuments to carpels.

Alleles of some whorl-identity genes, including *AP2* result in the formation of morphologically normal ovules on organs in the first three floral whorls. To test if *FTS* is required for development of ovules regardless of their location in the flower, fts/ap2-6 double mutants were constructed. Preliminary results suggest that the double mutant is unable to form morphologically normal ovules in any whorl.

The possibility that the mutant *fts* alleles are T-DNA tagged was investigated. Both Fts and Fms mutant phenotypes cosegregated with the T-DNA. Experiments to clone the *FTS* gene using the T-DNA as a marker are currently underway.

D 108 MOLECULAR STUDIES OF FLORAL MORPHOLOGICAL EVOLUTION, Hershkovitz, Mark A., and Elizabeth A. Zimmer, Laboratory of Molecular Systematics, MSC, Smithsonian Institution, Washington DC 20560.

We are studying phylogeny and floral/inflorescence evolution in Calandrinia s.str. (Portulacaceae), a temperate western American genus. Calandrinia includes rosettiform perennial herbs with solitary, axillary flowers (sect. Acaules; ca. 8 spp.), rosettiform annuals with axillary, branches bearing axillary, solitary flowers (sect. Calandrinia; ca. 5 spp.), and one rosettiform annual sp. with leafy, diffusely branched inflorescences and much reduced flowers (sect. Monocosmia). The morphological distinctions between sects. Calandrinia and Monocosmia parallel those between, respectively, wild type and leafy mutant Arabidopsis thaliana. We are using rDNA sequence data to corroborate morphological cladistic analysis indicating that sects. Acaules and Calandrinia are successively basal to sect. Monocosmia, as well as to address broader questions of Portulacaceae phylogeny. We also seek to isolate, sequence, and developmentally analyze Calandrinia orthologs of Arabidopsis floral/inflorescence homeotic/heterochronic genes, especially leafy and apetala-2, which are required in succession for determination of the Arabidopsis floral meristem and biseriate perianth, respectively. Our analysis of leafy in Calandrinia should reveal whether structural/regulatory modifications of this gene governed the evolution of sect. Monocosmia, and our analysis of apetala-2 may test the hypothesis that the biseriate perianth of Portulacaceae represents, phyletically, bracts and sepals. Through sound phylogenetic analysis, we aim to clarify the evolutionary significance of known floral homeotic/heterochronic genes, establish the taxonomic range of applicability of current molecular models of floral development, and reveal underlying genomic bases of morphological evolution.

D 109 CLONAL ANALYSIS OF THE HOMEOTIC PISTILLATA GENE IN ARABIDOPSIS.

Vivian Irish and Karim Bouhidel, Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511

Recessive mutations in the *pistillata* (*pi*) gene of Arabidopsis result in a dramatic transformation of the flower. Plants mutant for *pi* display a homeotic conversion of petals into sepal-like organs, while stamens are converted into carpelloid structures. We are examining the cellular basis of this transformation by constructing plants that are genetically mosaic for the *pi* mutation. Seed heterozygous for *pi* and a closely linked albino mutation are irradiated, and plants are recovered that contain albino sectors which can be scored in the internal L2 and L3 cell layers. Numerous sectors have been recovered that include part of an inflorescence, part of a flower, or a single floral organ. L3 sectors in the flower have not been recovered, suggesting that primarily L1 and L2 derived cells contribute to the flower. The sectored tissues are also mutant for *pi*, and such mosaics can be used to assess whether the *pi* gene functions in a cell-autonomous or non-autonomous manner. Preliminary examination of sectored flower buds indicates that the *pi* gene behaves in a non-autonomous fashion. This result suggests that the *pi* gene is necessary for cell-cell interactions required to establish the normal pattern of floral organ development. We are currently confirming this observation, and will discuss these results in the context of homeotic gene function.

D 110 **GENE TRANSFER TO FLOWER MERISTEM CELLS BY PARTICLE BOMBARDMENT.** Nathalie Leduc, Victor A. Iglesias, Roland Bilang, Shibo Zhang, Andreas Gisel, Christof Sautter and Ingo Potrykus, Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland.

Gene transfer to cells of immature flower meristems could lead to transgenic gametes. Fertilisation would then allow to recover transformed plants rapidly and with a limited tissue culture phase. In comparison to vegetative meristems, meristems closer to meiosis should have the advantage of minimizing possible sorting out of transgenic cells during plant development. We will report here that the microtargeting technique (Sautter et al. Biotechnology 9: 1080 [1991]) represents an efficient system for DNA delivery into flower meristem cells.

We have used wheat since it is an important crop, and it presents an inflorescence with numerous spikelets. Under long day culture conditions, we observe meristem transition towards flowering already 11 days after sowing. Flower meristems showing the double ridge stage to the differentiation of the anther primordia have been used for particle bombardment. We have shown that it is possible to target precisely to each individual spikelet primordium and to transfer particles and DNA into the L1 and L2 cell layers. Transient expressions of different reporter genes were observed with high frequencies. Several factors affecting efficient microtargeting will be presented. Advance in the culture of immature wheat flower meristems will be reported.

D 111 FUNCTIONAL ANALYSIS OF THE *ARABIDOPSIS* FLORAL HOMEOTIC GENE *AGAMOUS* IN TRANSGENIC PLANTS

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Genetic studies suggest that the *Arabidopsis* foral homeotic gene *AGAMOUS(AG)* is involved in several aspects of floral development: 1) *AG* functionally antagonizes *AP2*, another floral homeotic gene; 2) *AG* is required for normal identity of the third and fourth whorl organs (stamens and carpels, respectively); 3) *AG* is required for the formation of a determinant floral meristem. To investigate the *AG* functions at the molecular level, we have generated transgenic plants which carry fusions of the 35S promoter to the *AG* cDNA in either the sense or the antisense orientation. Here we present the characterization of these transgenic plants. Two types of floral phenotypes were observed in the transgenic plants carrying the sense constructs, *ap2*-like and *ag*-like. We reasoned that the *ap2*-like plants express the *AG* transgene ectopically, resulting in the inhibition of the *AP2* gene function. The elevated *AG* expression in both flowers and leaves supports this conclusion. The *ag*-like plants are most likely due to cosuppression of the *AG* transgene and the endogenous *AG*. The transgenic plants carrying the *AG* antisense construct also produce *ag*-like flowers, suggesting that the transgene inhibits the endogenous *AG* gene function. Both the *ap2*-like and the *ag*-like transgenic plants exhibit variable floral phenotypes, probably due to different levels of the transgene expression.

D 112 REGULATORY NETWORKS CONTROLLING PIGMENTATION IN MAIZE AND PETUNIA ARE CONSERVED

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Flavonoid flower pigments accumulate in specific cell-types of most floral organs making them excellent markers for the genetic programs controlling floral organ differentiation. Several genes encoding enzymes from the flavonoid pathway (structural genes) have been cloned and characterised in detail. Here we present an analysis of regulatory genes controlling expression of these structural genes.

By Northern blot analyses we show that four genes (*an1*, *an2*, *an4* and *an11*) control the mRNA levels of multiple structural genes from the pathway. Run on transcription assays show that the genes *an1*, *an2* and *an11* control expression of their target genes at the level of transcription initiation, which is in line with the observation that a GUS gene driven by the dihydroflavonol reductase promoter (dfrA-GUS) is transiently expressed in wild type corolla's after particle bombardment, but not in corolla's from $an1^-$, $an2^-$ or $an11^-$ mutants. These data show that the gene products of *an1*, *an2* and *an11* interact with the promoter of the dfrA gene to activate transcription.

When the *dfrA*-GUS fusion gene plus fusion genes expressing the maize regulatory factors *Lc* (a member of the *R*-family) and *C1* are co-introduced in *an1*, $an2^-$ or $an11^-$ corolla's, reactivation of the *dfrA* promoter is observed in all three cases. This indicates that *an1*, *an2* and *an11* encode or control factors similar to maize *Lc* and *C1*. When the *Lc* gene alone is co-introduced with *dfrA*-GUS, reactivation is observed in $an2^-$ corolla's, suggesting that *an2* encodes a transcription factor functionally homologous to the maize *R* genes. This conclusion is confirmed by the isolation via PCR of an *Lc* homologous sequence from Petunia whose mRNA is down regulated in $an2^-$ corolla's.

The *an1* and *an11* genes control pigmentation in all plant tissues (flower corolla and tube, anthers, seedcoat). The *an2* gene controls pigmentation of the flower corolla only, whereas *an4* appears to be active in the anthers mainly. Bombardment of $an4^-$ anthers with *Lc* also leads to complementation. This indicates that the genes *an2* and *an4* are members of the same family of regulatory genes, but differ in their expression pattern. This is remarkably similar to the R gene family from maize and further supports the view that these regulatory genes have a common ancestor.In maize, R genes control expression of genes acting early in the pathway (*chs*) as well as those acting late (*dfr*, *ufgt*), whereas the petunia *an2* gene controls only late acting genes (*dfr*, *ufgt*, *rt*) and not early genes such us *chiA* and *f3h*.

An evolutionary model will be presented, which explains the similarities and differences in the genetic control of the flavonoid pathway as they are found today in maize and petunia.

D 113 *APETALA2*: CHARACTERIZATION OF A NOVEL PLANT HOMEOTIC REGULATORY GENE FROM ARABIDOPSIS

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The homeotic regulatory gene *APETALA2 (AP2)* is a central player in the genetic control of flower development in Arabidopsis. The identity and development of all four types of floral organs can be dramatically affected in plants carrying an *ap2* mutation. Moreover, *AP2* has been implicated in the regulation of at least one other floral homeotic regulatory gene - *AGAMOUS*. As a first step towards understanding the cellular and molecular function of *AP2* we isolated this gene from Arabidopsis by T-DNA insertional mutagenesis. Molecular analysis of *AP2* gene structure suggests that this gene encodes a new class of plant regulatory protein. AP2-like genes have now been cloned from a variety of flowering plants. Comparison of these and wild-type and mutant Arabidopsis *ap2* gene sequences suggests that a 64 amino acid repeated motif, the AP2-domain, is necessary for normal *AP2* activity. Molecular analysis of *AP2* gene expression during flower development revealed the presence of AP2 RNA transcripts in immature floral buds, in all four types of floral organ primordia and in the inflorescence apex. Moreover, AP2 polysomal poly(A+) mRNA is detectable in nonfloral tissues including vegetative leaves and inflorescence stems. We are using both genetic and molecular strategies to determine whether *AP2* is required for normal vegetative growth and development.

D 114 CHARACTERIZATION OF TISSUE-SPECIFICALLY EXPRESSED NEW ZINC-FINGER PROTEINS FROM PETUNIA, Hiroshi Takatsuji, Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305, Japan

We have previously reported the characterization of the first Cys_2/His_2-type zinc-finger protein in Petunia[1]. This protein (EPF1) binds to a specific DNA sequence (EP1) in 5' upstream region of 5-enolpyruvylshikimate-3-phosphate synthase gene (EPSPS) that is specifically expressed in petal in mature plants and in root cortex, trichome and stem-meristematic region in seedlings. Some evidences suggested that EPF1 is responsible for the tissue- and developmental stage-specific activation of EPSPS. However, in vitro binding study implied the presence of additional factors that interact with the EPSPS 5' upstream region.

To clone the genes encoding these additional factors, I repeated Southwestern screening of a cDNA library from Petunia petal using EP1-tetramer as a probe. I obtained two cDNA clones (EPF2-5a, -5b) that show similar binding activity to that of EPF1. Furthermore, I isolated two additional homologous genes (EPF2-4,EPF2-7) by screening a Petunia genomic library using EPF2-5a coding sequence as a probe. The deduced amino acid sequences show that all of the four encoded proteins contain two repeats of zinc-finger motifs and a cluster of basic amino acids that are similar to those of EPF1. However, sequences other than these regions were completely different from that of EPF1. Northern analysis revealed that the expression patterns of EPF2-5s were quite similar to that of EPF1, implying that they interact with the EPSPS 5' upstream region cooperatively. Expression of EPF2-7 was stronger in root and stem than in petal, and that of EPF2-4 was undetectable. To reveal the possible roles of these proteins in flower development, over-expression and antisense-expression of the genes in transgenic Petunia are under way.

1) Takatsuji *et al.* (1992) EMBO J., 11, 241-249.

D 115 THE CONIFER PICEA ABIES HAS A HOMOLOGUE TO THE ARABIDOPSIS AGAMOUSLIKE GENES

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Our interest is in the development of the reproductive organs of conifers, the needlebearing gymnosperms. Most conifers are monoecious, with separate male and female organs. the pollen releasing male cone and the seed forming female cone. To investigate whether the system for control of the development of the reproductive structures in the conifers includes components that are similar to those of the hermaphroditic angiosperms we have used sequence from homeotic angiosperm genes to clone corresponding genes from Picea abies.

Some of the cloned angiosperm floral homeotic genes, AG, DEF A and AP3 code for proteins which share a well conserved 56 aa long MADS box. We used a degenerate oligonucleotide made to match a conserved sequence of the MADS box to screen a cDNA library constructed from female buds of Picea abies. 40 clones were isolated and 5 clones sequenced so far all represent the same gene. These Picea abies clones have a deduced aa sequence which is highly similar to that of AGL2, a member of a gene family with homology to AG, in Arabidopsis. Within the MADS box the identity to AGL2 is 93% and the position of the box in the N-terminal of the protein is the same as in AGL2. The similarity to the MADS box of AG is lower, 80%, and the 56 aa that preceed the box in the AG protein are missing in the conifer protein as well as in the AGL2 protein. The aa identity in the C-terminal of the protein is 56% as compared to AGL2.

D 116 TRANSGENIC PETUNIA OVEREXPRESSING A MADS BOX GENE PHENOCOPY THE PETUNIA *blind* MUTANT, Suguru Tsuchimoto, Sander van der Krol, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021

We have isolated and characterized a MADS box gene from Petunia which has striking homology with the *agamous* gene of Arabidopsis. This gene, designated *Pag1* , is expressed in stamens and carpels and not in sepals and petals of the wild type Petunia plants. In contrast to the wild type, we found that *Pag1* is expressed in all whorls of the Petunia *blind* mutant flowers, which have a partial conversion of limbs into anthers. We generated transgenic Petunia plants which constitutively express *Pag1* under the control of the CaMV 35S promoter. We found that wild type plants with ectopic expression of *Pag1* phenocopy the *blind* mutant. Our data suggests that *Pag1* is one of the floral homeotic genes in Petunia, and that it is involved in anther development.

D 117 CHARACTERISATION OF THE TEMPERATURE - SENSITIVE DEFICIENS MUTANT *DEFA-101*

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In Antirrhinum majus, floral organ identity is under control of homeotic MADS-box genes, like *DEFICIENS (DEFA), GLOBOSA* and *PLENA*. A temperature-sensitive (ts) *DEFA* mutant *(defA-101)* displays at the permissive temperature an almost wild-type like morphology. At the non-permissive temperature, where *DEFA* transcription is strongly reduced, petals are transformed into sepals and it resembles the morphology of a null mutant of *DEFA*. The ts-mutant is due to a three base-pair deletion in the K-box of the transcription factor and causes loss of a lysine in the protein.

Temperature-shift experiments were conducted with this mutant to define sensitive floral developmental stages during which *DEFA* is necessary to establish the identity of the second and third whorl organs, where *DEFA* is mainly expressed. As a prerequisite, wild-type and mutant developmental stages were defined and compaired using scanning electron microscopy.

Temperature-shifts from the non-permisssive temperature to the permissive temperature showed that second whorl organs can be restored even at late floral developmental stages. To restore the identity of the third whorl organs, *DEFA* has to be expressed at an very early floral developmental stage, where no floral organs are yet initiated. Quite surprisingly, it was found that a low level *DEFA* expression in the third whorl is a prerequisite for organ initiation in the fourth whorl, although it is not necessary for carpel organogenesis. Therefore, *DEFA* seems to control meristematic functions necessary for the development of the fourth whorl.

Floral Organ Development, Evolution and Function; Inflorescence Meristems and Cell Fate Choice

D 200 **Flower bud formation in the 'non-flowering' *mortalis* mutant of tomato.** Keith D. Allen, Ian M Sussex. Department of Plant Biology, University of California, Berkeley, CA 94720.

The vegetative to floral transition is one of the most important events in the life history of a plant. Thus, it is not surprising that considerable effort has been devoted to identifying mutations that completely block this transition. Several mutants have been identified that block the transition from inflorescence development to floral development. These include the well characterized *Leafy* mutation in *Arabidopsis*, and *Floricaula* in *Antirrhinum*. A mutant that that has been reported to block the earlier step from vegetative to inflorescence growth is *mortalis* (*mts*) in *Lycopersicon esculentum*. Based on the original description of this single gene recessive mutation (Stubbe, H. (1963) Kulturpflanze 11:603-644.), *mts* homozygotes are bushy dwarf plants that do not form inflorescences even if grafted onto flowering wild type hosts. This suggested that *mts* might mark a gene required for the reception of the floral stimulus by the shoot apical meristem (SAM). Therefore, we undertook a detailed study of development in *mts* homozygotes. To our surprise, although flowering in *mts* is somewhat delayed (in terms of the number of nodes prior to the first inflorescence), complete flowers are formed, but then abort, typically before becoming macroscopic (although occasionally a *mts* inflorescence may be several millimeters in diameter). Normal development in tomato is sympodial, with the SAM converting to an inflorescence meristem, and the nearest axillary meristem becoming the new SAM. In *mts* homozygotes this transition fails. The SAM is converted to an inflorescence meristem, but then both the inflorescence meristem and the axillary meristem that would have become the new SAM abort, terminating growth of the shoot. The development of axillary buds is dramatically enhanced in *mts* homozygotes, giving rise to very bushy plants that tend to expand laterally rather than vertically.

D 201 A MODULAR ENSEMBLE OF *CIS*-ELEMENTS CONTROLS THE EXPRESSION OF A NON STORAGE SEED PROTEIN GENE

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Different cell types of developing seeds realize complex interconnected gene expression programs. Underlying regulatory processes comprise the tissue specific and development dependent control of the transcription rate due to the interactions between DNA *cis*-elements and transcription factors.

The focus of this communication is a gene designated *usp*, coding for a functionally unknown non-storage seed protein of 30 kD. Its message dominates the poly A+ RNA fraction in cotyledons of the broad bean *Vicia faba* var. minor cv. Fribo. The 30 kD protein is found as monomer or dimer and turns over rapidly.

In contrast to the genes coding for the typical 12 S storage legumin, which become increasingly active towards later developmental stages, the *usp* gene expression peaks during the midst of the developmental time course.

Using transgenic tobacco seeds as an expression system we here show that the *usp* gene promoter contains a complex set of *cis*-elements differently involved in quantitative and qualitative aspects of expression and that the sequence motif CATGCATG often found in promoters active in developing seeds exerts a down regulating effect opposite to what is known from legumin-type promoters.

D 202 FUNCTIONAL COMPLEMENTATION OF POLY(A)-BINDING PROTEIN (PABP) ACTIVITY IN YEAST BY *ARABIDOPSIS THALIANA* FLORAL-SPECIFIC cDNA : EFFECT ON POLY(A) TAIL LENGTH AND TRANSLATIONAL INITIATION, Dmitry A. Belostotsky and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, GA, 30606

Poly(A)-binding protein (PABP) is considered an essential component of eukaryotic cells; deletion of a PABP gene in yeast is lethal. PABP has been implicated in a number of important cellular processes, such as mRNA turnover and translational initiation. Although it is generally recognized that PABP performs its functions as a complex with poly(A)-tails of mRNAs, molecular details of its action remain unclear.

Recently we have cloned several PABP genes from *Arabidopsis thaliana* using degenerate oligonucleotides and PCR. We estimate that small genome of *Arabidopsis* contains as many as 20 nonidentical PABP genes. Coding sequences so far analyzed show very high level of diversity suggesting functional specialization. Moreover, one of the genes, PAB5, is flower-specific and developmentally controlled [1]. To gain more insight into its mode of action, we achieved functional complementation of a yeast PABP mutation by PAB5 cDNA placed under the control of GAL1-10 promoter. This allows the mode of action of PAB5 to be studied in heterologous system. Growth of the resulting strain carrying PAB5 cDNA as the only source of PABP activity can be inhibited by GAL1-10 promoter inactivation which leads to the decreased PABP expression. Results of PABP depletion on polysomal profiles and poly(A)-tails lengths are being examined and will be discussed in regard with known activities of yeast PABP, a role in translational initiation and poly(A)-tail shortening.

1. Belostotsky and Meagher, PNAS, submitted

D 203 CELL ALLOCATION IN THE *Arabidopsis thaliana* MERISTEM, Mark Carrier and Ian Sussex, Department of Plant Biology, University of California, Berkeley,CA. 94720

The shoot apical meristem continuously generates new cells, and allocates a subset of these cells to organ generation. A fundamental question of meristem organization is how these processes are related, and how they are regulated. We are screening for mutants with abnormal patterns of organ generation. We have isolated and characterized several alleles of *clavata1*, which are altered both in their generation of organs and in meristem histology. We will use these mutants to ask questions about the relationship between meristem architecture and organ generation.

The vegetative and inflorescence meristems of *clavata1* are enlarged and produce more leaves and flowers than do their wild-type counterparts. The floral meristems of these plants are less obviously enlarged, but the mutant flowers contain more organs per whorl than wild-type flowers. The *clavata1* meristems also have severely altered histological zonation with an enlarged central zone which has displaced the flanking morphogenetic zone to a proximal location. *Clavata1* mutant plants also differ from wild type in their pattern of inflorescence organ system identity. Wild-type plants consistently generate all of their branches and subtending cauline leaves before they generate flowers, while on *clavata1* plants flowers and branches are often interspersed.

We are conducting comparative clonal analyses of wild-type and clavata1 mutant plants to answer specific questions about meristem function. These questions include: whether organ founder cell number is related to meristem size, whether cell number allocation is related to lateral organ identity, and how the allocation of cells is regulated in floral meristems. Fate maps of wild type and mutant dry seed and adult meristems are in progress.

D 204 TEOSINTE GLUME ARCHITECTURE (*TGA1*), A LOCUS DIFFERENTIATING MAIZE AND TEOSINTE, Jane Dorweiler[1], John Doebley[1], Adrian Stec[1], and Jerry Kermicle[2], [1]Plant Biology Department, University of Minnesota, St. Paul, MN 55108. [2]Laboratory of Genetics, University of Wisconsin, Madison, WI 53706.

The evolution of maize (*Zea mays* ssp. *mays*) from its probable progenitor teosinte (*Zea mays* spp. *parviglumis*) involved dramatic changes in inflorescence architecture. One of the features involved in this transformation was the architecture of the outer glume of the spikelets. Maize glume architecture (MGA) is represented by the glumes of the maize ear which are normally (1) relatively soft, (2) oriented perpendicular to the axis of the ear, i.e. reflexed, (3) relatively short, and (4) shovel-shaped. Teosinte glume architecture (TGA) is represented by the glumes of the teosinte inflorescence which are (1) highly indurated, (2) oriented parallel to the axis of the ear, i.e. upright, (3) relatively long, and (4) boat-shaped. We identified a factor on chromosome *4S* producing TGA, and transferred it from teosinte into maize inbred W22 by backcross breeding. Our analyses indicate that this factor behaves like a single Mendelian locus, which we have designated *TGA1*. *TGA1* is located approximately one cM proximal to the sugary locus (*su1*). Since *TGA1* affects several characteristics of the glume, it may represent a regulatory locus that controls the expression of several downstream loci involved in glume development. Differences in glume development between W22 and W22-TGA are not pronounced at the earliest stages of inflorescence development. Rather, the differences intensify gradually and are most dramatic in mature ears. The teosinte allele at *TGA1* behaves as a recessive in maize background; and preliminary data indicate that the maize allele behaves as a recessive in teosinte background. This suggests that recessiveness at this locus is a result of epistatic interactions, rather than a characteristic of the specific alleles. This locus significantly contributes to the difference between the female inflorescences of maize and teosinte, and represents a critical locus in the evolution of maize from teosinte.

D 205 CONTROL OF ORGAN FATE IN PRECOCIOUSLY-GERMINATING *BRASSICA NAPUS* EMBRYOS.

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Immature embryos of *Brassica napus* initiate apical growth when they are removed from the seed and placed on a basal medium lacking hormones. Young embryos produce a series of additional cotyledons at the shoot apex; older embryos produce a mixture of cotyledons, leaves, and chimeric organs with large sectors of leaf and cotyledon tissue; and the oldest embryos produce leaves. We have used probes for sequences characteristic of either embryo maturation or germination to analyze the overlap between these two developmental programs in different parts (cotyledons, axes, and epicotyls) of precociously-germinating embryos. Our analysis of mRNA accumulation patterns reveals that (1) at any one point in time, different parts of an embryo can be pursuing different developmental programs, and (2) immature embryos retain the ability to switch developmental programs (from embryo maturation to germination) in culture. The ability to make this switch varies not only with the stage at which the embryo is excised, but also with the particular part of the embryo being considered. We interprete our results as indicating that the sequential series of events involved in embryo maturation are inititated at different times in different parts of *Brassica napus* embryos. Since the ability to "switch" into germination is normally acquired during the course of this sequence, it is also achieved at different times in diferent embryo parts. In the shoot apex, it may also be achieved at different times in different populations of cells. We are currently using *in situ* hybridization techniques to extend our analysis to the cellular level; and, in particular, to examine the relationship between mRNA accumulation patterns and switches in organ identity at the shoot apex.

Supported by NSF DCB-8616198 and DCB-9105527.

D 206 **Characterization of a male flower gene from maize encoding a structural protein widely expressed in the spikelet**, Andrew J. Greenland, Philip J. Bell, Marie-Marthe Suner and Susan Y. Wright, ICI Seeds, Jealott's Hill Research Station, Bracknell, RG12 6EY, UK.

We have isolated a maize gene, *MFS18*, which is expressed specifically in developing male flowers throughout tassel growth up until mature pollen is produced in the anthers. *MFS18* mRNA accumulates in the glumes where it is particularly associated with the vascular bundle, and in anther walls, paleas and lemmas of mature florets. The *MFS18* ORF encodes a basic protein of 128 residues (isoelectric point = 9.54) with a predicted molecular weight 12.5 kd. MFS18 is rich in A, P, G and S residues and has similarities with other plant structural proteins. The protein is comprised of three distinct regions; a hydrophobic amino terminus which indicates that the protein may be secreted, a hydrophillic middle region and a non-polar region extending to the carboxy terminus. The latter contains the majority of the abundant residues which are arranged into 8 repeats of P G • *, where • can be P, S, G or F. In structure, but not in organisation of repeats, MFS18 is very similar to a G- and P-rich anther protein found in sunflower (Domon *et al.*, 1990) suggesting that these two proteins may be functionally related. We are currently using antisera raised against MFS18 to investigate the subcellular localisation of the protein.

Domon *et al.*, (1990) *Plant Mol. Biol.* **15**, 643-646.

