PLANT GENETICS

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April 13 — 19, 1985

Perspectives in Plant Genetics

1652 STRUCTURAL HETEROGENEITY OF CHROMOSOME 10 IN RACES OF MAIZE AND TEOSINTE AND ITS PHYLOGENETIC SIGNIFICANCE, M.M. Rhoades, Department of Biology, Indiana University, Bloomington, Indiana 47405.

Other than the remarkable range in knob number, site and size, little structural variation has been found in races of maize and teosinte. A striking exception is chromosome 10, the shortest member of the complement. The three types of chromosome 10, designated as N10, K10-I, and K10-II, differ primarily in the structure, length, and gene order in the distal portion of 10L. By means of a series of simple terminal deficiences of the K10-I chromosome, it has been possible to determine the complex structural changes differentiating the three types of 10. These include transposition, inversion, and the intercalation of a long segment of unknown origin possessing three small knobs. The ability of the K10-I and K10-II chromosomes to induce preferential segregation, meiotic neocentromere formation and to enhance recombination values resides in the distal segment which is not possessed by N10. The fact that N10 and K10-I exist both in races of maize and of annual diploid teosinte while all three types of 10 are found in certain races of teosinte suggests that modern maize evolved from the latter populations.

Plant Developmental Genetics

1654 PHENOTYPES IN CORN: CONTROL OF PATHWAYS BY ALLELES, TIME AND PLACE, Edward H. Coe, U. S. Dept. of Agriculture and Dept. of Agronomy, University of Missouri, Columbia, MO 65211.

The specification of a phenotype by a genetic constitution can be by control of single steps, by control of all steps coordinately, or by modulation of steps. In corn, studies with anthocyanin pigments, carotenoids, chlorophylls, storage proteins, storage carbohydrates, and specific isoenzymes have provided a framework from specific biosynthetic pathways for future dissection of processes that are more complex. Among the morphogenetic processes that are becoming subject to genetic dissection are the establishment and elaboration of form (especially leaf, kernel, tassel, ear), the sequence of embryogenesis, and the definition of pattern of growth and of expression. Certain loci (e.g., R, B, C, Pl) are attractive targets for molecular analysis of the specification of functions by alleles, time and place because they each express variations in these parameters at the phenotypic level. The special genetic properties of each locus suggest that we may or may not be able to make generalizations to other pathways and processes (especially to those, including pest resistances and stress responses, that are of utility in manipulation of the genome for efficiency and productivity), without more evaluation of existing germplasm for variants and genetic and cytogenetic analysis of their basis.

1655 ORGAN AND CELL SPECIFIC REGULATION OF Adh1 AND Kn1 IN MAIZE. Michael Freeling, Che-Hong Chen, Karen Oishi, Christie Karoly, Penny Globus-Norman & Deverie K.B. Pierce, Department of Genetics, University of California, Berkeley, CA 94720.

We are approaching developmental gene regulation in two different ways: from the outside in using mutants and variants of the alcohol dehydrogenase-1 gene (Adh1), and from the inside out, using the Knotted (Kn1) locus.

Among the approximately 100 mutants and variants that have been described at the Adh1 gene, several are regulatory in nature (altered transcription/processing/termination in all organs and at all times equally) but only a few behave in developmentally interesting ways. All of the naturally occurring variants tested show an organ-specific, reciprocal relationship in their quantitative expression: if an allele is over-expressed in the scutellum, it is necessarily underexpressed in the anaerobic root (Woodman and Freeling, 1978). We now know that this reciprocal phenomenon reflects message levels. We have recovered one spontaneous mutant that is underexpressed in the scutellum only, which violates the reciprocal relationship. Perhaps our most informative mutant was selected as an anaerobic-sensitive seedling rather than as an allyl rather than as an allyl alcohol resistant pollen grain; it is low in ADH expression in the seed and seedling, but expression in the pollen is normal. The lesion is a 1.4 kb insertion near the transcriptional initiation site. Although this mutant was recovered from a Robertson's Mu line, the insertion is dissimilar to Mu1 in sequence. Being most recent, many questions are now unanswered regarding how this insertion alters organ-specificity.

Our continued progress requires a transformation system for maize. We have developed current microinjection methods that can deliver about 200 pBR molecules per minute through cell walls and into the nuclei of cultured plant cells without retarding growth. Because Mu1 transposon (Freeling, 1984, Ann. Rev. Plant Physiol. 35, 277-298) jumps replicatively and regularly in the gametophyte of outcross progeny (see Alleman, this volume), unlike Ac or the copia-like element Bs1 (Johns et al., 1984, submitted), Mu1 should serve well as an integration module, and is being tested.

Our (Freeling and Hake, Maize Genet. Coop. News Ltr. 1984, 58, 23-26 & submitted) studies with the dominant,leaf-morphology locus--Kn1--indicate that mutants at this locus alter the normal course of differentiation of cells near presumptive lateral veins. Upper epidermal cells can divide excessively or generate out-of-place ligule, and the parenchyma that should differentiate into bundle-sheath and mesophyll cells appears to remain undifferentiated.

Plant Developmental Biology

1656 THE KNOTTED LOCUS IN MAIZE, Sarah Hake & Michael Freeling, Department of Genetics, University of California, Berkeley, California, 94720

The knotted locus of maize specifies unexpected divisions in the leaf blade. The resulting hollow protuberances, or knots, occur along lateral veins only. Kn/+ plants are also knotted but mild in comparison and altered in the timing of expression. There are 6 dominant knotted mutants that are closely linked to Adh1. These "alleles" differ in timing, severity, and the tissues affected. One of the knotted mutants, Kn1-O, specifies alteration of the ligule and a general distortion of the plant structure.

Segmental aneuploid experiments were designed to vary the dosage of the normal (kn) allele. Neither kn/- nor kn/kn/kn/kn plants are knotted. Plants with the genotype Kn/kn and Kn/kn/kn are approximately equally knotted. These results suggest that the knotted gene is a new function. Surprisingly, Kn/- plants are not knotted. Transvection has been ruled out as a possible explanation; perhaps a modifier on 1L required in 2 doses can explain this result.

The cell autonomy and timing of knotted are under investigation using X-rays to break the long arm of chromosome 1 carrying Kn. The resulting segmental monosomic chromosome carries kn and lw, a white cell marker. The white sectors of irradiated seedlings are then examined for knots. We have shown that the ligule alteration component of knotted is cell autonomous. Experiments to determine whether the knots are cell autonomous and whether they are specified by certain cell types are continuing.

An Adh1-negative mutant, 2F11, caused by the insertion of Ds into Adh1 is also mildly knotted. Revertants have been selected that have lost Ds at Adh1 but are still knotted. Thus the insertion into Adh1 is not the primary cause of the knotted expression. 2F11 plants with increased knotted expression have been selected, perhaps Ds is responsible.

1657 PHENOGENETICS OF LEAVES IN MUTANTS OF ANTIRRHINUM MAJUS L.

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In Antirrhinum many mutants are known whose phenotype involves a change of the form of the leaves. The statistical analysis of a series of experiments with measurements of thedevelopment of the leaves of 12 mutants and a standard form in different constant environments of controlled temperature and photoperiod made it possible to describe the development in quantitative terms by 2 functions: the developmental function, based on the allometric function, describing the relative growth of length and width, and the growth function for the length of the leaf. On a double-logarithmic scale the developmental function consists of two straight lines of different gradients b1 and b2 and the coordinates x,y of the turning point at the end of the primordial phase of leaf development. The mutants differ in the values of the parameters of the 2 functions and the changes of these values under the influence of the environment. Beside this, differences between the mutants are found in the rate of leaf appearance and its change during development of the plant, the time needed for flowering induction and in meristematic growth. In the narrow-leaf mutants the number of cells in the lamina is smaler than in the standard form. The final length of the leaf, an important parameter in the growth function, depends on the combined action of ageing of the meristem and the process of induction of flowering. The determination of the development of a leaf depends on the interaction of the genetic and non-genetic factors and is completed when the leaf primordium is separated from the apical meristem. The differences in gene action which are important for the development of the leaves must be located in the cells of the apical meristem. The mutants with narrow leaves react to different temperature, some with such serious disturbances of growth and morphogenesis that they can be called temperature sensitive.

1658 FLORAL DETERMINATION: STABILITY, TIMING, AND ROOT INFLUENCE

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Determination is a fundamental concept in developmental biology. Numerous plant tissues and organs have been shown to exhibit stable determination (1). Our laboratory is investigating floral determination in dayneutral and photoperiodic tobacco. Timing. Previous investigations with developmentally-arrested, axillary buds of dayneutal Nicotiana tabacum (2) and recent studies with developmentally-arrested axillary buds of long-day Nicotiana silvestris have shown that bud meristems become florally determined long before the formation of a flower: as many as ten nodes may be laid down by the meristem before it forms the terminal flower. Thus, in developmentally-arrested buds, floral determination occurs days before the expression of this determined state. In the growing terminal meristem of N. tabacum floral determination occurs just several nodes before the formation of the terminal flower indicating that determined axillary buds are not clonally derived from determined terminal meristems. Internode tissues from dayneutral tobacco also exhibit floral determination since tissues from the upper part of a flowering plant will produce de novo floral buds in culture (3). We are investigating on individual plants the spatial relationship between determined axillary buds and determined tissues. Although our analysis is incomplete, we are certain that at anthesis determined tissues are located in more basal portions of the plant than determined buds. Root influence. In both dayneutral N. tabacum (4) and long-day N. silvestris, continually rooted plants fail to flower indicating that an influence from the roots may maintain plants in a vegetative state. Stability. Floral determination appears to be more stable in N. tabacum than in N. silvestris since tissues from the inflorescence of N. tabacum form de novo floral buds in culture while tissues from N. silvestris do not (3). This interpretation is supported by the observation that rooted, determined, axillary buds of N. silvestris produced abnormal inflorescences: the floral branches have altered their developmental fate and grow as vegetative branches instead of floral branches.

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2) McDaniel, C.N. Dev. Biol. 66:250-255 (1978).
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Supported by NSF (PCM82-04491).

1659 EMBRYO-LETHAL MUTANTS OF ARABIDOPSIS THALIANA, David W. Meinke, Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078

Arabidopsis thaliana (Cruciferae) has been described previously (1-3) as a model system for the isolation and characterization of embryo-lethal mutants. The six mutants originally studied by Meinke and Sussex (2) were chosen because they had lethal phases during early stages of embryogenesis. The purpose of the present study was to isolate additional mutants with a wider range of lethal phases and a greater diversity of embryonic abnormalities. Thirty-two new mutants isolated following EMS seed mutagenesis have now been examined in detail. These mutants differ with respect to the stage of developmental arrest, the color of arrested embryos and aborted seeds, the percentage and distribution of aborted seeds in heterozygous siliques, and the extent of abnormal development. Unusual mutant phenotypes include the presence of abnormal suspensors, distorted embryos, and fused cotyledons. No examples of temperature-sensitive lethals were found. The extent of cellular differentiation in mutant embryos is being approached through analysis of the protein bodies and seed storage proteins characteristic of later stages of development. Microscopic and electrophoretic studies have shown that some mutant embryos contain protein bodies and the same abundant proteins found in wild-type seeds. The isolation of eight new mutants with a non-random distribution of aborted seeds in heterozygous siliques provides further evidence that many of the genes that control early stages of embryogenesis in plants are also expressed prior to fertilization. Reciprocal crosses between heterozygous and wild-type plants have shown that in at least one of these mutants, expression of the mutant allele disrupts pollen-tube growth but not the distribution of mutant ovules. The growth response of arrested embryos from 17 different mutant lines has also been examined on basal and enriched media. Most of the mutant embryos that arrest prior to the heart stage produce only a limited amount of callus. 122G-E mutant embryos arrest at a globular-cotyledon stage and grow only slightly on a basal medium but produce extensive callus and homozygous mutant plants on an enriched medium. Mutant plants transferred to pots without organics grow well at first but then turn pale before flowering and do not set seed. Arrested embryos from mutant 112A, which have fused cotyledons and a reduced hypocotyl, produce abnormal plantlets without roots when grown on a basal medium. 112A mutant callus also fails to produce roots on a variety of root-inducing media. Expression of this gene is therefore required for normal development of a root apical meristem during both embryogenesis in vivo and organogenesis in culture.

(1) Muller, A.J. (1963) Biol. Zentralbl. 82: 133-163.
(2) Meinke, D.W. and Sussex, I.M. (1979a,b) Devel. Biol. 72: 50-72.
(3) Meinke, D.W. (1982) Theor. Appl. Genet. 63: 381-386.

1660 HOMEOTIC MUTATIONS IN MAIZE, R. Scott Poethig, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Mutations of at least 3 different genes in maize (Corngrass, Teopod 1 and Teopod 2) are analogous to homeotic mutations in Drosophila in that they transform structures in one part of the plant into structures normally found elsewhere. All three of these dominant mutations transform reproductive structures into leaves, and cause upper vegetative nodes to acquire traits characteristic of lower nodes. This effect suggests that these mutations perturb the transition from a juvenile to an adult phase of development (Galinat, 1966). The fact that leaf-specific mutations are expressed in the tassel leaves of at least one of these mutations (Cg) supports this interpretation because it demonstrates that tassel leaves use the same developmental program as normal vegetative leaves. Additional support for this hypothesis comes from a dosage analysis of Tp2. Using B-A translocations we generated plants of the following genotypes: Tp2/- ; Tp2/+ ; Tp2/+/+. The phenotypes of these 3 classes were essentially indistinguishable. This result suggests that Tp2 is a constituitive mutation that causes the expression of a product at an inappropriate time, and is consistent with the observation that Tp2 mutants retain juvenile characteristics throughout development.

Galinat, W. C. (1966) The corngrass and teopod loci involve phase change. Maize Gen. Coop. News Let. 40:102-103.

Transposons

1661 THE MECHANISM, RATE, REGULATION, AND SPECIFICITY OF *Mu1* TRANSPOSITION IN MAIZE.
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Several independent mutations isolated in Robertson's *Mutator* lines of maize have been associated with the discrete insertion of the 1.4 kb DNA fragment *Mu1* (1,2). *Mu1* has the structure of a transposable element, including 213 to 215 bp terminal, inverted repeats and a 9 bp direct duplication of target DNA flanking the insert (3). Most *Mutator* lines have ten to thirty copies of an element structurally very similar to *Mu1* dispersed through the genome, while other corn lines have zero to two copies of *Mu1*-like DNA per genome (4). Robertson (5) has demonstrated that *Mutator* mutagenic activity can be lost on outcrossing and is rapidly lost upon repetitive inbreeding.

Recent investigations have indicated that *Mu1*-related DNAs are present at high copy numbers both in lines that have lost *Mutator* mutagenic activity due to outcrossing (10-15 copies of *Mu1*-like elements per genome) or due to inbreeding (50-80 copies). In these lines, however, the *Mu1*-like elements rarely transpose. There is no strict copy number control of *Mu1* function, since occasional plants with low (10-15) or high (60-80) copy numbers of *Mu1*-like DNAs show *Mutator* mutagenic activity and somatic mutability and frequent *Mu1* transposition.

In lines showing *Mutator* mutagenic activity and/or somatic mutability of a *Mutator*-induced lesion, transposition of *Mu1*-like elements is always observed. Southern analysis of active *Mutator* parents and outcrossed progeny indicates nearly one new, transposed copy of *Mu1*-like DNA per parental *Mu1*-like element. The parental elements segregate randomly, about half going to each offspring. Hence, the outcross of a plant with twenty copies of *Mu1*-like elements to a zero copy plant commonly yields progeny with 14-20 copies of *Mu1*-related DNAs. The net increase in *Mu1*-like elements in germinal tissues and, more importantly, the much higher level of transposition (about once per generation) than germinal reversion (one in 1000 or less) suggests that *Mu1* transposition does not occur by a simple excision process.

Analysis of the sequences surrounding *Mu1* insertion sites indicates that the element integrates preferentially into transcriptionally active, single copy DNA. This macro-preference for open DNA will be compared to observed micro-preferences in *Mu1* insertion.

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1662 MECHANISM AND REGULATION OF Tn10 TRANSPOSITION
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Two experiments suggest that Tn10 transposition may usually occur without extensive replication of transposon sequences. First, physical experiments show that Tn10 (IS10) transposase can promote the occurrence of double strand breaks at the ends of Tn10 or IS10 *in vivo*. Second, genetic experiments using heteroduplex donor transposons suggest that information from both strands of the donor transposon are recovered in the transposition product. These observations will be discussed in the context of specific models for Tn10/IS10 transposition.

A screen for *E.coli* mutants exhibiting increased Tn10 transposition frequencies has yielded mutations in the gene for DNA adenine methylase (*dam*). Analysis of these mutants has revealed that IS10 transposition is directly regulated by DNA adenine methylation. IS10 contains two *dam* methylation sites (GATC) which are normally symmetrically methylated on both strands. One of these GATC sites is in the promoter for the transposase gene, which is located at the "outside" end of IS10. Failure to methylate that site results in a ten-fold increase in activity of that promoter *in vivo* (D.E.R.) and *in vitro* (B. Hoopes and W. McClure). The second site is located 9 basepairs from the "inside" end of IS10, within the terminal sequences required in *cis* for IS10 transposition. Failure to methylate that site results in a twentyfive-fold increase in the activity of that inner terminus. Both genetic experiments and *in vitro* transcription experiments suggest that the two effects of non-methylation can be observed in hemi-methylated as well as fully unmethylated DNA; and genetic experiments suggest that in wild type (Dam^+) hosts, the two effects are temporally coupled. *E.coli* DNA is normally hemi-methylated immediately after passage of a DNA replication fork. We propose that the effect of Dam methylation of IS10 is to provide a burst of IS10 transposition immediately after replication of the element.

1663 PLANT TRANSPOSABLE ELEMENTS GENERATE THE SEQUENCE DIVERGENCY NEEDED IN EVOLUTION

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At least some of the plant transposable elements are thought to transpose by excision and reintegration. If this is so then the structure (DNA sequence) of the excision product may shed some light on the mechanism of transposition. Numerous revertants of transposable element induced mutations have been isolated, cloned and sequenced. Surprisingly the wildtype DNA sequence is not restored in these revertants. The rules according to which the excision events occur have been formulated into a model for transposition, which will be discussed. The ends of the integrated element provide the substrate for the transposase, which generates staggered nicks at which an error prone repair system can operate. The resulting product has certain similarities with the sequences flanking the integrated element. However, in most cases not a wildtype but a mutant sequence is formed in the excision process. Sometimes these mutant sequences result in an altered protein. Therefore, transposable elements while walking through the genome leave footsteps behind and thus generate the DNA sequence divergency needed in evolution.

Plant Developmental Biology; Plant Transposons

1664 CHARACTERIZATION OF A GENE FOR A DEVELOPMENTALLY REGULATED SULFUR-RICH PROTEIN FROM BRAZIL NUT, Susan B. Altenbach, Karen W. Pearson, Filomena Leung, and Samuel S.M. Sun, ARCO Plant Cell Research Institute, Dublin, CA

A 7Kd protein which is exceptionally rich in the sulfur amino acids methionine (19%) and cysteine (8%) has been identified in Brazil nut (Bertholletia excelsa). We have found that the 7Kd protein is synthesized during a specific developmental stage of seed maturation. Hybridization studies have demonstrated that a 700 base RNA species which encodes this protein is present in the nut tissue 9 to 10 months after fertilization but is not found at earlier stages or in the mature nut.

In vitro translation studies using 9 to 10 month old Brazil nut RNA have shown that the 7Kd protein is synthesized initially as an 18Kd precursor polypeptide. This 18Kd polypeptide is immunoprecipitable with an antisera made to the sulfur-rich protein. The 18Kd polypeptide is then trimmed post-translationally in at least two discrete steps before it reaches its mature form.

Using as a probe an 18 base synthetic DNA fragment which is complementary to amino acid residues #30-35 of the 7Kd protein, we have identified cDNA clones for the sulfur-rich protein. These cDNA clones are able to select a specific mRNA from preparations of 9 to 10 month old Brazil nut RNA which encodes the 18Kd polypeptide precursor of the 7Kd protein. The nucleotide sequence of the cDNA clone is in good agreement with amino acid sequence data obtained from the isolated 7Kd protein.

We have also identified and sequenced genomic clones for the sulfur-rich protein of Brazil nut. We hope that the transfer of the gene for the Brazil nut sulfur-rich protein and its subsequent expression in plants deficient in the sulfur-containing amino acids may present a molecular way to improve the nutritional quality of certain sources of vegetable proteins.

1665 Ac: A GENERALIZED PLANT MUTAGEN? Barbara J. Baker, Luis Herrera-Estrella, Nina Fedoroff, Host Lörz, Rüdiger Hain, Jeff Velten, Armin P. Czernilofsky and Jeff Schell, Max Planck Institut f. Züchtungsforschung, 5000 Cologne 30, West Germany.

We are testing the potential of a genetically and molecularly well characterized autonomous controlling element of maize, Activator (Ac), as a generalized plant mutagen. The goals of this research are to manipulate the element such that it can be introduced into the plant cells and subsequently mutagenize and mark genes whose inactivation can be scored visually or biochemically. Initial experiments have been designed to answer the question: can transposition of Ac occur in a plant cell other than maize? The Ac element has been placed within the coding region of the chimeric dominant selectable marker gene pNOS-NPT II in plasmid pLGV neo 1103.Insertion of the Ac element inactivates the NPT gene. Constructions have been designed such that if the Ac element is excised in vivo from the NPT gene its function should be restored and plant cells containing this construction would then revert to a Km resistant phenotype. We have utilized the cocultivation technique to transform tobacco protoplasts. We are replacing putative transcriptional promotors of the Ac element with those of well characterized T-DNA promotors.

1666 GENETIC ANALYSIS OF DEVELOPMENTAL CHARACTERS IN WHEAT ENDOSPERM, M W Bayliss, ICI Bioscience Group, Runcorn, UK; A J Chojecki, M D Gale, Plant Breeding Institute, Cambridge, UK.

The starch stored in wheat endosperm is one of the world's major food sources. We present evidence that the extent of starch accumulation is controlled by the processes of cell and organelle division occurring early during endosperm development before starch synthesis begins.

Using the technique of reciprocal monosomic analysis, we have identified the chromosomal locations of genes influencing cell and organelle division processes. Reciprocal crosses were made between monosomic sets of varieties Chinese Spring and Spica. Analysis of 42 chromosome F_3 lines showed that chromosome 1D of Spica carried genes increasing final endosperm cell number and the number and final starch content of their constituent amyloplasts. Chromosome 7A of Spica also carried genes increasing endosperm cell number and numbers of amyloplasts, without effect on final amyloplast size.

1667 LIGHT AND DEVELOPMENTAL REGULATION OF RuBPCASE GENES IN AMARANTH, J.O. Berry, B.J. Nikolau, J.P. Carr, and D.F. Klessig. University of Utah, S.L.C., UT 84132.

RuBPCase specific activity in light-grown amaranth cotyledons increases during growth to a level approximately 15-fold higher than in dark-grown cotyledons by the eighth day after planting. Whereas the levels of the LSU polypeptide are quite similar in both light- and dark-grown cotyledons, the levels of the SSU closely follow the changes in enzymic activity. In light-grown cotyledons synthesis of both subunits increases until the third day and then plateaus. In contrast, in dark-grown cotyledons synthesis of both subunits occurs only as a burst between the third and fifth day even though functional mRNA for, at least, the LSU is present until the eighth day. Between the second and seventh day, the LSU mRNA is 2-5 fold lower in etiolated compared to light grown cotyledons. On the eighth day the concentration of this RNA drops approximately 10 fold in dark-grown cotyledons. Transfer of dark-grown plants to light on the eighth day results in a marked rise in the level of LSU mRNA but only after a lag of 5 hours or more. However, the synthesis of the LSU (and SSU) polypeptides dramatically increases within just a few hours after transfer, long before an increase in mRNA levels is detected. Similarly, when light-grown plants are shifted into darkness, within a few hours LSU and SSU protein synthesis is markedly reduced without any concomitant change in, at least, LSU mRNA levels. Thus, in amaranth the evidence indicates that posttranscriptional as well as transcriptional regulation is occurring.

1668 DRYING AS THE "SWITCH" THAT TERMINATES DEVELOPMENTAL PROCESSES IN THE CASTOR BEAN ENDOSPERM AND PROMOTES GERMINATIVE EVENTS, J.D. Bewley and A.R. Kermode, Plant Physiology Research Group, Dept. of Biology, University of Calgary, Calgary, Alberta, Canada, T2N 1N4

Seeds removed from the pod at 50 days after pollination (DAP) will germinate when placed in water, whereas developing seeds at 25-45 DAP need to be dried before germination can occur. Hence premature drying elicits a germination response. Isolated endosperms of 30 and 40 DAP seeds, which have not completed all developmental events, will survive premature drying, and will respond upon subsequent rehydration in a manner which is characteristic of an endosperm from a germinating mature seed. In particular, the pattern of soluble and insoluble protein synthesis upon rehydration of dried 30 and 40 DAP seeds is identical to that of hydrated mature endosperms, and is distinctly different from the developmental pattern of synthesis after 30 and 40 days. Isocitrate lyase is virtually absent from developing endosperms, but those dried at 40 DAP are induced to synthesize the enzyme at levels comparable to normal germinated seeds. LeuNAase, a proteolytic enzyme, responds to drying in a similar manner.

1669 REGULATION OF RIPENING IN TOMATOES, Ann M. Callahan and Peter H. Morgens, USDA-AFRS, Kearneysville, W.V. 25430

While the literature reports many changes involved in fruit ripening, the regulation of the process has not been elucidated. Ripening is associated with increased ethylene levels. We are studying the regulation of genes that are either turned on or off during the ripening process in hopes of understanding the coordination involved in the developmental change. Our approach is to examine the problem at the levels of enzyme activity, protein synthesis and RNA synthesis. In comparing the different stages of ripening by examining total proteins, in vivo labeled proteins, and in vitro translated proteins we find many differences. The changes seen are not reflected by all three methods. This implies that the differences are not due simply to changes in the levels of transcription. The major example is the huge increase in total protein of polygalacturnoase that is not reflected by in vivo labeling or by in vitro translation of RNAs. We theorize that, in the case of polygalacturonase, it is being synthesized at about the time of ethylene production in at least one area of the mature green fruit. This is well before the time reported in the literature and leaves open the possibility that the action of polygalacturonase is associated with the induction of ethylene and subsequent ripening.
We are now building cDNA libraries from the different stages of ripening fruit. We hope to isolate two classes of genes whose products change during ripening: 1) those known to change in enzyme activity and 2) those that change in their relative levels of RNA. These may or may not be overlapping classes. Then questions such as whether polygalacturonase is sequestered before activity and whether ethylene production preceeds transcription or activation of ripening enzymes can be answered. We hope that the coordinated study of these genes and their respective products will further our knowledge of these developmental events.

1670 MUTATOR HOMOLOGOUS SEQUENCES IN INBRED & MUTATOR LINES. Vicki Chandler, Carol Rivin and Virginia Walbot, Dept. of Biological Sciences, Stanford Univ., Stanford Ca.

Robertson's Mutator is a transposable element system of maize, identified by its ability to increase mutation rates 20-50X. A 1.4 kbp transposable element has been cloned and designated Mu-1. (Bennetzen, et al PNAS 81:4125) Using the cloned Mu-1 element as a probe we find sequences homologous to Mu-1 in many non-mutator inbred lines of maize. Southern blot analysis reveals restriction pattern differences in the Mu-homologous sequences among the inbreds and these are different from those found in Mutator lines. We have isolated a clone homologous to Mu-1 from a non-mutator inbred B37C library. Comparison of the inbred Mu-homologous sequences with the active elements in Mutator lines may help to identify sequences involved in the activity and origin of the Mutator system. In order to analyze the molecular basis of the Mutator system we are examining the segregation of Mutator activity through several generations by following high mutation rate and continued somatic instability of a Mu-induced mutation at Bz2. When Mutator lines are outcrossed most of the F1 progeny retain Mutator activity. Occasionally, progeny are observed in which somatic reversion and high mutation rate have ceased. These plants were examined for Mu copy number and they appear to fall into 2 classes; low (<10) and high (>50). Southern blot analyses of high copy lines reveal the loss of the expected restriction fragments characteristic of Mu-1. Instead, we observe the presence of many larger Mu-homologous fragments. We are investigating the molecular nature of this alteration. Lines with altered elements have been crossed to normal copy number, active Mutator lines. Preliminary results indicate the F1 progeny contain no Mutator activity.

1671 ISOLATION AND CHARACTERIZATION OF ORGAN-SPECIFIC cDNA CLONES FROM TOBACCO, M.A. Conkling, C.L. Cheng, and H.M. Goodman, Department of Molecular Biology, Massacnusetts General Hospital, Boston, MA 02114. In order to investigate the nature of organ-specific gene expression in tobacco, we have constructed cDNA libraries from mRNA isolated from both leaves and roots. These have been screened with reverse-transcribed mRNA probes from the two organs. Differential hybridization was observed in about 5-10% or the clones analysed. Clones exhibiting differential hybridization were confirmed by Northern analysis of mRNA isolated from leaves, roots, and a tobacco suspension culture line. The results of these experiments show that among all of the leaf-specific cDNA clones, there are low but detectable levels of homologous mRNA present in both roots and suspension culture cells. Similarly, the root cDNA clones which exhibit preferential hybridization to root mRNA hybridize at low but detectable levels to both leaf and suspension culture mRNAs. Titration experiments demonstrate a 10 to 1000-fold enrichment in one organ over the other. To further characterize the transcriptional regulation of the genes corresponding to these clones, we are currently doing nuclear run-off experiments. Results from such experiments using nuclei isolated from leaves, suggest that some of the root-specific cDNAs are transcriptionally regulated and some are post-transcriptionally regulated.

1672 EXPRESSION OF THYLAKOID PROTEIN KINASE ACTIVITY DURING DEVELOPMENT IN WHEAT, Stephen J. Danko, Andrew N. Webber and John P. Markwell, University of Nebraska, Lincoln, NE 68583

Leaves of Gramineae, such as wheat (Triticum aestivum), are useful for studies on plant development since cell division occurs in a basal meristem. The result is that a gradient of cellular and plastid development exists from the base to the tip of the leaves. Using 4 and 7 day-old wheat leaves, we studied the activities of thylakoid protein kinase activity as a function of distance from the base. The thylakoid membranes isolated from various sections of these leaves had markedly higher levels of protein kinase activity in younger portions of the leaves. The thylakoid protein kinase activities from the leaf base are not light activated as is the case in more mature tissues. Furthermore, the kinase activities from the base and tip segments have differential ability to phosphorylate a variety of histone substrates. These data suggest that the thylakoid protein kinase enzymes expressed during plastid development in wheat are different from those present in more mature chloroplasts.

1673 THE ISOLATION OF TEMPERATURE-SENSITIVE MUTANTS IN ARABIDOPSIS THALIANA, Mark A. Estelle and Chris R. Somerville, MSU-DOE Plant Research Lab, Michigan State University, East Lansing, MI 48824

The isolation and characterization of mutants has been a productive approach to the study of a number of problems in plant biology. However, most conventional screening procedures are limited in the sense that only mutations in nonessential functions can be recovered. This limitation has been overcome in a variety of different organisms by the application of screening procedures designed to isolate mutations which are only conditionally deleterious or which are expressed only under certain conditions. The most frequently used non-permissive condition is high temperature. We have developed a similar screen for Arabidopsis thaliana using the failure to germinate at 32 degrees C. and subsequent germination at 22 degrees C. as a convenient screen for temperature-sensitive mutations. A preliminary screen of approximately 10,000 M2 seed from a EMS mutagenized population has produced 93 plants which are temperature sensitive for germination. We are currently expanding this collection and determining the effects of each mutation on germination, vegetative growth of intact plants, and cells in tissue culture. We believe that our approach will permit the recovery of a wide spectrum of mutations affecting plant growth and metabolism.

1674 CONTROL OF MATURATION OF RAPESEED EMBRYOS, Ruth Finkelstein and Martha Crouch, Indiana University, Bloomington, IN 47405

During seed maturation embryogenic development ceases as the seeds dry down and enter a period of developmental arrest preceding germination. Previous studies, using storage protein synthesis as a biochemical marker of embryogeny, have demonstrated that exogenous abscisic acid (ABA) can both maintain embryonic development and suppress precocious germination of cultured rapeseed embryos (Crouch and Sussex, 1981). We determined whether the transition from embryogeny to developmental arrest in maturing rapeseeds reflected changes in endogenous ABA levels, sensitivity to exogenous ABA, or both. ABA levels were measured by radioimmunoassay and sensitivity to ABA was assayed in terms of its ability to both suppress precocious germination and stimulate storage protein synthesis in cultured embryos. Endogenous ABA levels were highest at the time of maximum storage protein synthesis, just before the embryos began to desiccate. Following this peak, both endogenous ABA and ABA sensitivity declined. These results suggest that, although ABA plays a role in maintaining embryogeny and suppressing germination prior to desiccation, it does not perform these functions during the later stages of development. We are currently investigating the importance of desiccation to the maturation process.

1675 GENE EXPRESSION DURING PLANT DEVELOPMENT, Leona C. Fitzmaurice, Steven B. Ellis, John N. Bell, Chris J. Lamb*, Michael H. Harpold, and Greg C. Holtz, The Salk Institute Biotechnology/Industrial Associates, Inc., La Jolla, CA 92037

Plant development involves changes in gene expression. A comparison of in vitro translation products derived from different plant tissues at different times during development reveals constitutively-expressed as well as developmentally-regulated proteins. Tissue-specific protein synthesis also is apparent.

Two-dimensional gel electrophoresis of in vitro translation products and computer-assisted scanning and analysis of the resultant gels allows extensive comparisons of gene expression in different tissues. For example, expression of a phenotype may be viewed through expression of a protein or set of proteins during development. This information in turn allows identification of the genes associated with the phenotype.

Examples of data will be presented illustrating the applicability of these techniques to the molecular biological definition of plant development and the expression of phenotypes.

*Consultant

1676 STUDIES ON A PLANT HORMONE BINDING PROTEIN, C. Brinegar and J. E. Fox, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

A protein (CBF-1) which specifically binds cytokinins with relatively high affinity has been isolated from wheat embryos and appears to be a trimer of a single 54 kD subunit. During embryo development cytokinin-binding activity rose sharply at two weeks post anthesis, leveled off at four weeks and disappeared upon seed germination. Western immunoblotting and rocket immunoelectrophoresis indicated that changes in binding activity were correlated with the biosynthesis and degradation of CBF-1.

In vitro translation of polyadenylated RNA from developing embryos yielded an immunoprecipitable polypeptide which comigrated on SDS-PAGE with the CBF-1 subunit. Genomic libraries of wheat have been prepared and c-DNA libraries are under construction in order to study expression of the CBF-1 gene. Possible roles of this protein as a receptor or regulator of embryo cytokinin levels will be discussed.

1677 THE GENETICS OF Sn, A TISSUE SPECIFIC GENETIC ELEMENT IN MAIZE. Giuseppe A. Gavazzi, Milvia L. Racchi, University of Milan, Dep.of Biology, via Celoria 26 20133 Milan Italy.

Data are presented concerning the location and expression of Sn, a genetic element responsible of the production of anthocyanin in specific sporofytic tissues of maize. The expression of different Sn accessions is compared and a case of instability is described (Sn-bol3).
The effect of light on Sn expression is analized in terms of pigment accumulation following irradiations with light of different qualities as well as in terms of activity of enzymes of the flavonoid biosynthesis.
The results obtained are discussed in relation to the ways light might affect Sn expression.

1678 MOLECULAR ANALYSIS OF THE En (Spm) TRANSPOSABLE ELEMENT OF ZEA MAYS Alfons Gierl, Zsuzsanna Schwarz-Sommer, Andy Pereira, Ralf B. Klösgen, Heinrich Cuypers, Peter A. Peterson and Heinz Saedler, Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG

The autonomous transposable element En (En-1, 8 kb) and the receptor element Spm-I8 (2.2 kb) were isolated as insertions into the waxy structural gene. Heteroduplex analysis reveals that Spm-I8 is a deletion derivative of the autonomous Spm (En) element. The ends of En-1 and Spm-I8 can form a stem and loop structure. The few differences in the DNA sequence of the termini of both elements, occur exclusively in the loops. This may reflect a functional role of the double stranded stems.
In transcription analysis of the waxy m-8 allele, where Spm-I8 interupts the waxy transcription unit, we observed 3.5 kb chimeric transcript that is initiated at the waxy promotor and terminates in the inserted element beyond a polyadenylation site. In the presence of En this chimeric transcript disappears, although the inhibitor element is still present in 70% of the cells. This may be due to the transacting suppressor of En, inhibiting transcription into the element.

1679 MOLECULAR CLONING OF THE GENE FOR THE SYNTHESIS OF IAA-LYSINE FROM PSEUDOMONAS SYRINGAE PV. SAVASTANOI. N. Louise Glass and Tsune Kosuge, Plant Pathology Department, University of California, Davis, CA 95616

The phytopathogen, P. syringae pv. savastanoi, incites the production of galls on olive and oleander plants. Gall formation is dependent upon the bacterial production of the phytohormone indoleacetic acid (IAA). Two enzymes are involved in the conversion of tryptophan to IAA: tryptophan monooxygenase and indoleacetamide hydrolase. In oleander isolates of P. savastanoi the genes for IAA biosynthesis reside on a plasmid (pIAA) and are organized in an operon. In addition, oleander isolates further convert IAA to an amino acid conjugate 3-indole-acetyl-ε-L-lysine. IAA conjugates are ubiquitous in higher plants, and are thought to play a role in development by the regulation of free IAA pool sizes.

To facilitate research into the biological significance of IAA-lysine production on gall development, the IAA-lysine gene (iaaL) was cloned into E. coli (pLG87). The specific activity of IAA-lysine synthetase from the E. coli clone pLG87 appears to be significantly higher than the specific activity of the synthetase from P. savastanoi. TN5 mutagenesis of the P. savastanoi pLG87 DNA insert indicates that the iaaL locus is 2kb upstream from the IAA operon. The iaaL locus is not part of the IAA operon, although it is in close proximity to the IAA genes on the pIAA plasmid on all tested oleander isolates.

1680 TOWARDS UNDERSTANDING OF THE MOLECULAR BASIS OF SALT TOLERANCE IN ELYTRIGIA, Patrick Gulick and Jan Dvorak, University of California, Davis, CA 95616. Elytrigia elongata (=Agropyron elongatum) and a number of related species naturally occur on seashores and saline soils and are exceedingly salt-tolerant. This high salt-tolerance of Elytrigia is expressed even when the Elytrigia genome is combined with those of salt-sensitive bread wheat, as is in the amphiploid T. aestivum cv. Chinese Spring X E. elongata. This amphiploid was shown to be highly tolerant to Na^+, Cl^- Mg^{+2}, SO_4^{-2}, and sea-water. To investigate the molecular basis of this high salt-tolerance each of the 7 complete chromosomes and 13 of the 14 chromosome arms of E. elongata were added to and substituted for the chromosomes of Chinese Spring wheat. Since none of these stocks were more salt-tolerant than Chinese Spring wheat, E. elongata salt tolerance must depend on the expression of genes on several Elytrigia chromosomes. To clone some of these genes we hypothesized that the genetic system controlling salt-tolerance is induced by salt stress. Therefore, we isolated polyA+ RNA's from the roots of the amphiploid grown in a nutrient solution without salt and three days after salinity was progressively increased to 250 mM NaCl. The mRNA populations were in vitro translated with a rabbit reticulocyte cell-free translation system and synthesized proteins labelled with ^{35}S were fractionated by 2-D isoelectric focussing-SDS-PAGE. At least four major proteins occurred in the salt-treated plants that were absent in the nontreated control. A cDNA library, using mRNA isolated from the amphiploid acclimating to salt-stress is being made. The clones complementary to the E. elongata salt-tolerance genes will be identified by cross hybridizing the library with cDNAs of salt-treated and nontreated plants of the amphiploid.

1681 Genetic and Biochemical DIFFERENCES AMONG TWO ISOGENIC BEANS LINES DIFFERING IN THEIR RESISTANCE TO BEAN ANTHRACNOSE, V.K. GUPTA, DEPT. OF CROP SCIENCE, UNIVERSITY OF NAIROBI, NAIROBI, KENYA.

Studies were made to determine the genetic and biochemical differences, if any, between two isogenic bean lines differing in their resistance to bean anthracnose, This was based on two enzyme systems : peroxidase and esterase It was revealed that resistant and susceptible plants differed in their banding patterns and intensities. These differences were dependent upon the stage of development. There are specific isozymes which are present in susceptible line only at certain stage of development. These bands, C2 and C4, thus can be used as genetic/biochemical markers for screening the populations for resistance to bean anthracnose. The total peroxidase activity, especially in the primary leaves, did not show constant differences between resistant and susceptible lines. The trifoliate leaves showed that the susceptible lines had higher peroxidase activity.

1682 A LOW COPY NUMBER, COPIA-LIKE TRANSPOSON IN MAIZE, Mitrick A. Johns, John Mottinger and Michael Freeling, University of California, Berkeley, CA 94720

Bs1, a transposable element that moved into the maize Adh1 gene following barley stripe mosaic virus infection is present in 1-5 copies in all maize and teosinte lines tested. Bs1 sequences do not hybridize with the genome of barley stripe mosaic virus. The insertion of Bs1 is bounded by 304 bp perfect direct repeats, similar in structure to Ty1 in yeast, copia and related elements in Drosophila and vertebrate pro-retroviruses, but different from all other known plant transposons. No free copies of the terminal sequences or large internal deletions of Bs elements could be detected. Bs1 is apparently not related to several transposons which moved into the Shrunken gene in lines made genetically unstable by barley stripe mosaic virus infection, suggesting that this virus may cause genome shock, resulting in a general liberation of transpososns in response to environmental stress.

1683 ISOLATION OF DEVELOPMENTALLY REGULATED GENES FROM COTTON, Joseph C. Kamalay, Glenn A. Galau and Leon S. Dure III, University of Georgia, Athens,GA 30602

A characteristic set of gene products has been identified in cotton embryos which appears to be coordinately induced by the plant hormone abscisic acid (ABA). (Dure, et al 1981). cDNA copies of the hormonally regulated messenger RNAs were isolated and identified by hybrid-selected and hybrid-released in vitro translation. Using these cDNA clones as probes, RNA dot blot hybridization has shown that those gene sequences whose expression is controlled by ABA can be induced at mid-embryogenesis (ABA subset I) or at late embryogenesis (ABA subset II). Several members of these developmentally regulated gene subsets have subsequently been isolated from cotton genomic libraries in Charon bacteraphage. The genes coding for the major cotyledon storage proteins, whose timed expression is distinct from that of the ABA-induced genes in cotton, have also been isolated. The structure of each gene and its flanking sequences have been examined by restriction endonuclease mapping. DNA sequence analysis of the 5' upstream regions of several of the genes of each developmentally regulated subset will be presented.

1684 DEVELOPMENTAL REGULATION OF SOYBEAN UREASE ISOZYMES, R.W. Krueger and J.C. Polacco, Biochem. Dept., M121 Med. Sci. Bld., Univ. of Missouri, Columbia, MO 65212

There are at least two isozymes of urease in soybean. The seed-specific form is expressed only in developing embryos and comprises 0.2% of mature seed protein. The second form is termed the ubiquitous urease since it is found in all tissues examined, namely leaf, cell suspension culture and seed coat. Its presence can be detected also in developing embryos of a mutant lacking the seed-specific form. The ubiquitous urease is found at 1/500th the level of the seed-specific form. The two forms are nickel metalloenzymes and share subunit size and structure. However, they are easily distinguishable with respect to pH optimum and affinity for inhibitors (hydroxyurea and phenylphosphordiamidate) and seed urease antibodies.

A synthetic 21-mer oligonucleotide, based on a 7 amino acid sequence of the seed-specific urease, was used to probe a soybean genomic library in Charon 4A. Of the 20 clones recovered five were chosen for further characterization based on their homology to a pentadecamer primer extended on soybean seed poly(A)$^+$ RNA. The primer sequence was based on a published pentapeptide of Jack bean seed urease. These 5 clones hybridize a 3.3 KB RNA, which is the size of the 25S rRNA and mRNA for lipoxygenase and urease. However, these clones do not hybridize known clones for rRNA or soybean lipoxygenase.

We are currently attempting to align the nucleotide sequence of these clones with known amino acid sequence of soybean and Jack bean urease.

1685 T-DNA GENE TRANSMISSION AND EXPRESSION IN TOBACCO PLANTS REGENERATED FROM CELLS TRANSFORMED BY tmr-TiT37 T-DNA, Gary A. Kuleck and Andrew Binns, University of Pennsylvania, Philadelphia, Pa. 19104

T-DNA transformed plant cells provide an attractive model system for elucidating plant gene expression regulation. We have been examining T-DNA transmission and transcription in cells transformed by mutated TiT37 plasmid (the tmr locus is inactivated)(1) and the plants regenerated from these cells. The T-DNA in the plants is present in high copynumber(20-40 copies/haploid genome) and is stably integrated and inherited. Segregational analysis suggests that the T-DNA is present in eight linkage groups most of which have at least one copy capable of full activity. Some of these linkage groups are not inherited in a Mendelian fashion.

Steady state transcription from the nopaline synthase gene, which is normally constitutively expressed in tissue culture, are found to be greatly reduced in a percentage of these plants (nop-) and this reduction is maintained in a heritable fashion in some of the progeny from subsequent outcrossings. In some cases however,the level of transcription can increase during the growth and development of these nop- plants.Northern analysis suggests that while transcription from the auxin autonomy genes (the tms loci, transcripts #1 &2) is lower in the regenerated plants vs. the same cells in culture, the extent of this decrease may differ between the tms loci. Further experiments to determine the basis for these transcriptional patterns will be described.