D 207 **FLORAL EVOCATION IN ARABIDOPSIS: SIMULTANEOUS INDUCTION OF BOTH FLOWER AND INFLORESCENCE DIFFERENTIATION,** Frederick D. Hempel and Lewis J. Feldman, Dept of Plant Biology, University of California, Berkeley, CA 94720

Floral evocation occurs at the apex as a consequence of induction of the floral stimulus in the leaves. A necessary first step in analyzing evocation is to precisely characterize the morphogenetic changes which occur in the apices of florally induced plants. In this study, we have described the evocational morphogenesis of Arabidopsis thaliana (Landsberg *erecta* ecotype), a quantitative long-day plant, in inductive 16-hour daylengths. We found that all primordia initiated following photoperiodic induction became flowers and that the differentiation of paracladia (co-florescences) occurred *after* the initiation of 3-6 floral primordia. Paracladia were produced via the basipetal activation of buds in the axils of leaf primordia initiated *prior to* photoperiodic induction. We have shown that Arabidopsis inflorescences can be constructed completely from pre-existing, vegetative nodes which are recruited into the inflorescence following floral induction. Our results contradict models which suggest that "bract" leaves and their associated paracladia are initiated by a meristem which is in an intermediate developmental state between that of a rosette leaf producing meristem and a flower producing meristem. In our alternative model, no intermediate developmental state is required for the production of an Arabidopsis inflorescence. We suggest that the shoot apical meristem of an Arabidopsis moves directly from a vegetative state to a floral state; and that florally induced Arabidopsis plants construct inflorescences via the basipetal activation of buds in the axils of partially determined leaf primordia. Those activated buds subsequently differentiate into paracladia while their associated leaves are suppressed, becoming the so-called bract leaves of the inflorescence.

D 208 GENETIC ANALYSIS OF THE INITIATION OF FLOWERING IN *ARABIDOPSIS THALIANA*, Eva Huala and Ian M. Sussex, Department of Plant Biology, University of California, Berkeley, CA 94720.

Arabidopsis is a facultative long day plant in which the onset of flowering can be accelerated by long photoperiods or vernalization. The Landsberg ecotype flowers after 2-3 weeks under long (16 hr) photoperiods but under short (8 hr) photoperiods flowering is delayed until plants are 8-12 weeks old. One possible explanation for this flowering behavior is that two separate pathways for the initiation of flowering operate in Arabidopsis. One of these is triggered by a photoperiodic signal while another is activated in response to an age-dependent signal. Several mutants which may have defects in the photoperiodic pathway have previously been isolated. These include the late flowering mutants *fb* and *fg*, which flower after approximately the same number of vegetative nodes under long or short photoperiods[1]. We have observed that a previously isolated gibberellin responsive dwarf carrying a mutation at the *ga1* locus[2] flowers at the normal time when grown in long photoperiods but in contrast to wild type fails to flower for at least 6 months when maintained in short photoperiods. The *ga1* mutant has the phenotype expected for a mutant with a defect in the age-dependent flowering pathway.

To test whether the *ga1* and *fb* mutations define two separate pathways for the initiation of flowering, we have constructed double mutants between *ga1* and *fb*. Flowering of the *ga1 fb* double mutants grown in long days was significantly later than flowering of either *fb* or *ga1*, confirming that the GA1 and FB gene products act in two separate pathways. To further define these two pathways for the initiation of flowering we have constructed several other double mutant combinations including *ga1 fg*, *ga1 ft* and *ga1 fe*. Analysis of these double mutants is in progress. In addition, we are screening for new mutants with defects in flower initiation.

[1] M. Koornneef et al. (1991) Mol. Gen. Genet. **229**:57-66.

[2] M. Koornneef and J. H. van der Veen (1980) Theor. Appl. Genet. **58**:257-263.

D 209 **ANALYSIS OF A TAPETUM-SPECIFIC PROMOTER FROM MAIZE, Gary A. Huffman, Margit Ross, Joanie M. Phillips, and Lisa M. Marshall, Department of Biotechnology Research, Pioneer Hi-Bred Intl., Johnston, IA 50131**

The tapetum-specific regulation and expression of a cloned maize gene, designated SGB6, was studied. We have identified the start of transcription and subsequently fused the 5' flanking region to the β-glucuronidase (*uidA*) and luciferase (*luc*) marker genes. A transient assay for gene expression in maize anthers was developed, and the preferential expression of reporter genes in anthers at tetrad to uninucleate microspore stage was demonstrated. Deletion derivatives of the promoter were fused to the luciferase gene, and expression in appropriately staged anthers was analyzed. Successive reductions in expression were observed with deletions from the 5' end of at least three regions of the promoter. One of these regions included a 58-bp imperfect inverted repeat containing a 7-bp motif reported to enhance pollen specific expression of the LAT52 promoter from tomato ((Twell *et al.*, 1991, Genes & Development 5:496-507). Another region of the SGB6 promoter contained an imperfect (7/9bp) motif implicated in spatial (anther and pollen) expression but not temporal control of LAT52 and the petunia genes CHI-A and CHI-B (McCormick *et al.*, 1991, In Vitro Cell. Dev. Biol. 27P:15-20; van Tunen *et al.*, 1989, Plant Molec. Biol. 12:529-551; van Tunen *et al.*, 1990, Plant Cell 2:393-401).

D 210 ANALYSIS OF THE *SQUAMOSA* PROMOTER : AN APPROACH TO ISOLATE TRANSCRIPTION FACTORS REGULATING FLORAL TRANSITION

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SQUAMOSA (*SQUA*) is a homeotic gene, that regulates an early step in flower development (1). It codes for a MADS-box protein which is important to determine the identity of axillary meristems initiated on inflorescences. Lack of the gene product leads to indeterminate growth of these meristems: new inflorescences are formed instead of flowers. Occasionally a few, often malformed flowers are produced.

In the course of flower development SQUA, is one of the earliest expressed transcription factors so far known. Almost no insight into the molecular mechanisms directing steps preceeding inflorescence development has been gained. Analysing the transcriptional regulation of the *SQUA* gene, we hope to contribute to the elucidation of these processes.

The goal of the project is to isolate and characterize proteins, that bind to the *SQUA* promoter, thereby directly regulating its expression.

Two approaches are followed:

1. *in vivo* analysis of the *SQUA* promoter by promoter-reporter-gene constructs tested in transgenic plants (heterologous system),
2. *in vitro* studies utilizing promoter fragments and nuclear protein extracts in gel retardation assays. The binding factors will be isolated by screening an expression library with *SQUA* promoter elements as molecular probes or alternatively by purifying the proteins from nuclear extracts and subsequent microsequencing.

(1) Peter Huijser, Joachim Klein, Wolf-Ekkehard Lönnig, Hans Meijer, Heinz Saedler, Hans Sommer, 1992, EMBO J. Vol. 11, 1239-1249

D 211 THE ARABIDOPSIS fdh FUSION MUTANT: EVIDENCE FOR ALTERED CELL WALL POROSITY,

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Aerial organs of the Arabidopsis fdh mutant fuse[1] and support pollen growth[2]. In addition to these two carpel-like properties, fdh tissues are much more permeable than wildtype tissues as determined by susceptibility to fixatives and leaching of chlorophylls. This enhanced permeability may be due to (1) the absence of a cuticle on fdh epidermal surfaces or, (2) a difference in cell wall architecture and/or composition. Using cytochemical and microspectrophotometric techniques we have determined that the enhanced permeability of fdh tissues is due, at least in part, to differences in cell wall architecture and not to the absence of a cuticle. Our data show that the cell walls of fdh leaf epidermis contain nearly 2x as much free space as wildtype cells. Interestingly, our preliminary studies indicate that wildtype papillar cell walls also have a higher percent free space than leaf epidermal cells.

References

1. Lolle et al., 1992, Dev. Biol. 152, 383.
2. Lolle and Cheung, 1992, Dev. Biol., in press.

D 212 INFLORESCENCE DEVELOPMENT IN PEA: A COMPARISON OF ISOGENIC DETERMINATE (*det*) AND WILDTYPE PLANTS, Sonja L. Maki, Eugene L. Huang, and Susan R. Singer, Department of Biology, Carleton College, Northfield, MN 55057

Inflorescence architecture of *Pisum sativum* L. (pea) is altered by the *det* (determinate) mutation which limits the number of reproductive nodes. The terminal meristem senesces rapidly and forms a rudimentary stub rather than producing a terminal flower as implied by the gene symbol (Singer S.R. et al. 1990 Amer J. Bot 77:1330). Double mutants of *det* and *veg*, which blocks the formation of flower meristems, (Reid, J.B., Murfet, I.C. 1984 Ann. Bot. 53:369) reveal that *veg* is epistatic to *det*. This is consistent with the interpretation that *det* acts later in inflorescence development. Isogenic *det* and *Det* lines were developed to investigate the hypothesis that *det* is a heterochronic mutation affecting apex senescence in pea. Wildtype apices arrest with a viable meristem that will initiate further nodes if flowers and/or fruits are removed or if gibberellins are applied. Morphological characterization of deflowered/defruited plants revealed that terminal meristems eventually terminated in rudimentary stubs analogous to *det* stubs. This observation is consistent with the interpretation that flower/fruit controlled apex arrest is absent in *det* or obscured by rapid apex termination. The possibility that the missing steps in *det* inflorescence development are gibberellin regulated was investigated both genetically and physiologically using gibberellin-deficient mutants and exogenous gibberellin applications. Results indicate that reduced gibberellin levels further reduce the number of reproductive nodes, but are insufficient to explain the *det* phenotype. Supported by USDA grant 9103136.

D 213 NUCLEAR FACTORS INVOLVED IN ORGAN-SPECIFIC DIFFERENTIAL EXPRESSION OF *rbcS* GENES IN TOMATO, Iris Meier, Thianda Manzara and Wilhelm Gruissem, Dept. of Plant Biol., University of California, Berkeley, CA 94720.

The establishment of specific gene expression patterns during plant development and organ formation is a consequence of multiple events of transcriptional activation and inactivation. They are initiated by multiple, specific protein-DNA and protein-protein interactions within the promoters of the regulated genes. We are using the expression of the *rbcS* gene family in tomato as a model system for complex gene regulation. Transcription of the five genes (*rbcS 1, 2, 3A, 3B* and *3C*) is organ-specific and developmentally regulated in fruit, and light regulated in cotyledons and leaves. Of the multiple protein-DNA interactions detected within the five *rbcS* promoters many show organ-specific and developmentally regulated appearance. One protein was identified that interacts specifically with the *rbcS 3A* promoter in young tomato fruit. This promoter is, in contrast to the related and otherwise coordinately regulated *rbcS 1* and *rbcS 2* promoters, inactive in this organ. The protein was characterized and its DNA-binding activity found to occur only in nuclear extracts from young fruit. Its specificity for the *rbcS 3A* promoter is provided by a palindromic binding site unique to *rbcS 3A*. It is located within a region otherwise highly conserved between the three promoters. The protein binds directly adjacent to GBF, a general activating factor of several plant genes. In order to study the function of this potential organ- and gene-specific negative regulator *in vivo* and to further dissect the elements involved in differential activation of the *rbcS* promoters we established a microprojectile-based transient gene expression system which allows us to directly compare promoter activities in different organs. We have shown that the *rbcS* promoters fused to a reporter gene retain their differential activities, and we are now in the process of analysing the function of the individual protein binding sites.

D 214 A RAPID TECHNIQUE FOR ISOLATING TRANSCRIPTION FACTOR HOMOLOGUES FROM DIVERSE SPECIES

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Conventional approaches to cloning transcription factors in plants include transposon or T-DNA tagging, screening cDNA expression libraries, screening cDNA libraries with heterologous probes or degenerate oligonucleotides and sometime in the near future, chromosome walking. Recently, the PCR technique has allowed relatively rapid isolation of genes of known sequence. One problem when using PCR to clone genes of unknown sequence e.g., cross-species isolation of a particular gene, is that two separate conserved amino acid sequences are required to polymerise between. In the case of many transcription factors, only one small region of perhaps a few amino acids is conserved.

We have developed a RT-PCR technique employing just one set of degenerate oligonucleotides to efficiently clone specific families of transcription factor homologues. Using this approach we have isolated from mature petals two clones (with slightly different 3' ends) of a zinc finger protein from pea homologous to the EPF-1 clone from petunia, thought to be involved in the regulation of the shikimic acid pathway. We will present the sequence and characterisation of the pea zinc finger protein as well as describing clones representing other classes of transcription factor homologues from pea.

D 215 ANALYSIS OF AN OVULE SPECIFIC GENE,

Stephen B. Milligan and Charles S. Gasser, Section of Biochemistry and Biophysics, University of California, Davis, CA 95616.

The ovules of flowering plants are highly specialized structures contained within the carpels. They serve as the site of an array of complex functions essential to plant reproduction. Ovule function is dependent upon the expression of many unique gene products produced by the numerous differentiated cells and tissues which comprise this organ. The identity, tissue specificity, and function of these gene products remains largely unknown. To further our understanding in this area we are characterizing a gene, designated 9608, that is expressed exclusively in the inner few cell layers of the single integument of tomato ovules. These cell layers have previously been described as an integumentary tapetum or endothelium. In tomato the nucellus degenerates early in ovule development and the endothelium, therefore, lies directly in contact with the embryo sac. The endothelium is hypothesized to function in providing materials to the developing gametophyte or embryo, a role similar to that of the true tapetum in anthers. Accumulation of 9608 mRNA was shown to occur from around the time of anthesis to approximately five days post anthesis with decline in expression correlating with increased vacuolation and eventual disintegration of these cells. DNA sequence analysis shows 9608 to encode a 9 kd sulfur rich protein with a putative signal peptide. This gene is not similar in sequence to any previously reported protein. Northern blot analysis has shown this gene to be expressed in other diverged members of the Solanaceae. In tobacco, expression occurs in anthers as well as pistils. This expression may indicate a common tapetal-like function for this protein. In order to obtain additional clues as to the function of the 9608 protein we have produced antisense plants in which the levels of the 9608 transcript have been greatly reduced. The phenotype displayed by these plants will be discussed.

D 216 USING THE REGULATORY GENE *DELILA* TO INVESTIGATE THE EVOLUTION OF PLANT PIGMENT PATTERNS

Mark Mooney, Thierry Desnos, Rosemary Carpenter and Enrico Coen. John Innes Institute, Norwich U.K.

Plants exhibit a diverse array of pigment patterns, yet evidence from the cloning of the pigment regulatory gene *Delila (Del)* (Goodrich et al Cell 68, 1992) suggests that the genetic regulators responsible for these patterns are conserved. So how do diverse plant species utilise similar regulatory mechanisms to produce their individual phenotypes?

We are addressing this problem by introducing the *Del* gene from *Antirrhinum majus* into a range of plant species and monitoring the effects on pigmentation. We present data from transformations into both tobacco and tomato using the *Del* cDNA fused to the CMV35S promoter. With this construct we asked the following questions: 1. Can *Del* recognise the host biosynthetic genes? 2. Is *Del* the only factor required for pigment biosynthesis?

The phenotypes of the transformants suggested the following answers to these questions: 1. Both tomato and tobacco show enhanced pigmentation, indicating that the interaction with the biosynthetic genes is conserved between these species. 2. Pigmentation in the transformants is enhanced but still ordered in a distinct pattern and the phenotype is intensified by high light, suggesting that *Del* is not the only limiting factor.

These closely related species also exhibit differences in their response to the *Delila* gene and through further work we wish to investigate the reasons for this difference and relate it to the original question.

D 217 *CACGTG MOTIF IS A CIS-ELEMENT FOR DEVELOPMENTAL CONTROL OF THE BEAN ß-PHASEOLIN GENE.*

Norimoto Murai, Yasushi Kawagoe and Bruce R. Campell. Dept. Plant Pathology & Crop Physiol., Louisiana State University, Baton Rouge, LA 70803

Based on our cis- and trans-acting element analyses (Burow et al. 1992; Kawagoe & Murai, 1992), we propose a transcriptional activator/repressor competition model for phaseolin gene regulation. In this model, CAN is a transcriptional activator and is a member of the basic-helix-loop-helix protein family. AAAGA motif-binding protein AG-1 is a transcriptional repressor and is a member of a high-mobility-group or the histone H1 protein family. In the repressed state of the phaseolin gene, AG-1 promotes stable nucleosome formation by binding to two AT-rich regions (-191/-182 and -356/-347). In the active state of the phaseolin gene, CAN protein acquires the accessibility to CACGTG (-248/ -243) and CACCTG (-163/ -158) by an unknown mechanism, and activates transcription by cooperative interaction of CAN protein. We tested the validity of our model by determining the biological functions of CACGTG and other CANNTG and AAAGA motifs in phaseolin gene regulation. We substituted these motifs singly or in combination from the phaseolin proximal promoter (-391 or -295/+80), and tested the mutation effects by transient expression assay using bean cotyledon protoplasts. Mutation of the CACGTG motif significantly reduced expression of linked reporter gene, while substitution of nearby CACCTG and CATATG motifs had no effect. We concluded that CACGTG motif is a cis-acting regulatory element required for quantitative expression of the phaseolin gene in developing cotyledons. However, we were unable to demonstrate cooperative interaction of CACGTG and other CANNTG motifs in transient assay. We will discuss implication of the current results on our activator/repressor competition model.

D 218 COMPARISON OF TRANSACTIVATION OF DIFFERENT LC-CONSTRUCTIONS IN MAIZE PROTOPLASTS AIMING FOR GENETIC ENGINEERED CELL LINEAGE STUDIES IN MAIZE USING THE MICROINJECTION APPROACH, Gabriele Neuhaus-Url, Clotilde M. Lusardi, Ingo Potrykus and Gunther Neuhaus, Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland.

Lc gene, a member of the R-gene family of maize, activates structural genes which are involved in the anthocyanin biosynthesis. The feature of the Lc gene being a visible marker gene opens the possibility to use it for genetic engineered cell fate mapping in maize after microinjection of the appropriate Lc expression cassettes into meristematic cells. Different Lc constructions were designed which are classified into integrative and selfreplicating vectors. The integrative vectors result from fusion of the 2.4 kbp Lc cDNA to the CaMV 35S promoter, the same promoter with the Adh1 intron of maize and the 1kbp PEPC promoter. The selfreplicating vectors consist of the WDV shuttle vector containing Lc expression cassettes under the control of CaMV 35S promoter and Adh1 intron and the PEPC promoter aiming for transactivation possibility without integration pressure. Both categories of expression vectors were tested for their function in different maize protoplast systems by quantifying the red cells after PEG mediated direct gene transfer. The transactivation capacity differs due to the promoter, presence of intron, length of 5' UTR, presence of WDV sequences and different maize protoplast system (callus, mesophyll). Southern blot analysis revealed the evidence of selfreplication of the WDV-Lc constructions. Using these visible Lc marker constructions for cell lineage studies in maize by targeting defined cells in the apical meristem with the injection capillary, enables to follow up the fate of the genetic engineered cell. One important aspect respectively would be to define which cells in the meristem are responsible for the development of the generative tissue which give rise to the next generation. This knowledge would lead to substantial improvement for transformation experiments for recalcitrant species such as cereals, still lacking an efficient transformation method via protoplasts or biolistic approach.

D 219 **FLORAL MADS-BOX GENES IN POPLAR, PINE, AND DOUGLAS-FIR,** **Lorraine S. Nyers, Allen H. Doerksen, Alex B. Krupkin, and Steven H. Strauss, Department of Forest Science and Genetics Program, Oregon State University, Corvallis, OR 97331-7501**

The MADS box is a DNA-binding domain present in most floral homeotic genes isolated to date. Our work expands the study of MADS-box genes to two systems not yet explored: dioecious angiosperms and monecious gymnosperms. We used a set of degenerate MADS-box primers to amplify DNA isolated from the dioecious trees *Populus deltoides* and *Populus trichocarpa*, and from the gymnosperms *Pinus taeda* and *Pseudotsuga menzesii* using the polymerase chain reaction (PCR). The resulting products were cloned and T-track analysis was performed. Thirteen different MADS-box clones were obtained from *P. deltoides*, 9 from *P. trichocarpa*, 10 from *P. taeda*, and 17 from *P. menzesii*. The clones fell into subfamilies exhibiting similar T-track patterns. One or more clones from each subfamily was chosen for full DNA sequencing. Thirty of the 35 clones sequenced had deduced amino acid sequences with greater than 85 percent homology to the MADS-box domain of *AGAMOUS*, a floral homeotic gene isolated from *Arabidopsis thaliniana*, with similar residues considered homologous.

Analysis of MADS-box gene expression patterns and isolation of full-length clones is underway.

D 220 ISOLATION AND CHARACTERIZATION OF THE TOBACCO HOMOLOG OF THE *FLORICAULA* and *LEAFY* GENES, F. Bryan Pickett, Alan Kelly, Mark Bonnlander, and D. Ry Meeks-Wagner, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Loss of function mutations in the *LEAFY* (*LFY*) gene of *Arabidopsis thaliana* or the *FLORICAULA* (*FLO*) gene of *Antirrhinum majus* inhibit the formation of floral structures (1,2). The molecular characterization of *FLO* and *LFY* has shown that these genes share a high level of sequence similarity and are both transcribed in florally committed tissues. *In situ* analyses of *LFY* and *FLO* hybridization suggest that cells in which these genes are expressed form *anlagen* that contribute to the formation of a floral meristem in *Arabidopsis* and both floral and bract meristems in *Antirrhinum*. The sequences of these genes show the presence of acidic and proline rich domains in their putative translation products indicating that they may act as transcription factors (1,2).

Recently, cDNAs representing floral specific transcripts have been identified in libraries made from florally induced meristems of tobacco. cDNA clones homologous to *LFY* and *FLO* have been isolated from this library. Sequence analysis of these clones suggest that there is a single active *FLO* -like gene in tobacco. This gene has been designated tob-FLO. The message accumulation of tob-FLO in vegetative and florally committed apical meristems has been assayed by Northern analysis and by PCR amplification of tob-FLO cDNA. Preliminary results indicate that tob-FLO is expressed at low levels in the vegetative meristem and at higher levels in florally committed meristems. This may suggest that the tob-FLO gene is involved in developmental events occurring prior to flowering in tobacco.

1. Coen,E.S. et al. (1990) *floricaula* : A homeotic gene required for flower development in *Antirrhinum majus*. Cell **63** 1311-1322
2. Weigel, D. et al. (1992) *LEAFY* controls floral meristem identity in *Arabidopsis* . Cell **69** 843-859

D 221 OVULE DEVELOPMENT IN WILD-TYPE *ARABIDOPSIS* AND TWO FEMALE -STERILE MUTANTS, Kay Robinson-Beers, Robert E. Pruitt, Catherine M. Geil and Charles S. Gasser, Section of Biochemistry and Biophysics, University of California, Davis, CA 95616.

Ovules, the precursors of seeds, are contained within the carpels of flowering plants. The morphologic nature of the ovule and its constituent parts remains a major problem in structural and evolutionary botany. We are combining genetic methods with anatomical and morphological analyses to dissect ovule development. We have screened a mutagenized population for female-sterile mutants through 1) the failure of siliques to elongate after self pollination and 2) reciprocal crosses used to distinguish gynoecial vs. androecial defects. To date, several female-sterile mutants with altered ovule development have been isolated. The ovules of one mutant initiate a single integument-like structure whose further development is aberrant. It forms a collar of tissue surrounding an exposed nucellus, giving the ovule the appearance of a bell. Accordingly, this mutant has been named *bell* (*bel1*). Ovules of another mutant initiate two integuments which appear to undergo the normal pattern of cell division without the accompanying pattern of cell elongation that is necessary for the formation of morphologically normal integuments. This produces the *short integument* (*sin1*) phenotype for which this mutant is named. Neither of these mutants forms an embryo sac indicating that normal morphological development of the the integuments and proper embryo sac formation may be interdependent processes or in part governed by common pathways. Genetic analyses indicate that *bel1* and *sin1* define two loci which are essential for normal ovule development. Two additional female-sterile mutants with abnormal ovules have been identified and analyses of these are in progress. Our long range goal is to identify and characterize all loci required for normal ovule development and to examine the genetic interactions among these loci.

D 222 STRUCTURE AND EXPRESSION OF A *LEAFY* HOMOLOG FROM *POPULUS*. William H. Rottmann, Teresa K. Boes and Steven H. Strauss. Forest Science, Oregon State University, Corvallis, OR, 97331-5705.

Black cottonwood (*Populus trichocarpa*) and its hybrids are fast-growing trees having economic importance in the Pacific Northwest paper industry. The highly reduced male and female flowers are borne on inflorescences (catkins) of separate trees. The specialized floral structure and the dioecious character of the species make *Populus* a useful subject for comparative studies of gene expression during flower development. Degenerate primers derived from sequences conserved between the homeotic genes, *lfy (leafy)* and *flo (floricaula)*, were used to amplify a 161 bp gene-specific probe from *Populus* DNA, which was subsequently cloned. Several nearly full-length cDNA clones also have been isolated and characterized by sequencing. The *Populus* sequence more closely resembles *flo*, with an amino acid sequence identity of approximately 75%. Hybridization analysis of genomic DNA indicates that there are no other closely related genes, in agreement with the fact that there was no sequence variation among 24 clones of the 161 bp PCR fragment. On the basis of PCR analysis of cDNA libraries, the gene is expressed in both males and females weakly in vegetative and mature floral tissues, but strongly in developing inflorescences.

D 223 ISOLATION AND CHARACTERIZATION OF ANTHOCLADE MUTANTS IN THE *PETUNIA HYBRIDA* INFLORESCENCE, Jon Ruehle and Carolyn Napoli, Department of Environmental Horticulture, University of California, Davis, CA 95616

The inflorescence of wild type *Petunia hybrida* is produced by the sequential initiation of anthoclades under strict spatial and temporal control. We have isolated three distinct classes of Petunia mutants where this orderly pattern is disrupted. The mutations were induced using ethane methyl sulfonate. One classification is comprised of several independent mutant lines which demonstrate a terminal flower phenotype. The renewal of anthoclade initiation is blocked when the apex produces a flower. These lesions affect the inflorescence phyllotaxis preceding the terminal flowers, and resumption of growth from distal axillary buds results in characteristic branching patterns in each line. Allelism tests are underway with these mutants. A second mutant classification is called concentric (*con*) and has a mutation that may affect the temporal control of inflorescence initiation. The mutation is due to a single, recessive gene and causes precocious flowering and stunted anthoclades. Whorls are initiated as concentric rings, but the perianth undergoes only rudimentary development. A third mutant phenotype is similar to *floricaula* in Antirrhinum and *leafy* in Arabidopsis. In this mutant the production of flowers is completely blocked and difoliate sympods proliferate in their place. The genetic and morphological characterization of these Petunia inflorescence mutants will be presented.

D 224 MUTATIONS AFFECTING GYNOECIUM DEVELOPMENT IN *Arabidopsis thaliana*. Allen Sessions, Kenneth Feldmann *, and Patricia Zambryski. University of California, Dept. of Plant Biology, Berkeley, Ca 94720, and *University of Arizona, Dept. of Plant Sciences, Tucson, Az, 85721.