1. K.A. Barton, A.N. Binns, A.J.M. Matzke and M.-D. Chilton. Cell, Vol.32, 1033-1043, April 1983.

1686 ORGANIZATION OF THE 5SrRNA GENE FAMILY IN WHEAT, Michael W. Lassner and Jan Dvorak, University of California, Davis, CA 95616

We are investigating the organization of the 5S ribosomal RNA (rRNA) gene family in wheat in order to study the concerted evolution of a multi-gene family located on non-homologous chromosomes. We have utilized southern blot analyses of 60 aneuploid wheat lines, nulli-tetrasomics, ditelosomics and alien substitutions, by probing with plasmid pTA 794 (a 5S rRNA gene cloned from Chinese Spring wheat by W. Gerlach) and T. Dyer.

Like the wheat 18S and 26S RNA genes, the 5S rRNA genes are arrayed as tandem repeats; unlike their 18S and 28S counterparts, they are highly heterogenous for restriction enzyme recognition sites. There are 2 subfamilies of the 5S rRNA genes as defined by repeat length. A 410 base pair (bp) long family is present exclusively on the short arms of chromosomes 1B and 1D. A 500 bp family is found on the short arms of chromosomes 5A, 5B and 5D. Within each homoeologous group, the lengths of the repeats are conserved but the sequences, surveyed by restriction enzyme digestion, are diverged. Taq I cleaves the 5S rRNA repeats found on chromosomes 1D and 5B but not 1B, 5A or 5D. Amongst the group 5 chromosomes, only the 5A repeats have Hae III sites. Both the Hae III and Taq I sites are in the spacer. This clearly shows that there is little or no homogenization of the non-coding spacer sequences between non-homologous chromosomes. This is a marked contrast to the 18S-26S rRNA gene system in wheat; the spacers found in the 1B and 6B loci, despite their length polymorphims, are 99% homologous for each other in sequence. This suggests that one should exercise caution in generalizing about concerted evolution of all gene families based on a single study.

1687 ANABAENA VARIABILIS: A MODEL FOR THE STUDY OF DEVELOPMENTALLY REGULATED GENE EXPRESSION, Melanie E. Lynn & James D. Ownby, Okla. State Univ., Stillwater, OK 74078.

A. variabilis is a filamentous cyanobacterium that has served as a model system for the study of cellular differentiation by our laboratory. When cells are transferred to medium lacking NH_4^+ & NO_3^-, each filament differentiates specialized cells called heterocysts that occur in a regular pattern along the filament and which are the sites of nitrogen fixation. Several features of this process make it an attractive model system: 1) the genome is small and lacks repetitive DNA; 2) morphological development of the heterocyst can be monitored microscopically; 3) the mature heterocyst forms 48 hours after the onset of nitrogen starvation; 4) heterocyst N_2-fixation can be assayed by an C_2H_2-reduction technique; 5) the fully differentiated state consists of only two cell types which can be readily separated.

DNA:RNA hybridization techniques have been used by our laboratory to determine the number of genes required for heterocyst differentiation. We have found that 54% of the approx. 6000 genes present in the undifferentiated cells are transcribed. The mRNAs produced by these cells fall into 2 general frequency classes - an abundant class (18 copies/cell) and a rare class (< 1 copy/cell). The differentiated heterocyst expresses ca. 42% of the sense strand, equivalent to more than 2000 genes, 1500 nucleotides in mean length. The mRNAs produced by these cells are present in only one frequency class of low abundance. Only a fraction of these sequences, however, are unique to the heterocyst.

1688

TOMATO FRUIT SPECIFIC GENE EXPRESSION. Erik Mansson, Ellen Monson, Ann Pokalsky, Bill Hiatt and David Stalker.

Many changes in gene expression occur during tomato fruit ripening. In vitro translation of unripe and ripe fruit poly(A+)RNA indicated several distinct differences in the protein pattern when the translation products were analyzed.

A cDNA library was constructed from poly(A+)RNA isolated from ripe fruit. The library was screened with ^{32}P-cDNA synthesized from unripe and ripe poly(A+)RNA respectively. By combining this differential hybridization screen with Northern analysis genes expressed at specific stages of fruit development were identified. Data will be presented representing four cDNA's that, with respect to their developmental expression, cover varying stages of fruit development. These four cDNA's were used as probes against a tomato genomic library. The genomic organization of these genes will also be discussed.

1689

GENOME REARRANGEMENT IN THE EVOLUTION OF A DEVELOPMENTALLY-REGULATED MULTIGENE FAMILY IN WHEAT, R.A. Martienssen, D.C. Baulcombe and C.M. Lazarus, Plant Breeding Institute, Cambridge, U.K.

Cereal aleurone layers secrete two classes of α-amylase in response to gibberellic acid. cDNA clones of each class, which differ in timing of expression, starch specificity and genetic organisation, have been used to isolate nuclear DNA sequences corresponding to members of both related multigene families.

It has been possible to assign these clones to given chromosomes using DNA prepared from aneuploid lines of wheat. Using these assignments, the evolution of sub-family structures can be followed from diploid through tetraploid and finally to hexaploid wheats. The role of gene duplication and conversion as processes of sequence turnover between and within chromosomes is examined, along with restriction fragment length variants associated with these events.

Comparison of two members of such a sub-family by heteroduplex mapping and sequencing reveal the presence of a transposon-like insertion sequence in the 5'-flanking region of an α-Amy-1 gene in T. aestivum cv. Chinese Spring. Some other hexaploid varieties and the tetraploid progenitor allele appear to lack this insertion. The structure of the putative insertion sequence, with inverted repeats and direct target duplications, will be presented.

1690

PHOTOSYNTHETIC GENE EXPRESSION AND CELLULAR DIFFERENTIATION IN DEVELOPING MAIZE LEAVES, Belinda Martineau, Stephen P. Mayfield, and William C. Taylor, University of California, Berkeley, CA 94720

The basipetal pattern of maize leaf development results in a positional gradient of cell ages along the length of a young leaf. This simple pattern is somewhat complicated by the differentiation of bundle sheath cells; a photosynthetic cell type of maize leaves in addition to the leaf mesophyll. This "complication" of cellular dimorphism is interesting because the two cells functionally cooperate in the multi-step scheme of CO_2 fixation called C4 photosynthesis. Maize uses two CO_2-fixing enzymes (PEPCase and RuBPCase) which are located exclusively in mesophyll and bundle sheath cells, respectively, of the plant's leaves. I have found that PEPCase and RuBPCase mRNAs are also restricted to these respective cells types in mature leaf tissue. I have taken advantage of the leaf positional gradient to compare changes in gene expression involved in C4 photosynthesis with leaf morphological development monitored using light microscopy. We find accumulation of RuBPCase (large and small subunit) and PEPCase, and that of the mRNAs associated with these proteins, increases dramatically as bundle sheath cells become morphologically differentiated. Measurable quantities of all three mRNAs are, however, found in the relatively undifferentiated basal portion of the leaf. I am currently using in situ hybridization techniques to determine whether RuBPCase mRNAs are present only in cells predetermined to be bundle sheath.

1691 Newton, K.J. and E.H. Coe, Jr., Univ. of Missouri and USDA, Columbia, MO. Changes in mitochondrial DNA associated with NCS mutants of maize.--"Nonchromosomal stripe" (NCS) refers to unstable, maternally inherited mutants in maize which exhibit drastic and variable phenotypic effects including short stature, reduced viability and vigor, abnormal growth patterns and leaf striping (Shumway and Bauman, Genetics 55: 33; Coe, Maydica 28: 151).--Analyses of the mitochondrial genomes with restriction enzymes show that there are specific changes in the mtDNA of the NCS2 and NCS3 mutants, relative to the mtDNA of their common progenitor line. NCS2 has lost an 8 kb XhoI mtDNA band and gained a 20.5 kb XhoI fragment. NCS3 plants have a novel 20 kb XhoI band in their mtDNA, which is quantitatively correlated with a reduction in a 16 kb fragment. A new PstI fragment of 23 kb has been observed with mtDNA from NCS3, whereas NCS2 mtDNA contains a novel 7.6 kb PstI band and has lost a 3.7 kb band. The mtDNA changes in NCS probably do not result from simple losses in specific restriction sites.--The NCS mutations are unstable; that is, plants which only transmit normal phenotypes are occasionally found in mutant stocks. These normal derivatives of NCS2 and NCS3 have also lost the differences in mtDNA. We propose that the changes in mtDNA observed in the NCS mutants affect essential mitochondrial genes and that their drastic effects on plant growth are due to the sorting out of defective from normal mitochondria in dividing cells during development. If no mutant mitochondrial genomes are present in the cells giving rise to progeny, the aberrant plant phenotypes will no longer be transmitted.

1692 PLASTID GENE EXPRESSION DURING FRUIT RIPENING IN TOMATO

Birgit Piechulla, Karin Imlay, Margo Thelander, Wilhelm Gruissem
University of California, Berkeley, CA 94720.
The ripening process in tomato, L.esculentum, has been characterized by physiological and morphological changes. The differentiation of chloroplasts into chromoplasts occuring during fruit ripening has so far only been described by ultra structural changes. Here we present the results which indicate,that transcription of certain tomato plastid genes does occur throughout the ripening process (atp A, psb A : transcripts for these genes are found at all stages). Other genes, however, show a marked decrease in the level of transcription during the progression from green fruit to red fruit (rbc L, rrn, psaA). These results lead us to believe that transcription of chloroplast genome is differentially regulated during plastid conversion.

We have isolated clones from a plastid DNA library, in order to study DNA sequences which might be involved in gene regulation (transcriptional or translational level). Most of the isolated genes (psb A, rbc L, psb C, atp B, atp H, atp A, rrn, psa A) have been mapped to similar positions on the tomato plastid genome as are found on the plastid genome from spinach.

1693 MUTANTS WITH UNORDERLY CHLOROPLAST MORPHOGENESIS IN ZEA MAYS, Mary L. Polacco, University of Missouri, Columbia, MO 65212

Assembly of the major thylakoid complex, the chlorophyll (a/b) light harvesting complex (LHCII), is normally a late event in the development of eucaryotic photosynthetic membranes. A nuclear, virescent (v; slow-to-green) mutant (v*-424) has been found that permits LHCII to assemble earlier than usual. Most v-mutants do not uncouple LHCII assembly from the normal differentiation sequence. Three other, independently induced, virescent mutants allelic to v*-424 have been isolated from MG Neuffer's collection of M2 progenies. All were similar to v*-424 and permitted early assembly of LHCII during development. Curiously, some progenies for two of the mutants (v*-576, v*-588) also segregated v-siblings with normal, albeit delayed, thylakoid differentiation sequence. In the case of v*-588, I have confirmed that both v-phenotypes arise from the same locus.

Because no chloroplast encoded genes are known for LHCII assembly, the v*-424 phenotype could be attributed to a defect in any process that would slow protein synthesis by the developing plastid. However, finding that v-mutations at a single locus can generate two, distinct, assembly orders suggests that the locus is intimately involved in developmental timing. The mutated locus possibly (1) interacts with other loci that modulate developmental time or (2) is unstable, undergoing changes of state which may or may not perturb the differentiation sequence.

Current research is directed toward examination of plastid protein synthesis and LHCII turnover in mutant and normal tissue, and analysis of genetic interactions that generate the two virescent phenotypes observed for the v*-588 allele.

1694 PROPERTIES OF THE LARGE SUBUNIT RUBISCO BINDING PROTEIN COMPLEX, Harry Roy, Susan Cannon and Jane F. Koretz, Rensselaer Polytechnic Institute, Troy, New York 12180-3590

The control of chloroplast function by the nucleus may involve not only the supply of subunits for oligomeric chloroplast proteins and ribosomes, but also, in the case of ribulose bisphosphate carboxylase/oxygenase (RuBisCO), a 60 kDa protein which may facilitate assembly. Catalytic ("large") subunits of RuBisCO bound to this protein have been shown to participate in the assembly of RuBisCO in vitro (Milos and Roy, J. Cell Biochem. 24, 153-162). The oligomeric complex containing this protein has been purified and examined by high resolution electron microscopy. It presents a symmetrical rosette appearance with a central hole or depression; other views show a layered structure. The complex possesses a measurable ATPase activity which leads to complete dissociation into subunits. All the electron microscopic images are sensitive to ATP treatment, demonstrating their relevance to structural modelling of the complex. Research supported by NIH Grant to H. Roy.

1695 ANALYSIS OF BRASSICA NAPUS STORAGE PROTEIN GENES, Steven R. Scofield, Lorraine E. Solberg and Martha L. Crouch, Indiana University, Bloomington, IN 47405.

We are studying cloned Brassica storage protein genes to identify sequences responsible for their interesting pattern of expression. Expression of these genes is controlled at two levels. Expression is developmentally confined to embryonic tissue. Secondly, the rate of expression is modulated by the plant growth regulator, abscisic acid.

Brassica has two types of storage proteins, napin and cruciferin. Genomic Southern blots show that both napin and cruciferin are encoded by gene families. The napin family has at least twelve members and cruciferin has three.

Clones for six of the napin family and all of the cruciferin family have been recovered from genomic lambda libraries. None of these clones contains more than one storage protein gene. A napin gene, pgN1, is almost completely sequenced. It has a 71bp intron. S1 nuclease experiments indicate that transcription starts 33bp downstream from the TATA box. An enhancer-like sequence with 21bp of alternating purine-pyrimidine occurs between the AGGA and TATA boxes. A probe from the 3′ end of this gene has been shown to be specific for this family member on genomic blots. This probe hybridizes to a napin size RNA on northern blots suggesting that pgN1 is expressed.

1696 SELECTION OF MUTANTS IN IAA-CONJUGATE HYDROLYSIS, Janet P. Slovin and Jerry D. Cohen, Plant Hormone Lab. USDA, Beltsville, MD, USA.

The long-term objective of our laboratory is to understand the mechanisms by which plants control the level of the phytohormone, indole-3-acetic acid (IAA). It is implicit that if a hormone is to regulate plant growth processes then the concentration of the bioactive compound itself must be regulated. Many of the limits now preventing full implementation, in plants, of recent advances in biotechnology are probably the result of our inability to properly control the hormonal systems within plants and plant cell cultures.

One way by which plants are capable of increasing the level of free (active) IAA is by hydrolysis of amide or ester conjugates of the hormone. The conjugate is believed to be inactive hormonally but may have many different functions in the plant. For example, they appear to be storage forms of the hormone in corn kernels. We have developed a positive selection for screening for mutant plants or cell lines that cannot hydrolyze conjugates of IAA. The rationale for our selection involves making one or both of the moieties of the conjugate toxic. For this purpose we have synthesized, and assayed for toxicity, mono- and di-halogenated IAA analogs, and have synthesized IAA-analog conjugates as well as IAA conjugated by an ester linkage to the mycotoxin patulin. A plant that is capable of the hydrolysis reaction will release the toxic compound and its growth will be suppressed or it may die. The results of these selections on maize plants, Lemna plants and tissue cultures, and embryogenic carrot cell lines will be presented.

1697 STRUCTURE OF THE NIVEA LOCUS OF ANTIRRHINUM MAJUS AND CHRACTERISATION OF THREE TRANSPOSABLE ELEMENTS Tam1, Tam2, Tam3

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The nivea locus of A. majus codes for chalcon synthase (Chs), a key enzyme in flavanoid biosynthesis. Chs is light-inducible by the phytochrome system. DNA sequencing of genomic and cDNA clones allowed us to derive the structure of Chs: the coding region is interupted by two introns of 720 and 210 bp, respectively. A threefold repetition of an ACCA is found 5' upstream to the TATA-box which might be important for the light-inducibility.
Three nivea mutants were analysed. The two unstable mutants niv-rec53 and niv-rec98 carry DNA inserts in the promoter of the Chs gene: niv-rec53 a 17 kb long element, Tam1, niv-rec98 a 3.5 kb insert, Tam3. The elements prevent expression of the locus, but frequent excision during development leads to variegated phenotypes. In the stable white flowering mutant niv44 a 5 kb element (Tam2) is inserted close to the first exon/intron border. Tam1 and Tam2 induce a 3 bp duplication of the target site upon integration and possess the same inverted repeats at their ends. Both elements are members of the CACTA-family of plant transposable elements. Tam3 resembles the Ac/Ds elements in Zea mays: it makes a 8 bp duplication and 7 bp of its 12 bp inverted repeat are homologous to that of Ac/Ds.

1698 VARIABILITY AMONG MUTATOR ELEMENTS.

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The transposable element Mu-1 has been isolated from maize lines exhibiting Robertson's Mutator activity, a mutation rate 20-50X higher than normal. (Bennetzen, et al., (1984) PNAS 81:4125) Mu-1 is 1367 bp in length and contains terminal inverted repeats of 213 and 215 bp and four open reading frames. (Barker et al., (1984) NAR 12: 5955)

We have isolated 30 Mu-1 homologous clones from a Mutator line. Twenty-six contain elements of the same size class as Mu-1, however, four contain Mutator elements which differ in size and organization compared to Mu-1. Mu-L, a 1.7 kbp element contains 300 bp which are not present in Mu-1. Electron microscopy and hybridization experiments show that the additional 300 bp are not a duplication of sequences found in other Mutator elements nor are they highly represented in the DNA of non-mutator plants. The other three novel elements are smaller than Mu-1. Mu-S1 (1.0 kbp) has intact terminal repeats and internal segments which are homologous to Mu-1. Both Mu-L and Mu-S1 contain small deletions and/or restriction site polymorphisms which indicate they could not have arisen from Mu-1 by a single insertion/deletion event. Mu-S2 (800 bp) and Mu-S3 (650 bp) do not have intact terminal repeats and show less homology to Mu-1. We are sequencing the elements to determine their coding capabilities and their relationship to Mu-1. The 300 additional base pairs present in Mu-L may have the capacity to code for an additional protein. These unique sequences will also prove useful in following the segregation and transposition of Mu-L independent of other elements in the Mutator system.

1699 EARLY EFFECTS OF FLORAL INDUCTION ON SHOOT APICAL ACTIVITY IN GLYCINE MAX (L) MERR.

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Soybean is a quantitative SDP requiring two inductive cycles for floral initiation, which occurs first in the most undifferentiated meristem in an axil of a main stem leaf. Further progression of floral initiation to other meristems and its completion (signified by floral initiation at the main stem apex) requires additional inductive cycles. In chambers at the NCSU Phytotron with 26/22°C day/night temperatures and a 9-h photoperiod the main stem apex is committed to flowering after 8 SD with differentiation of the first flower after 10 SD. Changes in rate of leaf initiation, apical geometry, and nuclear DNA were followed daily during this period and compared with apices from plants kept under non-inductive LD. The LD photoperiod was given as a 3-h light break in the middle of the dark period. At emergence all plants had initiated 3 leaf primordia, and while vegetative maintained a plastochron interval of 2.0 days/leaf. On day 7 the plastochron was shortened to 1 day in SDP, followed on day 8 by the appearance of the floral primordium at the apex. Apical geometry remained unchanged until after 6 SD when the height of the dome from apex to point of insertion of the 2nd youngest leaf decreased due to less elongation in the rib meristem. Earlier events included a significantly lower amount of nuclear DNA in SD apices after 1 SD cycle, followed by a dramatic surge in DNA after 5 SD which peaked after 6 SD before declining. Shifts in increasing proportion of the population of nuclei from the 4C to 2C condition occurred after 1 SD and 3 SD. These events, though extended in time, resemble those which occur more rapidly during induction in apices of absolute photoperiodic species.

1700 BIOCHEMICAL AND CELLULAR PROPERTIES OF A SOMATIC EMBRYOGENIC, ANTHER DERIVED CELL LINE OF CORN, N.-S. Yang and T. Moen, Agracetus, Middleton, WI 53562

Corn YT10 cells were established from anther cultures of a hybrid corn. Through long-term cultivation and selection of these cells, we have developed a hard, friable callus cell line. Using a combination of dicamba and proline treatment in the tissue culture growth medium, we have induced high frequency of somatic embryogenesis and plantlet regeneration in these YT10 cultures. Light and electron microscopic studies showed that both normal and aborted embryos at various developmental stages were present as clusters arising from background, undifferentiated callus structures. Two dimensional SDS PAGE analysis revelaed that 1) two high pI, 40kd polypeptides and one lower pI, 50kd polypeptided are expressed in embryoidal tissues, but are absent in non-embryogenic callus; and 2) these three and six additional polypeptides of various molecular weights are expressed in regenerating leafy tissues and plantlets. These protein profile data thus partially define the biochemical complexity of the early somatic embryogenesis process in corn. Some information on the biochemical-genetic background of this cell line was obtained with tests on the expression of several genetically determined corn isozyme markers. The embryogenic callus tissue can be moderately digested with enzymes to release protoplasts. These various properties of the YT10 cell line make it a highly desirable culture system for use in genetic engineering of corn.

Developmentally Regulated Genes

1701 GENETIC CONTROL OF RHIZOBIUM-PLANT INTERACTIONS. Sharon R. Long, Department of Biological Sciences, Stanford University, Stanford CA 94305.

Rhizobium is a genus of bacteria which infect plants, stimulating the formation of symbiotic nitrogen fixing root nodules. Genes of both plant and bacteria are required for this process to occur (1). We have identified and characterized a cluster of Rhizobium meliloti genes involved in the infection of its plant host, alfalfa. Such genes have also been identified in other fast growing Rhizobium strains (2-5). Three genes, A, B, and C, are linked closely and read in the same translation direction; a fourth, D, begins near the 5' end of nodA and reads in the opposite direction. Tn5 insertions in A,B, and C result in a complete Nod$^-$ phenotype, while nodD mutations are phenotypically leaky Nod$^-$. These genes are functionally conserved in at least two other Rhizobium species, and do not affect the host range. All four genes are highly conserved at the amino acid sequence level in these different species as well, as determined by the work of the Stanford, Szeged, John Innes and ANU laboratories. Inspection of the sequence of nodC indicates possible signal peptide character, consistent with membrane localization of this of gene product. NodC has the further interesting property that it behaves as two genes in merodiploid complementations, which may indicate two functional domains in the protein or may reflect action of a recombinational hotspot. The protein products of these genes have been studied by overexpression in E. coli maxicells, minicells and in vitro (S-30) expression systems. The nodA gene product has been purified from overexpressing its gene in E. coli under the direction of the trp promoter; the protein has been used to generate antibodies for use in localization and expression studies. Similar approaches are being tried for studying the other nod gene products. The nod genes do not appear expressed in RNA from free-living bacterial cells, nor are there detectable nod gene transcripts in differentiated nitrogen fixing bacteroids. We have used gene fusions to provide an assay for expression of the nodulation genes. An in-frame translational fusion of nodC and lacZ has been obtained, and it demonstrates that there is increased translation of the nodC region in bacteria exposed to root extract. Thus the possibility exists that plants influence the activity of the bacterial genes used in plant invasion.

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1702 GENES FOR PHOTOSYNTHESIS IN ZEA MAYS, Donald Miles, Marjorie Hunt and Phillip McClean, Division of Biological Sciences, University of Missouri, Columbia, MO. 65211.

The development of the photosynthetic apparatus is regulated by structural genes in the nucleus and in the plastid genome. The expression of these genes can be altered by the activity of other genes both in the chloroplast and the nucleus. This is apparent when nuclear genes in maize were mutated by ethylmethane sulfonate (EMS) treatment. Nuclear gene mutants can cause the complete inhibition of photosynthesis by blocking the normal assembly or stabilization of thlakoid membrane protein complexes. Specific mutants were isolated which cause the complete loss of all polypeptides and functions of Photosystem II (1), other specifically cause the loss of the four components of the cytochrome b_6-f complex (2). It is clear that proteins coded in chloroplast DNA and synthesized in the chloroplast could be reduced or eliminated in response to mutation in a nuclear gene if these proteins were a component of a multi-polypeptide complex. There are several ways in which a nuclear gene could control the assembly of an organelle protein complex and through that a process such as photosynthesis. Our laboratory is investigating the mechanism by which such regulation could occur.

On approach is the use of transposon mutagenesis and tagging of sequences of nuclear DNA responsible for alteration of chloroplast proteins. Robertson's mutator (3) (Mu-1) has been used to induce high chlorophyll fluorescent (hcf) mutants in maize blocked in photosynthesis. Mu-1 nuclear mutants altering the Photosystem-I complex and another in the cytochrome b_6-f complex has been isolated and characterized. By selection of sequence of nuclear DNA it maybe possible to characterize the genes responsible. Another approach is to directly identify nuclear genes which code for a protein synthesized in the cytoplasm and transported into the plastid to become part of the photosynthetic electron transport chain. Oligonucleotide probes to specific coding sequences of the ferredoxin gene are being used to obtain clones for these genes in wheat. Similar selection are possible for other proteins such as plastocyanin or the nuclear coded subunit of the ATP synthetase, CF_1.

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1703 MUTANTS OF ARABIDOPSIS AS TOOLS FOR CELL AND MOLECULAR BIOLOGY. C.R. Somerville, P. McCourt, J. Browse, T. Caspar, N. Artus, M. Estelle, H. Zhang, M. Volokita. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

We have implemented a variety of selective and non-selective schemes which have permitted the characterization of mutants of Arabidopsis thaliana (L.) with biochemically defined alterations in photorespiration, dark respiration, carbohydrate metabolism and membrane lipid biosynthesis. These mutants have been useful in examining the functional significance of various metabolites and structural components, and in revealing the existence of metabolic regulatory mechanisms. For example, mutants lacking specific fatty acyl desaturases have been used to evaluate the role of lipid unsaturation in photosynthetic membranes, to discriminate among competing models of lipid biosynthesis, and to reveal the existence of regulatory mechanisms that sense and regulate the absolute concentration of specific membrane lipid species. The mutants have also been used to evaluate theories which invoke membrane composition as a key factor in chilling sensitivity, and may be useful as a means of physically identifying genes with potential practical applications in controlling the fatty acyl composition of oil seeds. Starchless mutants, which are conditional lethals, have been used to examine the functional significance of different modes of photosynthate partitioning. It appears that, because respiration is controlled by substrate supply rather than demand for ATP, the mutants may be used to select dirtectly for alterations in one of the two respiratory pathways thought to be operative in the leaves of the plant.

1704 PROMOTER REGIONS OF THE FOUR COORDINATELY REGULATED TUBULIN GENES OF CHLAMYDOMONAS AND THEIR USE IN THE CONSTRUCTION OF FUSED GENES WHICH ARE EXPRESSED IN ACETABULARIA. Donald Weeks, Karen Brunke and Nancy Beerman, Zoecon Corporation, 975 California Ave., Palo Alto, CA 94304 USA and Gunther Neuhaus, Gabriele Neuhaus-Url and H.G. Schweiger, Max-Planck-Institute for Cell Biology, D6802 Ladenburg-bei Heidelberg, FRG

DNA sequence analysis of the four tubulin genes (α_1,α_2,β_1, and β_2) of Chlamydomonas reinhardi has revealed a number of regions with potential regulatory significance. The most striking of these is a consensus sequence found in multiple copies immediately upstream of the TATA box in each of the genes. The consensus sequence is 16 bp base pairs in length [G C T C G/C A A G G C G/T G - C/A C/A G], the first 10 base pairs of which are the most highly conserved. An unexpected discovery is the presence of pseudopromoters in or near two of the transcribed tubulin genes. These regions closely resemble the authentic tubulin promoter regions but highly sensitive primer extension analyses have provided no evidence that these potential promoter regions are used at any point in the cell cycle or life cycle of Chlamydomonas. One pseudopromoter is located 400 base pairs upstream of the authentic α_2-tubulin gene promoter, whereas the other is located within the transcribed 5' noncoding region of the β_1-tubulin gene.

As an initial step toward determining which regions of the tubulin genes are important to their correct transcription and regulation we have constructed numerous vector plasmids containing various antibiotic resistance genes inserted between the 5' promoter region and 3' termination region of the Chlamydomonas β_2-tubulin gene. To test the functionality of such constructions, one vector carrying the coding region of the neomycin resistance gene of Tn5 and another vector with the coding region for the T-antigen gene of SV40 have been injected into the nuclei of Acetabularia and assayed for expression in vivo. Positive results were obtained with both vectors. Production of SV40 T-antigen was detected by indirect immunofluoresence. In cells which received nuclei injected with the vector containing the neomycin resistance gene in the correct orientation downstream of the tubulin gene promoter, more than 50% showed resistance to the antibiotic G418. In no case was expression detected when either structural gene was inserted into the vector in reverse orientation relative to the tubulin promoter. These data suggest that the vectors containing the Chlamydomonas tubulin gene promoters are functional in algal cells and that Acetabularia offers a useful system in which potential vectors for genetic engineering can be tested.

A new rapid and simple method for isolating DNA from algal cells and higher plant tissue also is discussed.

Somatic Cell Genetics

1705 A GENETIC APPROACH TO THE STUDY OF THE MECHANISM OF ACTION OF AUXIN IN **NICOTIANA**
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The induction of the division of tobacco mesophyll protoplasts requires high concentrations of ANA, an auxin analog, in the range of 5 to 15 µM. These concentrations are on the opposite extremely toxic for growth at low densities of protoplast-derived cells. This cytotoxicity of NAA is also observed for cells grown at low densities, derived from other tissues (pith, epiderm) or other species (**Nicotiana plumbaginifolia, petunia, chicorium, hyoscyamus**). Indoleacetic acid, the natural auxin, is also toxic under similar growth conditions thus suggesting that toxicity is not simply a side effect of NAA on cells, but one of the characteristics of the action of auxin.

In the hope of a better understanding of the basis of toxicity, mutants resistant to the toxic effect of NAA were isolated from mutagenized cultures of haploid tobacco mesophyll protoplasts and studied. Plants were regenerated from two independant clones, 35 and 36. These plants were unable to make roots. Protoplasts isolated from their leaves were confirmed to be significantly more resistant to NAA and IAA than the wild type. A genetic analysis of the transmission to progeny of NAA resistance and of the inability to root showed that both characteristics were cosegregating as a dominant monogenic nuclear marker. An increased rate of auxin degradation would be expected to be expressed as a dominant marker. However when mutant protoplasts were incubated in the presence of (^{14}C) NAA they were found to metabolize this molecule at the same rate as wild type protoplasts.

Agrobacterium rhizogenes is able to induce the hairy root transformation of many dicotyledones. Repeated attempts of transformation by the virulent strain A_4 of plants grown in the greenhouse or plantlets grown in sterile conditions were unsuccessful. NAA resistant clones are therefore naturally immune to the infection by this **Agrobacterium.** Crosses were performed between a NAA resistant plant and a plant regenerated from a hairy root induced on the tobacco wild type, carrying the T-DNA of **A. rhizogenes.** The NAA resistance trait was found to be dominant over the expression of the hairy-root promoting activity of the T-DNA in the progeny.

Agrobacterium tumefaciens was still oncogenous when tested on mutant plants. However octopine strains induced teratoma instead of non diferenciating tumors. Among **Agrobacterium tumefaciens** mutants affected in specific functions expressed by the T-DNA, a TMR mutant was unable to induce transformed roots when tested on clone 36. The implications of these results will be discussed.

1706 DEVELOPING A SOMATIC GENETIC SYSTEM FOR SOYBEAN, Karl G. Lark and E. Jill Roth, Department of Biology, University of Utah, Salt Lake City, UT 84112

The development of a somatic genetic system for soybean has followed the development of similar systems in yeast and fungi: Basically the system makes use of chromosome loss to express recessive characters. Soybean has 40 chromosomes, a DNA content of 5 to 6.5 pgm per somatic nucleus, and each chromosome contains about two linkage groups. Our experiments have concentrated on the properties of partial haploids produced by treating heterozygous cells with the herbicide CIPC (1,2). A variety of markers are available in the germplasm, because the heterozygous cells are derived from plant hybrids formed by a sexual cross between distantly related plant introductions. Expression of these markers will be discussed, together with interactions which suggest that genes on different chromosomes may be expressed as part of a set, and are modified or inactive if one or more chromosome within that set is missing. Quantitative variation in the 5S ribosomal RNA gene can be followed in this system as well as various physiological traits and isozymes.

The partial haploids have been used as starting material for mutant production and several such mutants have been isolated. Selective techniques for isolating RNA or nucleoside mutants and mutants requiring specific amino acids will be discussed.

Techniques for promoting somatic crossing over also are being developed and will be discussed.

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1707 INTERSPECIFIC CHLOROPLAST RECOMBINATION IN NICOTIANA, Pal Maliga*, Erzsebet Fejes* and Peter Medgyesy**, *Advanced Genetic Sciences, Inc., Oakland, CA 94608, USA and **Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary 6701

Chloroplasts do not mix upon sexual reproduction in crop plants since these organelles are inherited maternally. In the absence of physical contact, recombination of chloroplast genes can not occur. Crop improvement has been possible therefore, by combining nuclear genes, but not chloroplast genes, through crossing. Fusion of somatic cells made feasible to obtain cells with chloroplasts from two different species. Chloroplast recombinants could be identified in fused cells using an appropriate selection scheme, as described below.

The SR1-A15 Nicotiana tabacum line carried two cytoplasmic mutations, streptomycin resistance and pigment deficiency. Streptomycin resistant clones in cell culture are identified by their ability to form a green callus in a selective medium. Streptomycin resistance in the SR1-A15 line could not be expressed due to the pigment mutation. Selection of the chloroplast recombinants was based on the expression of streptomycin resistance from the SR1-A15 line through the separation of the cytoplasmic streptomycin resistance and pigment mutations by recombination. The SR1-A15 line was fused with the LR400 Nicotiana plumbaginifolia line, which is streptomycin sensitive, normal green and is resistant to lincomycin. The calli grown from the fused cell population were screened for the expression of streptomycin resistance. New mutants and recombinants were distinguished by the chloroplast DNA restriction patterns of regenerated plants.

Chloroplasts in the interspecific somatic hybrid pt14 were shown to be recombinant by the presence of species specific Sma I fragments from both parents, and by a new fragment in the Pst I restriction pattern. In order to localize the recombination sites, the Sal I fragments were cloned in pBR322 and compared by restriction mapping. So far three alternating Nicotiana tabacum-Nicotiana plumbaginifolia regions have been identified in the chloroplast genome of the pt14 line.

1708 DEVELOPMENTAL GENETICS OF SOMATIC EMBRYOGENESIS Z. Renee Sung, Departments of Genetics and Plant Pathology, University of California, Berkeley CA 94720

Early studies have shown that somatic embryogenesis can be manipulated by physiological and environmental factors such as fresh medium, cell density and hormones. However, these conditions merely initiate or inhibit the developmental events that are programmed within the cells. To study the biochemical and genetic mechanism of embryo development, we have examined differential gene expression during embryo development by 2 dimensional gel electrophoresis. Less than 2% of the relatively abundant proteins varies in quantity during embryo development. In an attempt to identify gene products present at low concentrations, we are searching for antigens and mRNAs preferentially accumulated in the embryonic teissues. Temperature-sensitive cell lines im paired in embryo development have been isolated. One ofthe cell lines was found to be altered in the ability to modify 2 heat shock preoteins post-transitionally. The results on the embryo-specific monoclonal antibodies and cDNA probes will be reported as well as the relationship between the developmental defect and the heat shock response in the temperature-sensitive cell line.

Mechanisms of Transposon Movement and Excision

1709 THE 'DOTTED' CONTROLLING ELEMENT SYSTEM IN MAIZE, A. Pryor, Division of Plant Industry, CSIRO, Canberra, Australia, 2601

Studies by Rhoades (1,2,3) on the interaction of Dt on chromosome 9 with the a allele on the long arm of chromosome 3 provided the first demonstration of the genetic control of mutability. Not only has Rhoades' interpretation of this phenomenon withstood the passage of time, but he also described most of the salient features of the system including somatic and germinal mutations to stable and mutable derivative alleles at the A locus, the specific dosage relationships between the Dt and a genes and the depressive effect of increasing temperature on mutability.

Subsequently features such as other chromosomal locations of Dt, highly mutable a alleles, phase variation and transposition of Dt and the induction of new Dt's by chromosome breakage events have been described (4,5,6,7,8).

More recently we have observed additional variants of these genes including transposition of Dt associated with a reduction in mutability at a, an autonomously mutating a allele and several new occurrences of Dt.

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1710 DIFFERENTIAL ACTIVITY OF TRANSPOSED wx-m9Ac DERIVATIVES ON VARIOUS DS ELEMENTS, Drew Schwartz, Department of Biology, Indiana University, Bloomington, IN 47405

Genetic analyses have been performed on 27 selected derivatives of the Ac element in the wx-m9 mutant. These derivatives are Ac elements which have been transposed away from the original site in the wx locus leaving behind a mutant, non-functional wx gene. These independent derivatives were designated "Ac-w" since they show a much reduced, weak transposase activity as judged by their action on the Ds element in the wx-ml controlling element mutant. They behave as State 1 Ac elements, causing dissociation of the chromosome with the resultant loss of distal markers and initiation of the breakage-fusion-bridge cycle. Finally, the Ac-w derivatives show differential transposase activity on the Ds element in wx-ml and on the Ds element in wx-m9Ds and the State 1 Ds in the standard position. Seventeen of the derivatives show strong transposase activity on the latter two while the remaining ten have no action on these Ds elements, even though they still transpose the wx-mlDs.

Genetic Analyses of Single Genes

1711 THE INVERSE EFFECT IN MAIZE AND DROSOPHILA, James A. Birchler, Department of Genetics, University of California, Berkeley, California 94720

The inverse effect was first observed in a dosage series of the long arm of chromosome one in maize(Birchler, 1979). The levels of certain enzyme activities were inversely correlated with the dosage of this chromosome arm. That is, in the one dose individuals the affected activities rose to levels approaching twice the diploid value and the same enzymes were reduced in three doses to a lower limit of two-thirds. This observation led to a systematic analysis of dosage responses first in maize and later in Drosophila. The generalizations from these studies are as follows: 1.) In any one tissue, the expression of a single structural gene can be inversely affected by multiple chromosomal regions. 2.) Any one region can affect varying numbers of structural genes from a very few to a substantial fraction of the genes expressed in a particular tissue. 3.) A dosage series surrounding a structural gene gives a directly proportional amount of gene product. 4.) When a structural gene and an inverse regulator are simultaneously varied, dosage compensation results(Birchler, 1981; Birchler and Newton, 1981). 5.) When a ploidy series is examined, a direct correlation with chromosomal dosage is observed. 6.) The inverse effect operates by modulating the level of messenger RNA. 7.) Mutations can be induced that behave as lesions in inverse regulators. 8.) When regulatory mutants of the monitored locus are combined with an inverse dosage series, the gene no longer responds.

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1712 HORMONAL, GENETIC AND ENVIRONMENTAL REGULATION OF ENZYME SYNTHESIS IN THE ALEURONE LAYERS OF CEREAL GRAINS, Tuan-hua David Ho, Department of Biology, Washington University, St. Louis, MO 63130

The synthesis and secretion of several hydrolytic enzymes, including α-amylase and protease, in the aleruone layers of barley and wheat seeds are regulated by two plant hormones, gibberellins (GA) and abscisic acid (ABA). The barley α-amylase consists of two types of isozymes (high and low pI) which are encoded by two groups of mature mRNA transcribed from two sets of structural genes located on chromosomes 1 and 6, respectively. The high pI isozyme is constitutively expressed, yet its synthesis is enhanced by GA. This enhancement requires the continuous presence of GA, i.e. midcourse removal of the hormone causes a decrease of high pI synthesis. In contrast, the expression of low pI isozyme is triggered by GA, but early withdrawal of the hormone does not affect the continuous synthesis of this isozyme. Genetic mutants of wheat and barley with altered synthesis of α-amylase have been isolated and characterized. Most of them are "early" mutants, i.e. the genetic lesions are on the early steps of GA action. A two-branched functional pathway concerning the mode of action of GA has been deduced from studying of these mutants. Among them, the D6899 wheat bearing a mutation in the Rht 3 gene (on chromosome 4A) fails to have any of the GA effects so far studied, indicating that a rate limiting step, which is common to many of the diverse GA resopnses, is partially blocked in D6899 wheat.

Abscisic acid is able to prevent the synthesis of high pI α-amylase when it is added to the aleurone cells either at the same time or many hours after GA addition. However, the synthesis of low pI α-amylase is significantly inhibited by ABA only when it is added very early. The action of ABA is most likely medicated by its first stable metabolite, phaseic acid (PA). Isolated PA is biologically active, and ABA self-induces its own metabolism to give rise to PA. At the same time, ABA induces a few new proteins, some of them are probably involved in the formation of PA. We have isolated cDNA clones coding for the ABA inducible proteins. The information obtained with the use of these clones will be discussed.

The synthesis of α-amylase is also subjected to the regulation of environmental stress conditions. Heat stress causes a fast disruption of ER which is essential for the synthesis of α-amylase. Consequently, the normally very stable α-amylase mRNA sequence is degraded. During the recovery from heat stress new α-amylase mRNA has to be synthesized to resume the synthesis of this enzyme.

1713 A RESERVOIR OF R GENE VARIABILITY IN MAIZE, Jerry L. Kermicle, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706

R interacts with a number of other genes to regulate anthocyanin production and distribution, plant part by plant part (1). R alleles differ both in the spectrum of parts affected and in the intensity of pigmentation in given parts, such that alleles collected from unrelated sources seldom are phenotypic duplicates (2). We are comparing the structure of representative alleles by mutational and recombinational analyses to discover the basis for diversity. Some alleles lose R function as a single mutational or recombinational step. Others mutate to loss of function stepwise, and the effects on different parts can be fractionated by crossing over. Sequential losses are explained by duplicate R elements. Such may be in tandem or displaced arrangement (3). The extent of the duplicated segment differs from allele to allele, sometimes encompassing the closely linked gene Isr (4). Chromosome segment duplication is a relatively common form of R polymorphism. Elements fractionated from allelic complexes, as well as simpler alleles which mutate as one unit, may pigment only the seed, particular plant parts, or a combination of seed and plant parts. Differences in such genic elements map to a point or within a very short genetic region (5). They share a longer homologous segment which can be substituted between elements by recombination (6). Eight different genic elements, involving four chromosome types, were identified in a collection of five alleles belonging to the colored-seed, colored-plant class. A limited number of variants at the genic and chromosmal levels can be recombined to give a wide array of allelic products.

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1714 THE WAXY LOCUS OF HIGHER PLANTS: MOLECULAR ANALYSIS OF NORMAL AND MUTANT ALLELES. Susan Wessler, Rita Varagona, George Baran, Ron Okagaki, and Stephen Dellaporta*, University of Georgia, Athens, GA 30602 and Cold Spring Harbor Lab*, Cold Spring Harbor, N.Y. 11724.

We are investigating the molecular basis of mutation in higher plants. The waxy (wx) locus of maize is the focus of our analysis because of its 45 mutant alleles. Eight of these have an unstable phenotype due to the insertion of the transposable element Ac or Ds. We and others have found that most or all of these insertions are dispersed throughout the wx transcription unit. One Ds insertion mutant, wx-m1, has a 392bp Ds element in the middle of the transcription unit. This Ds shares no sequence homology with the Ac element except for the short terminal inverted repeats. Excision of this element results in a series of stable alleles that display all levels of wx activity. Analysis of wx RNA and protein suggests that these stable derivatives produce defective wx enzymes.

The 35 stable mutant alleles have been examined by blot hybridization techniques. Over half of these alleles have gross chromosomal changes within the wx locus. Seven mutations are caused by DNA insertions ranging in size from 100bp to 8kb. In each case there is an excellent correlation between the physical lesion and the position of the mutant allele on a fine structure genetic map.

The waxy locus of rice is also well characterized genetically. Over 30 mutant alleles are available for analysis. The maize waxy clone has been used successfully as a probe to identify clones harboring the rice locus. Furthermore, Northern and Southern blot analysis reveals that this locus is highly conserved in many monocots.

Plant Gene Structure and Function During Development

1715 NUCLEOTIDE SEQUENCE ANALYSES OF THE LARGE SUBUNIT OF RUBISCO FROM ALFALFA AND PETUNIA AND OF THE PSBA GENE FROM ALFALFA, Jane Aldrich and Barry Cherney, The Standard Oil Company of Ohio (SOHIO), Cleveland, Oh. 44128-2837

Genes encoding the large subunit (rbcL) of ribulose bisphosphate carboxylase oxygenase from alfalfa and petunia as well as that of the 32 kd polypeptide psbA gene from alfalfa are currently being sequenced. Sequence analysis of that portion of the rbcL gene from petunia that has been sequenced shows that there is 97% homology at the nucleotide level and 95% at the amino acid level with Nicotiana tabacum. That portion of the alfalfa psbA gene that has been sequenced shows 95% homology at the nucleotide level and 99% homology at the amino acid level with the psbA gene from spinach. The sequences of these genes and that of rbcL of alfalfa will be compared to each other and to other published sequences for sequence homology comparisons.

1716 REGULATION OF PYRUVATE ORTHOPHOSPHATE DIKINASE GENE EXPRESSION. Kazuko Aoyagi and James A. Bassham, Lawrence Berkeley Laboratory, U.C. Berkeley, Berkeley, CA 94720.

Pyruvate Pi Dikinase (PPDK) is an essential enzyme in C_4 plants and some CAM plants. Studies have shown the presence of PPDK in various C_3 plants. PPDK gene in C_3 plants is developmentally regulated as in C_4 plant leaf. PPDK gene expression is also regulated at the organ level both in C_3 and C_4 plants. There are organ specific mRNAs; one that is expressed in photosynthetic tissues and the other which is expressed in non-photosynthetic tissues. PPDK enzyme activity of the photosynthetic type is regulated by light at the level of mRNA synthesis, processing of the precursor and phosphorylation of the enzyme. In contrast, the enzyme activity of the non-photosynthetic type is independent of light. Possible roles of PPDK in C_3 seeds and leaves will be discussed.

1717 α-AMYLASE AND OTHER CO-ORDINATELY REGULATED GENES OF WHEAT, David Baulcombe, Plant Breeding Institute, Cambridge CB2 2LQ, U.K.

The action of gibberellin on the aleurone cells of germinating wheat grains, results in production of mRNA for α-amylase and other hydrolytic enzymes. Evidence will be presented, based on hybridisation analyses with probes for mRNA precursors, that these are co-ordinately regulated genes controlled by the rate of transcription. The nucleotide sequences of two α-amylase genes and two other co-regulated genes have been determined and sequences which may be involved in the regulating process identified.