We are collecting and analyzing mutations that affect the formation of the crucifer (*Arabidopsis*) gynoecium. Our aim is to characterize these mutant lines at the ultrastructural, genetic, and molecular levels to determine when and where during normal gynoecium development the genes defined by these mutations are acting to direct cells toward their specific fates. The initial focus of this project has centered around describing the *ettin* mutation. Plants homozygous for a strong allele of this mutation are essentiallly female sterile and have septumless unilocular gynoecia bearing two stigmatic surfaces. Presented here is a comparison of wild type and *ettin* gynoecium development. This comparison reveals that *ettin* gynoecia fail to initiate a normal placental ridge early in stage 6 and subsequently undergo a markedly altered histogenic phase, resulting in gynoecia with altered transsectional symmetry and an overall disrupted anatomy. This analysis suggests that the *ettin* locus encodes a product necessary for the production of a normal placental ridge and the correct specification of cell fates during gynoecial development. The *et-1* mutant phenotype results from the insertion of a single T-DNA near the centromere on chromosome 2. We have isolated genomic clones spanning this insertion site and here present our initial molecular analysis of this region. Also presented are brief descriptions of a number of other mutations we have isolated that affect the development of the gynoecium .

D 225 CHARACTERIZATION OF A FLORAL MUTANT IN PEA AFFECTING THE TRANSITION FROM INFLORESCENCE TO FLOWER DEVELOPMENT, Susan R. Singer, Sonja L. Maki and Amy D. Horan, Department of Biology, Carleton College, Northfield, MN 55057

Apex culture experiments have demonstrated that determination for inflorescence development in wildtype *Pisum sativum* L. is separable from commitment to flower production (Ferguson, C.J. et al. 1991 Planta 185:518). Utilizing a spontaneous, recessive mutant that appeared in our early flowering stocks, we have further investigated the transition from inflorescence to flower production in pea. Our mutation is phenotypically similar to *leafy* in *Arabidopsis* and *floricaula* in *Antirrhinum* (Coen, E.S. et al. 1990 Cell 63:1311; Schultz, E.A., Haughn, G.W. 1991 Plant Cell 3:877). Inflorescence development in our mutation begins in the expected node for the flowering genotype. However, axillary meristems in the inflorescence initiate numerous leafy structures as well as aberrant flowers. Petals are frequently absent and pistils sometimes fuse to stamens. A large number of floral meristems are initiated by each axillary meristem, rather than the single flowers initiated by the wildtype. This extended production of reproductive nodes contrasts sharply with the reduced inflorescence development observed for the determinate (*det*) mutant (Singer et al. 1990 Amer. J. Bot. 77:1330). This raises questions regarding the role of these two genes in inflorescence development which we addressed by creating homozygous double mutants for the two mutations. The *det* gene was partially epistatic as double mutant plants produced fewer flowers in each axil with a more normal phenotype than the aberrant floral mutant. Thus the mutation may affect the inflorescence to flower transition with abnormal floral organs being indirect effects of extensive branching of the axillary inflorescence meristem. Supported by USDA grant 9103136.

D 226 CELL SPECIFIC REGULATION OF THE MITOCHONDRIAL GENOME DURING MALE FLOWER DEVELOPMENT IN SUNFLOWER. Christina.J.Smart & Christopher.J.Leaver, Department of Plant Sciences, University of Oxford,South Parks Road,Oxford OX1 3RB, UK.

Functional mitochondria are essential for the survival of higher plants, consequently very few non lethal mitochondrial genome mutations exist. However, the study of maternally inherited mutants like non chromosomal stripe in maize and cytoplasmic male sterility (CMS) can provide valuable insights into nuclear-mitochondrial interactions throughout plant development. CMS is a maternally inherited trait that results in pollen abortion, whilst maintaining normal vegetative development and female fertility. In sunflower (*Helianthus annuus*), as with many other species, the phenotype is associated with a mutation in the mitochondrial genome (mtDNA). A DNA insertion in the mtDNA of the sterile line has resulted in the creation of a novel open reading frame, ORFc, downstream of the *atp* A gene. In addition to the normal *atp*A transcript, in sterile and restored hybrid lines, the ORFc is cotranscribed with *atp* A. A novel 15kDa polypeptide is synthesised by mitochondria isolated from sterile and hybrid seedlings and could be the product of the ORFc (Laver et. al.,1991, *The Plant J.* 1(2);185).

In order to address the problem of why anther development is disrupted and yet the rest of the sterile plant is normal, the abundance of the normal *atp* A transcript and of the *atp* A-ORFc transcript have been analysed. Northern hybridization analysis shows that there is a specific decrease in the abundance of the chimeric *atp* A-ORFc transcript upon restoration of fertility in sunflower florets. *In situ* hybridisation analysis indicates that this reduction in transcript abundance in the restored hybrid occurs specifically in premeiotic pollen mother cells (PMCs). These results suggest a pollen mother cell specific action of the restorer genes in sunflower, possibly acting to reduce the stability and hence abundance of the chimeric *atp* A-ORFc transcript.

The expression of the mt genome has been investigated throughout normal microspore development, by *in situ* hybridization with various mt gene probes. In premeiotic anthers mt transcripts are most abundant in the PMCs, with little expression in the tapetum or parietal tissue. During meiosis transcript abundance increases in the tapetum, and by the tetrad stage the tapetal cells show high levels of mt transcripts. Some expression is also seen in the young microspores,but other anther tissues have low transcript levels. Whether these differences reflect changes in mitochondrial genome copy number during microsporogenesis is currently being investigated.

D 227 CLONING OF THE FLORAL HOMEOTIC GENE HOMOLOG OF *LEAFY/FLORICAULA* IN *EUCALYPTUS GLOBULUS*, Simon G. Southerton, Steven H. Strauss, Mark R. Olive and Elizabeth S. Dennis, CSIRO Division of Plant Industry, GPO Box 1600 Canberra, ACT 2601 Australia.

PCR primers based on the amino acid similarity between the *leafy* gene from *Arabidopsis* and the *floricaula* gene from *Antirrhinum* were used to amplify a 141 bp product from *E. globulus* genomic DNA. After subcloning into plasmid vectors a number of products were sequenced and found to have strong amino acid homology with the comparable region of the *leafy* and *floricaula* genes (Fig).