Previous molecular and genetical studies have shown that the α-amylase genes of wheat comprise two multigene families. Transcript mapping and nucleotide sequence analyses with clones of α-Amy 2 genes support a model which proposes that a subset of the genes are transcribed at an early stage of grain development as well as in germination. It is suggested that these different modes of expression use different promoters and that a differential RNA splicing mechanism is used so that the gene product is targeted to different cell membranes at the different stages of development.

1718 COPY NUMBER AND CHROMOSOMAL LOCATIONS OF NUCLEAR SEQUENCES HOMOLOGOUS TO RANDOM cDNA CLONES IN TOMATO. Robert Bernatzky & Stephen D. Tanksley, New Mexico State University, Las Cruces, NM 88003.

Nuclear probes, derived from poly-adenylated leaf mRNA, have been used to identify corresponding homologous fragments from digested total DNA preparations in tomato. Utilizing multi-locus segregation analysis with enzyme-coding genes and dosage analysis in aneuploid and chromosome substitution lines, we have been able to determine the copy number and chromosomal positions for the nuclear loci corresponding to most of the analyzed cDNA clones. Fourteen (78%) of the 18 clones which have been fully analyzed derive from single-copy loci and have been mapped to their respective chromosomal positions. Of the four clones corresponding to loci represented more than once in the genome, three were found to be two-copy and one is present in 4-6 dispersed copies. The latter was found to be a clone for the small subunit of ribulose bisphosphate carboxylase. Three clones were conserved and showed no variation for the eight restriction enzymes used. These data suggest that the majority of transcribed genes in tomato emanate from genes mapping to single chromosomal loci and which are present in single or low copy number. The new genetic loci provided by these clones are distributed on 7 of the 12 chromosomes and thus represent useful molecular markers for genetic and breeding experiments.

1719 In Vivo and In Vitro Transcription of Maize Zein Genes, Rebecca S. Boston and Brian A. Larkins, Purdue University, W. Lafayette, IN 47907

Synthesis of zeins, the major storage proteins of maize, is coordinately regulated during endosperm development. This synthesis accompanies an increase in accumulation of mRNAs for zeins and suggests that zein genes are under transcriptional control. We have used S1 nuclease mapping to locate the 5′ ends of mRNAs that correspond to genomic clones coding for 19,000- and 15,000-dalton zein proteins. These in vivo mRNA start sites have been reproduced in vitro using a HeLa cell transcription extract supplemented with truncated zein genomic clones. RNA products are analyzed by their migration on denaturing acrylamide gels compared to markers of known molecular weights in addition to S1 mapping and primer extension analysis. This in vitro system correctly transcribes zein genomic clones encoding both 19,000- and 15,000-dalton proteins. Studies on in vivo levels of zein mRNA are being extended to determine levels of zein mRNAs in nuclei of several maize mutants deficient in one or more of the zein proteins (eg. opaque-2).

1720 STRUCTURAL AND TRANSCRIPT ANALYSIS OF A NICOTIANA PLUMBAGINIFOLIA NUCLEAR GENE ENCODING THE β SUBUNIT OF MITOCHONDRIAL ATPase. Marc Boutry and Nam-Hai Chua, Laboratory of Plant Molecular Biology, Rockefeller Univeristy, NY NY 10021-6399

The plant mitochondrial ATPase is an abundant mitochondrial protein found in all plant tissues. To investigate the organization and structure of a "housekeeping" plant gene, we have selected from a genomic library of Nicotiana plumbaginifolia, clones containing the gene encoding the β subunit of the mitochondrial ATPase, using a yeast gene as a probe. Southern blot analysis indicated that two different β subunit genes are present in the N. plumbaginifolia genome. The nucleotide sequence of one of them has been determined. Unlike the yeast gene, the plant gene contains nine introns. The ninth intron contains 35 bp inverted repeats flanking a 800 bp loop. The inverted repeats are each bordered by a 5 bp direct repeat "AGTGG". This structural organization is reminiscent of that of a transposable element.

A partial sequence of the second β subunit gene revealed sequence conservation in the exons (89% in the nucleotide sequence and >99% in the amino acid sequence) but a high divergence in the introns (<40% homology). By Northern blot analysis, a 2.2 kb transcript has been identified in leaves, roots and calli, indicating that the mitochondrial ATPase β subunit gene is probably expressed in all tissues. We are currently preparing deletions in the 5' upstream region to identify control sequences of this "housekeeping" gene.

1721 ISOLATION AND CHARACTERIZATION OF A STARCHLESS MUTANT OF ARABIDOPSIS THALIANA, Timothy Caspar*, Steven C. Huber # and Chris R. Somerville*, *MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 and #USDA/ARS, North Carolina State University, Raleigh, NC 27606

A mutant of Arabidopsis thaliana which lacks leaf starch was isolated by screening for the absence of iodine reactive material. The starchless phenotype is due to a single recessive nuclear mutation which results in a deficiency of the chloroplast isozyme of phosphoglucomutase. Rates of growth and photosynthesis of the mutant and wild-type are indistinguishable when the plants are grown in constant illumination. However, in a short photoperiod, the growth of the mutant is severely impaired, the rate of photosynthesis is depressed and the rate of dark respiration, which is enhanced at the onset of the dark period, undergoes an uncharacteristic decay throughout the dark period. The unusual respiratory characteristics of the mutant are consistent with a proposal that an excess of soluble carbohydrate may stimulate non-productive respiration due to alternative oxidase activity. This excessive respiratory loss is, in turn, believed to be responsible for the low growth rate and poor photosynthetic rate of the mutant in a short photoperiod. We are currently attempting the isolation of revertants which show improved growth in a short photoperiod. Our goal is to isolate revertants which still lack starch but do not have enhanced respiratory rates due to loss of the alternative oxidase activity thought to be responsible for wasteful respiration in plants.

1722 THE CLONING OF THE PLANT NITRATE REDUCTASE GENE(S), C.L. Cheng, J. Dewdney, A. Kleinhofs*, H.M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114. *Department of Agronomy and Soils, Washington State University, Pullman, WA 99164.

Nitrate reductase (NR) is the first enzyme in the major nitrogen assimilation pathway in plants. Every year, there are 2 X 10^4 megatons of nitrogen assimilated into plants through this pathway. The nitrogen level, hence the growth and yield of crop plants highly correlates with the NR level of the plants. NR levels fluctuate in response to a number of environmental factors, i.e. temperature, light, etc. NR is inducible by nitrate and inhibited by ammonia.

We are interested in isolating cDNA clones which correspond to the NR mRNA. The cDNA can then be used for studies of the NR gene expression. To identify a cDNA clone encoding NR, we have constructed a cDNA library using mRNA isolated from nitrate induced barley. We are currently screening this library with synthetic oligonucleotide probes. Concurrent with this, experiments are underway to identify the NR mRNA by in vitro translation followed by NR antiserum immunoprecipitation. These technologies, combined with hybrid selection of mRNA, may then be used to confirm positive clones.

1723 CLONING OF GENES INVOLVED IN HIGHER PLANT NITROGEN ASSIMILATION
Gloria M. Coruzzi and Scott V. Tingey, Laboratory of Plant Molecular Biology
The Rockefeller University, 1230 York Avenue, New York, N.Y. 10021

In higher plants, nitrogen is assimilated into organic form via the glutamine synthetase (GS)//glutamate synthase, or glutamate dehydrogenase(GDH) pathways. To examine the relative importance of each pathway during plant growth and development, and to understand how the respective genes are regulated, we have isolated cDNA and genomic clones encoding GS and GDH from N.plumbaginifolia. Northern blot analysis has revealed the presence of two GS mRNAs in leaf (1.4 and 1.0kb) and one GS mRNA in root (1.4kb) of N.plumbaginifolia and G.maxima, while levels of GDH mRNA were undetectable in these tissues. Both GS mRNA and GDH mRNA (1.35kb) levels are induced in N-fixing nodules of G.maxima. The presence of GS polypeptide in these tissues was analyzed by two-dimensional gel electrophoresis followed by Western blot analysis. One form of GS polypeptide (45kd) occurs in leaf tissue of N.plumbaginifolia, while G.maxima leaves contain two isoelectric forms. Root tissue of both species contains two GS polypeptides which differ slightly in size (37-38kd) and charge. The correlation of the GS mRNAs with the various GS polypeptides will be elucidated by translation and immunoprecipitation of hybrid-selected mRNAs. Further characterization of the clones by DNA sequence analysis and plant transformation experiments, will enable us to define gene flanking regions that contain transcriptional regulatory elements.

1724 TEMPLATE SUPERCOILING STIMULATES PLASTID GENE TRANSCRIPTION IN VITRO,
Lyle D. Crossland, Steven M. Stirdivant, and Lawrence Bogorad, Harvard University, Cambridge, MA 02138

An in vitro transcription system using purified maize chloroplast RNA polymerase and cloned plastid genes has been used to invesitigate factors affecting plastid gene transcription. Because the in vivo template for plastid gene transcription is a supercoiled circle, the effect of DNA template supercoiling on transcription in vitro has been examined. Up to a point, increasing negative superhelicity stimulated promoter specific transcription of the gene for the large subunit of ribulosebisphosphate carboxylase (rcL) and the gene for the beta and epsilon subunits of chloroplast coupling factor (cflBE). Continued increase in negative superhelicity resulted in a decrease of rcL transcription, while cflBE transcription continued at its maximum rate. The DNA sequences responsible for these promoter specific effects are being investigated.

1725 PRODUCTS OF EARLY NODULATION GENES OF RHIZOBIUM MELILOTI, Thomas Egelhoff, Thomas Jacobs, John Mulligan, Robert Fisher, Sharon Long, Stanford University, Stanford, CA 94305

The bacterium Rhizobuim meliloti forms a symbiotic relationship with Medicago sativa (alfalfa) which results in the development of nitrogen-fixing root nodules. We have mapped and determined the nucleotide sequence of a 3.5kb cluster of bacterial genes that are involved in triggering the initial plant nodulation responses. The sequence contains four major open reading frames which correspond to our genetic data, which have been designated nodA, B, C, and D. Transcription studies using in vitro labeled RNA, and studies using RNA slot blots have indicated that nod gene transcripts are extremely rare or absent in free-living bacteria and in differenciated bacteroids. This is confirmed by behavior of nodC-lacZ fusions, which suggest that nod gene expression may be induced only in the presence of plant factors (J.Mulligan and S.Long, to be published). Using in vivo and in vitro expression systems, I have identified gene products corresponding to the nod genes. The nodA gene product was overproduced with an E.coli expression vector and used to raise antisera against the nodA protein. Antisera to nodC has been generated using carrier-coupled synthetic oligopeptides based on the nodC sequence. These antisera are being used to study the expression and localization of the nod gene products in Rhizobium. I have also constructed broad-host range plasmids that overproduce these products in Rhizobium. These strains are being used to study the effect of nod gene expression on plant root hair curling, infection thread formation and nodule development.

1726 MUTATOR GENE INDUCES PLASTOME MUTATIONS, Melvin D. Epp, ARCO Plant Cell Research Institute, Dubin, California 94568

A nuclear gene mutation in Oenothera hookeri increases the frequency of variegation sectors 10,000-fold. The gene is recessive; the variegation is cytoplasmically transmitted. Once the variegation is induced, the gene is not required for the continued expression of variegation. The variegation sectors may differ one from another and are phenotypcially maintained through sexual crosses. The genetic data implicate the chloroplasts as the site for the expression of variegation. Electron micrographs are used to describe the cytoplasmic phenotypes. Additionally, the chloroplasts of other Oenothera species, also mutate at an increased frequency when combined with the mutator gene.

1727 EXPRESSION OF ZEIN GENES, Günter Feix, Institut für Biologie III, University of Freiburg, 7800 Freiburg, F.R.G.

The zein genetic system is expressed in a highly regulated way in the developing endosperm of maize. Genomic clones representative for the major zein protein classes have been isolated and analyzed by a variety of techniques (in vitro transcription, S1-mapping, DNA sequencing etc.). A particular feature is the occurrence of several promoter structures up to 2 KB upstream from the coding sequences. For further assessment of the relevance of these and other regulatory sequences, in vivo test systems are required. As no homologous system is yet available, zein gene expression has been studied in yeast and the single cell alga Acetabularia. Successful expression of zein genes from their promoters was observed at the RNA level in yeast and at the protein level in Acetabularia. These systems are presently used to elucidate regulatory structures of the zein multigene family.

1728 THE MAIZE Adh1 GENE EXHIBITS MODIFICATIONS IN CHROMATIN STRUCTURE THAT CORRELATE WITH EXPRESSION, Robert Feri, Department of Botany, University of Florida, Gainesville FL

The maize Adh1 gene offers a convenient system to study the chromatin controlled parameters of gene expression. Adh1 is environmentally induced in roots by anaerobiosis and is developmentally inactivated in leaves. We can therefore compare and contrast the mechanisms of environmental and developmental gene regulation.

Using restriction enzymes as digestion probes on intact nuclei, we have been able to show that changes in the hypersensitivity of chromatin within the first few hundred bases 5' to the start of transcription correlate with the anaerobic induction of the gene. We have also shown that developmental inability to express the gene is correlated with the loss of hypersensitivity of sites 400-1100 bases upstream.

These results suggest that environmental and developmental controls over Adh1 gene expression are accomplished by different mechanisms acting at spatially separate sites.

1729 GENE ACTIVATION AND MOLECULAR MECHANISMS ASSOCIATED WITH DISEASE RESISTANCE RESPONSES OF PEA TISSUE TO FUNGAL AND BACTERIAL PATHOGENS. L. A. Hadwiger, B. Fristensky, D. F. Kendra, C. Daniels and W. Wagoner. Washington State University, Pullman, WA 99164.

The resistance responses of pea tissue to fungal and bacterial pathogens have similarities as well as characteristic differences. The phytoalexin, pisatin accumulates in both responses, but over different time regimes. In general, the protein synthesis response to the two pathogens differs. cDNA clones have been identified which cross hybridize with mRNAs coded by pea genes induced as the pea tissue resists Fusarium solani f. sp. phaseoli. These pea genes appear to be suppressed at about the time the true pathogen F. solani f. sp. pisi initiates active growth on pea endocarp tissue. Some, but not all, of the pea genes activated when resisting the fungus are activated in the resistance response of pea tissue to races of Pseudomonas syringae pv. pisi. The fungal wall carbohydrate, chitosan, has been shown to induce the same pea genes and enhance the synthesis of the same pea proteins as live F. solani fungal spores. Chitosan's potential action on the DNA of plant chromatin will be discussed. The stimulatory action of chitosan, a cationic polymer, in plant tissue, is not mediated by membrane destruction and reduced cell viability, however, its antifungal action against F. solani is associated with reduced cell viability as determined by fluorescence after treatment with fluorescein diacetate.

1730 IMMUNOLOGICAL AND GENETIC ANALYSIS OF CONFORMATIONS OF MAIZE ALCOHOL DEHYDROGENASE, Erin E. Irish and Drew Schwartz, Indiana University, Bloomington, IN 47405.

The enzymes specified by several variant and mutant alleles of the maize gene alcohol dehydrogenase-1 were analyzed immunologically. Alcohol dehydrogenase (ADH) enzyme with low activity was activated to wild type levels by precipitation with certain anti-ADH antisera. Forms of ADH with no detectable activity could be activated by immunoprecipitation with certain antisera in the presence of the enzyme's cofactor, NAD. All of these null enzymes were unable to bind NAD, which accounts for their lack of ADH activity. It is proposed that specific activity is directly related to the conformational equilibrium of active and inactive enzyme molecules in solution. Antibodies raised against active forms of ADH can increase the specific activity of low activity enzyme forms by binding enzyme molecules with low or no activity and inducing a conformational change, thus shifting the equilibrium toward active conformations.

One antiserum raised against an active form of ADH was observed to inactivate any form of maize ADH that it bound. The mechanism of inactivation was analyzed. It is proposed that the immunoglobulins of this antiserum bound ADH at the coenzyme binding domain of the enzyme, blocking the isomerization that normally follows NAD binding during catalysis. Preincubation with NAD (or NADP, which is not a coenzyme for ADH) protected the enzyme from inactivation, presumably by affecting the conformation of the enzyme.

1731 REGULATION OF LEGHEMOGLOBIN GENE EXPRESSION, Jens Stougaard Jensen, Kirsten Bojsen, Erik Ø. Jensen, and Kjeld A. Marcker, Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark

Leghemoglobins (Lbs) are hemoproteins synthesized exclusively in the root nodules of plants during symbiosis with nitrogen fixing bacteria. Soybean nodules contain four major Lbs components called Lba, Lbc_1, Lbc_2, and Lbc_3. The Lbs are encoded by a family of genes arranged in two clusters. DNA sequence analysis revealed four active genes coding for Lba, Lbc_1, Lbc_2, Lbc_3 and demonstrated a primary structure with three intervening sequences. Consensus CT/AG dinucleotides were found at the borders of the intervening sequences. Putative regulatory sequences (CAP site, TATA box, CAT box, poly A site) similar to other eukaryotic gene sequences were also found. Transcription of the Lb genes was measured with specific probes These probes distinguish the mRNA precursors from the different Lb genes, and it was demonstrated that the Lbc_3 and Lbc_2 genes are transcribed before the Lbc_1 and Lba genes, during nodule development. The Lba gene was transcribed at the highest level followed by the Lbc_3, Lbc_1 and Lbc_2 with the lowest level. Induction followed exponential kinetics and all four genes remained transcriptionally active. An Agrobacterium based transformation system has been modified for legumes. Tissue specific transcription, transcript processing and the Lb protein synthesis can therefore be followed after transfer of complete soybean Lb genes to other legumes. Chimeric genes that allow easy detection of Lb gene activity has been constructed. Regulatory sequences required for Lb gene activity can therefore be defined by deletion and site directed mutagenesis.

1732 ANALYSIS OF A PLASTOME MUTATOR INDUCED CHLOROPLAST MUTANT OF OENOTHERA, Ellen M. Johnson*, Barbara B Sears and Charles J. Arntzen*, Department of Botany and Plant Pathology, *MSU-DOE Plant Research Laboratory, Michigan State Univ. E. Lansing, MI

An enhanced rate of chloroplast gene mutation has been observed in Oenothera johansen when plants are homozygous for a recessive nuclear gene, "plastome mutator" (pm) (1). Plants exhibiting these mutations were crossed to return the mutant plastids to a stable (heterozygous) nuclear background. Seed from these crosses was sterilely germinated and the homoplastidic mutant tissue was established in shoot culture. Further screening was performed using antibodies against a number of chloroplast-encoded proteins. Stromal and thylakoid proteins of pm-induced mutants were analysed by immunoblotting. One mutant (pm7) appears to accumulate a high molecular weight protein which is antigenically related to cytochrome f. This could be due to a lesion which directly affects cytochrome f gene expression or affects some processing function, as cytochrome f is known to be chloroplast-encoded and translated as a precursor in spinach and pea (2,3); it also contains a covalently bound heme. Heme-staining of pm7 thylakoid proteins shows altered mobility of heme-containing proteins as well. Analysis of restriction patterns of chloroplast DNA has shown altered mobility of particular fragments. Fine structure restriction mapping is being used to determine the nature of these DNA alterations.

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1733 ACCELERATED POST-INSERTIONAL TURNOVER OF CHLOROPLAST-ENCODED PHOTOSYSTEM II POLYPEPTIDES IS A CONSEQUENCE OF NUCLEAR MUTATION, Kenneth J. Leto, Erin Bell*, and Lee McIntosh*, Central Research and Development, DuPont Co., Wilmington, De. 19898 and *MSU/DOE Plant Research Laboratory, Michigan State University, E. Lansing, Mi. 48824

We have studied the synthesis and accumulation of a chloroplast-encoded 48kDa chla-reaction center protein and the 34.5kDa "atrazine binding" protein in a nuclear maize mutant which fails to assemble photosystem II reaction centers. The failure of these polypeptides to accumulate in mutant thylakoids is not due to direct nuclear control over their synthesis but is rather due to their specific, accelerated turnover following insertion into the thylakoid membrane. The accelerated turnover of these polypeptides in mutant thylakoids is largely independent of illumination conditions, as accelerated turnover occurs in the dark as well as in the light. In contrast to wild type, the 48kDa and 34.5kDa polypeptides are preferentially associated with stroma, rather than grana, lamellae in mutant membranes, suggesting that turnover occurs before these polypeptides become enriched in the grana. The nucleus thus plays a role in the stabilization of newly inserted chloroplast-encoded photosystem II reaction center polypeptides.

1734 CHARACTERIZATION OF THE MULTIGENE FAMILY ENCODING THE LIGHT-HARVESTING CHLOROPHYLL A/B-PROTEIN (LHCP) IN ARABIDOPSIS THALIANA, Leslie S. Leutwiler and Elaine M. Tobin, UCLA, Los Angeles, CA 90024

The haploid genome size of Arabidopsis thaliana, a member of the mustard family, is exceedingly small--approximately 7×10^7 nucleotide pairs. Arabidopsis is unusual in that it lacks detectable levels of middle repetitive DNA (based on Cot curves); additionally its single copy component may be smaller than that of other plants. For instance, the nuclear genes encoding the LHCP are present as multigene families containing at least 8 members in pea, 10-12 in Lemna, and 16 in petunia. In contrast, Arabidopsis contains only three coding sequences all clustered on an 11.0 kb Eco RI fragment.

Two of the coding sequences have identical restriction maps, are oriented in the same direction and separated by 1.9 kb. The third coding sequence is oriented in the opposite direction about 1.3 kb upstream from the others. Since the amount of LHCP mRNA is regulated by red light, the expression of these individual coding sequences is being studied using probes derived from their unique 3' untranslated regions.

1735 STRUCTURE OF THE TRIOSEPHOSPHATE ISOMERASE GENES OF ZEA MAIZE. Mark A. Marchionni and Walter Gilbert. Biogen Research Corp., Cambridge, MA 02142

Triosephosphate isomerase (TIM) is a ubiquitous enzyme essential to several pathways of carbohydrate metabolism: glycolysis, gluconeogenesis and the dark reactions of photosynthesis. Two identical 21K dalton subunits comprise the active TIM enzyme, whose structure has been described by crystallographic studies as a beta barrel. That TIM is highly conserved across divergent as E. coli and chicken enabled us to use a chicken cDNA clone to probe a maize root cDNA library. Among 40K lambda gt10 recombinants screened 18 positives were detected. The first such clone sequenced contained a portion of a maize TIM cDNA- the carboxyl 2/3 of the protein-coding region and 300 bp of 3' untranslated DNA. Predicted amino acid sequences were identical to chicken at 75% of the residues compared. Furthermore, with the single exception of a glu to gln switch at position 133, the invariant amino acids from residues 103 to 245 matched those of all 6 species studied previously. The sequence was used to screen 10^6 charon 4A recombinants, and we detected 28 positives. These genomic clones fell into 3 groups, depending on which portions of the cDNA sequence hybridized to them. Restriction mapping of the phage and of maize DNA suggested the presence of at least 2 genes. Detailed mapping and nucleotide sequencing now in progress shall provide a picture of the structure of the maize TIM genes and permit comparison with the chicken gene, as well as alignment of the introns with the well-characterized protein structure. This work supported in part by NRSA grant 5 F32 CA07048-03 awarded to MAM.