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floricaula      LHCLDEEASNALRRAFKERGENVGS
leafy           ......A.................A
          -----> primer                  <----- primer
Eucleaf 1       ......H......KS.......I.L
Eucleaf 2       ......H......KS.........A
Eucleaf 3       ......H......KS.......I.A
```

Eucleaf 2 was used as a probe to screen a *E. globulus* genomic library constructed in EMBL4 and 4 hybridizing clones were isolated. DNA from these clones was subcloned and mapped. Fragments hybridising to the Eucleaf 2 probe also hybridized to the *leafy* cDNA at low stringency.

The hybridization pattern of the four genomic clones suggested there are at least two copies of the gene in *Eucalyptus*. A 1.4 kb KpnI fragment spanning the hybridizing region from one genomic clone was used to probe EcoR1 and HindIII digested *E. globulus* DNA. Three EcoR1 and two HindIII fragments hybridized to the probe confirming that there are two copies of the *leafy/floricaula* homolog in *E. globulus*.

D 228 **CHARACTERIZATION OF DIFFERENT GENE EXPRESSION PATTERNS DURING INFLORESCENCE DEVELOPMENT IN THE *COMPOSITAE* FAMILY.**
Teemu H. Teeri, Paula Elomaa, Yrjö Helariutta and Mika Kotilainen, Institute of Biotechnology, University of Helsinki, Karvaamokuja 3, 00380 Helsinki, Finland

The inflorescence of the *Compositae* family is typically a large regulatory unit with developmentally polymorphic flowers, the organs of which are evolutionarily highly specialized. We are focusing on corolla differentiation related gene expression in *Compositae* using gerbera (*Gerbera hybrida*) as a model.

In gerbera there are three flower types (ray, trans and disc florets), all with distinct corolla anatomy in some varieties. All the corolla forms can be divided to tube (unpigmented) and zygomorphically bilabiate lips (epidermally anthocyanin and carotenoid pigmented).

We have approached the corolla development of gerbera by two ways: 1) by analyzing the regulation of flavonoid genes by comparing the chalcone synthase (*chs*) and dihydroflavonol-4-reductase (*dfr*) gene expression in different gerbera varieties, and 2) by analyzing the expression of other specifically corolla abundant genes isolated by differential screening techniques.

In the varieties with fully red corolla pigmentation, the expression of both *chs* and *dfr* follows the anthocyanin accumulation pattern. In the varieties with spatially restricted color patterns, only the expression of *dfr* follows the anthocyanin accumulation patterns, *chs* expression being uniform. This suggests that specifically *dfr* is a target of several regulatory signals regulating gene expression spatially during inflorescence development.

In the analysis of other corolla abundant cDNAs, we have found genes the expression patterns of which are different from that of the flavonoid genes. The most abundant of these encodes a protein with 40-50% homology to plant lipid transfer proteins.

The analysis of the expression of these genes indicates that there are several different regulatory patterns of the corolla abundant genes at cellular, regional (within the corolla), organ and floret type levels during inflorescence development.

D 229 EXPRESSION OF TOBACCO ANTHER-SPECIFIC GENES, Ann Tuttle, Sue Kadwell, Linda Mercer Dawson, Susan Jayne, Tina Grater, Trang Le and Lyle Crossland, CIBA-GEIGY Corporation Agriculture Biotechnology Research Unit, P.O. Box 12257, Research Triangle Park, NC 27709-2257

Anther-specific cDNAs from tobacco have been isolated by differential screening of a subtracted, anther cDNA library and the developmental expression and sequence of several clones has been determined. In-situ hybridization studies of the ant32 cDNA have shown that the mRNA is localized to the tapetal cell layer of the anther. Genomic clones corresponding to the ant32 and ant43D cDNAs have been isolated and the promoter regions have been fused to the β-glucuronidase and diphtheria toxin A-chain genes. β-glucuronidase activity in transgenic plants has been assayed fluorometrically and histochemically and has been shown to be present only in the tapetum with ant32 fusions, and in the tapetum as well as other anther tissues with ant43D fusions. However, diphtheria gene fusions of both promoters destroyed only the tapetum of the anther in transgenic plants.

D 230 GENETIC AND MOLECULAR ANALYSIS OF MAIZE INFLORESCENCE DEVELOPMENT, Bruce Veit and Sarah Hake, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710

We are using a combination of genetic and molecular analyses to describe maize inflorescence development. This process begins with the formation of a ramified structure bearing perfect floret primordia. Subsequently, two inflorescence types, the tassel and ear, differentiate from each other through respective abortion of pistil and stamen primordia. To better understand how a sequence of gene interactions brings about these events, we have subdivided these phases further by detailed analysis of several mutant loci. These mutants were first differentiated according to when their pattern of development diverges from normal. *ramosa* (*ra*) perturbs one of the earliest stages, causing reiterations of primary branching patterns. *branched silkless* (*bd*) affects later stages to cause reiterations of a morphologically distinct pattern of branching that immediately precedes floret formation.

We have constructed double mutants to more clearly define functional relationships between inflorescence mutants. The *bd*; *ra* double mutant displays an additive phenotype, suggesting the two types of branching affected by these mutants can be regulated independently. The two mutants also interact differently with mutants that affect other aspects of inflorescence development such as sex determination. To distinguish whether these differences are due to temporally distinct expression patterns of *bd* and *ra* versus qualitative differences in their function we have begun to clone genes by transposon tagging and interspecific homology. The characterization of cloned genes obtained by both approaches is described.

D 231 GAMETOPHYTIC GENE EXPRESSION IN MAIZE Philip R. Wakeley and Patrick J. Hussey
Royal Holloway and Bedford New College, University of London, Egham, Surrey, U.K. TW20 OEX.

The objectives are (i) to isolate cDNA clones expressed in maize pollen and/or germinating pollen (ii) to characterise the temporal and spatial expression of the transcripts corresponding to these cDNAs (iii) characterise the spatial distribution of the gene products of selected cDNA clones in pollen and germinating pollen.

Comparison of 2-D gel fractionated *in vitro* translation products of shoot, pollen and germinating pollen mRNAs reveals significant differences in the quality and quantity of gene products. Between shoot and the late stages in pollen development there is a marked increase in the expression of cytoskeletal proteins, presumably reflecting the importance of the cytoskeleton in pollen function. In particular the four electrophoretically separable α-tubulin isotypes show a coordinate increase in abundance in pollen development. The marked increase of α-tubulin expression is apparent post pollen mitosis 2.

A differential screening procedure has been undertaken in order to identify genes expressed exclusively in the pollen of maize. A germinating pollen cDNA library has been screened with radiolabelled first strand cDNA generated from pollen, germinating pollen and shoot mRNA. Differentially expressed clones have been identified for 4 of the 10 size fractions of the germinating pollen cDNA library. Northern analysis reveals that several of these clones are either specific or abundant in the late stages of pollen development. The specific and the abundant clones correspond to transcripts that are expressed only or abundantly, post pollen mitosis 2. The pollen abundant clones mimic the expression programme of total α-tubulin gene expression.

We are currently preparing antibodies to the gene products of selected cDNAs in order to perform immunolocalization studies.

D 232 THE ROLE OF THE MUDDLED LOCUS IN DETERMINATION OF SHOOT TYPE, Mark D Wilkinson, George W. Haughn,
Dept. of Biology, University of Saskatchewan, Saskatoon, SK, Canada, S7N OWO

A novel gene has been identified in Arabidopsis thaliana which appears to play a role in both floral and inflorescence development. This gene has been designated *MUDDLED* (*MDL*) due to the highly variable phenotype caused by the recessive mutant allele *mdl-1*. Mutant plants exhibit abnormal floral development under continuous light conditions. Mutant flowers have an altered organ number in all whorls, and organs in the second and third whorls frequently display variable homeotic transformations. The transformations observed in both the *mdl* single mutant and in combination with *agamous* (*ag*), *apetala2* (*ap2*), *pistillata* (*pi*), or *apetala3* (*ap3*) indicate that the changes are the result of a reduction in the activity of the *AP3/PI* genes and ectopic expression of both the *AG* and *AP2* genes. Thus in mdl mutants the control and coordiantion of floral homeotic gene expression is altered. In addition to the effects in the flower, primary and coflorescence meristems terminate in floral-like structures. The gene *APETALA1* (*AP1*) is believed to play a role in determination of shoot meristem fate. *mdl ap1* double mutants are unable to produce flowers, but continue to produce coflorescence-like structures in their place which suggests that the *MDL* gene may play a role in determination of shoot type. This hypothesis is supported by short-day studies. Preliminary results under short daylength conditions suggest that both floral phenotype and lateral meristem type are affected in *mdl* homozygotes, indicating that the *MDL* gene responds to floral-pathway induction signals. Mutant plants may bolt significantly earlier than wild type although the time to bolting ranges over several weeks. *mdl* individuals produce a highly variable number of coflorescences, where the number increases in relation to the time of bolting. The decision to produce flowers is frequently unstable such that a lateral shoot may select the coflorescence fate though previous laterals have selected the floral fate. On the basis of these observations we propose that the *MDL* locus is involved in activating the floral developmental program.

D 233 MOLECULAR MARKERS FOR EARLY EMBRYOGENESIS, J.L. Zimmerman, X. Lin, and G.-J. Hwang, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228

The early molecular events which direct the development of higher plant embryos remain largely unknown, due in part to the difficulty associated with identifying genes which are regulated during this process. We have taken a new approach to the problem of isolating genes which are up-regulated early in embryogenesis using the carrot somatic embryo system. We prepared a cDNA library from polysomal mRNA of globular staged embryos of carrot, and screened it with a "subtracted cDNA probe" in which globular cDNA was prehybridized with polysomal mRNA from zygotic seedlings to remove all common sequences. Using this approach, we have been successful in isolating 50 different clones, all of which are enhanced in embryos compared to seedlings.

DNA sequence analysis reveals that we have isolated clones corresponding to many of the previously identified "embryo" genes, as well as many other known clones which have never been analyzed during embryogenesis. Moreover, we have identified at least 11 novel clones which are now being characterized. Analysis of polysomal and total RNAs from several plant tissues and embryo stages has revealed interesting patterns of temporal/developmental regulation for this set of genes. Each of these genes is also being analyzed by *in situ* hybridization in order to visualize their spatial regulation in the developing embryos. These analyses will result in the characterization of collection of molecular markers for specific events in embryo development and differentiation.

Vegetative Meristems

D 300 ISOLATION AND MOLECULAR CHARACTERIZATION OF PROTEIN KINASES FROM ARABIDOPSIS THALIANA

Nazeem Ali and Nam-Hai Chua
Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021

The development of multicellular organisms requires extensive cell-cell communication and quick response to changes in extracellular cues. Receptor-type protein kinases are known to play an important role in relaying extracellular information to the inside of the cell. These membrane spanning proteins contain an extracellular ligand-binding domain, a transmembrane region and a cytoplasmatic kinase domain. Binding of the ligand to the receptor activates the latter and enables it to pass on the signal to the cell inner via a cascade of intracellular kinases.

In plants only a few examples of protein kinases have been reported up to date and little is known about the involvement of protein kinases in signal transduction and development of plants .
We have cloned different types of protein kinases from Arabidopsis. Data on the molecular characterization, expression pattern of these kinases and their role in early Arabidopsis development will be presented.

D 301 ANALYSIS OF STRESS INDUCED EMBRYOGENESIS IN *BRASSICA NAPUS* MICROSPORES, Angelis K.J. and P. Pechan, Max Planck Institut für Züchtungsforschung, D-5000 Köln 30, FRG

Brassica napus microspores is a well defined highly embryonic system where up to 70% of microspores can undergo embryogenesis (Pechan and Keller 1988, Phys. Plant. **74**, 377). Redirection of pollen development to the embryo developmental pathway can be induced in freshly isolated microspores in late mononucleate to early binucleate (corresponding to 3 - 5 mm bud size) stage by a 32°C temperature treatment of minimum 8 hr duration. However, when cultured at 18°C, microspores continue to develop into pollen grains.

cDNA libraries were constructed from temperature induced and uninduced microspores isolated from 4 mm buds and differentially screened for clones associated with induction. 100 clones were identified, sequenced and analyzed in detail.

They were grouped into 3 categories: app. half of the clones were ribosomal sequences and 45 % of clones contained stretches of simple sequences (Tautz and Renz 1984, NAR **10**, 4127). Other identified clones did not showed any sequence homology in GenEMBL data base.

D 302 TWO PUTATIVE PROTEINASE INHIBITOR GENES ARE EXPRESSED IN SPECIFIC CELL LAYERS OF THE TOMATO SHOOT TIP. Jörg Brandstädter, Claus Roßbach, Karin Schumacher and Klaus Theres, Institut für Genetik, Universität zu Köln, D-5000 Köln 41, F.R.G.

In higher plants the shoot apical meristems are of fundamental importance for postembryonal development. Several models describing organization and function of the shoot apex are based on morphological and physiological observations. Although it is generally believed that the processes of organogenesis and differentiation are initiated by the onset of specific gene expression patterns nearly nothing is known about genes regulating these processes. With the goal of identifying and studying developmentally relevant genes we are trying to isolate genes that are either preferentially or exclusively expressed in the shoot apical meristem of the tomato plant *(Lycopersicon esculentum)* using the method of differential cDNA hybridization. So far two genes (AT1 and AT2) preferentially expressed in the shoot apex have been characterized in more detail. RNA in situ hybridization experiments revealed that AT1 is strongly expressed in corpus cells of the shoot apex whereas no hybridization signal is detectable in tunica cells. Furthermore AT1 is also expressed during flower development in all organs of the flower. Sequence analysis revealed that the deduced AT1 protein shows significant similarities to the tomato proteinase inhibitor II. Expression of AT2 was found to be restricted to the outermost cell layers of about 4 -5 leaf primordia preceding the formation of an inflorescence. No hyrbridization signal was observed in the apical meristem itself during vegetative growth whereas after transition to reproductive growth AT2 is expressed at the flanks of the floral meristem and later in the outermost cell layers of all floral organs. Sequence analysis revealed that AT2 is related to a flower specific tobacco thionin and a potato gene which itself is similar to the soybean proteinase inhibitor CII (Bowman-Birk). Presently we are trying to influence the expression of AT2 in vivo by exogenously introduced copies of the AT2 gene that can be transcribed either in sense or in antisense orientation.

D 303 CHARACTERIZATION OF TWO FUNCTIONS IN THE VEGETATIVE SHOOT APEX OF ARABIDOPSIS, Joseph D. Callos, Bruce Link, and June I. Medford, Department of Biology, Penn State University, University Park, PA 16802

We have analyzed the Forever Young (Fey) mutant in Arabidopsis that affects the position of leaf initiation and maintains the meristem as a formative region. In wild-type plants, leaves are initiated at a divergence of 136.4° (SE±1.6; N=152) with a plastochron ratio of 1.20 (SE±0.02; N=152). Using these values we determined the phyllotaxis index of 3.02 corresponding to the third orthogonal parastichy (Richards 1951). In contrast to the 3/5 parastichy pattern seen in wild-type, we were unable to find any characteristic pattern in Fey apices. In Fey, the meristem itself is very disorganized, zonation is disrupted and leaf primordia are initiated with an abnormally large number of cells. Further, leaf primordia are frequently misshapen or centric and are initiated without a discernible pattern. Sometimes two primordia are subsequently produced adjacent to each other on the same side of the apex. The simplest interpretation of our data suggests that Fey disrupts a "repressor-function" acting in the meristem that maintains the meristem as a formative region and prevents the initiation of leaf primordia.

We have also analyzed the action of the meri-5 gene (Medford et al, Plant Cell 3: 359-70, 1991). Transgenic plants were generated by separately introducing the meri-5 gene in both the sense and anti-sense orientations. Both classes of transformants show an abnormal phenotype. The abnormal phenotype can be identified by improper leaf expansion. Further, the leaves frequently show necrosis at the distal ends. I have used an RNase protection assay to verify that these abnormal phenotypes correspond to changes in the steady-state levels of the meri-5 mRNA.

D 304 ISOLATION AND ANALYSIS OF EXPRESSION OF GROUP 1 LATE EMBRYOGENIC ABUNDANT (Lea) GENES IN SOYBEAN (Glycine max L.)

Eberson S. Calvo, Eve S. Wurtele and Randy C. Shoemaker, Iowa State University and USDA-ARS-FCR, Ames, Iowa, 50011.

Lea proteins can be classified into at least three distinct groups (1, 2, and 3) based on the comparison of their sequences. Here we report the isolation of a group 1 soybean late embryogenic cDNA and genomic clones using the carrot EMB-1 cDNA as a heterologous probe in a soybean seed cDNA library. The soybean cDNA, SLE, codes for a 112 amino acid, highly conserved, hydrophilic, glycine- rich polypeptide. An interesting characteristic of the SLE protein is the presence of a 20 amino acid highly hydrophilic motif that is present only once in most homologs but occurs twice in SLE as an imperfect tandem repeat. The SLE mRNA is seed-specific and is present as a single-sized message at similar levels in both the embryo axis and the cotyledons. SLE mRNA levels increase dramatically at later stages of seed development. SLE mRNA accumulation is also developmentally regulated in soybean somatic embryos. However, somatic embryos at the globular stage have levels of SLE mRNA similar to those found in the mature seed. Abscisic acid (ABA) increases SLE mRNA levels both in zygotic and somatic embryos. Neither ABA or desiccation induce *sle* expression in 4 week old soybean plants. Southern blot and RFLP analysis suggest that at least three independent copies of the *sle* gene are present in the soybean genome. Screening of a soybean genomic library resulted in the isolation of three distinct genomic clones. Data on the mapping, sequence, and expression of these clones will also be presented.

D 305 MOLECULAR CHARACTERIZATION OF AN ARABIDOPSIS FUSCA GENE, Linda A. Castle and David W. Meinke, Department of Botany, Oklahoma State University, Stillwater, OK 74078 USA

The fusca mutant phenotype is unique to Arabidopsis. It is characterized by accumulation of anthocyanin in cotyledons of developing embryos. Fusca embryos are morphologically normal but die soon after germination. There appear to be 10-15 fusca genes in Arabidopsis. In a genetic background in which anthocyanin biosynthesis is blocked, homozygous mutant seedlings still die. Thus, lethality is not caused by the presence of anthocyanin. We propose that pigment accumulation is a secondary stress effect brought on by the primary defect of the mutated gene. To study this phenomenon, we have cloned and characterized the fusca gene EMB78. emb78-1, which maps adjacent to cer7 on chromosome 3, is a T-DNA insertion allele. emb78-1 has a complex T-DNA insert composed of at least four T-DNA units. We recovered a genomic fragment of emb78-1 by plasmid rescue. This fragment was used to recover genomic and cDNA clones. EMB78 is a single copy gene that encodes a 1.75 kb transcript. The cDNA clones were recovered from a library made from mRNA isolated from immature siliques. Sequence analysis of EMB78 has not revealed homology to known genes. We have an additional allele (emb78-2) tagged by a small T-DNA fragment that is being recovered by library construction. We also have an EMS allele (fusca-1) that is less severe than the tagged alleles. Results of protein analysis, tissue specific and light regulation of transcription, and complementation of the mutant phenotype will be presented. We are also investigating several other fusca mutants. Models of gene action leading to this unique phenotype will be discussed. Supported by NSF grant # DCB-8905137.

D 306 CHARACTERISATION OF GENES EXPRESSED SPECIFICALLY IN EMBRYOS DURING EARLY EMBRYOGENESIS IN *ARABIDOPSIS THALIANA*, Martine Devic, Michel Delseny and Patrick Gallois, Department of Plant Physiology and Molecular Biology, University of Perpignan, Avenue de Villeneuve, 66860 Perpignan cedex, FRANCE.

Our strategy consists in inserting a T-DNA containing a beta-glucuronidase reporter gene devoid of its promoter into the genome of *Arabidopsis* such that, in some cases, its expression will be driven by plant endogenous promoters. In a preliminary experiment on 100 individual transformants, we estimated by fluorometric measurements that 62% of the transformed plants expressed GUS in at least one organ. In our transformant population, the number of T-DNA copies is on average 2 to 3 per genome. Therefore it is possible to find more than one T-DNA copy as a transcriptionally active GUS fusion in the same plant. 27% and 26% of the transformants expressed GUS respectively in flowers and young siliques but only 3% and 2% did so exclusively in these organs. Up to now, 250 transformants have been carefully screened for expression in embryos at early stages of development, firstly by fluorometric assays on the whole flower and young siliques and, secondly, by *in situ* localisation using x-gluc. We have found several transformants containing transcriptional fusions with plant promoters active in early embryogenesis. Even though most of them showed expression in other part of the plant, a few transformants showed expression restricted only to the seed and embryo and one only to the heart stage embryo. The sites of T-DNA insertion have been determined by IPCR and we are now proceeding to clone the corresponding wild type genes. We also screened the transformed plants for abnormal phenotype (embryo-lethal and pattern formation mutants).Transformant 124 presented a characteristic embryo-lethal phenotype with the presence in each silique of the same plant of 1/4 of aborted seeds. We are currently analysing the linkage and tagging by the T-DNA insertion.

D 307 SPATIALLY REGULATED EXPRESSION OF THE PARSLEY 4CL-1 GENE DURING VASCULAR SYSTEM DIFFERENTIATION. Carl J. Douglas, Stephen P. Lee, David Neustaedter, and Karl Hauffe*. Department of Botany, University of British Columbia, Vancouver, BC Canada V6T 1Z4. *Present address: Institut für Biologie II, Universität Freiburg, Freiburg, FRG

The enzyme 4-coumarate:CoA ligase (4CL) catalyzes the formation of activated thiol esters of cinnamic acid derivatives, the last step of general phenylpropanoid metabolism. These esters are required as substrates for the the synthesis of many phenylpropanoid compounds, including flavonoids and lignin. Expression of the parsley 4CL-1 gene is activated in parsley cells by elicitor and light, and in transgenic tobacco plants by elicitor, light, and wounding. In addition, the 4CL-1 promoter specifies a complex developmentally-regulated pattern of cell- and tissue-specific expression in transgenic tobacco and *Arabidopsis* plants.The ability to specify expression in developing xylem, root tips, nectaries, pollen, and in the epidermal cells of stigmas, ovules, and petals, was localized to a 210-bp 4CL-1 promoter fragment, within which several sites of protein-DNA interaction in parsley tissue-cultured cells had been identified by *in vivo* footprint analysis. Analysis of internal deletions of the 4CL-1 promoter indicated that a 42-bp region located between -78 and -120 is critical for vascular-specific expression and for the quantity of expression in parsley protoplasts and tobacco seedlings. Gel retardation experiments showed that nuclear extracts from parsley tissue-cultured cells contain a factor or factors which interact specifically with sequences in this 42-bp region. Nuclear extracts from tobacco and *Arabidopsis* seedlings, in which vascular tissues are rapidly developing, are being tested for the presence of a similar factor. Site-directed mutagenesis has been used to alter potential *cis*-acting elements in this region, and these constructs are currently being tested for expression in vascular tissues of transgenic tobacco and parsley protoplasts, and for their ability to interact with factors in nuclear extracts. The information from these studies will be used to attempt to obtain a cDNA clone encoding a potential transcription factor critical in activating gene expression during vascular system differentiation.

D 308 RADICLE ZONATION PATTERN WITHIN DEVELOPING BRASSICA NAPUS L. EMBRYO: PRESUMPTIVE MERISTEM AND ELONGATING ZONE, Joseph G. Dubrovsky[1] and Teresa Tykarska[2], [1]The Center for Biological Research (CIB), La Paz, B. C. S., A. P. 128, Mexico 23000 and [2]Botanical Institute of Warsaw University, 00-35 Warsaw, Poland

Developmental events in Brassica napus L. embryogenesis are characterized by transverse segmentations, however the exact hypocotyl/radicle boundary, and thus the radicle territory within embryo axis, is not obvious. To reveal this boundary in the developing embryo from the torpedo stage to maturity, we stained the embryos in toto with the reactive dye, Procion Blue MX-R. Our method showed a definite organ-specific staining pattern: the embryo hypocotyl was unstained, and the stained region revealed the radicle territory proper. This was proven experimentally by radicle decapitations at different levels and direct observations of the developmental fate of cells. It was concluded that the determination of the cellular developmental fate in files of radicle cells is position-dependent and polar-organized and that the radicle zonation pattern is the consequence of this determination. Cells located between the basal boundary of the stained region and the basal radicle cap boundary (level L1), are the cells of the prospective root elongating zone, while cells below level L1 are components of the prospective meristem. Therefore, the radicle consists of the presumptive meristem and the presumptive elongating zone. This zonation pattern is established during embryo development, at the torpedo embryo stage or earlier. Mother cells of the presumptive elongating zone in the rhizodermis lose their meristematic activity after 4 or 5 rounds of division, at which point they are committed to elongation. It is possible that the principle of "presumptiveness" in embryogenesis works similarly in plant and animal development.

D 309 DOMINANT MUTATIONS IN THE *ARABIDOPSIS CDC2* GENE CAN AFFECT THE NORMAL PLANT DEVELOPMENT, Paulo C.G. Ferreira, Adriana S. Hemerly, Marc Van Montagu and Dirk Inzé, Laboratorium voor Genetica, Universiteit Gent, B-9000 Gent, Belgium

In plants, development is mostly post-embryonic. Plant organs are formed continuously as a result of cell division and differentiation in meristematic regions. The control of cell division is regulated by an intrinsic developmental program and by environmental signals as well. The activity of the regulatory molecules that regulates the cell cycle must be under stringent control and perturbations in this regulation can change the normal development program of the plant. Therefore, de-regulation of meristematic activity, caused by abnormal cell divisions, could help to reveal some of the controls of plant development.

One of the key regulators of the cell cycle is the product of the *cdc2/CDC28* genes, known as $p34^{cdc2}$. In the yeasts, several dominant mutations in the *cdc2/CDC28* genes have been isolated. These mutations interfere with the normal controls of cell cycle progression, causing either cell cycle arrest – the $Cdc2^-$ phenotype – or accelerate cell division leading to small, aberrant cells, the Wee phenotype.

We have previously isolated an *Arabidopsis cdc2* functional homolog cDNA [1]. Point mutations that caused dominant mutant phenotypes in the yeasts were introduced in the plant cDNA. *Arabidopsis* plants were transformed with the wild-type and mutant forms of the *cdc2* cDNA under the control of the 35S and the 2S seed storage promoters. The transgenic plants obtained display abnormal patterns of development. An analysis of the phenotype of the plants will be presented.

Reference

1. Ferreira, P.C.G., Hemerly, A.S., Villarroel, R., Van Montagu, M., and Inzé, D. (1991). The *Arabidopsis* functional homolog of the $p34^{cdc2}$ protein kinase. *Plant Cell* 3, 531-540.

D 310 MICROSURGICAL AND HORMONAL ANALYSES OF AN ARABIDOPSIS MUTANT ALTERED IN ORGAN ELONGATION,

Roxanne H. Fisher[1], Kathy Barton[2], Jerry D. Cohen[3], R. Scott Poethig[2], Todd J. Cooke[1] [1]Dept. of Botany, Univ. Maryland, College Park, MD 20742; [2]Dept. of Biology, Univ. Pennsylvania, Philadelphia, PA 19104; [3]Horticultural Crops Quality Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705

We have isolated a seedling-lethal mutant called gordo following EMS mutagenesis of Arabidopsis seeds. gordo segregates as a nuclear recessive. If grown on enriched medium, all the multicellular organs of this mutant exhibit radial expansion instead of uniaxial elongation and its stunted inflorescence fails to set seed. Wild-type and gordo embryos were dissected out of developing seeds in order to perform microsurgical and hormonal studies. The roots of gordo torpedo embryos surgically separated from the apex elongate three times as much as those roots which remain attached to the apex. Wild-type heart embryos treated with napthalene acetic acid mimic the gordo phenotype, but other auxins and auxin antagonists have no significant effect on the length-to-width ratios of wild-type and gordo embryos. The free auxin levels (ng/g f.w.) of gordo seedlings are more than eight times the levels of free auxin found in wild-type seedlings. The ethylene levels (nmole/plant) of gordo seedlings are three times those found in wild-type seedlings. These results indicate that the mutation in gordo may lie in a gene encoding an auxin conjugating enzyme, leading to the higher levels of free auxin present in this loss-of-function mutant. The higher auxin levels may, in turn, induce the higher levels of ethylene which may cause the radial expansion of the mutlicellular organs in the mutant. This work was supported by USDA-CRGO-89-37261-4791 and USDA-CRGO-91-37304-6655.

D 311 GENETIC AND MOLECULAR ANALYSIS OF DOMINANT LEAF MUTATIONS IN MAIZE HOMEOBOX GENES, John Fowler, Phil Becraft and Michael Freeling, Department of Plant Biology, University of California, Berkeley, CA 94720

We have focused on characterizing mutants which affect the morphology of the maize leaf in order to begin to understand the genes which are involved in regulating its development. The boundary between the leaf blade and sheath is sharply defined by the ligule and the auricle. Dominant *Rough sheath-1, Liguleless-3* and *Liguleless-4* mutations alter the position of this boundary and eliminate or displace the ligule and auricle in specific regions, or domains, of the leaf. Tissue-specific genetic markers and morphological characterization of mutant plants shows a transformation of cells which normally adopt a blade cell fate into ones which instead adopt a more sheathlike cell fate. *Rs1* is defined by three mutant alleles with similar phenotypes, and *Lg3* and *Lg4* are each defined by single alleles, however, new revertant alleles of both *Rs1* and *Lg3* have been recovered from *Mutator*-transposon lines. Certain genetic interactions of these mutations with each other, and with the *Kn1-O* mutation, suggest that all four function in a similar manner.

The similarity of the phenotypes of these mutations to that of *Kn1* mutants (reviewed in Freeling, Devel. Biol. 153, 1992) suggested that all four genes might encode proteins with similar protein motifs. The KN1 protein has recently been shown to contain a homeodomain and to be part of a larger gene family (Vollbrecht, *et al.*, Nature 350, 1991). Using populations which were segregating for *Rs1*, *Lg3* and *Lg4*, we have shown that all three genes are closely linked with homeobox sequences, and therefore may be mutations in these homeobox genes. Both the *Rs1*- and *Lg3*-linked homeobox genes have been cloned, and the identity of the two clones has been confirmed by Southern analysis of *Mutator*-induced revertant alleles. Comparison of predicted amino acid sequence in the homeodomain region shows a high degree of conservation among the KN1, RS1, and LG3 proteins. RNA expression analysis suggests that the mutant phenotypes result from ectopic expression of these genes in the leaf. J.F. is an HHMI Predoctoral Fellow.

D 312 DEVELOPMENTAL CONTROL OF CELL DIVISION: SPATIAL AND TEMPORAL REGULATION OF *CDC2* EXPRESSION, Adriana Hemerly, Paulo Ferreira, Janice Engler, Gilbert Engler, Marc Van Montagu and Dirk Inzé, Laboratorium voor Genetica, Universiteit Gent, B-9000 Gent, Belgium

Cell division in plants is subjected to multiple levels of regulation. At cellular level, there is an intrinsic biochemical and molecular mechanism controlling all dividing cells that seems to be similar in all eukaryotes. At the whole plant level, there are also external controls that strictly regulate cell divisions, temporally and spatially, according to the developmental plans and programs the organism follows.