1736

ANALYSIS OF AN INTERSPECIFIC SOMATIC HYBRID OF DAUCUS,
Benjamin F. Matthews[1] and Jack M. Widholm [2], (1) U.S. Department of Agriculture, Beltsville, MD 20705, and (2) University of Illinois, Urbana, IL 61801.

The chloroplast and mitochondrial DNA compositions of cell suspension cultures of Daucus carota, D. capillifolius and a somatic hybrid were determined. The chloroplast DNA of the hybrid and D. carota were identical and were different from that of D. capillifolius. The mitochondrial DNAs of both parental lines were different. The somatic hybrid contained DNA fragments in common with one or both parents and also contained unique fragments.

Enzymes in the pathway leading to the synthesis of the essential amino acids lysine, threonine and methionine were measured and characterized. Two major forms of homoserine dehydrogenase were present in the hybrid, including one unique form which was not present in either parent.

1737

TWO NODULIN GENES OF SOYBEAN, Vincent P. Mauro, Panagiotis Katinakis, Sui-Lam Wong and Desh Pal S. Verma, Plant Molecular Biology Laboratory, Biology Department, McGill University, Montreal, Canada H3A 1B1

Nodulins are specifically induced during the development of root nodules in legumes following infection by Rhizobium. We have identified two nodulin genes (Nodulin-23 and Nodulin-24) which are abundantly transcribed in soybean nodules. DNA sequence analysis of Nodulin-23 gene showed that this gene is interrupted by a single intron and codes for a 23.5 kd protein. The deduced protein sequence suggests the presence of a signal sequence. Nodulin-24 gene contains four introns and encodes a protein of 15 kd. This gene has an unusual structure consisting of three tandomly repeating units containing intron and exon sequences. It appears to have been generated by duplication of a sequence resembling an insertion element. Immunological studies suggested that this gene codes for a peribacteroidal membrane protein. Little sequence divergence in the duplicated introns suggests that this gene was generated recently in the evolution of soybean. Sequence comparison of the 5' ends of Lbc_3, Nodulin-23 and Nodulin-24 genes revealed three conserved seqeunces. These are arranged in a unique fashion and have a spatial organization both with respect to order and position. In vitro transcription studies of nodulin-23 suggested the presence of two promoters. In addition, an antisense promoter (with its transcription direction opposite to that of the nodulin) was observed in this gene. This feature may be involved in regulating the expression of this gene during nodule development.

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SEQUENCE OF A GENOMIC DNA CLONE FOR THE SMALL SUBUNIT OF RIBULOSE BIS-PHOSPHATE CARBOXYLASE-OXYGENASE FROM TOBACCO, Barbara J. Mazur and Chok-Fun Chui, E. I. du Pont de Nemours & Co., Central Research & Development Department, Experimental Station, 328, Wilmington, Delaware 19898

We have cloned and sequenced a gene for the small subunit of ribulose bis-phosphate carboxylase-oxygenase (RUBISCO) from Nicotiana tabacum. The gene was isolated from a genomic library of tobacco, using a pea cDNA clone and a soybean genomic RUBISCO clone as hybridization probes. We isolated two distinct clones carrying RUBISCO genes; neither clone carried additional RUBISCO genes. The tobacco gene is most closely related to the small subunit genes from the dicots soybean and pea, and less so to the monocots wheat and Lemna. The sequence of the mature protein is in all cases more closely conserved than is its chloroplast transit sequence. Unlike the two monocots, which have one intron in their genomic sequences, and the two other dicots, which have two introns in their genomic sequences, the tobacco genomic clone has three introns. The third tobacco intron lies 3' to the two conserved introns, in the middle of one of the most invariant regions of the small subunit gene. The 5' end of the gene and the first and third introns together carry six sequences which are identical to consensus mammalian enhancer signals. Two of the 5' enhancer-like sequences are embedded within a 67 bp inverted repeat. These inverted repeats flank a series of eight direct repeats of a 43 bp sequence and the direct repeats themselves each carry inverted repeats. Thus this region 5' to the promoter is capable of assuming considerable secondary structure. Putative regulatory sequences at the 5' end of the gene are conserved, whereas the poly A recognition sequence is not.

1739 MUTANTS OF ARABIDOPSIS WITH ALTERED CHLOROPLAST MEMBRANE LIPID COMPOSITION, Peter McCourt, John Browse & Chris Somerville, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824

Chloroplast membranes of all higher plants contain a high proportion of polyunsaturated fatty acids suggesting an important functional role for these lipids in supporting the light reactions of photosynthesis. Although many, primarily biophysical, approaches have been used to investigate the functional significance of membrane lipid unsaturation, an explanatory model has not emerged. In order to approach this problem from a new perspective we have isolated mutants of Arabidopsis thaliana with altered membrane composition by directly analysing the fatty acid composition of individual leaves from a mutagenized population. Ten lines which have deficiencies in five distinct enzymatic reactions associated with leaf lipid metabolism were identified from among approximately 2000 plants. Although several of the mutants show dramatic alterations in chloroplast membrane composition, genetic studies indicate that these alterations are due to single simply inherited nuclear mutations. Preliminary biochemical characterization of the mutants suggests that each of the mutants exhibits a deficiency in one of the following enzymes: 1) a chloroplast 16:0 desaturase; 2) a chloroplast 16:1/18:1 desaturase; 3) a chloroplast 16:2/18:2 desaturase; 4) a chloroplast phosphatidyl glycerol specific desaturase; 5) a chloroplast phosphatidic acid phosphatase. All of the mutants appear phenotypically normal when grown at 21°C under low light, with the exception of the 16:1/ 18:1 desaturase mutant which exhibits normal growth only at temperatures above 26°C. The remaining mutants show only subtle differences in growth and photosynthetic function at a wide range of temperatures.

1740 CHANGES IN GENE EXPRESSION INDUCED AS A RESULT OF PREMATURE DESICCATION OF P. VULGARIS SEEDS, S. Misra and J.D. Bewley, Department of Biology, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

For most seeds, drying is the terminal event in development, leading to a state of metabolic quiescence. During development of P. vulgaris seeds, one of the major storage proteins to be synthesized in the cotyledons and axes is phaseolin, representing about 50% of total protein in mature seeds. Utilizing a cloned cDNA gene from P. vulgaris homologous to β-phaseolin mRNA (gift of Dr. J. Slightom, Agrigenetics Advanced Research Lab), we have examined the fate of the phaseolin message in normally matured, prematurely dried, and dried-rehydrated seed axes. Northern blot analysis show that the mRNA for phaseolin is preserved following normal maturation and premature desiccation of seeds. Following rehydration of immature seeds at the desiccation-tolerant stage the message is no longer detectable in the axes. This mimics the pattern observed in mature germinated seeds. Thus the changes observed in protein profiles in response to premature desiccation of P. vulgaris seeds seem to occur at the level of transcription. Whether this is effected by the direct action of drying-rehydrating events on the genome, or whether other changes such as hormones (or their receptors) essentially involved with development or germination are involved remains to be elucidated.

1741 HORMONAL REGULATION OF β-1,3-GLUCANASE mRNA IN CULTURED TOBACCO TISSUE Debra Mohnen, Hideaki Shinshi and Fred Meins, Friedrich Miescher-Institut, Basel, Switzerland.

Accumulation of the abundant enzyme β-1,3-glucanase (EC 3.2.1.39) in cultured tobacco tissues is regulated by the hormones auxin and cytokinin (c.f. poster by Felix and Meins). As judged by immunoprecipitation and partial proteolysis of the in vitro translation product, poly (A) RNA isolated from induced tissues contains mRNA for a ca.38Kd precursor of the mature 33-34Kd enzyme. This mRNA was not detected in non-induced tissues. The time course of mRNA induction measured by Northern and dot blot analysis of total RNA using a glucanase cDNA probe was similar to the time course of glucanase induction. This indicates that the regulation of β-1,3-glucanase by auxin and cytokinin in culture is at the mRNA level.

1742 THE EXPRESSION OF PEA rbcS GENES IN PEA PLANTS AND IN TRANSFORMED PETUNIA
Giorgio Morelli, Phyllis Moses, Ferenc Nagy, Gloria Coruzzi, Robert Fluhr, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, NY, NY 10021-6399.

We have isolated four pea genomic clones which encode five different members of the multigene family for the small subunit of ribulose-1,5-bisphosphate carboxylase (rbcS). DNA sequence analysis of three genes revealed a high degree of homology at the nucleotide level in the coding sequence, but both the promoter and the 3' non-coding regions are divergent. All three genes are interrupted by two introns located in the same relative positions; however, only the second intron is conserved. Northern and S1 nuclease analysis revealed that all three genes are expressed in a tissue specific fashion, and the ratio of transcripts encoded by the three genes are different in different tissues. Furthermore, all the genes are induced by light and respond to phytochrome regulation. These results indicate that transcriptional controls of individual rbcS genes vary. In collaboration with R. Fraley and S. Rogers of the Monsanto company, we have utilized Agrobacterium mediated transformation to study the regulatory regions of one of these genes, rbcS-E9. This gene is expressed in a light-dependent and tissue-specific manner under the transcriptional control of its own promoter in petunia cells. A series of 5' deletions in the promoter region and a series of chimeric genes have been constructed to define the DNA sequences required for light-induction and maximal expression of rbcS-E9.

1743 REGULATION OF CHLOROPLAST PROTEIN SYNTHESIS, John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

Plastids in higher plants have been found to exhibit two distinct protein translation modes. The first type is found in photosynthetically competent plastids and is characterized by the dependence of protein translation on light as a source of energy. Proteins translated within these plastids range up to 70 Kd in size and in general radiolabeled products comigrate with polypeptides which accumulate to significant levels (i.e. rbcL gene product). A second translation mode is found in developing plastids, etioplasts and carotenoid deficient-bleached plastids. Proteins translated in these plastids range up to 200 Kd in size and translation does not require light as an energy source. RNA which is translated in each translation mode have been characterized. It has been found that translation of the light-dependent type can be switched to the light-independent type without major changes in RNA populations. These data demonstrate that a significant regulation of plastid gene expression occurs at the level of protein translation.

1744 NODULIN-35, A NODULE-SPECIFIC URICASE OF SOYBEAN: STRUCTURE, BIOSYNTHESIS AND LOCATION, Truyen Nguyen, Victoria Foster, Hanna Bergmann, Maria Zelechowska and Desh Pal S. Verma, Plant Molecular Biology Laboratory, Biology Department, McGill University, Montreal, Canada H3A 1B1

Nodulin-35 represents a 35 kd protein which is induced about ten days after infection of soybean by Rhizobium japonicum. It was shown to be a subunit of the nodule-specific uricase. Subcellular localization studies done using nodulin-35 specific antibody and protein-A gold revealed the presence of this protein in the peroxisomes of the uninfected cells of nodules. This protein is found to be synthesized on free polysomes and thus appears to be transported to peroxisomes post-translationnally. A cDNA clone (pNod35) was isolated using mRNA from immuno-precipitated polysomes. Hybridization of this clone to soybean genomic DNA revealed four EcoRI bands, suggesting multiple copies of this gene. A genomic clone corresponding to two of these EcoRI bands was isolated from a soybean genomic library. The structure of this gene was determined by DNA sequencing and compared with other nodulin and leghemoglobin genes of soybean.

1745 REGULATION OF TRANSCRIPTION IN THE AZOLLA-ANABAENA SYMBIOSIS, Sandra A. Nierzwicki-Bauer and Robert Haselkorn, Univ. of Chicago, Chicago, IL. 60637

Azolla is a small water fern which is symbiotically associated with the filamentous nitrogen-fixing cyanobacterium Anabaena azollae. The Anabaena species, which occurs as a symbiont in the leaf cavity of Azolla, can fix enough atmospheric nitrogen to satisfy both its own requirement for combined nitrogen and that of its host. The Azolla-Anabaena association provides an interesting system for studying the regulation of transcription because the processes of protein synthesis and heterocyst differentiation in the Anabaena symbiont vary considerably from those seen in free-living forms of Anabaena, probably as a result of the fern's influence on the cyanobacterium. Using cloned genes from Anabaena 7120 for glutamine synthetase (GS), ribulose-1,5-bisphosphate (RuBP) carboxylase, nitrogenase, and the 32-kD protein of photosystem II, transcription of the corresponding genes in the endosymbiont was studied by Northern hybridization. The most striking result is that message for GS is not present in RNA isolated from the endosymbiont. Thus we conclude there is transcriptional regulation of GS synthesis within the endosymbiont. Additionally, in the endosymbiont there is greater than a two-fold increase in the amount of message for the 32-kD protein, and greater than a two-fold decrease in the amount of message for RuBP carboxylase, compared with transcript levels in the free-living Anabaena azollae. Northern and Southern blots hybridized with specific nitrogenase probes indicate that, as in Anabaena 7120 (J. Golden, personal communication), there are rearrangements involving the genes coding for nitrogenase in both the endosymbiont and in heterocysts of the free-living Anabaena azollae.

1746 CHARACTERIZATION OF cDNA CLONES CODING FOR MAIZE GLUTELINS. P.Puigdomènech, S.Prat, J.Cortadas and J.Palau. Institut de Biologia, CSIC Jordi Girona Salgado, 18, 08034 Barcelona, Spain.

Clones corresponding to glutelins from maize have been obtained by screening cDNA libraries from immature endosperm. Immunodetection of clones has been done using antisera raised against the main component of the glutelin-2 fraction, the 28 kd protein. Due to the immunological cross-reaction with another endosperm protein, the 14 kd zein-2, clones corresponding to the two proteins have been isolated and characterized. The 28 kd glutelin-2 protein has a sequence presenting at least four well-defined domains. The main domain is a Pro-Pro-Pro-Val-His-Leu sequence repeated eight times. It is preceded by a short fragment containing two Cys residues and a 19-residue signal peptide. After the repetitive sequences and alternative stretch Pro-X is present and the C-terminal part contains most of the Gln and Cys residues. This part has a strong degree of analogy with the 14 kd zein-2. Both proteins show analogies with storage proteins from other cereals such as gliadins and hordeins. A composite function for these proteins structural and storage is proposed.

1747 IDENTIFICATION OF AN ABUNDANT, HORMONALLY REGULATED POLYPEPTIDE IN TOBACCO TISSUE CULTURE AS β-1,3-GLUCANASE, Georg Felix and Frederick Meins, Friedrich Miescher-Institut, Basel, Switzerland.

We have purified an abundant, 33-34Kd polypeptide (P33) from cultured tissues of Havana 425 tobacco. The P33 content, measured immunologically, increases from >1% to 10% of the soluble protein when tissues are transferred from medium containing auxin and cytokinin to media with one or both hormones deleted. P33 also exhibits a specific distribution in the plant. It makes up 5-10% of the soluble protein in lower leaves and roots, but is not detectable in leaves near the top of the plant. Based on its hydrolytic activity P33 was identified as endo-β-1,3-glucanase (EC 3.2.1.39), an enzyme implicated in the response of plants to pathogens. The results show that this enzyme is regulated by hormones in culture and developmentally regulated in the plant.

1748 BARLEY ∝-AMYLASE GENES ARE TRANSCRIBED ON BOTH STRANDS, John C. Rogers, Washington University, St. Louis, Mo 63110.

We have cloned and sequenced full-length cDNAs for each of the two different barley ∝-amylase gene families; these are located on two different chromosomes and the coding sequences of the mature secreted enzymes are only 74% identical. Nevertheless, both cDNAs have an open reading frame on the strand opposite the ∝-amylase sequence that begins at an identical position and extends from the first ATG for 1.1 kb. This unusual finding suggested that the opposite strands might code for another mRNA. The high-pI cDNA was cloned in both orientations behind the SP6 polymerase promoter, and labeled RNAs synthesized from that promoter were used as hybridization probes. The strand-specificity of the probes was proved on blots carrying high-pI sequences cloned into M13. On northern blots, the strand-specific probe hybridizing to authentic ∝-amylase mRNA demonstrated that mRNA is present in unstimulated aleurone cells, and increases with GA and decreases with ABA stimulation. In contrast, opposite strand RNA identical in size to ∝-amylase mRNA is present at the same levels regardless of hormonal manipulation, and is present in higher quantities in leaf and root cells. M13 DNA carrying ∝-amylase mRNA sequence was used to hybrid select opposite strand RNA from ABA-treated aleurone cells and from leaf cells. This selected RNA, when translated in a wheat germ system, generated a specific peptide of 27 kd. Thus the opposite strand RNA appears to be mRNA. Preliminary S1 experiments indicate that the opposite strand RNA is essentially perfectly complementary to the ∝-amylase mRNA. These findings may be relevant to GA regulation of ∝-amylase gene expression.

1749 ISOLATION AND CHARACTERISATION OF cDNA AND GENOMIC CLONES FOR TOMATO POLYGALACTURONASE. Wolfgang Schuch[1], Lorraine Clarke[1], John Ray[1], Keith Edwards[1], Adrian Slater[2], Martin Maunders[2] and Don Grierson[2].

[1] ICI Corporate Bioscience Group, Runcorn, U.K., and [2] Dept. of Agriculture, University of Nottingham, Sutton Bonnington, Loughborough, U.K.

Gene expression during tomato fruit ripening was investigated by cDNA cloning. A cDNA library was prepared from ripe tomato polyA$^+$ RNA and ripening-related cDNA clones were selected. Clones for polygalacturonase, a major cell wall softening enzyme, were identified by hybrid-selection and immunoprecipitation.

Genomic clones for polygalacturonase were isolated from a partial library prepared in λgt ıwes.

Data will be presented on the structure of the cDNA and genomic clones, and on the expression of PG during the ripening process.

1750 TANDEM REPETITIVE ELEMENTS IN CUCURBITACEAE SPECIES. Albert Siegel and Robert Leclerc, Wayne State University, Detroit, MI 48202

Approximately 6% of Cucurbita pepo nuclear DNA is a 351 base tandem repetitive element. Most of the elements contain a single Hind III site but this site is occasionally blocked or absent, giving rise to ca.21% dimers and ca. 9% trimers upon extensive Hind III digestion of genomic DNA. The elements also contain an Hpa I site and some contain two. Several Hind III repeat units were cloned and sequenced in order to assess variability among them. Each of the units is unique, differing at from 10 to 19 base positions from all of the other cloned units. Nuclear DNA of another Cucurbita species, foetidissima, also has a 350 base tandem repetitive element most of which contain both Hind III and Hpa I sites but other Cucurbita, maxima and moschata, do not. C. foetidissima has, in addition, a shorter, 200 base, Hind III containing element. The C. pepo tandem repetitive element is present in about 107,000 copies per haploid genome. It bears no detectable homology to a sequenced 352 base tandem repetitive element found in a species of another Cucurbitaceae genus, Cucumis melo (Brennicke and Hemleben, Z. Naturforsch. 1983, 38c: 1062-1065), and does not hybridize to a similar element detected in Cucumis sativa. It also fails to hybridize with either of the two elements detected in C. foetidissima.

1751 ALTERATION OF THE HIGHER PLANT NITRATE ASSIMILATORY PATHWAY, John Smarrelli, Jr., Michelle T. Watters, and Luke Curtis, Department of Biology, Loyola University of Chicago, Chicago, Illinois 60626

The majority of the 10^{10} tons of nitrogen fixed annually into plant material is derived from the assimilation of nitrate. Nitrate reductase is the first enzyme of this pathway, and generally regarded to catalyze the rate limiting step of the pathway. Squash (Cucurbita maxima L.cv. Buttercup) and soybean (Glycine max var. Williams) cells in culture have been used to examine nitrate assimilation in higher plants. Two approaches have been undertaken. First, the effect of various nitrogen sources on nitrate reductase activity has been examined. This work will focus on glutamine, a known repressor, and nitrate, a known inducer, of nitrate reductase activity in lower eukaryotes. Second, the generation of mutants defective in the nitrate assimilatory pathway in the squash tissue will be described. Based on these studies, the regulation of nitrate assimilation in higher plants will be discussed. (Supported by National Science Foundation PCM 8404243)

1752 MONOCLONAL ANTIBODIES FOR BARLEY CELL SURFACE ANTIGENS, Shauna Somerville and John Sherwood, DOE - Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

The interaction of barley (Hordeum vulgare L.) with Erysiphe graminis f.sp. hordei, the causal agent of powdery mildew disease, is typical of host-pathogen relations described by the "gene-for-gene" hypothesis. The highly polymorphic Reg 1 locus of barley is one of six loci known to mediate reaction to E. graminis. Under the assumption that the Reg 1 locus encodes a component of a host recognition mechanism, we have initiated a project to identify the Reg 1 gene product using monoclonal antibodies to detect subtle antigenic differences between congenic barley lines, resistant and susceptible to E. graminis race CR3. BALB/c mice have been injected with a crude preparation of plasma membranes from both resistant and susceptible barley lines. The supernatants from the resulting hybridomas were screened for reactivity to glutaraldehyde-fixed nongreen protoplasts. None of the 19 hybridoma lines, retained in an initial screen, secreted antibodies which discriminated unequivocally between tissue from resistant and susceptible lines. However, by immunofluorescence microscopy, some of these antibodies seem to recognize plasma membrane-specific antigens. Such monoclonal antibodies are potentially useful for 1) the identification and purification of epidermal tissue and plasma membranes; 2) the characterization of the developmental fate of specific cell types during ontogeny and; 3) for the identification and purification of polypeptide constituents of plasma membrane-localized enzymes.

1753 GENETIC ANALYSIS OF THE RUBP CARBOXYLASE SMALL SUBUNIT TRANSIT SEQUENCE. Les J. Szabo, Mark Hand, Anthony R. Cashmore, Rockefeller University, New York, NY 10021.

Although chloroplasts contain their own genetic system, the majority of chloroplast proteins are encoded by nuclear genes, and are the products of protein synthesis on free cytoplasmic ribosomes. Many of these polypeptides are synthesized as soluble, higher molecular weight precursors which are processed during or after translocation into the chloroplast. The most extensively characterized of these translocated polypeptides is the small subunit of ribulose-1,5-bisphospate carboxylase/oxygenase (Rubisco). DNA sequence analysis of the Rubisco small subunit from Pisum sativum showed that the precursor contains a 57 amino acid N-terminal transit sequence, which is presumed to direct translocation. We have developed an in vitro system to analyze geneticaly altered Rubisco small subunit transit sequences. A full lenght cDNA clone was constructed and subcloned into a vector containing the Salmonella SP6 promoter. Capped RNA obtained by run off transcription was efficently translated in wheat germ. The resultant polypeptides were properly transported and processed, by isolated chloroplasts in a translocation assay. A series of transit sequence deletions have now been constructed and will be used to determine functional domains of the small subunit transit sequence.

1754 LIGHT AND DNA GYRASE INHIBITORS AFFECT CHLOROPLAST TRANSCRIPTS IN CHLAMYDOMONAS REINHARDTII, Robert J. Thompson and Gisela Mosig, Vanderbilt Univ., Nashville TN.