To understand how cell division is coupled to plant development, we have studied the regulation of the *cdc2* gene - a key regulator of the cell cycle – in *Arabidopsis thaliana*. Firstly, the control of expression at cellular level was characterized. *Arabidopsis* cell suspensions blocked in different phases of the cell cycle have only a small decrease in *cdc2* mRNA level when compared with actively cycling cells. However, upon an increasing period of starvation, the amount of *cdc2* transcripts gradually decreases and will raise again as the cells reinitiate division. To investigate the *cdc2* gene regulation during development, we have fused the *Arabidopsis cdc2* promoter with ß-glucuronidase (GUS) as a reporter gene and transferred it into *Arabidopsis* plants. Histochemical analyses of GUS activity in transgenic plants showed that the *cdc2* gene is developmentally regulated, being expressed mainly, but not exclusively, in dividing cells. Roots were used as a model system to investigate the effect of plant hormones in the *cdc2* expression. Treatment with different hormones demonstrated that *cdc2* transcription can be induced or inhibited, and furthermore, these responses can be triggered in different regions of the root according to the hormone applied.

D 313 VP1 IS A REPRESSOR AS WELL AS AN ACTIVATOR OF TRANSCRIPTION IN THE DEVELOPING SEED. Ute Hoecker, Leonard Rosenkrans, Indra K. Vasil and Donald R. McCarty. Horticultural Sciences Department, University of Florida, Gainesville, FL 32611.

The *Vp1* gene of maize encodes a functionally complex transcription factor that is required for activation of abscisic acid (ABA) regulated genes expressed during maturation of the seed. In addition to blocking maturation related gene expression, mutations in *vp1* cause or allow derepression of the aleurone germination response during seed development. In order to investigate VP1's role in inhibiting hydrolase gene expression, 35S-Sh-VP1 and a barley high pI α-amylase promoter-GUS reporter construct (JR254, a plasmid here after referred to as Amy-GUS, was kindly provided by John Rogers, Washington U.) were introduced by particle bombardment into aleurones of developing and germinating seeds of maize and barley. Over-expression of VP1 caused a 90-95% reduction in Amy-GUS expression in maize aleurone and specifically blocked gibberillic acid (GA) induction of Amy-GUS in barley. The repressor activity was not affected by deletion of the acidic activation sequence in VP1 suggesting that VP1 mediated repression does not require activation of intermediate genes. The VP1 protein, therefore, apparently functions directly as a repressor. Deletion analysis of VP1 allowed further discrimination of sequences required for gene activation and repression. Significantly, certain internal deletions of VP1 converted it from a repressor to an activator of Amy-GUS. A model for activation/repression based on these results will be presented.

D 314 THE SHOOT MERISTEM OF IMMATURE WHEAT EMBRYOS: A MODEL SYSTEM FOR MERISTEM STUDIES, Victor A.Iglesias, Andreas Gisel, Nathalie Leduc, Shibo Zhang, Christof Sautter and Ingo Potrykus, Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland.

Key areas of research in meristems (e.g. competence for stable transformation, cell fate, virus replication, gene expression and specific promoters) suffer from the lack of a system which can transform exclusively meristem cells. It is expected that using ballistic micro-targeting (Sautter et al., Biotechnology 9: 1080 [1991]) on apical meristems of immature wheat embryos such a transformation can be achieved and would serve as an excellent tool for these studies.
Immature wheat embryos can be obtained all the year round, from greenhouse grown plants. They are relatively easy to excise and manipulate. Seven day old embryos have a newly formed and exposed meristem and can germinate to normal fertile plants. The exposed meristem is an ideal target for microprojectile-mediated transformation using micro-targeting. Micro-targeting allows bombardment of meristem cells, excluding other cell types such as the scutellum or coleoptile. In the case of stable transformation, a cell lineage beginning at this point would , theoretically, involve a large part of the shoot.
Anatomical studies of immature embryos, optimisation of particle delivery (to exclude or include other tissues), and transient expression in meristem cells using different marker genes and promoters will be presented. Variation in expression patterns between meristem cells and other cell types (scutellum and coleoptile) will be reported.

D 315 EXPRESSION OF HOMEOBOX CONTAINING GENES DURING MAIZE DEVELOPMENT. David Jackson and Sarah Hake, U.S.D.A. Plant Gene Expression Centre, 800 Buchanan St., Albany, CA 94710.
A set of dominant mutations of the *KN1* gene in maize lead to abnormal leaf development, where extra cell divisions in the leaf blade lead to distorted growth or knots and photosynthetic development around the veins is altered. Isolation of the *KN1* gene using a Ds2 tag revealed that the encoded protein contains a homeodomain. The KNOTTED protein is expressed in vegetative and floral meristems as well as in developing veins in the stem, but is not expressed at all in lateral organs, suggesting that the *KN1* gene product is involved in the maintainance of the undetermined state of plant meristems.

A number of clones related to *KN1* by the homeobox were isolated from cDNA libraries. Two of these also correspond to known dominant leaf mutations (Freeling lab., pers. comm.). We have used *in situ* hybridisation to determine the sites of expression of members of the *KN1* class of homeobox genes, and will present data showing that, like *KN1*, they are also expressed in meristems and in the stem, but not in lateral organs. The patterns of expression in meristems and stem, however, differs for the different homeobox genes.

D 316 THE TERMINAL SHOOT MERISTEM OF SUNFLOWER IS DETERMINED FOR INFLORESCENCE FORMATION IN THE DEVELOPING EMBRYO BY SEVEN TO NINE DAYS AFTER ANTHESIS, Dorothy E. Jegla and Stacey R. Allen, Department of Biology, Kenyon College, Gambier, OH 43022

The terminal shoot meristem of sunflower initiates a predictable number of vegetative nodes and then produces an inflorescence. Culture and grafting experiments indicate that even the meristems of young seedlings can make fewer but not more the expected number of vegetative nodes before producing the inflorescence. This suggests that the sunflower apex is already determined for inflorescence formation in the young seedling. In order to ascertain the time at which the apex becomes determined for inflorescence development, we cultured apices of dry seed embryos that were imbibed for 12 hours, and apices of developing sunflower embryos ages 7-9, 10-12, and 13-15 days post-anthesis. Dry seed embryos generally have two pairs of leaf primordia. Microscopic examination indicated that the first pair of leaf primordia appear between 7 and 9 days; the second pair between 12 and 13 days. Explants included the meristem, the leaf primordia, in some cases the basal 1/4 of one cotyledon, and 0.5mm or less of the axis below the meristem. Apices of embryos of all ages produced inflorescences in culture after producing fewer than the predictable *in situ* number of vegetative nodes. Thus the sunflower apex is determined for inflorescence formation in the developing embryo by 7-9 days after anthesis.

D 317 MAIZE ADH2 EXPRESSION RESPONDS TO DEVELOPMENTAL-STAGE, TISSUE- AND CELL-SPECIFIC SIGNALS. Barbara Kloeckener-Gruissem and Michael Freeling, Department of Plant Biology, University of California, Berkeley, CA 94720.

In anaerobic seedling roots alcohol dehydrogenase enzyme activity derives from dimerized monomeres encoded by two known unlinked genes, *Adh1* and *Adh2*, which are both simultaneously expressed in all cells. Using in situ staining method we studied the pattern of ADH2 activity in roots of one week old seedlings which carry a null mutation in the *Adh1* locus. Surprisingly, we detected a new pattern of ADH2 expression which appears disconnected from the expression of ADH1. Unexpectedly, ADH2 activity is localized to only epidermal cells and is confined to a short region of about 0.7 mm along the longitudinal axes of root development and growth, that reaches from the meristematic region of the root tip to the differentiated region of the central vascular strands. **Since this very specific ADH2 activity** pattern is localized to cells that are actively dividing and are at the developmental stage just before first signs of cellular differentiation, we will investigate a potential function during the establishment of developmental patterns.

D 318 **THE PATTERNS OF GENE EXPRESSION IN THE VEGETATIVE SHOOT APICAL MERISTEM**

Cris Kuhlemeier, Andrew J. Fleming and Therese Mandel, Institute of Plant Physiology, University of Berne, CH-3013 Berne, Switzerland.

The vegetative shoot apical meristem consists of several hundred small, densely cytoplasmic cells. We are interested in studying how the cells in the apical meristem differentiate to give rise to an exactly determined pattern of leaf primordia. In order to do this it is necessary to know in detail the organization of the meristem. In classical studies the meristem has been subdivided either into three concentric layers (L I, L II, L III) or into three zones (central, peripheral, rib zone). Since changes in gene expression are likely to underly cellular differentiation, we used in situ hybridization to study whether the cells of the meristem could be classified based on differences in the expression of genes. We constructed a cDNA library from carefully dissected tomato shoot apical meristems and used clones from this library, and other published cDNAs, to construct a refined molecular map of the meristem. The data collected for over 20 genes indicate that (1) no genes are expressed exclusively in the meristem (2) most genes are expressed in the meristem either uniformly or in layered patterns (3) zonal patterns appear to be rare.

D 319 PATTERN FORMATION IN THE *ARABIDOPSIS* LEAF EPIDERMIS: GENETIC INTERACTIONS BETWEEN *GL1* AND *TTG* RESULTING IN CLUSTERS OF TRICHOMES. John C. Larkin[1], Ellen T. Paparozzi[2], and M. David Marks[1], [1]Division of Biological Sciences, and [2]Department of Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

Two of the most fundamental problems in developmental biology are: 1. How do cells aquire different developmental fates? and 2. How is the spatial arrangement of different cell types achieved? Trichome differentiation in the *Arabidopsis* leaf provides an opportunity to examine both of these questions at the level of the differentiation of a single cell type. Approximately one in 600 epidermal cells on the adaxial surface of the *Arabidopsis* leaf is a trichome. Recessive mutations in two genes, *GL1* and *TTG*, block the initiation of trichomes. The *GL1* gene has been cloned by T-DNA tagging, and exhibits sequence similarity to the Myb class of transcriptional regulators (Oppenheimer et al., 1991). Transgeneic plants containing the *GL1* gene expressed under the control of the CAMV 35S promoter (35SGL1) were crossed with *ttg* mutant plants to test hypotheses about order of function of *GL1* and *TTG*. Plants of the genotype *ttg/ttg*; 35S*GL1*/35S*GL1* failed to produce trichomes, indicating that *TTG* does not act upstream of *GL1* in the trichome initiation pathway. Unexpectedly, plants of the genotype *TTG/ttg*; 35S*GL1*/+ in the F1 generation of our original cross had an unusual phenotype. 30 % of the leaf trichomes of these plants occurred as clusters of 2-5 trichomes. In wild-type plants, only 0.6% of the trichomes occur in clusters, and we have never seen a cluster on a wild-type plant with more than 2 trichomes. Segregation ratios in the F2 are consistent with the hypothesis that a single recessive gene in the *ttg* parent is responsible for the "clustering" phenotype. We are currently testing whether the *ttg* mutation is responsible for this phenotype by examining F3 families. These results suggest that the *TTG* gene may play a role in selecting which protodermal cells are selected to differentiate as trichomes.

D 320 CHARACTERIZATION OF A KNOTTED-1 HOMOLOGUE IN ARABIDOPSIS, Cindy Lincoln, Lauren Hubbard and Sarah Hake, U.S.D.A. Plant Gene Expression Center, Albany, CA 94710 . Dominant mutations of the maize homeobox gene Knotted-1 (Kn1) alter the timing of cellular differentiation during leaf development. Although multicellular development in plants and animals differs dramatically, both systems require regulatory mechanisms that control the proper specification of cell fate. Homeobox genes play a major regulatory role in animal development and are likely to have a similar function in plants. In order to gain insight into the function of the Kn1 gene, a putative Kn1 homologue in Arabidopsis is being characterized. This gene was identified by screening an Arabidopsis cDNA library using a maize Kn1 hybridization probe. Demonstration that a functional Kn1 homologue exists in Arabidopsis will require sequence comparisons, careful examination of RNA and protein expression patterns and creation of "mutant" phenotypes through the use of overexpression or antisense expression experiments. A long term goal is to identify genes which interact with the Kn1 gene. The isolation of extragenic suppressors, a powerful technique for identifying additional genes in a specific developmental pathway, may lead to the identification of genes which interact with or bind the Kn1 gene product. Therefore, once a "mutant" phenotype in Arabidopsis has been generated, screens for extragenic suppressors will be carried out. Analysis of Kn1 gene action in Arabidopsis combined with knowledge already gained from a thorough genetic and phenotypic characterization of the Kn1 gene in maize will greatly enhance our understanding of the role of this homeobox gene during plant development.

D 321 Vegetative Meristem Genes, Bruce Link, Bibo Xu, June Medford Department of Biology Pennsylvania State University, University Park Pa 16803

We are working on how vegetative meristems function in plant development. Meristems have four primary functions: initiating new organs, initiating new tissues, sending signals to the rest of the plant, and maintaining themselves as formative regions. We are using both classical genetic studies and molecular biology approaches to construct a model for meristem function.

The vegetative shoot meristem goes through distinct stages: juvenile, and adult. The products of the juvenile and adult meristem can differ greatly in their form suggesting that there are underlying differences in gene regulation. We are currently analyzing a clone that was isolated by differential screening of a juvinile meristem PCR library. Expression analysis suggests that it is specific for the juvenile meristem. Further analysis indicates that this is a single copy gene in Arabidopsis. Work is in progress to localize gene expression by in situ hybridization.

We have also been using a forward approach by analyzing vegetative meristem mutants. One particularly interesting cross is the cross between the Forever young mutant (Fey) and the Fully fasciated mutant (Fuf). While the Fuf mutant has an enlarged meristem the Fey mutant appears to have a reduced ability to maintain itself as a formative region. Double mutants of Fey/Fuf lead to a multiple shoot phenotype, and disruption of product of the floral meristem. We will present our most recent analysis of this work.

D 322 GENETIC AND MOLECULAR CHARACTERIZATION OF ABNORMAL SUSPENSOR MUTANTS OF ARABIDOPSIS THALIANA, Tammy L. Lynam, Linda A. Castle, and David W. Meinke, Department of Botany, Oklahoma State University, Stillwater, OK 74078.

The zygote in flowering plants divides to form an embryo composed of two parts: the embryo proper and suspensor. The suspensor in Arabidopsis contains six to eight cells arranged in a single column. Abnormal development of the suspensor is a characteristic feature of many embryo-defective mutants of Arabidopsis. One model proposed to explain this pattern of abnormal development [Marsden and Meinke (1985) Amer J Bot 72: 1801-1812] is that continued growth of the suspensor is normally inhibited by the embryo proper; abnormal growth of the suspensor may result when this inhibitory effect is indirectly removed by a mutation lethal only to the embryo proper. We have begun to analyze in detail several T-DNA insertional mutants with abnormal suspensors in order to test this model and characterize developmental interactions between the embryo proper and suspensor. Emphasis has been placed on two tagged mutants (emb76 and emb177) generated by Ken Feldmann following Agrobacterium-mediated seed transformation. These mutants have been mapped to opposite ends of chromosome 1: emb76 is located within 1 cM of an whereas emb177 is within 2 cM of gl2. Mutant embryos become arrested at the globular (emb76) or late globular-heart (emb177) stages. A second allele of emb76 (untagged) with a similar pattern of development has been identified. Plasmid rescue has been used to recover plant sequences flanking the T-DNA insert in both emb76 and emb177. Probes made from these sequences hybridize to DNA isolated from both heterozygous and wild-type plants. Initial sequencing has failed to reveal any significant homologies with known genes. We have prepared a cDNA library from immature siliques to facilitate molecular analysis of these mutants. We recently initiated a collaboration with Gerard Lazo (Plant Biology Division, SR Noble Foundation, Ardmore, OK), who has identified a putative clone of EMB76 in an Agrobacterium library of Arabidopsis genomic sequences. Details of ongoing molecular analysis will be described. Supported by NSF grant # DCB-8905137.

D 323 TISSUE - AND ORGAN- SPECIFIC EXPRESSION OF HOMEOBOX-CONTAINING GENES FROM *Arabidopsis thaliana* AND CARROT. Jim Mattsson, Eva Söderman, Marie Svenson and Peter Engström, Dept of Physiological Botany, University of Uppsala, Box 540,75121, Uppsala,Sweden

To test the hypothesis that plants, like other eukaryotes use homeobox-containing genes to control developmental processes, we set out to isolate such genes from plants. Complementary DNA libraries made from rosette stage *Arabidopsis thaliana* plants and somatic carrot embryos, were screened with oligonucleotides matching the most conserved part in the homeodomain of the Drosophila gene *Antennapedia*. One clone was isolated from each library. The clone from *A. thaliana* was then used in a second screen to isolate additional clones from this plant. At present, six different homeobox-containing clones have been sequenced.

The deduced amino acid sequencees of these clones shows that: (1) The sequence similarity in the homeodomain ranges from 50-80% between the clones and at most is 30-40% to any homeodomain from the animal kingdom. (2) All have a leucine zipper motif carboxy-terminal to the homeodomain. (3) Although there is very little sequence similarity outside the homeodomain and leucine zipper regions, all proteins contain stretches rich in prolines, acidic residues or residues with hydroxyl-groups.

Northern hybridization experiments showed that the carrot clone is expressed at low levels during all stages of embryo development. Further, one gene from *A. thaliana* is expressed only in the leaf, wheras two others are expressed in all organs, although at different levels. A fourth gene from *A. thaliana* is specifically expressed in the cortex cells of the root as well as the stem.

D 324 THE LAM-1 GENE CONTROLS BLADE FORMATION THROUGH REORIENTATION OF DIVISION IN THE L3 CELL LAYER, Neil A. McHale, Department of Biochemistry and Genetics, The Connecticut Agricultural Experiment Station, New Haven, CT 06504

The leaves of angiosperms display a wide morphological diversity at maturity, but the early stages of development are strikingly similar. Conserved patterns of cell division in three cell layers (L1,L2,L3) control initiation of the primordium and formation of the blade. The molecular mechanisms specifying the position and polarity of these meristematic domains in the developing leaf remain unknown. We have initiated a genetic dissection of cellular mechanisms of leaf development in *Nicotiana* and *Arabidopsis*. We have isolated a bladeless mutant (*lam-1*) in *N. sylvestris*. Mutant leaves grow to adult length but remain bladeless. Anticlinal divisions are initiated in the two surface cell layers (L1, L2) at the normal blade initiation site, but mutant primordia fail to generate periclinal divisions in the underlying L3-derived cell layer. These divisions normally generate an advancing core of two cell layers destined for the middle mesophyll in proximal regions of the blade. These observations suggest that the onset of anticlinal divisions in L1 and L2 constitutes selection of a blade initiation site on the flank of the primordium, but that outward progress of the blade hinges on a LAM-mediated induction of periclinal cell division in the L3 founder cells of the middle mesophyll. Anticlinal divisions in L1 and L2 show an early attenuation in the mutant, suggesting that sustained division in surface layers requires communication with actively dividing L3 cells.

D 325 DO FLAVONOIDS PLAY A ROLE IN ALFALFA ROOT NODULE DEVELOPMENT? Heather I. McKhann and Ann M. Hirsch, Dept. of Biology, University of California, Los Angeles, Los Angeles, CA 90024

During the symbiosis bewteen legumes and bacteria of the genus *Rhizobium*, a novel structure is formed on the plant root, a nitrogen fixing root nodule. This symbiosis therefore provides a model system for understanding events in plant development. Flavonoids released from alfalfa seeds and roots are known inducers of *Rhizobium* nodulation genes. Flavonoids can also compete with the synthetic auxin transport inhibitor, NPA (*N*-1-(naphthyl)phthalamic acid) for binding to its receptor. NPA is a molecule which has been shown to elicit the formation of nodule-like structures on alfalfa roots. We therefore hypothesized that flavonoids, acting as natural auxin transport inhibitors, may be involved in nodule development by altering the endogenous hormone balance locally. To gain a better understanding of the roles that flavonoids play in nodulation, we have cloned cDNAs for two key enzymes in flavonoid biosynthesis, chalcone synthase and chalcone isomerase, and are studying their spatial and temporal patterns of expression in alfalfa roots. Four different chalcone synthase cDNAs have been isolated and characterized from an alfalfa nodule cDNA library. The differential expression of the chalcone synthase gene family members has been studied with northern analysis and RNase protection studies using gene-specific probes. At least one gene family member shows symbiosis-enhanced expression. The expression pattern of chalcone synthase will be compared in uninoculated roots versus roots inoculated with wild type or mutant *R. meliloti*. In contrast, chalcone isomerase is encoded by 1-2 genes in alfalfa. Its expression in uninoculated and inoculated roots has also been examined using northern analysis.

D 326 Mutants Disrupting the Arabidopsis Vegetative Shoot Apex. June I. Medford, and Fred Behringer. Department of Biology, The Pennsylvania State University, University Park PA 16802

We are focusing our studies on a molecular and genetic study of plant meristems. In order to understand meristem functions we have identified a number of mutants that disrupt, or alter, meristem tissue and organ formation.

One of the mutants, Schizoid (Shz), disrupts the ability of the meristem to form cells and tissues of the stem. The Shz disruption is seen in the main stem but not in any axillary stems, indicating that there is either a tight temporal control of the Shz gene product or that there is a difference between the main apical meristem and axillary meristems. This mutant was generated by T-DNA insertional mutagenesis (Feldmann, 1991). Linkage analysis indicates that the schizoid gene is tagged by a T-DNA insert. Work is in progress to clone the Shz gene.

Another mutant causes leaves to form in a cup or grail shape and was named Holy grail (Hgl). This remarkable phenotype was previously observed by workers microsurgically dissecting the shoot meristem and was attributed to a disruption in communication between the meristem and leaf primordia. We are dissecting the phenotype and found that it may be the result of lesions in more than one gene. One mutant phenotype segregating from the population produces plants where the leaves are centric i.e. lack a dorsiventral signal. The meristem in this mutant greatly enlarges yet no organs with a determinate growth pattern (e.g., leaves) are produced. Work is in progress to further characterize the Holy grail or related mutants and to determine if any are tagged with a T-DNA insert.

D 327 A HOMEOTIC MUTANT OF ARABIDOPSIS THALIANA PRODUCES LEAFY COTYLEDONS, David W. Meinke, Department of Botany, Oklahoma State University, Stillwater, OK 74078

Cotyledons are specialized leaves produced during plant embryogenesis. Cotyledons and leaves typically differ in morphology, ultrastructure, and pattern of gene expression. The leafy cotyledon (lec) mutant of Arabidopsis thaliana described in this poster fails to maintain this distinction between embryonic and vegetative patterns of development. Mutant embryos are phenotypically abnormal, occasionally viviparous, and desiccation intolerant, but can be rescued in culture prior to desiccation. The resulting seedlings appear normal except for their cotyledons, which contain trichomes characteristic of leaves, lack protein and lipid bodies characteristic of cotyledons, and exhibit a vascular pattern intermediate between that of leaves and cotyledons. These results suggest that lec cotyledons are partially transformed into leaves, and that LEC functions to activate a wide range of embryo-specific pathways in higher plants. Although many homeotic aberrations have been observed in plants, mutations that transform embryonic cotyledons into foliage leaves have not been previously reported. Leafy cotyledon demonstrates that a single gene controls many of the important differences between leaves and cotyledons in Arabidopsis. The LEC gene product appears to be required for proper expression of embryo-specific functions during plant development. In the absence of LEC function, mutant cotyledons revert to a primitive developmental state that is surprisingly leaf-like. Mapping with visible markers has demonstrated that lec is not a severe allele of an existing ABA mutant. Mutant embryos are also not resistant to ABA in culture. The discovery by J. Giraudat that ABI3 shares regions of high sequence similarity with VP1 of maize and may perform a related function in Arabidopsis is consistent with the view that basic mechanisms of embryonic maturation are conserved among angiosperms and that LEC performs a critical function not previously described in higher plants. Molecular isolation of LEC and overexpression in transgenic plants could thus lead to enhanced maturation of somatic and zygotic embryos from a variety of plant species. Supported by NSF grant # DCB-8905137.

D 328 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN MAIZE VIVIPARY MUTANTS, Kathryn B. Moore[1] and Karen K. Oishi[1,2,3], [1]Department of Molecular and Cellular Biology, [2]Department of Plant Sciences, [3]Committee on Genetics, University of Arizona, Tucson AZ 85721

End products of the isoprenoid biosynthetic pathway play major roles in metabolism, growth and development of plants. There is evidence that isoprenoid compounds are necessary during seed development and may function to regulate specific developmental processes. In particular, the growth regulator abscisic acid (ABA) has been shown to be absolutely required for the seed to control or maintain aspects of dormancy and water relations. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) catalyzes the first committed step in isoprenoid biosynthesis, the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate. We have shown that the specific activity of HMGR during Zea Mays seed development is regulated in both seed tissues, the embryo and endosperm. To further investigate the regulation of HMGR activity during normal seed development, we have examined whether increased ABA content in the seed tissues correlates with observed decreases in HMGR activity. Several maize vivipary mutants, which block steps in ABA biosynthesis and do not undergo dormancy, have been used to directly test whether ABA regulates HMGR activity during seed development. To determine the in vivo effect of ABA levels on HMGR activity, endogenous ABA concentrations as well as microsomal HMGR activity have been measured during seed development in these maize vivipary mutant lines. HMGR activity in vp2 and vp7 embryos has been shown to be greater than wildtype activity from 14-16 days after pollination, during early maturation. In later seed development, from 20 to 30 days after pollination, HMGR activity in mutant embryos decreases below wildtype levels. In endosperm, HMGR activity in vp2 seeds are significantly higher than levels in wildtype seeds from 14-25 DAP. This may be the first indication that ABA may be involved in the regulation of HMGR activity during seed development.

D 329 IDENTIFICATION AND CHARACTERIZATION OF DECREASED APICAL DOMINANCE MUTANTS IN PETUNIA HYBRIDA, Carolyn Napoli, Department of Environmental Horticulture, University of California, Davis, CA 95616

A mutational analysis of plant growth habit has been carried out in an inbred *Petunia hybrida* line using the chemical mutagen ethane methyl sulphonate. Two genetic loci have been identified which control the degree of axillary branching and result in the phenotypic expression of decreased apical dominance (dad). The parental inbred Petunia line typically demonstrates an unbranched growth habit, when plants are grown under conditions that promote flowering. However, Petunia dad mutants have a compact and highly branched growth habit under the same environmental conditions. Dad mutants mature slower than parental plants and require a longer time to flower. A total of six independent dad mutants have been identified from approximately 950 separate M2 families. The phenotypes of all six mutant plants are indistinguishable from each other. All mutations are due to single recessive genes which map to two unlinked genetic loci called *dad1* (two alleles) and *dad2* (four alleles). All four *dad2* alleles demonstrate phenotypic instability and revert at a high frequency to the wild type "increased apical dominance" phenotype. The nature of this instability is under investigation. The genetic analysis of these mutant phenotypes and preliminary studies toward a characterization of the mutant phenotype will be presented.

D 330 THE PROMOTER OF THE ALEURONE SPECIFIC BARLEY *Ltp2* GENE CONTAINS A FUNCTIONAL MYB BINDING SITE. Peter S. Nielsen, Roger Kalla, Robert Potter, Casper Linnestad, Danny N.P. Doan and Odd-Arne Olsen. Plant Molecular Biology Lab. Agricultural University of Norway, P.O.Box. 5051, N-1432 Aas, Norway.

As part of our efforts to understand barley aleurone cell development, we have previously cloned the aleurone specific gene *Ltp1* (Linnestad et al., Plant Physiol. **97**, 841, 1991), which encodes a 10 kD non-specific lipid transfer protein (ns-LTP). Here we present a second aleurone specific gene, *Ltp2*, that encodes a novel barley 7 kD ns-LTP. The *Ltp2* transcript accumulates in the endosperm from 9 days after pollination (DAP), which coincides with the appearance of the first recognizable aleurone cells in the barley grain, and levels off about the mid-stage of seed development. By *in situ* hybridization analyses we have shown that the *Ltp2* transcript represents a molecular marker for the onset of aleurone cell differentiation. The *Ltp1* transcript appears around 20 DAP, and is present in the aleurone layer of mature as well as germinating seeds.

The *Ltp1* and *Ltp2* promoters both contain consensus binding sites for the Myb and Myc transcription factors, in a similar position relative to the transcription start sites and with equal spacing in both genes. In maize, the Myb and Myc binding sites in the promoter of the anthocyanin structural gene *Bz1* are essential for the C1 and B mediated *trans*-activation of *Bz1*. The *Ltp2* promoter furthermore contains the sequence element CATGCATG, which is required in the promoter of the maize C1 gene for *trans*-activation of *C1* by VP1 (Hattori et al., Genes & Dev. **6**, 609, 1992). Transient expression studies, using biolistic bombardment of barley endosperm, have shown that the -805 bp promoter of *Ltp2* is active in aleurone cells. Moreover, in gel shift assays the Myb binding sites from the *Ltp1* and *Ltp2* promoter were shown to form specific complexes with a truncated chicken c-Myb protein. Studies, using promoter deletions, to test the significance of the Myb, Myc, and VP1 binding sites for the expression of *Ltp2* in transient assays are currently undertaken. In addition, progress on the isolation of barley Myb and Myc genes expressed in immature endosperm tissues will be presented.

D 331 ISOLATION OF TRANSCRIPTS INVOLVED IN CELL WALL FORMATION DURING THE SYNCYTIAL STAGE OF BARLEY ENDOSPERM DEVELOPMENT. Odd-Arne Olsen and Danny N.P. Doan, Plant Molecular Biology Laboratory, Agricultural University of Norway, P.O.Box. 5051, N-1432 Aas, Norway.

Based on microscopy studies of wild type and sex-mutant endosperms we divide endosperm development into four stages, each marking major events in the differentiation process (Bosnes et al., Plant J., Sept, 1992). During the **Syncytial** stage (I), a syncytium containing the endosperm nuclei is formed in the periphery of the embryosac (IA). Preceding endosperm cell wall formation, the syncytium becomes vacuolarized (IB). In the **Cellularization** stage (II), the cell walls first appear as projections between individual endosperm nuclei perpendicular to the embryosac wall. Later, cell walls from opposite sides of the embryosac meet, leading to the cellular endosperm. During the **Differentiation** (III) and **Maturation** (IV) stages the two endosperm tissues, ie the aleurone layer and the starchy endosperm, differentiate and mature.

The poster presents our efforts to clone transcripts involved in endosperm cell wall formation. In our view, the endosperm is a good model-system for the study of cell-wall formation for several reasons. First, the transcription-rate in the endosperm nuclei increase at least six-fold during stage IB prior to cell wall formation (Bosnes and Olsen, Planta 1992,vol. 186:376). Thus, a large fraction of the transcripts at the beginning of stage II may encode proteins involved in cell wall formation. Second, embryosacs containing the developing endosperm during stage I and II can be isolated, allowing efficient differential screening of cDNA-libraries for relevant clones. Thirdly, barley sex-mutants in which cell wall formation is either missing or delayed are useful both in differential screening strategies as well as in ultrastructural and histochemical characterization of the growing cell wall.

D 332 ROLE OF VIRAL MOVEMENT PROTEINS IN THE PATHOGENESIS OF SQUASH LEAF CURL VIRUS,
Erica J. Pascal, Paige E. Goodlove, and Sondra G. Lazarowitz, Department of Microbiology, University of Illinois, Urbana, IL 61801

The bipartite geminiviruses such as squash leaf curl virus (SqLCV) encode three proteins essential for systemic movement and disease development in the plant. While one of these proteins (AL2) has been implicated as a transactivator of viral gene expression, the role of the movement proteins BR1 and BL1 in pathogenesis remains to be elucidated. To investigate both the function and potential interactions of these proteins with host and viral proteins, we have constructed transgenic plants expressing these movement proteins in various combinations. Plants expressing AL2 and/or BR1 appear to be phenotypically normal. Rather unexpectedly, plants expressing BL1, either singly or in combination with other viral proteins, phenocopy the viral disease symptoms, which include leaf epinasty (curling under) and mosaicism. In addition to these characteristics, the BL1-expressing plants exhibit a "slow-grow" phenotype in which they are slow to shoot and slow to root, and exhibit decreased fertility, traits that appear to segregate in a Mendelian fashion. Thus, it appears that BL1 is at least in part responsible for disease symptoms in the infected plant. Cellular fractionation of transgenic plants and immunoblot analysis demonstrates that BL1 is localized to cell wall and cell membrane fractions. Electron microscopic analysis of thin sections to identify potential sub-cellular alterations and more precisely localize the viral movement proteins within the plant are currently in progress. These, as well as biochemical analyses to delineate interactions with other viral or host proteins and the role of the movement proteins in determining viral host range, will be presented.

D 333 GENETIC AND MOLECULAR ANALYSIS OF THE *de-etiolated-1, (det1)* MUTANT of *ARABIDOPSIS THALIANA*, Alan Pepper, Terry Delaney, Dan Poole, Tracy Washburn and Joanne Chory. Plant Biology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92186-5800.

Light plays a critical role in directing the development of higher plant seedlings. Etiolated (dark) growth in angiosperms is characterized by an arrested state of leaf development. Dicots, such as *Arabidopsis thaliana*, also display exaggerated axial growth of the hypocotyl or epicotyl. Light stimulates a switch in developmental programming called 'de-etiolation', during which axial growth is inhibited, leaf development is stimulated, gene expression patterns are altered, and etioplasts differentiate into chloroplasts. In normal development, light signals are integrated with intrinsic signals defining temporal and spatial specificity of gene expression and cell differentiation.

In *Arabidopsis*, we have identified a class of mutants that displays many of the characteristics of a light grown plant, when grown in the dark. These mutants designated *det* (for *de-etiolated*) are recessive, and fall into four complementation groups. Epistasis analysis suggests that the *DET1* gene acts downstream from phytochrome and other photoreceptors. The *det1* mutant is also defective in temporal and spatial regulation of gene expression and chloroplast development. We propose that *DET1* is a negative regulator of chloroplast and leaf development. The evolutionary acquisition of photoreceptor control over the activity of a spatial and temporal regulator of leaf development may have led to the profound photomorphogenic transition seen in seedlings.

In order to study the *DET1* gene product, we are in the process of cloning the *DET1* gene by chromosome walking followed by complementation of the mutant with cloned wild-type DNA sequences. In an effort to identify additional genes which act in the de-etiolation pathway, including those which interact directly with the *DET1* gene product, we have isolated second-site genetic suppressers of the *det1* mutation. A homozygous *det1-1* strain was mutagenized and screened in the dark for plants which exhibited some or all of the characteristics of the etiolated wild-type plant. Recessive and dominant mutations, a subset of which are extragenic to the *det1* locus, were obtained. The phenotypes of these mutants, which we have designated *teds* (since they reverse the *det1* phenotype) in both the dark and the light, will be discussed.

D 334 **GENETIC ANALISYS OF A HETEROCHRONIC ECOTYPE OF Arabidopsis thaliana THAT HAS DELAYED CONVERSION OF LATERAL MERISTEMS FROM THE VEGETATIVE TO THE REPRODUCTIVE PHASE,** **Vojislava Popadić-Grbić and Anthony Bleecker, Department of Botany, University of Wisconsin, Madison, WI 53 705.**

The shoot apical meristem of *Arabidopsis thaliana* goes through 3 phases during development: the phase 1 meristem is defined as a vegetative meristem that forms leaves organized into a rosette; the phase 2 meristem is the initial reproductive meristem that gives rise to cauline leaves and lateral inflorescence meristems; the phase 3 meristem produces flowers. At the transition from vegetative to reproductive development in wild type Landsberg erecta (LER), the primary meristem and all lateral meristems are coordinately converted into phase 2 meristems. The late flowering ecotype of Arabidopsis (SY-0) exhibits delayed conversion of the lateral meristems from vegetative to reproductive phase which results in aerial rosette formation at the nodes of the stem. Data from F1 crosses between SY-0 and LER, and analysis of segregating F2 and test cross (BC1) populations reveal that the observed heterochronic phenotype is a result of two dominant genes. Mapping of the two loci by RFLP analysis of F2 and BC1 segregating population is in progress.

D 335 CATALASE ISOZYMES ARE USEFUL MARKERS OF DIFFERENTIATION IN MAIZE TISSUE CULTURES.
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In maize initiation and maintenance of embryogenic culture is now well established if immature embryo is used as primary explant. The difficulties encountered with other tissues may be due to genetic constrains to gain totipotency correlated to developmental stages of the primary explant. Understanding the early steps of somatic embryogenesis the acquisition of "competence" of cell and meristem differentiation is important and could help to explain difficulties as genotype and primary explant dependence.

Searching for indicators of differentiation and regeneration at cell level we focused our attention on isozymes. The potential of isozymes as biochemical markers in maize tissue culture can be further exploited by an enzyme such as catalase that has many isozymic forms. In addition, there is exquisite tissue-and cell-specificity of these isozymes; this diversity presents a potentially powerful tool for analyzing the induction phase of an embryogenic culture and classifying the early stage of regeneration. The aims of this study were to correlate developmental phases with the presence of different catalase forms and to establish a biochemical markers for specific stages of maize tissue culture. We have examined callus induction from immature embryo and leaf tissue and early phases of plant regeneration in different inbred lines. The results obtained indicate that different catalase isozymes are developmentally expressed at tissue culture level. Callus initiation and subsequent plant regeneration involve the promotion of meristematic activity, its maintenance and its suppression where differentiation takes place. This process can be easily monitored by means of catalase isozymes, Cat-2, in particular, is especially useful in following the transition process from the undifferentiated to the differentiated state. Consequently, the changes in catalase isoforms can be used to define the developmental stages of the maize cultures.

D 336 Isolation of the *Glabra2* gene in *Arabidopsis thaliana*
William G. Rerie and M. David Marks. School of Biological Sciences, University of Nebraska, Lincoln, NE. 68588-0118.
Trichomes are cells which differentiate from the protodermal tissue of the apical meristem and develop to form visible hairs on the surfaces of leaves and stems of most plants. Genetic analysis of trichome mutants in Arabidopsis indicates that the genes involved in trichome development can be considered in two classes. One class determines which protodermal cells are destined to develop into trichomes, while the second subset of genes is responsible for establishing the final size and shape of the developing trichome cell. We consider this second class of genes to be an excellent model system for studying events which control cell expansion and hence cell shape in the plant. Plants homozygous for the glabra2 (gl2) mutation initiate trichome formation, but their trichomes appear to abort during the cell expansion phase, producing only stumps on the leaf surface while the stems appear completely glabrous (hairless). Interestingly, the phenotypic consequences of the gl2 mutation are most severe on the first leaf pair of the rosette. The gl2 mutation is also pleiotropic, in that plants homozygous for the gl2 allele do not produce a seed coat mucilage. Screening a population of Arabidopsis plants mutagenized by Agrobacterium T-DNA, we identified several mutants which were allelic to gl2. Genetic analysis of one particular line indicated co-segregation of the gl2 allele with the T-DNA insert. The presumptive T-DNA tag of the gl2 allele was used to clone approximately 24kb of wild-type genomic DNA at this locus. Molecular complementation of a gl2 mutant has delimited the Gl2 gene to a 6kb SmaI-EcoRV fragment within this region. Although the SmaI-EcoRV fragment successfully restored trichomes on the leaves and stems of the transformant, the trichome phenotype on the leaves was underbranched. Additional sequences adjacent to the EcoRV site resulted in wild-type trichomes developing on the leaves. The 6kb SmaI-EcoRV also complemented the seed-coat mucilage minus phenotype of the gl2 mutant. We are presently determining the nature of the Gl2 gene product and the localization of its expression within the leaf primordia.

D 337 DIFFERENTIAL CONSTITUTIVE EXPRESSION OF GLUCANOHYDROLASE GENES IN TISSUES OF UNINFECTED POTATO PLANTS REVEALED BY *IN SITU* HYBRIDIZATION AND IMMUNOLOCALIZATION

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We have analyzed the tissue-specific expression of chitinase, ß-1,3-glucanase and, for comparison, phenylalanine ammonia-lyase in healthy, unstressed potato plants, using RNA-RNA hybridization and immunostaining techniques to localize the respective mRNAs and proteins *in situ*. Organs examined included leaf, flower, apical meristem, node and axillary bud, stolon, tuber and root. While ß-1,3-glucanase and phenylalanine ammonia-lyase were present to varying extents and uniformly distributed in most tissues of all organs examined, chitinase exhibited a distinctive and characteristic expression pattern in being confined to the outer epithelia of all aerial parts of the plant. More complex patterns of chitinase expression were observed in stolon, tuber and root. These unexpectedly distinctive patterns of expression suggest fundamentally different roles for the enzymes in the metabolism of unstressed plants and, by extension, in the defense response.

D 338 A MUTANT AFFECTING THE DEVELOPMENTAL TIMING OF THE LEAVES AND SHOOTS OF MAIZE,
Denise Schichnes and Michael Freeling, Department of Plant Biology, UC Berkeley, Berkeley, CA 94720
Lax-Midrib 1-O (Lxm1-O) is an EMS induced dominant mutant first described by Dr. M.G. Neuffer, that primarily affects the midrib portion of the maize leaf, as well as all other regions of the maize plant. The Lxm1-O mutation expresses a heterochronic phenotype. Heterochrony can be defined here as a novel phenotype caused by a change in the developmental timing of the plant. The severity of the phenotype varies in different time-to-flowering backgrounds, being most severe in an early background. Lxm1-O heterozygotes, compared to their wild type siblings, have fewer nodes, particularly fewer juvenile nodes. Placement of reproductive structures along the main axis in Lxm1-O plants is also drastically different from wild type. These and other characteristics suggest that Lxm1-O could be abbreviating the juvenile stage. The Lxm1-O overall phenotype is similar to that of the heterochronic, systemic mutant, Hsf1-O (Bertrand & Freeling, 1990), but where Lxm1-O appears to abbreviate juvenility, Hsf1-O prolongs juvenility. While Lxm1-O is suppressed in a late-flowering background, Hsf1-O is exacerbated. Lxm1-O affects the middle lateral region of the blade (midrib), Hsf1-O affects the margins. We will argue that heterochronic, systemic mutants such as these could be important for fueling novelty during evolution.

D 339 AN ANALYSIS OF ARABIDOPSIS HOMEOBOX-CONTAINING GENES WHICH ARE MEMBERS OF THE *KN*-CLASS,
Kyle A Serikawa, Erik Vollbrecht, Sarah Hake and Patricia Zambryski. Department of Plant Biology, University of California, Berkeley, CA 94706
Homeobox-containing genes are firmly entrenched within the process of development in many different eukaryotic organisms Our expectation is that the same will hold true for plants. Already several different classes of homeobox genes have been isolated in plants. These include: the *Knotted* gene in maize and related sequences in maize and Arabidopsis; the HD-Zip homeobox genes in Arabidopsis; and the *Zmhox1a* and *1b* genes in maize. Our lab is studying three *Kn*-like genes in Arabidopsis. These sequences were originally isolated from Arabidopsis cDNA libraries using the *Kn* homeobox as a probe and all of our sequences show strong homology with *Kn*. We are undertaking analyses of both sequence data and expression patterns. Comparisons of cDNA sequences thus far demonstrate very high amino acid and nucleotide identity within the homeobox region as well as strong homology in regions 5 to the box proper. We have performed northern analyses on these genes and each displays a different pattern of expression in different gross tissue types. A more detailed analysis of one of our genes using *in situ* hybridization has shown that its expression is specifically localized to regions within the pedicels of the inflorescence and the pith of the stem of the developing plant. These patterns may indicate a role for this gene in vasculature differentiation or cell elongation Future directions for our work include greater characterization of the expression patterns of all of these genes and overexpression of these cDNAs in Arabidopsis to examine the effects of ectopic expression

D 340 CLONING, MAPPING, AND CHARACTERIZATION OF HOMEOBOX CONTAINING SEQUENCES IN TOMATO, Neelima Sinha*" and Sarah Hake^", * Biology Department, Boston University, Boston, MA 02215, ^ USDA Plant Gene Expression Center, Albany, CA 94710, and " Department of Plant Biology, U. C. Berkeley, Berkeley, CA 94720.

The homeobox, first described in Drosophila homeotic genes (Gehring, W. J. Science, **236**:1245-1252, 1987) encodes a sequence-specific DNA binding motif, the homeodomain. We have used the homeobox sequence isolated from the maize Knotted-1 gene in our laboratory (Vollbrecht et al., Nature, **350**:241-243) to clone homologous genes expressed in immature tomato fruit. A characterization of four of these cDNAs reveals that they are highly expressed in the female floral parts, post-fertilization ovaries, and immature tomato fruit. Partial sequencing has revealed the presence of Kn1-like homeodomains in two cDNAs. The sequence similarity in the homeodomain between the tomato cDNAs from tomato and Kn1 ranges from approximately 50-80%. This homology extends into the 5' region beyond the homeobox and may signify a conserved plant motif.

We have used Northern blotting and in situ hybridization techniques for the clones isolated to determine timing and cell specificity of expression. Three cDNAs have been mapped to chromosome positions using F2 populations from a cross between L. esculentum and S. pinnellii (Paterson et al., Nature, **335**:721-726). Two clones map to regions containing dominant leaf mutations in tomato and one of the cloned cDNAs identifies a DNA restriction polymorphism that cosegregates with a dominant leaf mutation on chromosome 2.

D 341 *ARABIDOPSIS THALIANA* LIGHT-REGULATED GENES ENCODING DNA-BINDING PROTEINS. Sjef Smeekens, Nicolette Quaedvlieg, Jan Dockx and Peter Weisbeek, Dept. of Molecular Cell Biology, University of Utrecht, Padualaan 8, NL-3584 CH Utrecht.

In plants light is an important signal for differentiation and development. We are interested in the signal-transducing mechanisms which couple light to development. In animal and plant systems developmental processes are often controlled by genes encoding transcription factors (TF). The approach we have taken is to isolate light-dependent TF-genes. For this purpose a TF-gene library was constructed by screening an *A. thaliana* genomic library with probes encoding known DNA-Binding motifs. This TF-library was screened with radiolabeled cDNA made from mRNA of seedlings which have been treated with different light-regimes. In this way over 25 clones have been identified whose expression is induced or suppressed by light.

D 342 THE EXPRESSION AND FUNCTION OF THE *KNOTTED-1* HOMEOBOX GENE DURING MAIZE EMBRYOGENESIS, Laurie G. Smith and Sarah Hake, U.S.D.A. and Univ. California Berkeley Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710

Dominant mutations in the maize homeobox gene, *KNOTTED-1*, alter patterns of cell division and differentiation in the maize leaf. We have previously shown that in wild-type plants, the *KN-1* gene is expressed at high levels in vegetative and floral shoot apices, but not in developing leaves. In dominant *KN-1* mutants, the *KN-1* gene product appears normal, but the gene is expressed ectopically in developing leaves, where it apparently interferes with the progression of leaf cells to their normal fates. We have proposed that in shoot apices, *KN-1* contributes to the maintenance of a relatively undetermined state, thus playing an important role in shoot apical meristem function. Consistent with this model, we show here that *KN-1* expression is initiated at the end of the transition stage of embryogenesis in a spatial pattern that is co-incident with the first morphological signs of shoot meristem formation. Embryos lacking *KN-1* function due to the presence of a deletion of the entire locus are developmentally arrested early in embryogenesis, lacking a shoot meristem and its products. However, further evidence will be required to prove that this phenotype is due only to the loss of *KN-1* function.

D 343 MECHANICAL FORCES IN THE SHOOT APEX COULD DEFINE SITES OF LEAF PRIMORDIA DEVELOPMENT, Guy L. Steucek[1], Jeanne Lang Selker[2], and Wolf Ernst Reif[3], [1]Department of Biology, Millersville University, Millersville, PA 17551, [2]Department of Biology, University of Oregon, Eugene, OR 97403 and [3]Institut und Museum für Geologie & Paläontologie, Universität Tübingen, Tübingen, BRD

Leaves and floral parts are formed in regular patterns by meristematic tissue in the apex of the plant shoot. Turgor and biophysical compliance of plant cell walls determine the morphology of cells and tissues. Since the outer walls of epidermal cells of the shoot apex must bear most of the turgor load of the tissue, the distribution of mechanical stress in the epidermis influences the external form of the shoot apex and may direct formation of leaf primordia. Hence the shoot apex can be modeled as an architectural shell. Finite element models of the shoot apex were used to predict the distributions of mechanical stresses within the outer walls of epidermal cells. The shoot apex of *Vinca minor* with a decussate phyllotaxy provided geometrical input for our models.

Early in the plastochron, islands of high shear stress and low plane stress are located at spots which will become the flanks of new leaf primordia. Changes in the geometry of the shoot apex, cellulose orientation in the outer walls of epidermal cells, and forces generated by the growth of existing leaves during the course of the plastochron enhance this effect in complementary fashion. Models of stresses in the shoot apex were validated by observing responses of the apex to microsurgery. With the use of sub-modeling, the distribution of stresses in all walls of epidermal cells was modeled at various locations on the shoot apex.

D 344 A GYMNOSPERM HOMOLOGUE TO THE MAIZE KNOTTED-1 GENE

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In an attempt to clone genes which potentially take part in the regulation of meristem activity and organ development in Picea abies (Norway spruce) we have isolated clones related to the Kn1 gene from maize. Kn1 is a homeobox-containing gene, which encode a putative transcription factor expressed in the apical meristem.
We used the Kn1 clone to screen a cDNA library constructed from spruce embryos treated with cytokinin to induce the formation of adventitioud buds. In a first screen we have isolated a clone (Kno13A) that most likely represents a homologue to the Kn1 gene. Partial sequence analysis of Kno13A revealed that it contains a homeobox with a high degree of similarity to the Kn1 homeobox. The deduced amino acid sequence of the homeodomain of Kno13A shows 84% identity to the Kn1 homeodomain but only 18-28% identity to homeodomains of other classes. Twelve amino acids in the homeodomain has been shown to be conserved among all or a majority of identified homeodomains. In three of these positions Kn1 deviates from the consensus and in all these positions Kno13A is identical to Kn1.

D 345 *bumbershoot*: AN *ARABIDOPSIS THALIANA* VEGETATIVE TO INFLORESCENCE TRANSITION MUTANT

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We have identified an *Arabidopsis thaliana* mutant, *bumbershoot* (*bum*), that affects the vegetative to inflorescence transition. In wild-type plants the vegetative meristem, which produces shoots and leaves, is transformed into the inflorescence meristem which gives rise to the floral meristems that eventually form the floral organs. This progression can be summarized as Vegetative -->Inflorescence -->Floral (V-->I-->F).

Initially *bum* mutant plants produce a regular rosette of vegetative leaves and an apparently normal shoot emerges from the rosette leaves. However after the inflorescence raceme has elongated several inches a new secondary rosette of leaves is produced instead of a coflorescence. Often two shoots emerge from this secondary rosette. The shoot, rosette leaves shoot pattern is repeated several more times before the individual shoots fully complete the transition to inflorescence growth. These same *bum* plants fail to produce any axillary shoots. Also common in *bum* mutant plants is the the development of rosette leaves attached to the side of a growing stem and/or the presence of a split meristem (i.e. several main shoots originate from a single stem which splits). The above phenotypes indicate that the *bum* mutation affects greatly the V-->I transtion, however the mutation also affects flower development as the mutant plants fail to produce functional carpels.

It is likely that *bum* plants are either unable to complete the V-->I transition initally and instead enter a V-->I-->V-->I developmental loop, or alternatively the mutation results in an imposition of the vegetative phase on inflorescence development. The latter possibility is reminescent of the *teopod* mutants of maize.

To analyze the *bumbershoot* mutant we are genetically mapping the locus, utililizing light microscopy and SEM to determine the cellular organization of the meristemic cells, and generating double mutants between *bum* and other vegetative and inflorescence meristematic mutants. We are particularly interested in the epistatic relationship between the inflorescence mutant *tfl1-1*, which leads to an early V-->I transition, and *bum* because these two mutants appear to have nearly the opposite phenotype.

D 346 CHARACTERIZATION OF *AGL3*, A MADS-BOX GENE WITH NON-FLOWER-SPECIFIC EXPRESSION.

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The *Arabidopsis* floral homeotic *AG* gene was previously found to be a member of a gene family, whose protein products share a sequence motif with known transciption factors SRF and MCM1 from humans and yeast, respectively (Yanofsky et al., Nature, 346:53-39). This motif, which is also been found in the *Antirrhinum* floral homeotic gene *Deficiens A*, has been called the MADS box. Six additional members of *Arabidopsis* MADS-box gene family have been identified and were called *AGL1* to *AGL6* (*AG*-like) (Ma et al., Genes & Dev., 5:484-495). These gene are preferentially expressed in flowers with the exception of *AGL3*, for which only partial sequence was obtained. We have now isolated and characterized the complete *AGL3* genomic and cDNA sequences, which indicate that *AGL3* shares the same general stucture with the other members of the *Arabidopsis* MADS-box family and that it has an glutamine-rich region near the C-terminus. We have analyzed *AGL3* expression using Northern and *in situ* RNA hybridizations, and the results show that *AGL3* is expressed in rosette leaves, stems, cauline leaves and seed pods and at a lower level in flowers, but is not expressed in roots. The fact that *AGL3* is expressed in a number of tissues suggests that it has a more general function that its floral counterparts. To characterize *AGL3* expression further and to study *AGL3* function, we have began experiments using promotor-GUS fusions and transgenic plants. Results from these experiments will also be presented.

D 347 GENES ESSENTIAL FOR EMBRYOGENESIS IN *ARABIDOPSIS THALIANA*

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Embryogenesis in higher plants is a complex process during which the tissue and organ systems are differentiated and the root and shoot apical meristems are specified to establish the polarity of the plant body. Virtually nothing is known of the molecular details involved in the organization of the primary tissue and organ systems during embryogenesis, or of the genes essential for these processes. Because the first ultrastructural indications of tissue differentiation are observed in globular to heart transition stage embryos, we have focused our studies on this stage of embryogenesis. We have taken a molecular genetic approach to study processes involved in cellular differentiation and organogenesis by identifying an array of *embryo lethal* mutants of *Arabidopsis thaliana* that produce morphologically abnormal embryos that are unable to complete the transition from the globular to the heart stage. Our objective is to identify and characterize genes that are essential for progression through the transition stage. Because these *embryo lethal* mutants are obtained from populations of plants that have been mutagenized insertionally with T-DNA, we have excellent prospects of isolating at least some of these genes.

Our specific aims are to morphologically characterize the effects of these *embryo lethal* mutations on cell differentiation during embryogenesis, to examine the effects of these mutations on the expression of stage specific genes during embryogenesis, and to isolate and characterize the genes that give rise to these morphologically abnormal phenotypes when inactivated. In the long term, we will analyze the temporal and spatial patterns of expressions of these genes in order to gain clues about their roles in embryogenesis.

D 348 DIFFERENTIAL RESPONSE OF MAIZE CATALASES TO ABSCISIC ACID: VP1 TRANSCRIPTIONAL ACTIVATOR IS NOT REQUIRED FOR THE POSITIVE ABA-MEDIATED REGULATION OF Cat1. John D. Williamson and John G. Scandalios. Department of Genetics, North Carolina State University, Raleigh, NC 27695.

Aerobic organisms have evolved enzymatic as well as nonenzymatic systems to minimize damage by externally and internally generated oxygen radicals. Enzymatic systems, such as the catalases and superoxide dismutases, scavenge oxygen radicals and convert them into less reactive species. The superoxide dismutases ($\cdot O_2^- : \cdot O_2^-$ oxidoreductase; EC 1.15.1.1; SOD) catalyze the disproportionation of two superoxide radicals ($\cdot O_2^-$) into hydrogen peroxide (H_2O_2), whereas catalases ($H_2O_2 : H_2O_2$ oxidoreductase, EC 1.11.1.6; CAT) catalyze the dismutation of H_2O_2 into H_2O and O_2. Working in concert, these enzymes convert the superoxide radical to water and molecular oxygen.

Analysis of RNA and protein accumulation in excised embryos of the maize inbred line W64A indicated that each catalase responds differently to exogenously applied ABA. Accumulation of *Cat1* transcript and protein is rapidly upregulated. In contrast, levels of *Cat2* transcript and protein are down regulated, while *Cat3* transcript levels are not affected. In developing embryos of the ABA deficient viviparous mutant *vp5*, lower endogenous ABA levels correlated with lower *Cat1* RNA accumulation. The maize viviparous mutant *vp1* is phenotypically insensitive to normal endogenous levels of ABA. Analysis of the response of *Cat1* to exogenously applied ABA in mutant and wild type *vp1* sibling embryos suggests that, unlike other ABA-responsive genes analyzed to date, the *Vp1* gene product is not essential for the ABA-mediated regulation of *Cat1*. Sequence analysis of the *Cat1* promoter revealed the presence of tandem *Em1a*, ABA concensus sequences, but no embryogenesis/ *Sph* concensus.

While all three catalases convert H_2O_2 to less toxic molecules, the individual maize catalases exhibit dramatically different patterns of developmental and tissue-specific expression, as well as differential response to various environmental signals. This differential response implies that promoter regions of each catalase gene contain unique sets of control elements resulting in a complex, multilayered pattern of response particular to each catalase. Mobility shift assays are being performed to evaluate involvement of various promoter elements in these responses.

Ref: Williamson, J & Scandalios, J (1992) *PNAS* (in press).

Evolution and Organization of Plant Genomes; Unifying Cellular Mechanisms

D 400 A MITOCHONDRIAL MUTATION ASSOCIATED WITH VARIEGATION AND ABNORMAL DEVELOPMENT IN TOBACCO, H. T. Bonnett and K. Glimelius, Department of Biology, University of Oregon, Eugene, OR 97403, and Department of Plant Breeding, Swedish University of Agricultural Sciences, 750 07 Uppsala Sweden

A variegated mutation appeared in the leaves of a tobacco cybrid plant resulting from fusion of protoplasts from tobacco with Petunia. This mutation was inherited maternally. The light green coloration of leaf sectors resulted from a substitution of spongy parenchyma for palisade parenchyma. No defects were detected in the chloroplasts of the plants, which were derived from Petunia. The mitochondria, as judged by the electrophoretic pattern of the DNA after digestion with restriction endonucleases, were very similar to mitochondria of tobacco, although with some unique cybrid-specific fragments. A second round of fusions was performed to confirm that mitochondria, not chloroplasts, were associated with the variegated phenotype. In these fusions, the Petunia chloroplasts of the variegated plants were replaced by tobacco chloroplasts. The mitochondria, according to the DNA restriction pattern, retained all or some of the unique cybrid-specific fragments found in the original variegated tobacco cybrid. Since the variegated phenotype remained after the chloroplast exchange, the chloroplast DNA cannot be the site of the mutation which is responsible for the mutant phenotype. This result eliminates the chloroplast and confirms that the mitochondrial genome is associated with the mutant phenotype.

D 401 USE OF PCR TO OBTAIN cDNA LIBRARIES FROM YOUNG CORN EMBYOS.

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We are interested in the genetic program governing early embryogenesis in plants. Among the different models available in our laboratory, we have focused our work on corn zygotic embryogenesis (1, 2). Sampling young embryos represents time consuming dissections. The use of PCR to amplify cDNA populations has allowed us to work with less material and selected embryos. mRNAs were extracted from different developmental stages and the corresponding cDNAs were amplified using non-selective primers (3). Amplified cDNAs appeared as smears after electrophoresis and EtBr staining. Amplification was obtained from as little as 1/10.000 of the cDNA from 1 mature embryo (30 Days After Pollination) after a second round of PCR and from 5 to 10 embryos of 10 DAP. When ABA responsive gene Rab 17 was used as a probe on Southern filters presenting the whole embryo development, the corresponding cDNA was only detected 30 DAP or later. This agreed with the published data and was confirmed by Northern hybridizations (4).We are currently preparing amplified cDNA libraries from fertilized embryo-sacs (5 DAP) to be screened for early embryogenesis specific genes.

(1) Gaillard et al., 1991, Plant Cell Rep. 10: 55-58
(2) Mol et al., 1992 in Press (Planta)
(3) Tam et al., 1989, Nucl. Acids Res. 16: 1269
(4) Vilardell et al., 1990, Plant Mol. Biol. 14: 423-432

D 402 ORGANELLAR AND NUCLEAR RIBOSOMAL GENE SEQUENCES WITHIN ONAGRACEAE: PHYLOGENETIC AND EVOLUTIONARY CONSIDERATIONS, Carol J. Bult and Elizabeth A. Zimmer, Laboratory of Molecular Systematics, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560

The primary structure for regions within the small subunit ribosomal RNA gene from nuclear, chloroplast and mitochondrial genomes is presented for species within the plant family, Onagraceae. The sequence data are used to assess the congruence of nuclear and organellar rDNA-based phylogenies within Onagraceae and reveal fundamental aspects of the pattern and process of rDNA evolution among the different genomes on a fine time scale.

D 403 MOLECULAR ANALYSIS OF SEQUENCES HOMOLOGOUS TO THE TRANSPOSABLE ELEMENT dTph1 IN *PETUNIA HYBRIDA*. Victoria Carollo and Carolyn Napoli, Department of Environmental Horticulture, University of California, Davis CA 95616