We have previously shown (Plant Physiol. 76:1-6, 1984) that a family of overlapping transcripts originating from the Hpa II 5 region of the chloroplast chromosome overaccumulate when certain strains are grown in the dark as compared with the light. These same transcripts overaccumulate when light-grown cells are treated with novobiocin or nalidixic acid, two inhibitors of bacterial DNA gyrase. A comparison of the effects of these inhibitors on the Hpa II 5 transcripts with their effects on other chloroplast transcripts suggests a unique regulation for the Hpa II 5 transcripts.

We have found that Chlamydomonas cells contain an ATP-dependent topoisomerase which supercoils DNA in vitro. This supercoiling activity is inhibited by novobiocin (0.1 μM) and by nalidixic acid (0.1 mM). Together, our results strongly suggest that the supercoiling topoisomerase plays an important role in chloroplast transcription. Experiments to define the relationship between the effects of light/dark and the effects of inhibitors of this topoisomerase on chloroplast transcription are in progress.

We suspect that the algal supercoiling topoisomerase resides in the chloroplast because preliminary hybridization experiments suggest that an E. coli restriction fragment containing the gyrB gene hybridizes to chloroplast DNA. Similar hybridization experiments employing the E. coli gyrA gene are in progress.

1755 CHROMATIN STRUCTURE OF A STORAGE PROTEIN GENE OF SOYBEAN UNDER THE CONTROL OF A DEVELOPMENTAL AND CONSTITUTIVE PROMOTER. Mary L. Tierney, Michael A. Lawton, and Roger N. Beachy, Washington University, St. Louis, MO 63130.

Studies on the chromatin structure of developmentally regulated genes indicate that transcription is frequently correlated with a local enhanced sensitivity of the gene sequence to specific nucleases. We have been studying the chromatin structure of the 7S α' gene in soybean during seed development and germination. This gene is under tight tissue-specific and developmental control, being expressed only during mid-maturation of the seed. In these studies, nuclei were isolated from developing seeds and cotyledons of DNase I. The DNA isolated from these samples was then restricted and probed for the nuclease sensitivity of known restriction fragments within and outside of the α' gene. In developing seeds, the restriction fragments within the coding sequence were more sensitive to DNase I than the restriction fragments flanking the gene. However, in cotyledons of germinating seeds, the α' gene no longer displayed an enhanced sensitivity to DNase I. This indicates that the coding sequences of the α' gene are more sensitive to DNase I when the gene is transcriptionally active and that the chromatin shifts from an active to an inactive conformation in the cotyledon during the final stages of seed development and/or dessication. The structural sequences of the α' gene have also been transformed into petunia under the control of a CaMV promoter. This gene has been shown to be expressed in both callus tissue and leaves of petunia. Experiments are in progress to analyze the chromatin structure of this gene in a foreign environment, now under the control of a constitutive promoter.

1756 NUCLEOTIDE SEQUENCES REQUIRED FOR PHOTOREGULATION OF NUCLEAR GENE EXPRESSION, Michael P. Timko, Albert P. Kausch, and Anthony R. Cashmore, Rockefeller University, New York, NY 10021

We have examined the DNA sequences required for high levels of light-inducible expression of two photoregulated nuclear genes from pea, the small subunit of ribulose-1,5-bisphosphate carboxylase and the major polypeptide of the light-harvesting chlorophyll a/b-protein complex. Chimaeric genes consisting of promoter fragments of varying length of the pea ss-3.6 gene linked to the coding sequences of the bacterial cat (chloramphenicol acetyltransferase) gene were constructed and introduced into tobacco cells by Agrobacterium-mediated cell transformation. The expression of these genes was studied in light and darkness. Such studies have shown that nucleotide sequences residing within 973 bp upstream from the cap site for the ss-3.6 gene are required for high levels of light-inducible expression. Deletion of sequences from the 5' end of this promoter (-973 to -722 bp upstream from the cap site) result in a 60 % reduction in levels of light-inducible expression. Nucleotide sequences within 92 bp of the cap site are still capable of light-inducible expression, but at levels significantly reduced from the level of the 973 bp promoter. We are presently analyzing the nucleotide sequence requirements for light-inducibility of the chlorophyll a/b-binding protein genes. The results of these studies will be presented.

1757 RNA POLYMERASE FROM ANABAENA: PURIFICATION AND CHARACTERIZATION OF START SITES FOR IN VITRO TRANSCRIPTION, Nilgun E. Tumer, Catherine Richaud, George Borbely and Robert Haselkorn, University of Chicago, Chicago, IL 60637

RNA polymerase was purified from the vegetative cells of Anabaena and was used to transcribe cloned glutamine synthetase (gln A) and RuBP carboxylase (rbc L and rbc S) genes. The gln A gene is transcribed abundantly in vegetative cells, in heterocysts and in cells induced for nitrogenase. During growth on ammonia, transcription initiates at several sites, all of which are "E. coli-like." These transcripts are absent from cells induced for nitrogenase but are replaced by mRNA whose promoter is "nif gene-like". A DNA fragment containing all these promoters was transcribed in vitro. The run-off transcripts correspond to initiation at each of the E. coli-like promoters but not the nif-like promoter.

The 3kb transcript of the rbc L-rbc S operon is abundant in ammonia grown cells but absent from cells starved for nitrogen. The in vivo start site for these genes has an E. coli-like -10, however, the sequence around -35 is very different from the E. coli consensus. A DNA fragment containing this sequence was used as a template for in vitro transcription with RNA polymerase from E. coli or Anabaena. The E. coli enzyme does not transcribe this fragment, while the Anabaena enzyme transcribes efficiently from the promoter used in vivo. The Anabaena enzyme is completely sensitive to rifampicin. A mixture of Anabaena enzyme plus rifampicin resistant E. coli enzyme will transcribe from the rbc L promoter in the presence of rifampicin, suggesting that Anabaena σ- factor can direct E. coli core to transcribe from an Anabaena promoter.

1758 CHROMATIN STRUCTURE OF THE MAIZE Adh 1 GENE, Michael E. Vayda and M. Freeling, U. California at Berkeley, California 94720

Maize is an excellent system in which to correlate the chromatin structure of a plant gene and the transcriptional activity of that locus. Expression of the Maize Adh 1 gene is inducible in root and scutellar tissues subjected to anaerobic stress and constitutive in pollen. By contrast, Adh 1 is not expressed in leaf tissues under neither aerobic nor anaerobic conditions. Further, several mutant alleles of Adh 1 exist which have resulted upon the insertion of a transposible element within the transcriptional unit; the phenotypes of these mutants include reduced levels of cytoplasmic Adh 1 mRNA and abolition of Adh 1 expression. We have investigated the sensitivity of Adh 1 alleles to nucleases under varied physiological conditions, and have attepted the isolation of chromatin subunits .

1759 METHYLATION AND SEQUENCE DIFFERENCES OF CHLOROPLAST DNA BETWEEN FERTILE AND CYTOPLASMIC MALE STERILE GOSSYPIUM AND PETUNIA. FANG-SHENG WU, ZOECON CORP., PALO ALTO, CA 94304.

Chloroplast (ch) DNAs of Gossypium hirsutum and Petunia parodii were isolated and restricted with 14 restriction enzymes including isoschizomers which recognize the same DNA sequences but digest differentially depending upon the methylation status of the DNA. In G. hirsutum, the ch DNA of fertile (B), cytoplasmic male sterile (A or CMS) and restorer (R) lines all have different restriction patterns when they are digested by HpaII and MspI. However, a single enzyme give similar restriction patterns for the A and R lines but a different one for the B line. Similar phenomena were observed with EcoRII and BstNl digests. Other restriction enzymes (TaqI, Sau3Al, HaeIII, BamHl, Bgl II, EcoRl, SmaI, XmaI, and HhaI) all produced the same restriction patterns for the A and R lines which were differed from B line. In Petunia, the ch DNA of either line 3699 (fertile) or line 3688 (CMS) differed in its restriction patterns between HpaII and MspI and between EcoRII and BstNl digests. Other restriction enzymes tested which cut irrespective of DNA methylation, only Bgl II, EcoRl and BamHl differentiated between the restriction patterns of fertile and CMS lines.

These data indicate that the ch DNAs of fertile or CMS Gossypium and Petunia, have methylation sites at cytosine residues, and the sequences vary between fertile and CMS lines. The extent of such differences was greater in Gossypium than in Petunia.

1760 THE MITOCHONDRIAL ATPASE SUBUNIT 9 GENE OF PETUNIA, Ellora Young and Maureen Hanson, University of Virginia, Charlottesville, VA 22903

We have isolated a DNA segment of the mt genome of Petunia hybrida line 3704 that contains the entire coding sequence of subunit 9 of the F_o ATPase. The coding region DNA sequence is 59% homologous with the mt ATPase proteolipid-like gene of Neurospora crassa. The predicted Petunia protein of 70 amino acids is highly hydrophobic and contains a glutamic acid at position 50 that has been shown to be involved in DCCD-binding and proton-translocation in other organisms. This predicted polypeptide sequence compares favorably with the protein sequences of these other proton-translocating subunits.

S1 nuclease protection studies indicate that this gene is transcribed in P. hybrida and P. parodii. Three transcripts of varying 5' non-translated lengths are found.

Two sterile-specific point mutations internal to the leader occur in CMS P. parodii line 3688 but do not appear to alter the relative proportions or the various lengths of the RNAs.

Quantitative Aspects of the Genome

1761 NUCLEOTYPIC DIMENSIONS AND PLANT GENETICS, Michael D. Bennett, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England.

Interspecific variation in nuclear genome DNA amount is more than 1000-fold in angiosperms[1] (from 1C = 0.125 pg in Rosa wichuraiana to 127.4 pg in Fritillaria assyriaca). Such variation has far-reaching biological consequences[2]. It is causally correlated with several biophysical characters at the chromosome phenotype level, e.g. the total length, volume and mass of the haploid complement. Moreover, it is correlated, perhaps sometimes causally, with many widely different nuclear, cellular, tissue and even organismal phenotypic characters including: the number of chloroplasts per stomatal guard cell, rates of nuclear and cellular processes, pollen volume, seed weight, habit and minimum generation time, radiosensitivity, and the optimum environment and geographical range of crop and non-crop species. Nuclear DNA influences the phenotype both directly by its genic content, and indirectly by the physical effects of its mass. The term 'nucleotype' defines those conditions of the nucleus which affect the phenotype independently of the encoded informational content of its DNA. Significantly, the nucleotype limits the range of phenotypic expression which can be achieved by gene action[3].

Apart from a few treasured exceptions (e.g. flax genotrophs[4]) most early work concentrated on the extent and consequences of major interspecific variation, partly because of difficulty in detecting or conclusively demonstrating intraspecific variation, and partly because of the once widely held view that genome size is constant (hence the term 'C-value') within a species. However, several new examples of intraspecific variation in genome size have been found recently, so that considerable and increasing interest has focused on the origins, extent, distribution and significance of the phenomenon. Evidence for angiosperms (including: Poa annua, Microseris douglasii[5], Zea mays and Secale cereale) suggests that intraspecific variation in genome size has detectable biological consequences of interest to plant geneticists. Thus, it may be important to consider: first, whether total genome size is amenable to controlled change in response to selection and/or manipulation; and second, whether there are constraints or controls which limit or direct the distribution of DNA gain or loss within and between chromosomes[6]. Ability to design and construct useful artificial chromosomes in plants may depend on our understanding a possible nucleotypic relationship between stability and size, and the mechanism(s) of 'counters' which may quantify and adjust intranuclear genomic characters at various levels from DNA sequence copy number to chromosome arm size.

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1762 A HYPOMORPHIC MUTATION AT THE SHRUNKEN LOCUS IN MAIZE, John P. Mottinger*, Stephen L. Dellaporta** and Patrick B. Keller*
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Mutations at the shrunken locus in maize which are due to insertions of transposable elements have been recovered in descendants of plants infected with barley stripe mosaic virus[1,2]. In one of these, sh-5584, the insert is at the 5' end of the gene near the promotor. The mutation exhibits a dosage effect in some genetic backgrounds wherein one dose produces a shrunken kernel while two or three doses result in an intermediate or plump phenotype. Shrunken is a structural gene for sucrose synthetase[3]. Analyses of endosperm extracts on polyacrylamide gels show a direct relationship between the dose of sh-5584 and the amount of Sh protein. Densitometer readings of gels indicate that the amount of gene product in kernels with three doses of sh-5584 is about 30% of that in kernels with one dose of the normal Sh allele (Sh sh sh). Hence the amount of enzyme needed to generate a plump kernel is substantially less than the quantity produced by a single dose of the dominant allele.

Enzyme studies have shown that the specific activity of sucrose synthetase produced by sh-5584 is approximately 30% that of wild type for the synthetic reaction and 10% for cleavage. Northern blot analyses of poly A RNA from normal Sh and sh-5584 homozygotes show a five-fold reduction in the amount of message produced by the mutant relative to the normal allele. The transcript of the mutant allele is wild type in size and no altered messages are apparent. These observations indicate that sh-5584 is a regulatory mutation. The location of the insert at the 5' end of the gene near the promotor is consistent with this interpretation.

In some genetic backgrounds, one dose of sh-5584 produces a fully plump kernel. This suggests that another gene (or genes) functions as a second site suppressor of the mutation at the shrunken locus.

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1763 DEVELOPMENTALLY-TIMED CHANGES IN MAIZE ENDOSPERM DNA, Ronald L. Phillips, Michael D. McMullen, Shinichiro Enomoto, and Richard V. Kowles, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

Maize endosperm development progresses rapidly after the formation of a triploid nucleus from the fusion of the two polar nuclei of the central cell of the embryo sac with a sperm nucleus. Cell divisions occur in the non-peripheral regions of the endosperm through about 12 days post-pollination establishing the cell population of the central zone. At about the time cell division ceases, the size of the nuclei and the nuclear DNA content begin to increase in parallel. The DNA content of inbred A188, measured by Feulgen microspectrophotometry, is estimated to be 4.5 C during the first 10 days of endosperm development. Presumably, this DNA level is reasonable since the tissue is triploid. At about 12 days post-pollination, the mean level of DNA per nucleus increases in the absence of cytokinesis. The DNA amplifies to a peak of 90 C by 16 days post-pollination. This pattern of DNA amplification has been consistent over four seasons in field grown material. Other inbreds may amplify their DNA to even higher levels. Hybrids have DNA levels that approximate the expected values relative to the parental inbreds.

The question can then be asked: Are the developmentally-timed changes in endosperm DNA levels a result of amplification of the total genome or of specific regions? Polyploidization does not appear to be indicated because in situ hybridization with labelled rRNA produces only three silver grain clusters over the nucleolus showing the presence of only three chromosomes 6 as expected in triploid cells. Saturation hybridization of labelled rRNA to DNA taken from endosperm tissue 4 to 24 days post-pollination indicates that the proportion of rDNA is constant through day 10, increases about 50% by day 16, and returns to the initial level or lower by day 20. An interpretation is that the rDNA is among the first DNA portions to amplify and its proportion is gradually decreased as other genomic regions amplify. Other information indicates that the overall amplification process cannot be entirely accounted for by an increase of only a few sequences.

Control of gene expression generally is thought to involve regulation of transcription, processing, translation, or post-translational modifications. The dramatic amplification of endosperm DNA, whether selective or not, leads to the possibility that endosperm gene expression is regulated importantly by alterations in gene copy number. Total soluble proteins, various starch synthesis enzymes, zeins, and other proteins increase in a parallel manner to DNA amplification. Tests are underway to correlate gene copy number with the activity of various proteins.

1764 **RAPID GENOMIC CHANGE IN MAIZE,** Virginia Walbot, Vicki Chandler, Carol Rivin*, Chris Cullis**, and Loverine P. Taylor, Department of Biological Sciences, Stanford University, Stanford, CA 94305. *current address: Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR. **current address: John Innes Institute, Colney Lane, Norwich, UK.

We are interested in understanding how genome organization is regulated in maize and whether rapid genomic change is a feature of the adaptation of plants to continuous development in a variable environment (1,2). McClintock has also proposed that stress may trigger a variety of rapid genomic changes including the release of cryptic transposable elements (3). We have investigated the organization and stability of the copy number of 9 cloned repetitive sequences in maize representing sequences present in low (<50), middle (~ 1,000) and high (>50,000) number. We have discovered that each inbred line of maize tested has a characteristic copy number for each sequence class (4). When two lines are crossed the F1 progeny frequently contain an unexpected copy number (not the mean of the parental values) for one or more repetitive sequence types. Instability in copy number is also detected in embryos of inbred lines placed in tissue culture.

In all inbreds tested (8) we find sequences homologous to Mu-1, a transposable element family found in Robertson mutator lines of maize (5); however, these sequences do not appear to vary significantly in copy number among different inbreds. In contrast, in crosses with lines containing an active mutator system we find progeny with widely different copy numbers of Mu-1 and Mu-L. Mu-L is a 1.7 kb element highly homologous to Mu-1 found in many mutator lines. At very low copy number (<10) no new mutations are recovered and mutable "reporter" genes show no somatic reversion. Some higher copy number (>50) lines also yield no new mutations and show no somatic instability. Surprisingly, in these higher copy lines the mutator elements no longer show the typical restriction pattern of Mu-1 and Mu-L, but appear much larger. We are investigating whether this modification of element organization is due to DNA modification, amplification, or rearrangement of sequences within the element. The change in element restriction pattern is progressive; individuals have been identified that contain both the normal Mu-1 and Mu-L patterns as well as the larger fragments. Lines containing these altered elements have been crossed to lines that contain only the normal Mu-1 and Mu-L. Genetic and molecular results from these crosses will be discussed.

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Enlightened Mutagenesis and Mutant Recovery

1765 BIOCHEMICAL GENETICS OF THE ASPARTATE-DERIVED AMINO ACIDS IN PLANTS.

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The branched pathway of synthesis of aspartate-derived (lysine, methionine, threonine, isoleucine) and related amino acids (valine, leucine) has been studied for two reasons. Firstly these are nutritionally essential and sometimes limiting amino acids in animal nutrition. Secondly two new classes of herbicides inhibit one of the pathway enzymes, acetolactate synthase. Mutants have been sought as an aid to understanding and as a way of changing this area of plant metabolism in a directed way.

Mutants resistant to lysine plus threonine have been selected at differing levels of organisation in various systems. Where established, a lysine-sensitive form of the enzyme aspartate kinase has been identified with altered regulatory properties. Barley mutants at two loci correspond to two lysine-sensitive isoenzymes, which can be combined in double mutants with the expected enzyme phenotypes. The mutants have characteristic facets of enzyme regulation and patterns of soluble amino accumulation. Two mutants of Nicotiana sylvestris selected with aminoethylcysteine each accumulate lysine. Dihydrodipicolinic acid synthase regulation by lysine is reduced [1]. Resistant tobacco mutants have been identified amongst protoplast-derived cells cultured with valine. Such mutants fall into two classes [2] with one having altered regulatory properties of acetolactate synthase. This enzyme is also altered in tobacco mutants resistant to chlorsulfuron [3].

Auxotrophic mutants requiring threonine, methionine, isoleucine, leucine or isoleucine plus valine have recently been identified after screening mutagenised haploid cells or protoplasts [4-6]. We are investigating the enzyme lesions in some of these lines. Detailed characterisation of the biochemistry and genetics of all these mutants will enhance the understanding of the regulation of metabolism in vivo and the consequences of perturbing it by genetic engineering.

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1766 STRATEGIES TO SELECT BIOCHEMICAL MUTANTS WITH POTENTIAL USE IN GENETIC AND CELLULAR ENGINEERING.

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The recent avaibility of stable and well characterized selectable markers and the ability to combine genomes parasexually have opened new perspectives in the genetic manipulation of higher plants.

We have obtained several types of biochemical mutants using protoplast cultures (Nicotiana sylvestris and N. plumbaginifolia), embryogenic cell suspensions (Daucus carota) and embryos (Arabidopsis thaliana and Hordeum sativum). Resistance dominant markers, with emphasis on lysine plus threonine and S-aminoethylcysteine resistant mutants were isolated and characterized at the enzyme and genetic level. Resistant recessive, deficient mutants were selected in the case of alcohol dehydrogenase and nitrate reductase. Auxotrophs for several amino acids were obtained after enrichment selection and recovery of surviving clones. For mutants requiring histidine, isoleucine (ileu$^-$) and methionine, enzyme lesions were determined, they concern respectively histidinol phosphate transaminase, threonine deaminase and -cystathionase. Their genetic analysis based on sexual transmission or/and somatic fusion shall be presented.

Some of the described mutants are used to develop efficient systems of gene transfer and to analyse the organisation of plant genes. DNA-mediated transfer is performed using threrefore Ti plasmid vector to introduce coding sequences of the yeast threonine deaminase gene ILV-1 into ileu$^-$ protoplasts of N. plumbaginifolia. Transfer of genes by fusion of protoplast with donor cells inactived by gamma irradiation was attempted using complementation of deficient or sensitive receptor cells by the corresponding selectable markers of the donor partner. The experimental systems concerns the following marker genes : nitrate reductase, 5-methyltryptophane ands S-aminoethylcysteine resistance, tryptophane auxotrophy and octopine synthesis. Results tend to show that relatively small fragments of chromosomes can be transfered and expressed in the regenerated plants.

Such results due to the use of selectable markers may offer new possibilities to integrate valuable traits into crop plants.

1767 THE EN TRANSPOSABLE ELEMENT SYSTEM, P. A. Peterson*, Zs. Schwarz-Sommer**, A. Gierl**, A. Pereira**, H. Saedler**, *Dept. of Agronomy, Iowa State Univ., Ames, IA 50011, **Max-Planck-Institut, 5 Koln-30, West Germany

The En transposable element system in maize originated as a pale-green mutable (pg-m) allele from seed that was exposed to the Bikini tests in 1946. The controller of the mutability of pg-m was named Enhancer (En). From plants containing pg-m, a plant was found to have an unstable allele at the Al locus (a-m) also controlled by En. This original allele was autonomously mutable (self control of mutability of Al) and gave rise to a large number of En-controlled derivatives. Among the derivatives was a colorless allele, identified as a-m(r)[2]. This response in the combination of receptor (an I element at the a-m(r) allele) to a regulatory element En is specific and identifies a system and in this test the En transposable element system. Spm[3] generates a similar response and therefore is homologous to the En system. The lack of response in such a test identifies non-homologous separate systems[4]. En like Spm expresses two gene functions termed S and M; S suppresses the phenotypic expression of a locus and M causes excision events of the I elements or of En itself. These functions became visible at the molecular level where we could show quantitative and qualitative differences between wx-specific RNAs originated from plants carrying the wx^{m-8} allele in comparison with plants also containing En[7].--There is an assortment of distinguishable alleles of En and these represent the states of the element at particular loci. These could include changes in En-encoded S and M functions in addition to I receptivity to S and M.--From a transposition event of En, a wx-844 allele was uncovered and this proved to have an 8.4 kb insert identified as En-1. From sequence analysis of the terminal inverse repeat (TIR) of En-1, it was found to be identical to the TIR of the previously isolated Spm-I8 element from the wx-m8 allele[5]. Reversion events (eg wx→Wx) have the host duplicate repeat variously modified that lead to speculations on the evolutionary implications of these elements.--With the use of En-1 the Al locus was rescued by transposon tagging of the a-m(papu)[6] allele.