The transposable element dTph1 in *Petunia hybrida* is a nonautomonous element 284 basepairs (bp) in length. It is flanked by perfect 12 bp GC-rich inverted repeats and creates an 8 bp target site duplication upon insertion. In order to clone dTph1, or related elements, from inbred line V26, a 12 base oligonucleotide corresponding to one strand of the 12 bp repeat was used as a primer in polymerase chain reaction (PCR) experiments. At low primer annealing temperatures, a number of sequences of varying length were amplified. Increasing annealing temperatures to 60 degrees resulted in preferential amplification of products smaller than 300 bp. Amplified fragments in this size class were subcloned and sequenced. These elements shared from 65% to 94% homology with the published sequence of dTph1. The alignment produced by the PCGene program Clustal organized the sequences into three distinct groupings which we designate as families. A striking feature of dTph1 and the elements from V26 was the presence of the sequence $^{5'}$-AAAGGGGTTCAA-$^{3'}$, which we named Motif I, repeated as subterminal repeats at both ends and near the middle of the elements. A subset of the aforementioned motif, $^{5'}$-AAAGGG-$^{3'}$, is similar to the proposed binding site of ORFa, a protein encoded by the maize transposable element Activator (*Ac*). The reverse of this sequence $^{5'}$-TTGACACCCCTTT-$^{3'}$, named Motif II, is immediately adjacent to Motif I at the 3' terminus and appears between the 5' and center Motif I sequences. These sequences and PCR data will be presented.

D 404 EXPRESSION OF SOYBEAN SEED STORAGE PROTEIN GENES IN TRANSGENIC PLANTS; THEIR EFFECTS ON EXPRESSION OF A NEIGHBORING GENE AND POSITION DEPENDENCY, Toru Fujiwara[1] and Roger N Beachy[2] Department of Biology, Washington University, St. Louis, MO 63130, USA. [1]Current address: Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan; [2]Current address: Division of Plant Biology, Department of Cell Biology MRC7, The Scripps Research Institute, 10666 No. Torrey Pines Rd., La Jolla, CA 92037.

The β-conglycinin genes, encoding the 7S seed storage protein of soybean, were shown to be relatively independent of "position effects" in transgenic plants, i.e., expression levels of the α' and β subunit genes of β-conglycinin were shown to be relatively consistent on a per gene basis compared with other plant genes. We tested whether the promoter region of the α' subunit gene confers position independency by introducing a fusion gene comprised of the α' subunit promoter and the *gus*A (encoding β-glucuronidase) gene and found that the α' subunit promoter lost its position independency. We also tested the possibility that the β-conglycinin genes may have an ability to neutralize "position effects" by placing a position dependent gene next to the β-conglycinin genes. Genes comprised of the 35S promoter of the cauliflower mosaic virus or the α' subunit promoter and the *gus*A coding region were employed as a position dependent reporter gene. Neither the α' nor the β subunit genes effectively altered position dependency of the CaMV 35S promoter or of the α' subunit promoter. Nevertheless the α' subunit gene acts as an enhancer on the CaMV35S promoter when the promoters are adjacent to each other.

D 405 CONSERVATION OF PROTEINS INVOLVED IN POLAR AXIS FORMATION - FUCUS MEETS YEAST, Brad Goodner and Ralph S. Quatrano, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

We are using the brown alga Fucus as a model to study polar axis formation in plants. The first cell division of the Fucus zygote is asymmetric and results in two cells which differ dramatically in their developmental fates. Previous work in our laboratory indicated a role for the actin cytoskeleton in setting up the polar axis and a role for the cell wall in stabilizing that axis. To identify other proteins involved in polar axis formation, we have taken an approach based on the idea that many of the mechanisms involved in polar growth and asymmetric cell division, and the proteins involved in those mechanisms, have been conserved during eukaryotic evolution. Budding in the yeast Saccharomyces cerevisiae, an organism very distant phylogenetically from plants, shares many similarities with the first cell division in Fucus. Genetic and molecular studies in other laboratories have identified many proteins involved in selecting the site of budding, setting up the polar axis, and bud growth. We have used some of the same antibody and gene probes to look for homologous proteins in Fucus. We have concentrated on two families of proteins. One is the family of ras-like proteins. Two ras-like proteins, BUD1 and CDC42 are involved in the budding process. Antibodies raised against BUD1 recognize at least one protein in Fucus embryos. We have used PCR to clone the genes for the ras-like proteins and are currently sequencing them and studying their expression patterns. The other proteins we are studying are the neck filament proteins. These proteins were first described in S. cerevisiae as a new cytoplasmic cytoskeletal system localized around the site of bud growth. Antibodies raised against one of these proteins, CDC3, recognize a single protein of the same MW in Fucus embryos. We are also cloning the gene(s) for these proteins.

D 406 PATTERNS OF AC/DS TRANSPOSITION IN TOMATO

Jacques Hille, George Rudenko, Tatyana Glagotskaya, Erik van der Biezen, Karin Blok, Bert Overduin, Caius Rommens, John Nijkamp and Mark van Haaren. Department of Genetics, Free University Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Certain autonomous transposable elements can cross the borders between plant species and carry out the transposition process successfully in heterologous plant species. This has opened the window to study various aspects of transposable element behaviour in the new host. The challenge has been to use well characterized transposable elements to develop transposon tagging in plant species that lack such transposon systems. This will allow the isolation of genes that can only be recognized by the phenotype they confer on plants when mutated as a consequence of transposable element insertion. In developing transposon tagging in transgenic plants many aspects of the transposition process are being studied in detail like rearrangements induced by the transposable elements, patterns of transposition and regulation and biochemistry of the transposition process. In this work we describe our research on the behaviour of the Activator (Ac) element and an Ac-derived Ds element in tomato. Furthermore, progress in transposon tagging of the Alternaria stem canker disease resistance locus in tomato will be presented.

D 407 **HIGH FREQUENCY GERMINAL TRANSPOSITION OF Ds^{ALS} IN *Arabidopsis*.**

Mary Honma, Candace Waddell, and Barbara Baker
Plant Gene Expression Center, UC Berkeley-USDA, Albany, CA 94710

We have established an efficient transposon tagging system in *Arabidopsis* based on the maize transposon family *Activator/Dissociation (Ac/Ds)*. Our system has two components: a stable *trans*-activator, Ac^{st}, and a *cis*-responsive *Ds* element. *Ds* transposes to new sites when *trans*-activated by Ac^{st} but is stabilized when Ac^{st} segregates away from *Ds*. *Ds* and Ac^{st} elements carry different selectable and screenable markers with which to monitor *Ds* transposition. For example, *Ds* carries an *ALS* gene, to facilitate testing for cosegregation of a transposed *Ds* and a mutant phenotype. The starting position of Ds^{ALS} is within a kanamycin resistance gene that serves as an excision marker. Thus, both excision and reintegration of Ds^{ALS} is easily assessed. Ac^{st} transposase expression is driven by one of three strong promoters: the 35S promoter from CaMV, or promoters from the *Arabidopsis rbcS* or *CHS* genes. The Ac^{st} constructs carry a GUS marker to follow the segregation of Ac^{st} in *Ds*-containing plants. The GUS marker facilitates the identification of those plants in which a transposed *Ds* is stable (Ac^{st} has segregated away, Gus^-), and those plants in which the *Ds* element will continue to transpose (Ac^{st}-containing, Gus^+). We have generated 10 independent Ds^{ALS} lines as well as 43 Ac^{st} lines carrying one of the three Ac^{st} constructs.

Our results show that Ds^{ALS} transposes at a high frequency in *Arabidopsis*. 43 independent Ac^{st} lines were crossed to two independent Ds^{ALS} lines, and the resulting F_2 populations were assayed for germinal transposition of Ds^{ALS}. *Trans*-activation of Ds^{ALS} by Ac^{st} resulted in germinal excision frequencies of up to 64% using 35S transposase fusions, up to 28% using *rbcS* transposase fusions, and up to 1% using *CHS* transposase fusions. Induction of the *CHS* transposase fusion with high intensity light increased the germinal excision frequency up to 15%. Amongst progeny bearing germinal excisions, 55% from 35S-Ac^{st} crosses carried reintegrated Ds^{ALS} elements and 28% from *rbcS*-Ac^{st} crosses carried reintegrated Ds^{ALS} elements. Plants carrying transposed *Ds* elements will be used as starting material for mutant screening.

D 408 RELATIONSHIP OF SOYBEAN AND ITS WILD RELATIVES REVEALED BY SEED MATURATION PROTEINS, Jaw-shu Hsieh and Kim-leiong Hsieh, Graduate Institute of Agronomy, Naitional Taiwan University, Taipei, Taiwan, ROC

The *Glycine* species, soybean and its relatives, comprises many wild annual or prennial herbaceous species distributed mainly in Australia, North to South China, and in the southwest rim of the Pacific. A wide variety of systematic approaches, including cytogenetics, morphology, isozyme, and RFLP, have been used to define relationships within the taxon.

In this study, we used the cultivated soybean, *Glycine max* and its wild relatives *G. soja*, *G. tomentella*, and *G. tabacina*. The former two are annual and the latter two are prennial. The antibodies against 20, 22, and 130 kD soybean seed maturation proteins as well as lectin were used to check the presence and changes of molecular mass of these proteins. PCR analysis for the messages of 20 and 22 kD proteins were also carried out. The detail results will be presented at the meeting. These results confirm and extend the present model for the taxonomic relationships. Screening of seed maturation proteins thus has a potential for revealing the relationships of the member species of the *Glycine* taxon.

D 409 SOYBEAN SEED MATURATION PROTEINS: CLONING AND CHARACTERIZATION, Yue-ie Hsing, Institute of Botany, Academia Sinica, Taipei, Taiwan, ROC

To study soybean seed maturation protein, we have selected clones from a pod-dried seed cDNA library by differential screening. The clones, pGmPM 1 through 9, whose mRNAs are abundant in the dry seed, were characterized. GmPM stands for *Glycine max* physiological maturation. These clones hybridized to RNA classes of approximately 800 to 2400 nulceotides whose accumulation are detected in pod-dried as well as mature soybean seeds. These cDNA clones correspond to soybean seed maturation proteins of 18, 19, 20, 22, 31, 32, 41, 48, 50, 52, 60, and 70 kD, as revealed by hybrid select translation.

The pGmPM 1 and 9 correspond to 22 and 20 kD proteins, respectively. Both of them have high pI value, are group 1 *Lea* proteins, and belong to the same gene family, with a 23 amino acid deletion from each other. pGmPM2 bolongs to group 3 *Lea* protein, and has long tandem repeats. The genomic clones of GmPM9 had been pulled out and sequenced. The antibodies against 20, 22, and 130 kD maturation proteins are also available. The *in situ* localization study indicated that some of the proteins are present only in seed coat while others are present only in embryonic axis and cotyledon.

D 410 A RICE RIBOSOME-INACTIVATING PROTEIN GENE, [1]Ju-Kon Kim, [1]Young-Soo Hwang and [2]Ray Wu, [1]Department of Biochemistry, Agricultural Biotechnology Institute, RDA, Suwon, Korea 441-707, [2]Section of Biochemistry, Cell and Molecular Biology, Cornell University, Ithaca, NY 14853.

Ribosome-inactivating proteins (RIPs) are a class of plant protein that are inactivators of eucaryotic protein synthesis. To use as a defense molecule in transgenic rice, we have isolated ten different RIP clones from a rice (*Oryza sativa* L.) genomic DNA library. Nucleotide sequence analysis of one (*Rrip 1*) of those genes revealed that the rice gene is closely related to a maize RIP gene that encodes a protein called b-32. The protein, deduced from the nucleotide sequence has many features which were previously implicated as being important for RIP activity. These include: three conserved amino acids(E, R, and W), which are known to be located within proposed active site of ricin A-chain and required for RIP function of the protein in Yeast; two putative RNA-binding domain of the ribonucleoprotein consensus sequence; three short repeats, which are also present in the maize b-32. A full-length cDNA clone corresponding to *Rrip 1* was also isolated and *in vitro* translation product of the cDNA was tested for RIP activity in *in vitro* translation systems. As a preliminary result, the rice *Rrip 1* product appears to inhibit translation in rabbit reticulocyte, but not in wheat germ cell-free systems.

D 411 ISOLATION OF PHENYLALANINE AMMONIA-LYASE REGULATORY MUTANTS IN *ARABIDOPSIS*. B. Kraft, R.A. Dixon, S. Ohl*, J. Chory*, and C.J. Lamb*, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402; * Plant Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037.

Phenylalanine ammonia-lyase (PAL) is a key regulatory enzyme in the biosynthesis of a wide variety of phenylpropanoids in plants. PAL expression is highly regulated throughout plant development and in response to environmental stress, but little is known about the cellular components involved in this regulation. To facilitate the identification of these components in *Arabidopsis thaliana* the transgenic line PAG1 was constructed in an *adh*⁻ strain. The PAG1 line contains a chimeric T-DNA insert which contains two 1.8 kb copies of the *Arabidopsis* PAL1 promoter. One copy of the promoter regulates the expression of an intact *adh* gene and the second copy regulates the ß-glucuronidase (GUS) gene. The restoration of *adh* expression in this strain makes treatment with allyl alcohol lethal. This enables the selection of putative mutants with decreased PAL1 promoter activity by screening for decreased sensitivity to allyl alcohol. Mutants defective in trans-acting components of PAL1 regulation can then be verified by testing for decreased GUS activity.

Seeds from a homozygous PAG1 line were mutagenized with either γ-irradiation or EMS, sown to soil, and the M2 seed collected. Numerous putative PAL1 regulatory mutants exhibiting decreased sensitivity to allyl alcohol have been isolated. A number of these exhibit decreased GUS activities. Further characterization of the putative mutants is in progress and will be presented.

D 412 **CHARACTERIZATION OF A MUTATION (*bsd1*) THAT DISRUPTS CELLULAR DIFFERENTIATION IN MAIZE LEAVES. Jane A. Langdale**, Dept.of Plant Sciences, University of Oxford, South Parks Rd., Oxford, England.

Our understanding of events that regulate cellular differentiation in the developing maize leaf is limited. In order to address this issue, I am characterizing a mutation that disrupts the differentiation of a single cell-type within the leaf. The mature maize leaf is characterized by a series of parallel veins running the length of the leaf. Surrounding these veins are concentric circles of two morphologically and functionally distinct photosynthetic cell-types. These photosynthetic bundle sheath (BS) and mesophyll (M) cells develop co-ordinately to interact at maturity in the fixation of CO_2 in the C4 photosynthetic cycle.

Using the maize transposable element *Spm* as an insertional mutagen, I have isolated a mutant that disrupts BS cell development. The *bundle sheath defective (bsd1-m1)* mutant allele is unstable such that dark green revertant sectors can be observed in the leaves of pale green mutant plants. In the absence of large revertant sectors the mutation is lethal. I will present data to show that despite the presence of normal M cells, BS cells do not develop chloroplasts nor do they accumulate photosynthetic enzymes. Since M cells develop appropriately, it is clear that the absence of a functional *BSD1* gene product perturbs the tightly co-ordinated development of BS and M cells that is essential for photosynthetic function. This suggests that during normal development the *BSD1* gene product may play a role in BS-M cell communication processes. Progress towards cloning *BSD1* will be presented.

D 413 MOLECULAR CHARACTERIZATION OF THE AXR1 GENE OF*Arabidopsis thaliana*, Ottoline Leyser, Cindy Lincoln, Candace Timpte, Jocelyn Turner, Doug Lammer and Mark Estelle. Indiana University, Department of Biology, Jordan Hall 142, Bloomington, IN 47405.

We are taking a genetic approach to study the mode of action of the plant hormone auxin. We have used a variety of screens to identify genes involved in auxin action. One such gene, AXR1, is defined by a series of mutant alleles conferring resistance to normally inhibitory levels of exogenously added auxins. The mutations are all recessive and pleiotropic. Homozygous axr1 plants are dwarfed and show reduced apical dominance and defective root gravitropism. The degree of auxin resistance conferred by the different axr1 alleles correlates with the severity of other aspects of the phenotype. This implies that the AXR1 gene product is involved in auxin perception or response.

In the expectation of learning more about auxin action in plants, we have cloned the AXR1 gene by chromosome walking. We have identified a 16.5 kb DNA segment which, when transformed into axr1-3 mutant plants, rescues the mutant phenotype and restores wild-type levels of auxin sensitivity. By screening cDNA libraries and carrying out RNA blot analysis we have identified a transcript from this region which shows quantitative and, in one case, qualitative differences in expression in plants homozygous for various axr1 mutant alleles. Sequence analysis has shown that this gene has 14 exons and its predicted protein product has significant homology to ubiquitin-activating enzyme E1. However, because the protein lacks residues known to be essential for E1 activity, we believe that it does not function as an E1 enzyme but rather has a novel activity. We are currently taking a variety of approaches to understand how the AXR1 protein mediates auxin responses.

D 414 ORIGIN OF ANGIOSPERMS INFERRED FROM DNA SEQUENCES
Wen-Hsiung Li, Center for Demographic and Population Genetics, University of Texas, PO Box 20334, Houston, TX 77225; Kenneth H. Wolfe and Paul M. Sharp, Department of Genetics, Trinity College, Dublin 2, Ireland

The origin of angiosperms was dubed "an abominable mystery" by Darwin. The fossil record shows a vast increase in the numbers and distribution of angiosperm species in the mid-Cretaceous period, around 100 million years (Myr) ago, but the earliest reliable angiosperm macrofossils are only ~120 Myr old. Although angiosperms probably descended from the progymnosperm lineage, there is little agreement as to when they arose. Since the progymnosperm lineage extends back to at least 370 Myr ago, there is an enormous range of time during which angiosperms might have had their beginnings. There are two competing hyptheses for the origin of anygiosperms: either angiosperms did not exist until the early Cretaceous and then radiated explosively, or pre-Cretaceous angiosperms lived in habitats so refractory to fossilization that they left no record. We attempt to decide between the two hypotheses by analyzing plant DNA sequences, which can be used to estimate the date of divergence of monocotyledons and dicotyledons, and hence to provide a minimal age for angiosperms themselves.

We estimate this date by reconstructing phylogenetic trees from chloroplast DNA sequences, using two independent approaches: the rate of synonymous nucleotide substitution was calibrated from the divergence of maize, wheat, and rice, whereas the rate of nonsynonymous substitution was calibrated from the divergence of angiosperms and bryophytes. Both methods lead to an estimate of the monocot-dicot divergence at 200 Myr ago (with an uncertainty of about 40 Myr). This estimate is also supported by analyses of the nuclear genes encoding large and small subunit ribosomal RNAs. These results imply that the angiosperm lineage emerged in Jurassic-Triassic time, which considerably predates its appearance in the fossil record (~120 Myr ago). We estimate the divergence between cycads and angiosperms to be ~340 Myr, which can be taken as an upper bound for the age of angiosperms.

D 415 GENE EXPRESSION DURING THE TRANSITION FROM MATURATION TO GERMINATION OF SOYBEAN SOMATIC EMBRYOS , Wennuan Liu, David F. Hildebrand and Glenn B. Collins, Department of Agronomy, University of Kentucky, Lexington, KY 40546

Soybean somatic embryos from embryogenic suspension cultures of cv "J103" usually do not germinate even when grown on medium containing ABA. Desiccation of the somatic embryos at 76% relative humidity for 96 h results in more than 90% germination. Germination frequency is dependent on the percent fresh weight loss of the somatic embryos. In order to identify genes involved in the transition from the maturation to the germination phases, we investigated the expression of the *Mat1* gene that encodes a 31 kD polypeptide present in later stages of soybean embryo development and is induced by desiccation of immature soybean seeds. We also investigated the genes *SC514* and *LOXB2* which encode germination-associated lipoxygenase isozymes whose expression in soybean seedlings is increased by water deficit. *Mat1* expression was induced in both cotyledons and hypocotyl/radicle tissues of somatic embryos after 72 h desiccation and a loss of fresh weight of about 30%. The transcript level rose as water loss from the embryos increased up to 96 h desiccation. *Mat1* expression was not induced by exogenous ABA supplementation in the maturation medium. Desiccation did not directly induce the expression of *SC514* and *LOXB2* transcripts or proteins in somatic embryos. However, upon rehydration for 24 h and prior to any visible developmental change in embryos on the germination medium, the transcripts and proteins of *SC514* and *LOXB2* became detectable in somatic embryos desiccated for 96 h but not in somatic embryos desiccated for 48 h. The data suggest that: 1) the expression of *Mat1* correlates with the achievement of competence for germination of somatic embryos but *Mat1* is apparently not involved in the germination process since it is turned off as germination is initiated; 2) *Mat1* expression does not require a maternal factor or if there is a maternal factor involved it can be induced by desiccation; 3) the expression of *SC514* and *LOXB2* is indicative of activation of root initiation and/or hypocotyl elongation.

D 416 EXPRESSION OF PHYTOCHROME GENES IN DOUGLAS-FIR, Kimberly A. Marshall, M. Carol Alosi, and David B. Neale, Institute of Forest Genetics, USDA Forest Service, P.O. Box 245, Berkeley, CA 94701

We have begun to study the structure and expression of phytochrome genes in Douglas-fir (*Pseudotsuga menziesii*(Mirb.) Franco). We constructed two degenerate oligonucleotides based on consensus sequences of three phytochrome genes of Arabidopsis (Sharrock and Quail, Genes & Development 3:1745-1757). The 5' oligo was from the chromophore attachment site; the 3' oligo was approximately 400 bp downstream. We used these oligos to amplify a 400 bp PCR product from total RNA of Douglas-fir seeds germinated in total darkness. Subsequently, we amplified a 400 bp fragment from Douglas-fir genomic DNA. This PCR product was cloned and partially sequenced. Sequence comparisons confirmed that the PCR product was phytochrome. We are now using the phy PCR clone to study expression of phytochrome genes in Douglas-fir. Data on tissue-specific (shoot and root) and light-regulated (light, dark, R, FR, R/FR) will be presented. Southern hybridization of genomic DNA with the 400 bp phy PCR probe suggests that there are 2-4 phytochrome genes in Douglas-fir.

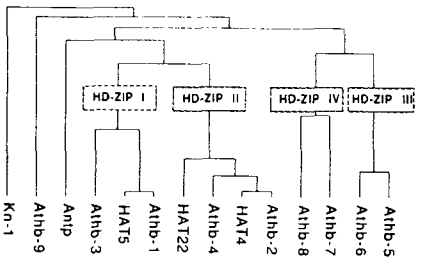
D 417 THE STRUCTURE AND EVOLUTION OF THE ACTIN GENE FAMILY IN *ARABIDOPSIS THALIANA*. J. McDowell, S. Huang, E. McKinney, Y.Q. An, and R. Meagher. Dept. of Genetics, The University of Georgia, Athens, GA 30602. There is a considerable body of evidence which associates actin with several critical structures and processes in a variety of plant cell types. Actin has been implicated in processes as diverse as cell plate location and formation, cell elongation, cytoplasmic streaming, tip growth, organellar movement (e.g. light-responsive chloroplast orientation), and the gravitropic response in roots. The diversity of potential roles for plant actins is paralleled by the divergence within plant actin gene families. Partial surveys of several large actin gene families have revealed distinct actin subclasses which are conserved among plant species that have been separated for hundreds of millions of years. We have proposed that these ancient subclasses of plant actin genes have been conserved due to a requirement for specific functions and/or differential regulation in the complex tissues and organs present in higher plants.

We are analyzing the actin gene family in *Arabidopsis thaliana* as a model for actin gene families in higher plants. Extensive screening of several genomic and cDNA libraries has resulted in the isolation of 12 distinct *Arabidopsis* actin genes. It is quite likely that this collection contains most, if not all of the family members. The nucleotide sequence of the entire coding region of each gene has been determined, as well as ~1000 bp of 5' flanking sequence and 400 bp of 3' flanking sequence. The coding sequences are 1-16% dissimilar in D_N (non-synonymous nucleotide substitutions). Little similarity is seen among non-coding regions. The intron-exon organization of each gene is completely conserved with respect to all other plant actin genes with the exception of *AAc2*, which is missing one of four introns. A neighbor-joining tree has been reconstructed from 22 plant actin coding sequences. Conserved subclasses which contain orthologous members from several plant species are apparent. Representatives from the *Arabidopsis* actin family are found in most subclasses.

Data from studies which are focusing on the expression patterns, regulation, and function of *Arabidopsis* actins will be presented. We hope to integrate information from these studies with knowledge of the evolutionary structure of plant actin gene families in order to better understand the forces which have affected plant actin gene family evolution. The interaction of functional and regulatory constraints which produced the actin gene family will be discussed.

D 418 GENOTYPIC AND DEVELOPMENTAL REGULATION OF TRANSIENT EXPRESSION OF A REPORTER GENE IN SOYBEAN ZYGOTIC COTYLEDONS,
Patricia J. Moore, Allen J. Moore and Glenn B. Collins, University of Kentucky, Lexington KY 40546.
The use of recombinant DNA technology to genetically modify responsive plant species has proven valuable in studies of plant growth and development. Plants transformed with chimeric genes containing plant promoters and reporter genes allow scientists to generate information about the timing and tissue specific expression of developmentally important genes. The key to these studies is the ability to transform a plant species of interest. Many studies in developmental biology have used soybean (*Glycine max*) as a model system. However, it has been difficult to regenerate transformed soybean plants. One hypothesis to explain the difficulty of regenerating transformed soybean plants is that, to date, all regeneration systems for soybean utilize embryonic or meristematic cells that may have evolved mechanisms to protect themselves from integrative transformation (Potrykus, I. 1991. Gene transfer to plants: assessment of published approaches. Annu. Rev. Plant Mol. Biol. **42**; 205-225). In order to study the processes involved in transformation of soybean explants capable of regeneration, the transient expression of a chimeric gene including the 35S promoter, GUS coding region and 3' end from the soybean 7S storage protein was assayed in immature zygotic cotyledons. The plasmid containing the chimeric gene was delivered to the cotyledon cells via particle bombardment using the Dupont PDS1000 particle delivery system with the helium attachment. The cotyledons were bombarded once at 1100 or 900 psi. Zygotic cotyledons from six soybean varieties were tested for transient expression of the GUS gene. The level of expression of GUS differed between genotypes. The genotypes could be classified as high, fair or poor expressers. The ability of cotyledon tissue to express the GUS gene also depended on the developmental stage of the seed from which it was excised. Cotyledons from seeds 3mm in length expressed GUS at a lower efficiency than cotyledons from seeds 5mm to 8mm in length. Factors influencing the developmental specificity of transient expression of the reporter gene will be discussed.

D 419 IDENTIFICATION OF DISTINCT FAMILIES OF HD-Zip PROTEINS IN *Arabidopsis thaliana*,
Giorgio Morelli, Simona Baima, Monica Carabelli*, Sabrina Lucchetti, Fabio Nobili, Giovanna Sessa* and Ida Ruberti*, Unità di Nutrizione Sperimentale, INN Roma and *Centro di Studio per gli Acidi Nucleici, CNR Roma.
We have recently identified other genes encoding HD-Zip proteins from *Arabidopsis thaliana*. DNA sequence analysis revealed that five of them, designed Athb-4, -5, -6, -7, -8, code for HD-Zip proteins, whereas another one, named Athb-9, codes for a HD protein. While this work was in progress other members of the HD-Zip class of proteins have been characterized: Athb-3 (Mattsson et al., 1992), HAT4, 5 and 22 (Schena and Davis, 1992). On the basis of sequence homology, we propose to group the *Arabidopsis* HD-Zip proteins, identified so far, into four distinct HD-ZIP families. The dendrogram shows the order of the pairwise alignment of the homeodomain of the *Arabidopsis* proteins, the maize protein *Knotted* (*Kn*) (Vollbrecht et al., 1991) and the Drosophila protein *Antennapedia* (*Antp*).
Data on the expression pattern of genes coding for members of the HD-ZIP families will be presented.
Mattsson et al.,(1992) Plant Mol Biol **18**, 1019-1022
Schena and Davis, (1992) PNAS **89**, 3894-3898
Vollbrecht et al., (1991) Nature **350**, 241-243



D 420 THE REGULATION OF EMBRYO SPECIFIC GENES BY SULPHUR NUTRITION IS CONSERVED BETWEEN PEA AND TOBACCO AND IS MEDIATED BY 3' UNTRANSLATED SEQUENCE
Roger L. Morton, William G. Rerie and T.J.V. Higgins, CSIRO Division of Plant Industry, Canberra, Australia.

Legumin and Pea albumin 1 (PA1) are embryo specific proteins which contain a large proportion of sulphur amino acids. The steady state levels of these proteins and their mRNAs are severely reduced when plants are grown under sulphur deficient conditions. This phenomenon appears to be widespread: sulphur deficiency has been reported to affect the balance between the sulphur-rich and the sulphur-poor seed proteins in lupins, wheat, barley, soybean, cowpea, rape and sunflower. *In vitro* transcription assays using pea seed nuclei, indicate that the poor sulphur nutrition exerts its effect on sulphur-rich-protein gene expression by reducing the stability of the mRNAs encoding the proteins. When the unmodified legumin gene was introduced into tobacco plants the gene was expressed in the tobacco seed and was regulated by sulphur nutrition indicating that the *trans*-acting factors responsible for this regulation were conserved between pea and tobacco seeds. A chimeric gene containing a leaf specific promoter, a reporter-gene coding region and 500bp of PA1 3' flanking region was expressed in the leaves of tobacco and was regulated by sulphur while a similar gene without the PA1 sequence was not. This indicates that factors capable of regulating the sulphur-rich proteins are also found in the leaves of tobacco. The evolutionary implications of these results will be discussed.

D 421 THE TRADE SYSTEM: APPLICATIONS FOR THE USE OF TRANSPOSITION AND SITE-SPECIFIC RECOMBINATION IN PLANTS,
H.John J. Nijkamp, Jeroen Stuurman, Jacques Hille and Mark J.J. van Haaren, Department of Genetics, Free University Amsterdam, The Netherlands
To study the plant genome architecture and to optimize plant gene isolation strategies, we developed the TRADE (transposition/deletion) system. This system, based on the combination of transposition and site-specific recombination, allows the induction of specific deletion or inversion events in the plant genome. The induction of chromosomal deletions will be a very usefull tool in several aspects of plant molecular biology, including identification and isolation of genes of interest to plant science.
To develop an efficient system for the induction of chromosomal rearrangements, we choose Ac/Ds transposable elements in combination with the site-specific recombination system Cre/loxP of the *Escherichia coli* bacteriophage P1. Both the transposable element and the recombination system have seperately been shown to function efficiently in transgenic tobacco plants (Dale and Ow 1991; Rommens et al. 1992).
In the plant one of the recombination sites will be mobile because of its presence within the transposable Ds-element, while a second recombination site will remain at the original site of T-DNA integration. After transposition of the Ds-element has occurred recombination can be induced, resulting in an inversion, deletion or translocation. Different strategies for the detection of these rearrangement events will be discussed, the progress made with the trade-system will be presented as well as a strategy for the cloning of plant genes using this system.

Dale, E.C. and Ow, D.W. (1991) Gene transfer with subsequent removal of the selection gene from the host genome. Proc.Natl. Acad. Sci. USA 88, 10558-10562.
Rommens, C.M.T., Rudenko, G.N., Dijkwel, P.P., Haaren, M.J.J. v., Ouwerkerk, P.B.F., Blok, K.M., Nijkamp, H.J.J. and Hille, J. (1992) Characterization of the Ac/Ds behaviour in transgenic tomato plants using plasmid rescue. Plant Mol. Biol. 20, 61-70.

D 422 THE ORGANISATION OF THE AMPHIDIPLOID *Brassica napus* GENOME, Isobel Parkin, Deborah Keith, Andrew Sharpe and Derek Lydiate, Cambridge Laboratory, John Innes Institute, Colney Lane, Norwich, United Kingdom.

Brassica napus is an amphidiploid species arising from the hybridisation of two closely related diploid ancestors, *Brassica oleracea* and *Brassica rapa*.

We have developed two detailed RFLP maps of this species. The first map was generated from a cross between two 'natural' oilseed rape cultivars which are known to have structurally stable *B. napus* genomes. The second map was generated from a cross between one of the above cultivars and a 'synthetic' *B. napus* line. The 'synthetic' line was the product of a new cross between the diploid parental species and is known to have a genome which suffers from frequent rearrangements which are caused primarily by recombination between homoeologous chromosomes.

By comparing the maps derived from the two crosses we have established that the structure of the present day *B. napus* genome has evolved very little from a simple hybrid between the two diploid ancestors. The data from the cross with the 'synthetic' *B. napus* has allowed the ancestral origins of the ten *B. rapa* chromosomes and the nine *B. oleracea* of *B. napus* chromosomes to be established.

Most 'synthetic' *B. napus* genomes are known to be structurally unstable. Therefore, it is likely that genes which promote stringent chromosome pairing were very important in the establishment of a successful *B. napus* plant. We are in the process of mapping the genes which determine stable chromosome pairing in oilseed rape using a set of microspore-derived doubled haploid lines produced from the oilseed rape x 'synthetic' *B. napus* cross.

D 423 THE C1-I ALLELE IN MAIZE: ITS ORIGIN VIA A TRANSPOSITION EVENT, Peter A. Peterson, Department of Agronomy, Iowa State University, Ames, IA 50011-1010

The *C1-I* allele in maize is highly pervasive in a number of native populations, especially in South America. It was the first maize dominant allele that was molecularly described following its availability after the *C1* locus was cloned. (Paz-Ares *et al.*, 1987 EMBO J. 6:3353). It is a **dominant negative** and produces transcripts, one of which that yields a 252 amino acid protein that differs from the wild-type *C1* protein at its carboxy terminus. It is postulated (Paz-Ares *et al.*, 1990 EMBO J. 9:315) that this resulting reduced acidity of this protein manifests itself as a repressor function (negative dominance).--A newly induced *C1-I* allele has evolved from a wild-type *C1* allele via a transposition event suggesting that the original *C1-I* allele (*C1-I°*) originated similarly. The origin of this newly originated *C1-I'''* allele is from the mutable *c-m 11702* allele. This unstable allele has an *En* insert that is located at a position of the *C1* locus near the site of the transcript truncation of *C1-I°*. It is postulated that the *c-m1 1702* allele yields an abortive transposition event that results in a truncated transcript lacking the carboxy terminus. Tests with several of these germinal derivatives indicate that *C1-I'''* does not have the potency of *C1-I°*. *C1-I'''* is not expressed when transmitted as a male. The likely explanation of this lack of male transmitted expression is that *C1-I°* has an altered sequence in box II of the promoter that differs from that of *C1-I'''*. *C1-I°* has a "stronger" promoter resembling *'C1*-a super *C1* allele known for more abundant RNA and more competitiveness against *C1-I°* in color suppression.--Likely, the evolution of the *C1-I°* allele came from two events, the event leading to a truncated transcript and the second, by the change in the promoter sequence in box II.--This demonstration of an evolving allele supports the role of transposons in gene changes

D 424 **ANALYSIS OF THE MAIZE *SUPPRESSOR-MUTATOR* TRANSPOSIBLE ELEMENT'S PROMOTER,** Ramesh Raina, Douglas Cook and Nina Fedoroff, Carnegie Institution of Washington, Department of Embryology, Baltimore MD. 21210

We developed a transient assay system to define and study the *Suppressor-mutator* (*Spm*) promoter. The 5' end of *Spm* was fused to the luciferase reporter gene and introduced into NT1 tobacco suspension culture cells by microprojectile bombardment. Luciferase activity was normalized to a chloramphenicol acetyl transferase (CAT) internal control, thus providing a means to quantify *Spm* promoter activity. Results will be presented indicating that basal *Spm* promoter activity is contained within its 5' 220 bp. The *Spm* promoter is roughly two to three orders of magnitude weaker than the cauliflower mosaic virus (CaMV) 35S promoter. Deletion studies reveal that minimal promoter activity can be deteced using deletions to within -35 from the transcription start site. Deletions including the putative TATA box abolish promoter activity. When the 35S enhancer sequence was introduced upstream of the *Spm* 5' end, enhancement of luciferase activity was minimal. Up to 30-fold enhancement of the measured promoter activity was obtained by altering the sequences both upstream of the transcription start site and in the untranslated leader, suggesting that the *Spm* promoter's sequence buffers it from nearby enhancers, rendering expression relatively independent of the insertion site in the genome.

D 425 **GLUTAMINE SYNTHETASE GENE EXPRESSION IN ROOT NODULE DEVELOPMENT OF SOYBEANS IS REGULATED AT THE TRANSCRIPTIONAL LEVEL AND AT THE LEVEL OF ENZYME ACTIVATION.** Dominique Roche, Stephen J. Temple, Champa Sengupta-Gopalan. Plant Gen. Eng. Labs., Dept. of Agron. and Hort., Mol. Biol. Program, New Mexico State Univ., Las Cruces, New Mexico, 88003, USA.

The onset of N_2 fixation during root nodule development in soybeans is accompanied by a significant increase in glutamine synthetase (GS) activity. This increase in activity can be attributed to a novel GS isoenzyme. Sequence analyses of GS cDNA clones isolated from a nodule cDNA library allowed for their categorization into two classes, each with a unique 3' untranslated region. Using the two 3' untranslated region as gene specific probes in northern analysis and hybrid select translation experiments, one class has been shown to be expressed in a nodule specific manner independent of N_2-fixation. The second class is transcribed constitutively at a basal level in roots, leaves, cotyledons and nodules, but its expression is greatly enhanced following onset of N_2-fixation. While nodule specific GS genes can be transcribed and the transcripts translated in the absence of N_2-fixation, nodules formed by bacteria defective in structural nitrogenase, did not show enzyme activity attributable to nodule specific GS isoenzyme. Native PAGE of nodule proteins followed by western analysis for GS protein showed that the inactive GS holoprotein had a slower electrophoretic mobility than the active GS enzyme and that N_2-fixation was required to activate the inactive nodule GS isoenzyme.

D 426 THE *Arabidopsis thaliana* Athb-1 AND -2 PROTEINS HOMO-DIMERISE, FORMING COMPLEXES WHICH RECOGNISE DYAD-SYMMETRIC DNA SEQUENCES, Ida Ruberti, Giorgio Morelli* and Giovanna Sessa, Centro di Studio per gli Acidi Nucleici, CNR Roma and *Unità di Nutrizione Sperimentale, INN Roma.

Homeobox (Hb) genes have recently been identified in two plant species, maize (Vollbrecht et al., 1991) and *Arabidopsis* (Ruberti et al., 1991). Maize and *Arabidopsis* homeodomains (HDs) contain the highly conserved amino acid residues that have been shown to be involved in protein-DNA contacts. Furthermore, the *Arabidopsis* Athb-1 and -2 hb genes contain a second element that potentially codes for a leucine zipper motif (Zip). The exact spatial register between the HD and the Zip motif in the Athb-1 and -2 proteins is similar to that observed between the DNA binding and the dimerisation domains in the b-Zip proteins. We have previously proposed that the HD-Zip proteins might use the dimerisation domain to closely juxtapose a pair of DNA contacting surfaces, each of which fits into half of a dyad-symmetric recognition sequence (Ruberti et al., 1991), in analogy with the DNA binding model proposed for the b-Zip transcription factors (Vinson et al., 1989).
Protein dimerisation and DNA binding studies have now established that Athb-1 and -2 are able to form homo-dimeric complexes *in vitro* and that these complexes interact specifically with dyad-symmetric DNA sequences.

Vollbrecht et al., (1991) Nature **350**, 241-243
Ruberti et al., (1991) EMBO J. **10**, 1787-1791
Vinson et al., (1989) Science **246**, 911-916

D 427 CHARACTERIZATION OF AN *ARABIDOPSIS THALIANA* HOMOLOG OF THE TRANSCRIPTION FACTOR c-myb. Diane M. Ruezinsky[1], Susan A. Hedrick[2], Richard A. Dixon[1], Christopher J. Lamb[2] and Peter W. Doerner[2], [1] The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402; [2] The Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037.

Members of the *c-myb* proto-oncogene family have been shown to be required for cell differentiation and proliferation in several species. The protein product of the first *myb* homolog identified in plants, the maize C1 gene, activates transcription of genes involved in anthocyanin biosynthesis. The *Arabidopsis* GL1 gene product is required for trichome formation. Additional *myb* homologs have been cloned from *Arabidopsis*, maize, barley, petunia, *Antirrhinum* and carrot. We have cloned seven *myb* homologs from an *Arabidopsis thaliana* genomic library. The amino-terminal end of the protein encoded by one of these genes, Atmyb4, shares from 56 to 73% amino acid identity with other plant *myb* genes. In addition, the intron/exon boundaries of C1, GL1 and Atmyb4 are positioned at the same amino acid residues. To determine the expression pattern of the Atmyb4 gene product, we linked the Atmyb4 promoter to the b-glucuronidase (GUS) gene and introduced the construct into *Arabidopsis*. In order to determine what role the product of this gene plays in plant development, a series of antisense, dominant negative and overexpression constructs have been introduced into *Arabidopsis*. Phenotypic analysis of transgenic plants is currently underway.

D 428 CHARACTERIZATION OF THE MADS BOX GENE FAMILY FROM BLACK SPRUCE USING PCR CLONING, Bob Rutledge, Chantal Côté, Jack Pitel and Glen Sunohara. Petawawa National Forestry Institute, Forestry Canada, Chalk River, Ontario, Canada, K0J 1J0.

We have used PCR (polymerase chain reaction) for the rapid and detailed analysis of MADS box genes from the gymnosperm, black spruce (*Picea mariana*). Construction of two opposing universal primers targeted to highly conserved regions within the MADS box, allowed the simultaneously *in vitro* amplification of a 60 bp segment from multiple gene members. Combined with a routine subcloning into M13 and sequence analysis of individual subclones, this approach has provided extensive amounts of DNA sequence data. Forty distinct gene fragments have been identified, indicating that black spruce has a large and complex MADS box gene family that may contain over a 100 genes. This approach thus provides a rapid and powerful method for the characterization of multigene families and clearly has potential for applications in other gene characterization projects, particularly for novel species. DNA sequence comparison suggests that the black spruce MADS gene family can be subdivided into 10 to 15 subfamilies, one of which shares extensive amino acid sequence homology to *agamous;* another shares homology with *squamosa;* several others are highly distinctive and appear to be unrelated to any other previously characterized MADS gene. DNA sequence differences between subfamilies range from 30-50%, precluding cross hybridization and the isolation of diverse MADS gene members using heterologous probing. PCR cloning thus provides the ability to identify distantly related members of a gene family. PCR cloning using cDNA derived from an embryogenic culture, indicated that a large proportion of the identified MADS genes are transcribed. Preliminary evidence also indicates that one *agamous*-like gene is expressed in proembryos, suggesting a potential role for *agamous*-related genes in somatic embryogenesis.

D 429 **TnpA *trans*-activates *de novo* methylated *Suppressor-mutator* transposable elements in transgenic tobacco**, Michael Schläppi, David Smith and Nina Fedoroff, Carnegie Inst.Wash., Department of Embryology, Baltimore MD. 21210

We report that the maize *Suppressor-mutator* (*Spm*) transposable element is subject to epigenetic inactivation in transgenic tobacco, as it is in maize. *Spm* inactivation in tobacco is correlated with increased methylation of sequences near the element's transcription start site. To determine whether element-encoded gene products can promote the reactivation of an inactive element, as has been reported in maize, we investigated the effects of introducing individual cDNAs for TnpA, TnpC, and TnpD, three of the element's four known protein-coding sequences. Each cDNA was expressed from the strong 35S CaMV promoter and introduced into plants containing one or more copies of the *Spm* element and an excision assay plasmid with an internally deleted *dSpm*-disrupted ß-glucuronidase (GUS) gene. Introduction of the CaMV 35S-*tnpA* cDNA into the transgenic tobacco plants promoted the reactivation of the inactive resident *Spm* element, as judged by the appearance of regenerants with very early excision events and transposed elements. By contrast, neither the CaMV 35S-*tnpC* nor the CaMV 35S-*tnpD* cDNAs affected the activity of the resident *Spm* element. Similar results were obtained when the element-encoded cDNAs were introduced either by *Agrobacterium*-mediated retransformation or by a genetic cross. Reactivation of an inactive *Spm* by TnpA is accompanied by reduced methylation of several methylation-sensitive restriction sites near the element's transcription start site. Maintenance of the reactivated *Spm* element in an active state requires the continued presence of the CaMV 35S-*tnpA* cDNA. Elimination of the CaMV 35S-*tnpA* cDNA locus by genetic segregation generally results in decreased element activity, as judged by its ability to promote excision of the *dSpm* element from the excision assay plasmid, and is accompanied by increased methylation of the element's 5' end. Exceptions resembling the phenomenon of "presetting" are also observed in which progeny plants that did not receive the CaMV 35S-*tnpA* cDNA locus maintain high excision activity and exhibit low methylation levels.

D 430 A SYSTEM FOR DETECTING TRANSPOSED ELEMENTS IN ARABIDOPSIS, David L. Smith and Nina Fedoroff, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210

Insertional mutagenesis is a successful and widely adopted method for identifying and studying genes that play a role in an organism's development. Therefore, much effort has recently been placed on developing and using T-DNA and transposable element based mutagenesis systems to study plant development. Using the Ac element and several bacterial and plant marker genes, we have devised a versatile system for identifying plants in which a transposon has excised and reinserted elsewhere in the genome. The transposons have been designed to facilitate the identification of insertions downstream of promoters and in the vicinity of enhancers by the inclusion of a ß-glucuronidase (GUS) gene either lacking a promoter or having a minimal promoter sequence. The system permits a transposase-deficient transposon and a non-mobile source of transposase to be maintained either stably in separate plants or together in the same plant. Plants in which transposition is occurring can be identified by frequent somatic activation of the GUS gene. The herbicide chlorsulfuron is used as a selective agent to identify progeny plants in which the transposon has excised from its original insertion site within a chlorsulfuron-resistant acetolactate synthase gene. Additional selectable markers permit the identification of plants containing a transposed element, but which lack the source of transposase. We have recently described our initial characterization of the system and demonstrated its reliability and efficiency in identifying plants with transposed elements (Fedoroff, N. and Smith, D. 1992. The Plant Journal in press). We are continuing to accumulate plants containing transposed elements and will present data on the efficiency and frequency of GUS gene activation.

D 431 EXPRESSION OF FLAVERIA TRINERVIA C_4 PEPCase IN TRANSGENIC PLANTS

Jörg Stockhaus, Michael Esser and Peter Westhoff
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C_4 plants are superior to C_3 plants by their high rates of photosynthesis combined with an efficient use of water and nitrogen resources. It is generally accepted that the C_4 pathway has evolved from the C_3 pathway independently in a number of distantly related plant families. The genus Flaveria is used as a model system for the analysis of the transition from C_3 photosynthesis to C_4 photosynthesis because this genus includes C_3-, C_4- as well as species characterized by C_3/C_4 intermediate photosynthesis. The C_3/C_4 intermediate species represent an ideal model system for the analysis of the evolution of the C_4 pathway especially because this transition is rather complex and involves a number of morphological and metabolic changes which can not be reconstituted easily in C_3 plants. The most prominent changes are: Establishment of a C_4 like Kranz anatomy, reduced rates of photorespiration by differential compartmentation of photorespiratory enzymes, enhanced levels of PEPCase to increase recycling of photorespired CO_2, elevated levels of the other enzymes of C_4 photosynthesis and establishment of a C_4 cycle.

It is our goal to shift the characteristic C_3/C_4 intermediate photosynthetic properties towards C_4 photosynthesis by the expression of genes involved in C_4 photosynthesis. Here we describe C_4 PEPcase expression constructs which were introduced into the C_3 plants tobacco and potato. The characterization of the expression of the C_4 PEPCase is presented. Transformation of various C_3/C_4 Flaveria intermediate species is currently being established and the progress of this work is summarized.

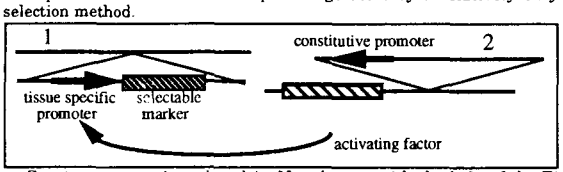
Supported by the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung und Technologie

D 432 IDENTIFICATION AND CHARACTERIZATION OF REGULATORY GENES INVOLVED IN PLANT SEED DEVELOPMENT, Clemens Suter-Crazzolara, Josefa-Maria Balsalobre, Jeff Schell and Bernd Reiss, Max-Planck-Institute, Carl-von-Linneweg 10, 5000 Köln 30, Germany

In the last years much has been learned about factors involved in differential gene expression, mainly from mammals, yeasts and insects. Most of these factors bind to the regulatory sequences of the target gene and influence its expression. Characterization of these factors has mainly taken place by biochemical means or methods like transposon tagging. Factors were also identified through analysis of mutants with altered phenotypes, as was the case for many homeotic genes of Drosophila.

Our research is mainly concerned with transcriptional regulation in plant seeds. During seed formation, a well-coordinated expression of storage proteins takes place. Regulation of this process operates through differential gene expression, which acts predominantly at the level of transcription.

We have developed a novel approach to identify transcription regulators. Basically, a reporter gene is coupled to a seed specific promoter, and this construct is introduced into the plant (construct "1", figure). Secondly, a construct containing a strong, constitutive promoter is integrated into the genome (construct "2"). This promoter can lead to the production of an anti-sense RNA, or switch on the silent gene of a transcription regulator. If the associated gene product interacts (either directly or indirectly) with the seed specific promoter of the first construct, the reporter gene will be switched on, enabling selection. It is thus possible to isolate transcription regulators by a relatively easy selection method.



Constructs were introduced in *N. tabacum* with the help of the Ti plasmid of *Agrobacterium tumefaciens*. We have obtained data which indicate that the silent marker gene is indeed activated by the independent integration of the second construct. Currently, genes linked to the constitutive promoter of this T-DNA are being isolated and analyzed.

The advantages of this approach are: 1. the use of a straigthforward selection scheme, 2. the possibility to characterize several members of a hierarchical regulatory cascade, and 3. the possibilty to adapt the system with some modifications to different organisms.

D 433 IAA METABOLISM IN LAND PLANTS, A. Ester Sztein[1], Jerry D. Cohen[2], Janet P. Slovin[1] and Todd J. Cooke[1]. [1]Department of Botany, University of Maryland, College Park, MD 20742, [2]Horticultural Crops Quality Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705

Major evolutionary differences among the land plants involve vascular tissue organization, meristem structure, leaf type, root initiation and embryo development. Studies performed on several higher plants suggest that most of these developmental phenomena are primarily regulated by the phytohormone auxin (IAA). IAA concentration is largely controlled by conjugation to amino acids or sugars in addition to possible feedback mechanisms. The purpose of this investigation was to determine whether different land plants have the biochemical ability to produce IAA conjugates, and to study the complexity of their IAA conjugation patterns. Vigorously growing shoot tips from 24 land plants ranging from liverworts to angiosperms were incubated with ^{14}C-IAA for 22 hours, after which IAA conjugates were separated using thin layer chromatography. The conjugates were analyzed using radioimaging techniques (AMBIS). In general, our results show that the complexity of the overall metabolic pattern is positively correlated with morphological complexity: more primitive plants produce fewer conjugates than more advanced plants. The charophycean alga Nitella does not produce any conjugates. Liverworts do not show any conjugation potential, whereas mosses and hornworts produce a few amide and ester conjugates, respectively. Lower vascular plants, such as club mosses and horsetails, produce an intermediate number of both amide and ester conjugates. Ferns, gymnosperms and angiosperms tend to produce the largest number of different conjugates. These results suggest that changes in IAA regulation accompanied the major evolutionary events in the land plants.

This work was supported by USDA-CRGO-89-37261-4791 and USDA-CRGO-91-37304-6655.

D 434 *IN VITRO* RNA DEGRADATION SYSTEMS DERIVED FROM SOYBEAN AND PETUNIA FAITHFULLY DEGRADE A rbcS mRNA INTO DISCRETE PRODUCTS, Matthew M. Tanzer and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, GA 30602

Light is a major stimulus in development of plants. It has been previously shown in soybean seedlings that the genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (rbcS) are controlled at the transcriptional and posttranscriptional levels in response to light. In soybean seedlings transcription of rbcS genes is 32 fold higher in the light than in seedlings treated with 48 hours of darkness, whereas the steady state RNA levels are only eight fold higher in light versus dark adapted soybean seedlings. These data indicate that the rbcS mRNA is less stable in the light than in the dark (Shirley and Meagher (1990) NAR, **18**, 3377-3385).

It has been shown in light grown soybean seedlings and in transgenic petunia that the mRNA encoding the rbcS gene SRS4 degrades into a set of discrete lower molecular weight (LMW) products in the light. These products generally lack the 3' end of the RNA consistent with mRNA decay data from bacterial and mammalian systems, and they also lack poly (A) tails. In order to more closely study the mechanism of decay of the SRS4 mRNA, we have developed an *in vitro* degradation system derived from light grown soybean seedlings and from mature petunia plants. In the soybean system endogenous SRS4 mRNA levels decrease over a six hour time course while the LMW products decrease at different rates. When *in vitro* synthesized SRS4 RNA is added to the *in vitro* degradation system derived from soybean seedlings or mature petunia plants, the RNA is degraded into a similar set of LMW products. With this data we can examine various models for rbcS degradation by determining precursor/product relationships during degradation of the RNA. We hope to use this system to examine the determinants and mechanism of degradation of the SRS4 mRNA and its modulation by light and other developmental stimuli.

D 435 CHARACTERIZATION OF IDIOBLASTS DIFFERENTIATED FOR CALCIUM OXALATE FORMATION IN *VITIS*, Mary Alice Webb[1], John Cavaletto[1], Nicholas Carpita[1], and Howard Arnott[2], [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907 and [2]Biology Department, University of Texas at Arlington, Arlington, TX 76019

Many plants contain idioblasts, cells that differentiate from surrounding cells to become specialized in structure and/or content. In members of the Vitaceae, idioblasts that develop within the leaf mesophyll are much larger than the cells around them, and they are specialized to accumulate substantial crystalline deposits of calcium oxalate. We have begun to characterize the structural and biochemical features of these cells in several species of *Vitis*, as a step towards understanding the role of idioblast differentiation in leaves of these plants. In each idioblast hundreds of crystals of calcium oxalate develop inside the vacuole within membrane-bound chambers. All of the crystals have a uniform and characteristic raphide morphology, suggesting that the crystallization process is controlled within the vacuole. We have shown that the chambers surrounding the crystals contain a complex assortment of polypeptides, and we have some evidence to indicate that a vacuolar-type ATPase may be present. In the vacuole around the crystal chambers is a water-soluble matrix containing a variety of carbohydrates, some of which are constituents of glycoprotein. In all species of *Vitis* studied, the vacuoles of crystal idioblasts contain a characteristic set of carbohydrates, including the sugars arabinose, mannose, galactose, and glucose, and a substantial amount of glucuronic acid. This carbohydrate material persists throughout the development of the cells, such that the main constituents of mature idioblasts are the carbohydrate matrix and the calcium oxalate crystals. The *Vitis* idioblasts have some features in common with crystal-containing idioblasts in certain monocots, suggesting possible conservation of processes that influence the development of these specialized cells in divergent plant taxa.

D 436 TOWARDS AN UNDERSTANDING OF THE MOLECULAR EVOLUTION OF C_4 PHOTOSYNTHESIS, Peter Westhoff, Kann Ernst, Winfried Poetsch & Elke Rosche, Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität, D-4000 Düsseldorf 1, Germany

C_4 plants are of polyphyletic origin, i.e. they have evolved several times independently from C_3 ancestral species during the evolution of angiosperms. This suggests that the changes required to convert a C_3 into a C_4 species must have been quite readily achieved in evolutionary terms. Indeed, the enzymes known to be typical for C_4 photosynthesis are also found in C_3 species. By implication, these C_3 genes have served as a starting point for the evolution of the C_4 isoform genes. At least three major events can be identified which must have occurred during evolution in order to shape a C_4 isoform gene starting from a C_3 ancestral gene: (1) alteration of the kinetic and regulatory properties of the encoded enzymes, (2) increase of expression levels by at least one order of magnitude and (3) confinement of the expression to the mesophyll or bundle-sheath cells of the leaf. To unravel the molecular changes leading to the formation of the C_4 isoform genes a comparative approach was followed using two closely related C_3 and C_4 species of the genus *Flaveria*, i.e. *F. pringlei* (C_3) and *F. trinervia* (C_4). This study focusses on the phosphoenolpyruvate carboxylase (*ppc*) and the pyruvate orthophosphate dikinase (*pdk*) genes whose C_4 isozymes both accumulate in a mesophyll-specific manner. The gene familes encoding the C_4 as well as the C_3 isoforms have been isolated from *F. trinervia* (C_4) and *F. pringlei* (C_3). The closest neighbour of the C_4 isoform *ppc* gene (=*ppcA*) was not found in *F. trinervia*, but in *F. pringlei* indicating that the least common ancestor of the two species contained already a *ppcA* gene. In contrast, the *pdk* C_4 isoform gene evolved after the two plant lineages were separated. To analyze the expression characteristics of the C_4 and C_3 promoters the 5' flanking regions of the genes were fused to the GUS reporter gene and are currently used in transformation experiments.

Supported by the Deutsche Forschungsgemeinschaft.

D 437 SOMATIC MUTATION IN WOODY PLANT DEVELOPMENT AND EVOLUTION, Ross Whetten, Jose Chaparro, Dennis Werner, David O'Malley and Ronald Sederoff, Departments of Forestry and Horticulture, North Carolina State University, Raleigh NC 27695-8008

The role of somatic mutation in plant development and evolution has been debated and modeled, but rarely directly tested. Vegetatively propagated horticultural plants can accumulate somatic mutations, and provide a system in which to test the frequency and mechanisms of somatic mutation in plants. We are investigating the inheritance of naturally occurring morphological variants of peach [*Prunus persica* (L.) Batsch] and characterizing an unstable mutation in the gene encoding UDPG:flavanol glucosyltransferase (UFGT). This enzyme catalyzes a late step in anthocyanin biosynthesis, thus mutant and revertant sectors are readily distinguishable. The somatic reversion rate of the unstable mutant allele has been measured for both the LI and LII histogenic layers. We are also inducing mutations using gamma rays and EMS, in order to compare background mutation rate, mutation rate in the presence of an active transposon, and mutation rate in the presence of known mutagens.

D 438 PROTEIN FARNESYLTRANSFERASE IN PLANTS: MOLECULAR CLONING AND EXPRESSION OF A HOMOLOG OF THE β SUBUNIT FROM PEA, Zhenbiao Yang, Carole L. Cramer, and John C. Watson, Department of Botany, Maryland Agricultural Experiment Station, and Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742-5815; Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330 (C.L.C.)

Protein farnesyltransferase is a heterodimeric enzyme that attaches a farnesyl moiety to C-terminal cysteine residues. The farnesylation is required for membrane association and thus *in vivo* activity of an increasing number of regulatory proteins such as *ras* and related small GTP-binding proteins. Both the α and β subunits have recently been cloned and sequenced from yeast and rat. Degenerate oligonucleotides, corresponding to conserved regions of the β subunit, were used as primers for the polymerase chain reaction to amplify cDNA synthesized from total cellular RNA from the apical buds of pea seedlings. The 171 bp fragment obtained encodes an open reading frame of 57 amino acids showing 65% identity to the rat β subunit. Using this fragment to screen a pea cDNA library, one full-length cDNA clone was obtained and sequenced. The predicted amino acid sequence of 419 amino acids exhibits 48% and 40% identity to the rat and yeast β subunits, respectively, indicating that this cDNA encodes a pea homolog of the β subunit of farnesyltransferase. To our knowledge, this is the first protein farnesyltransferase homolog cloned from plants. Gel blot hybridizations show that the pea β subunit homolog is likely to be encoded by a single copy gene and is expressed as a transcript of approximately 1.7 kb. During photoregulated leaf development in continuous white light, transcript levels within apical buds increase rapidly and transiently and then decline steadily. This transient increase precedes an increase in mitotic activity, suggesting that protein farnesyltransferase may play an important role in the control of cell division cycle in plants, analogous to its role in yeast and animals.

Late Abstracts

MOLECULAR CHARACTERIZATION OF TWO GENES FOR MAIZE MITOCHONDRIAL Cpn60.

P.S. Close[1,2], B.A. Bowen[1], R.G. Hallberg[3] and S.P. Briggs [1], [1] Pioneer Hi-Bred International, Inc., Johnston, IA; [2]Iowa State University, Ames, IA; [3]Syracuse University, Syracuse, NY.

Cpn60 (Hsp60) is an abundant mitochondrial protein first described in *Saccharomyces cerevisiae* and *Tetrahymena thermophila* cells. It belongs to a family of proteins homologous to the *E. coli* protein GroEL. This family also includes the chloroplast Rubisco-binding protein. These proteins are designated **'chaperonins'** because they are implicated in the folding of polypeptides and the assembly of oligomeric protein complexes. Genomic Southerns reveal two copies of cpn60 in most maize inbreds screened. One copy is present in the *Arabidopsis* genome. Segregation patterns indicate that both maize genes are encoded in the nuclear genome. The chromosomal location of both cpn60 genes have been determined by RFLP mapping. A maize B73 genomic library has been constructed and screened for cpn60 clones. Two major families of overlapping clones have been identified corresponding to the two gene copies identified on genomic Southern blots. Nucleic acid sequence has been determined for these two genes. The sequence of genomic cpn60I and cpn60II correspond to the sequence of two cpn60 cDNA's which have been identified and sequenced. Four minor families of genomic clones which show varying degrees of homology to the cpn60 cDNA have also been identified. An *Arabidopsis* cpn60 genomic clone has also been isolated. Sixteen introns have been identified in each maize gene, introns are also present in the Arabidopsis cpn60 gene. Expression of maize cpn60 mRNA and protein is developmentally regulated in young seedlings. Expression in seedlings also increases several fold in response to cold and heat shock treatments. Results of analyses of cpn60 I and cpn60II gene specific expression will be presented.

Cpn60, also known as heat shock protein 60 (Hsp60) is an essential protein in yeast. The maize cpn60 II cDNA was transformed into *Saccharomyces cerevisiae*. Maize cpn60 gene product is properly imported into yeast mitochondria and processed to the correct size mature protein. Maize Cpn60 protein was capable of sustaining yeast cells in the absence of yeast Hsp60 protein. Data from transient expression studies in maize cells will be discussed.

A NOVEL HOMEOTIC MUTANT IN *ARABIDOPSIS THALIANA* AFFECTING ROOT, STEM AND FLORAL MERISTEMS, Peter Morris, Gisela Felix, Thomas Altmann, Alison Jessop, and Lothar Willmitzer, Institut für Genbiologische Forschung Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, Germany

Plants with mutant phenotypes were screened for in progeny derived from a two-component transposon tagging system in transgenic *Arabidopsis thaliana*. Lines bearing an immobilised source of Ac transposase and the non-autonomous transposon Ds were crossed and progeny arising from the crosses selected for mobilised Ds through the activation of a selectable marker. 593 independent lines of Arabidopsis bearing mobilised transposons were screened and at least 47 stable mutant lines were found. One mutant in particular was found to be altered in both floral and vegetative tissues. This recessive mutation is however not thought to be due to insertion mutagenesis by the Ds element. The phenotype is of a highly reduced apical dominance with many short, branched shoots arising from the base, and the formation of secondary rosettes where normally axillary buds and branches would be present. The leaves are of abnormal shape. Stems arising at the base of the plant occasionally have disturbed gravitropism, grow downwards into the soil and even flower underground. Some roots "forget" their identity and turn into stems, growing upwards to produce secondary plants. The flowers are never normal and very variable, consisting primarily of sepal-like and carpel-like tissues, but frequently other entire or hybrid organs are present. We present initial data on the morphological and genetic characterisation of the mutant, and phenotypic aspects of double mutants with other described homeotic mutants in Arabidopsis.