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1768 THE MU MUTATOR IN MAIZE FROM A GENETIC PERSPECTIVE, Donald S. Robertson, Department of Genetics, Iowa State University, Ames, IA 50011

The Mutator system of Maize (Mu) was first described genetically (1) but recent work in several laboratories have provided evidence suggesting transposable DNA is involved (2) and recently the transposon responsible (Mu1) has been sequenced (3). Studies on the transmission of the mutator phenotype have revealed that about 10 percent of the outcross progeny of a Mutator plant do not have mutator activity. Unlike their Mutator parents, these Mu-loss plants have lost the ability to induce new mutants. This represents a germinal loss of Mutator activity. Mutable Mutator-induced aleurone mutants (e.g., al Mum1) sometimes lose somatic mutability. This represents a somatic loss of Mutator activity. This report will deal with the relationship between these two classes of loss events. Tests of somatic-loss lines reveal that they also have lost germinal Mutator activity. Crosses between somatic-loss lines and standard Mutator stocks result in the restoration of somatic mutability in most instances tested. These genetic results will be discussed in light of the reported behavior of the Mu1 element.

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Genetic Instability

1769 FLUIDITY OF THE FLAX GENOME. C.A.Cullis and W. Cleary, John Innes Institute, Colney Lane, Norwich NR47UH, England.

It has been shown that the interaction between the flax variety 'Stormont Cirrus' and the environment in which it is grown can result in the production of genetically distinct stable lines termed genotrophs. These stable lines can differ from one another and the original line from which they were derived in a number of characteristics including plant weight, plant height, peroxidase isozyme band pattern and the total nuclear DNA amount.

Much of the difference in the nuclear DNA amount resides in the highly repetitive fraction of the genome which in flax is organised in a long period interspersion pattern. Representative clones from all the highly repetitive sequence families have been isolated. The copy numbers of these families have been determined in the genotrophs, other flax and linseed varieties and the supposed primitive progenitor of flax, Linum bienne. It was found that all the families except one (that being the major component of the light satellite) could vary. However within a family, for example the 5S genes, a particular subset was shown to be preferentially affected when the changes occurred.

The DNAs from normal seed derived plants, callus tissue and the progeny of regenerated plants (derived from the genotrophs) have been compared using the same set of probes, namely all the highly repetitive families in the flax genome. Again variation was observed for all these families except for the light satellite. The variation observed between plant, callus and regenerated plants was comparable to that observed when the genotroph DNAs were compared, including the particularly variable subset of the 5S genes.

From these results it is proposed that the flax genome is compartmentalised into two parts, one of which is variable and the other is constant. The interaction with the external environment can be such that a change can be generated in the variable component. This change is responsible for the phenotypic variation subsequently observed. The mechanism by which variation in the repetitive components of the genome can mediate phenotypic variation is unknown but one possibility is via some form of position effect.

1770 CELL HERITABLE CHANGES DURING DEVELOPMENT, Frederick Meins, Jr. Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

During development of multicellular plants, structures become determined for specific fates. Tissues and organs obtained from different parts of the same plant can exhibit different phenotypes when serially propagated in culture. This indicates that at least some forms of determination persist in populations of dividing cells. The present experiments were undertaken to find out whether determination can be inherited by individual cells.

Tissue derived from the leaf lamina of tobacco requires exogenous cytokinin for continuous growth on an otherwise complete culture medium containing auxin. Tissue derived from the cortex of the stem, on the otherhand, is cytokinin autotrophic. The leaf- and cortex-specific states of cytokinin requirement persist in cloned lines isolated from the two tissues indicating that both phenotypes are inherited at the cellular level. Therefore, cell-heritable changes occur during the development of the tobacco plant. Tissues cultured from plants regenerated from cortex and leaf cells exhibit the same cytokinin requirement as comparable tissues from seed-grown plants. This shows that different cell-heritable states can be "reset" at some point during the plant regeneration process. The fact that resetting is a directed process and occurs at rates $>5x10^{-3}$ per cell generation provides strong evidence that alterations in cytokinin requirement have an epigenetic basis. Although the changes in heredity are stable they are not permanent.

We have also shown that cultured tobacco cells can undergo genetic changes in cytokinin requirement. By culturing cloned lines of leaf cells on media containing successively lower cytokinin concentrations, lines were obtained which exhibit the cytokinin-autotrophic phenotypes. Leaf tissues from plants regenerated from cloned lines of these cells are cytokinin autotrophic. This phenotype, called habituated leaf (Hl), seggregates in sexual crosses as a single, dominant Mendelian factor. Measurements of the equilibrium distribution of cytokinin-requiring and cytokinin-autotrophic cells in leaf-derived tissues grown on different concentrations of cytokinin suggest that changes in the Hl locus occur in the forward and back direction at high rates, greater than 10^{-3}-10^{-2} per cell generation. Apparently the Hl locus is destabilized in culture and then "locked" into stable states during plant regeneration.

Mutagenesis; Genetic Instability; Transformed DNA Expression

1771 MODIFICATIONS OF THE CaMV GENOME FOR USE AS A PLANT MOLECULAR VEHICLE Gail Baughman and Stephen H. Howell, Univ. of Calif., San Diego, La Jolla, CA 92093

Cauliflower mosaic virus (CaMV), a circular double-stranded DNA virus, has been the focus of considerable attention as a potential vector for the introduction of foreign DNA into plant cells. Viral DNA can be introduced directly into plants by mechanical inoculation and results in a systemic infection. The CaMV genome is densely packed with essential coding regions and this, in addition to size constraints for encapsidation and subsequent propagation of the virion, has severely restricted the size of foreign DNA successfully introduced into the genome. Attempts to circumvent these problems through the development of a helper genome system have been hampered by recombination between vector and helper virus genomes. To prevent this recombination, critical homologous sequences that permit exchange between input genomes were deleted in various constructs. In addition, selectable markers (ie. drug resistance genes) were introduced in the CaMV genome at unique restriction sites created by oligonucleotide-directed site specific mutagenesis to ensure the maintanence of the individual coinfecting genomes.

1772 MAIZE CHROMOSOMAL DNA SEQUENCES WHICH REPLICATE AUTONOMOUSLY IN YEAST, Roberta E. Berlani, Ronald W. Davis, and Virginia Walbot, Stanford Univ., Stanford, CA 94305

We have characterized three independently isolated maize chromosomal DNA fragments which enable chimaeric plasmids to replicate autonomously in yeast. These autonomously replicating sequences, or ARSs, serve as the origin of replication for the yeast/E. coli shuttle vector YRp14/cen3. The best of the maize ARSs is comparable to yeast-derived ARS1 in efficiency of initiating plasmid replication.

ARS-containing DNA fragments were isolated by screening EcoRI restriction fragments of maize nuclear DNA for ARS activity in YRp14/cen3. Within each of the primary ARS fragments (2.5, 2.8, and 5.5 kbp in length) full ARS activity was confined to a single short (500-700bp) subcloned restriction fragment. DNA fragments up to 2kbp away from each ARS-containing subclone were also isolated and shown to have no ARS activity. The ARS subclones are 65-70% A+T and have the consensus sequence proposed for yeast ARSs.

The three ARS subclones represent two distinct families of repeated sequences in maize. Quantitative analysis shows that there are over 10^4 copies of each family per haploid genome. Thus, these two families could account for one ARS roughly every 160kbp in the maize genome of 5.7×10^6kbp, if the members are evenly dispersed and all have ARS activity. Non-ARS fragments 2kbp away from the ARSs have at least 10-fold lower copy numbers, indicating that the ARSs are not in a simple tandem array. Furthermore, there is a broad distribution of lengths of the restriction fragments which contain ARSs of a particular family. Studies are underway to determine approximately how many of each family's members have ARS activity.

1773 MOLECULAR BASIS FOR AN UNSTABLE PHENOTYPE IN PETUNIA HYBRIDA, Bharat B. Chattoo and Barbara Hohn, Friedrich Miescher-Institut, P.O.Box 2543 CH-4002 Basel, Switzerland

An X-ray induced recessive nuclear mutation in Petunia hybrida results in a stable periclinal chimera when the nuclear background is heterozygous, but shows forward and back-mutations at a high frequency when the nuclear mutation is homozygous. A detailed comparison of the chloroplast genome organization did not show any gross changes. However, a number of chloroplast proteins are affected; being present in low or undetectable levels in the mutant lines. Furthermore, a repeated nuclear sequence shows organizational differences in several lines into which the mutation has been introduced through crosses.

1774 CHROMATIN STRUCTURE OF TRANSFERRED T-DNA IN CROWN GALL TUMORS, David Coates, Earl W. Taliercio, and Stanton B. Gelvin, Purdue University, West Lafayette, Indiana 47907

We have investigated the chromatin structure of the integrated T-DNA in two established transformed Nicotiana tabacum tissue culture lines, using the enzymes deoxyribonuclease I (DNase I) and micrococcal nuclease (MN). Nuclei isolated from E9, and octopine-producing plant cell line, and HT37, which synthesizes nopaline, were treated with increasing amounts of either DNase I or MN. The DNA was subsequently purified and the relative abundance of the integrated T-DNA measured by dot blot analysis. MN treated samples were analyzed on Southern blots to determine the nucleosome arrangement. DNase I treated samples were restricted with Eco RI, and the fragment patterns of specific probes analyzed after electrophoresis and Southern transfer. Our data suggest that the T-DNA is not found in a classical nucleosome conformation, and shows a greater sensitivity to DNase I, relative to the bulk of the chromatin and to the sequences homologous to the chlorophyll a/b binding protein and calmodulin genes. No DNase I hypersensitive sites have been found associated with integrated T-DNA.

1775 DNA SEQUENCE ORGANISATION IN THE HOLOCENTRIC CHROMOSOMES OF THE GENUS LUZULA. C.A. Cullis, B.A.Bowen, G.P.Creissen,S.R.Turner and G.E.Marks, John Innes Institute, Colney Lane, Norwich NR47UH, England.

Holocentric chromosomes have multiple kinetochores (i.e. microtubule attachment sites), and yet such chromosomes are faithfully transmitted through both mitosis and meiosis. We are investigating the organisation of Luzula chromosomes to see whether they differ from those of other monocots which udergo classical breakage-fusion-bridge cycles when their chromosomes contain more than one kinetochore. The genome of Luzula pilosa is similar in size to Arabidopsis and contains approximately 0.3pg of DNA per haploid nucleus. Unlike the dicot Arabadopsis, however, it contains a very high proportion of highly methylated tandemly repeated sequences. We have characterised at least 5 different families of these sequences by Sothern and 'slot-blot' hybridisation, and we estimate that these families alone account for 40-50% of the L.pilosa genome. The most highly repeated sequence in the L.pilosa genome occupies about 30% of the genomein this species but only a much lower proportion of the genome in 9 other Luzula species analysed. In contrast another tandem repeat is the most highly conserved sequence reiterated at high copy number in 8 of the other species, all of which have similar C-values. Cross-hybridisation between L.pilosa DNA and L.elegans indicates that apart from the rDNA there are relatively few repeated sequences shared by these two species. L.elegans (n=3) has a C-value 15 fold greater than L.pilosa (n=33). We are currently screening genomic libraries of L.elegans and L.pilosa for conserved repeated sequences which are dispersed in the genome of each species.

1776 TRANSFORMATION OF TOBACCO PROTOPLASTS BY A Ca PHOSPHATE MEDIATED PRECIPITATION TECHNIQUE AND SUBSEQUENT CELLULAR CONCATEMERIZATION OF THE INPUT DNA, Armin P. Czernilofsky, Rüdiger Hain, Barbara Baker and Jeff Schell, Max Planck Institut, 5000 Cologne 30, West Germany

We are investigating the cellular concatemerization of selectable marker DNA (Tn5-Km) in tobacco protoplasts introduced by the Ca phosphate mediated precipitation technique. Preliminary data suggest that the mechanisms involved in the construction of arrays of DNA are similar to the mechanisms we and others observed for mammalian cell transformation after microinjection or Ca phosphate mediated transformation. We suggest that the input DNA fragment is multiplied in a directional manner resulting in a 20-50 times repeated structure of altered and/or unaltered original input DNA which are predominantly arranged head to tail. A comparison of the integrated DNA structures obtained by this technique to the Ti-plasmid mediated cocultivation procedure indicates that the DNA in the cells transformed by the former technique results in a variety of structural changes and concatemer formation whereas the DNA integrated by the latter procedure is mostly unaltered and only one or a few copies can be detected in high molecular weight plant specific DNA. Presently we are investigating the chromosomal and extrachromosomal distribution of the input DNA after transformation and the possibility to utilize transposable like structures for low copy number integration of unaltered selectable marker genes. We are exploring the possibilities for site specific integration and we are also studying the expression of cotransfected nonselectable marker genes.

1777 DNA INSTABILITY DURING DIHAPLOID FORMATION IN TOBACCO, S. S. Dhillon[1], M. A. Torres[1], J. P. Mikschel[1] and E. A. Wernsman[2], Dept. of Botany[1] and Crop Science[2], North Carolina State University, Raleigh, NC 27695-7612

Nuclear DNA contents of a conventionally inbred flue-cured tobacco (Nicotiana tabacum L.) cultivar, Coker 139, and doubled haploid lines developed from it by anther culture as well as maternal source were compared. The nuclear DNA contents determined by Feulgen cytophotometry were in close approximation to DNA determinations made by flow cytometry using DNA specific fluorescence stain mithramycine. Mean nuclear DNA contents of fully formed leaves of anther-derived dihaploid lines were 10.62 pg by Feulgen-cytophotometry and 10.43 pg by mithramycin-stained flow cytometry, while nuclei of the parental cultivar contained 9.32 and 9.38 pg by Feulgen and mithramycin-fluorescence methods, respectively. The anther-culture process and/or the nature of vegetative nuclei of pollen grains from which haploid plants originate have been suggested as possible contributing factors to higher DNA values in dihaploids. The flow cytometric studies on suckered leaves of anther- as well as maternal-derived dihaploids, however, indicated a decline in nuclear DNA amounts of dihaploids as compared to parental cultivar. Normal leaves of maternal-derived dihaploids also showed a decline in DNA values as compared to parental cultivar. The significance of the lower DNA values in maternal-dihaploids as well as suckered leaves of dihaploids in both maternal- and anther-derived plants in contrast to normal leaves of anther-derived dihaploids is not clear, and is being explored at a molecular level.

1778 TRANSCRIPTION OF THE RI T-DNA IN TRANSFORMED TOBACCO PLANTS,Mylene J. DURAND-TARDIF,Richard BROGLIE and David TEPFER,ROCKEFELLER UNIV.,NY 10021

Tobacco plants were regenerated from adventitious roots incited on stems by Agrobacterium rhizogenes.These transformed plants exhibit an altered phenotype that is characterized by the presence of wrinkly leaves and a highly branched root system.This altered phenotype was shown to be correlated with the presence of a segment of RI plasmid:the T-DNA(transferred) in the nuclear genome.To study the molecular basis of the transformed phenotype,we have utilized molecular clones of RI plasmid as hybridization probes to detect transcripts of the T-DNA in the transformed plants.The tobacco clone utilized in these studies contain two segments of T-DNA ,termed T_L (12kb) and T_R.Northern blot analysis of RNA from roots and leaves reveal that six polyA$^+$ mRNA are transcribed from T_L.No transcripts were detected from T_R.One of the transcripts is expressed in a tissue specific manner,and the overall level of T-DNA transcription is higher in roots.We have also found an additional transcript in leaves of plants exhibiting the supertransformed phenotype wich appear at a low frequency in progeny of regenerated plants.

1779 DESTABILIZATION OF PRODUCTION AND GROWTH CHARACTERISTICS IN CLONING OF ROSMARINIC ACID PRODUCING CULTURES OF ANCHUSA OFFICINALIS. BRIAN E. ELLIS, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada.
Cell suspension cultures of A. officinalis produce rosmarinic acid (α-O-caffeoyl-3,4-dihydroxyphenyllactic acid) as their main phenylpropanoid secondary metabolite. Individual cells within such a suspension vary in their metabolic commitment to rosmarinic acid production as measured by microspectrophotometric evaluation of the vacuolar contents. Single-cell cloning from such a mixed population yields clonal lines whose rosmarinic acid production levels appear to be unrelated to the production observed in the original mother cell. The clonal lines also display differences in growth rate and degree of cell aggregation. Further cloning results in a new set of line-typical values for these characteristics. To determine whether the isolation and/or manipulation of the single cells leads not only to phenotype alterations, but to genotype modification, the rate of appearance of restriction endonuclease polymorphisms within cell populations raised from single cells is being compared with that within populations derived from multi-cell isolates.

1780 CONTROL OF KANAMYCIN RESISTANCE IN SUNFLOWER, Nic Everett, Bob Nutter Dottie Pierce, Irv Mettler, Pat Okubara, Lootsie Panganiban, Jill Johnson, Rosanna Lachmansingh, Alex Kostrikin, Lu Pomeroy and John Howard, Stauffer Chemical Company, Richmond, CA 94804.
Kanamycin resistance is now being used in many laboratories as a selectable marker for plant cell transformations. We have constructed a number of chimeric genes comprising coding sequences of neomycin phosphotransferase under the control of a number of different promoters and with modifications to the sequences upstream of the coding region. The relative activities of these constructions have been assessed by introducing them, via A.tumefaciens, into inbred sunflower tissue. The results will be discussed in terms of both the most active construction and the regulation of foreign gene expression in sunflower.

1781 CYTOKININ-MODULATED REGULATION OF mRNA LEVELS FOR THE CHLOROPHYLL a/b PROTEIN IN LEMNA, Susan Flores and Elaine M. Tobin, UCLA, Los Angeles, CA 90024

It has previously been observed that light and cytokinins are synergistic in promoting chloroplast development in greening tissues. We have found that in Lemna gibba this interaction extends to the level of mRNA coding for the chlorophyll a/b protein. When plants grown in white light are placed in the dark for 6 days, Northern blots show that the proportion of total RNA which hybridizes to a probe for this message declines dramatically. Reappearance of this mRNA can be induced by a one-minute light treatment, acting through the phytochrome system. However, this red-light mediated induction is maximally observed only in the presence of exogenously supplied cytokinins such as kinetin or benzyladenine. In their absence, only a slight increase in response to light is observed. We also have found that 24 hours after addition of cytokinin, an increase in the level of this mRNA has occurred in plants kept in the dark. However, light treatment always leads to a further increase in message level even at the highest concentration of benzyladenine tested (3 μM). Preliminary results indicate a similar role of cytokinins in the light-induced increase of mRNA for the small subunit of RuBP carboxylase which, like the chl a/b protein, is a nuclear encoded chloroplast protein. The percentage of total RNA hybridizing to a probe for β-actin was not altered by the addition of cytokinins either in dark maintained tissue or in red light treated plants. This suggests that the increase in the proportion of total RNA hybridizing to probes for the chl a/b protein or the small subunit is not merely due to a general increase in the ratio of total message to total RNA.

1782 MOLECULAR CLONING AND SEQUENCING OF GLYCININ A_3B_4 SUBUNIT cDNA, Chikafusa Fukazawa Takayuki Momma, and kyoko Udaka, National Food Research Institute, Tsukuba, Japan, The cDNA clones encoding the precursor form of glycinin A_3B_4 subunit have been indentified from a library of soybean cotyledonary cDNA clones in the plasmid pBR322 by a combination of different colony hybridization and then by immunoprecipitation of hybrid-selected translation product with A_3-monospecific antiserum. A recombinant plasmid, designated pGA_3B_41425, from one of six clones covering codons for NH_2-terminal region of the subunit was sequenced and the amino acid sequence was inferred from the nucleotide sequence, which showed that the mRNA codes for a precursor protein of 516 amino acids. Analysis of this cDNA also showed that it contained 1786 nucleotides of mRNA sequence with a 5'-terminal nontranslated region of 46 nucleotides, a signal peptide region corresponding to 24 amino acids, A_3-acidic subunit region corresponding to 320 amino acids followed by B_4-basic subunit region corresponding to 172 amino acids, and a 3'-terminal nontranslated region of 192 nucleotides, which contained two characteristic AAUAAA sequences that ended 110 nucleotides and 26 nucleotides from a 3'-terminal poly(A) segment, respectively. Our results confirm that glycinin is synthesized as precursor polypeptides which undergo posttranslational processing to form the nonrandom polypeptide pairs via disulfide bonds. The inferred amino acid sequence of mature basic subunit, B_4, was compared to that of basic subunit of legumin, Leg ß, which contained 185 amino acids. Using an alignment that permitted a maximum homology of amino acids, it was found that overall, 42% of the amino acid positions are indentical in both proteins. These results led us to conclude that both storage proteins have a common ancestor and gene rearrangement has occurred on evolution.

1783 **DEFENSE GENES IN BEAN SEEDLINGS: CHARACTERIZATION OF AN ETHYLENE-REGULATED cDNA CLONE FOR CHITINASE[1]**, John J. Gaynor[2] and Richard Broglie, Laboratory of Plant Molecular Biology, Rockefeller University, NY, NY 10021-6399.

Ethylene production has long been associated with stress responses in plants. Likewise, the appearance of particular "defense" proteins shows a strong correlation with increased ethylene levels under various stress conditions. To learn more about these host defense proteins and to understand the mechanism of ethylene regulation of gene expression, we have characterized the response of Phaseolus vulgaris seedlings to ethylene. By the use of in vivo labeling and 2-D PAGE, we have catalogued those proteins which are either induced, repressed or unaffected by ethylene treatment. For example, the synthesis of RuBPCase in the leaf is repressed by ethylene whereas other proteins are induced. In this latter category, we have identified and isolated several cDNA clones for chitinase, which is putatively involved in the host defense mechanism. Chitinase is a basic 30 kd polypeptide which is capable of hydrolyzing the ß-1,4-linkages of N-acetyl-D-glucosamine polymers common to fungal cell walls. We have shown by immunoblotting and Northern blot analysis that chitinase is induced following ethylene treatment in both leaves and roots and that this induction is regulated at the level of steady-state mRNA. The chitinase clone pCh4 (ca. 1200 bp) was identified by hybrid selection from translation products. Current efforts are directed at the sequence analysis of cDNA and genomic clones of chitinase and in examining the putative ethylene promoter of this gene. [1]This work supported by NIH grant GM-31500. [2]Present address: Dept. of Botany, Rutgers University, Newark, NJ 07102.

1784 TRANSFER OF THE Ti-PLASMID FROM AGROBACTERIUM TO PLANTS, Stanton B. Gelvin, Elizabeth L. Virts, and Bradley W. Goodner, Purdue University, West Lafayette, IN 47907

We have investigated the early events in the transfer of the Ti-plasmid from Agrobacterium tumefaciens to cultured Petunia cells. Co-cultivation of regenerating Petunia protoplasts with Agrobacterium for 0-12 hours was followed by reisolation of the protoplasts, DNA purification and Southern blot analysis. Control experiments indicated that we were not detecting DNA leaking from Agrobacterium cells, and that infected protoplasts could go on to form tumors with a normal complement of T-DNA sequences. Results from these experiments indicate that multiple copies of the T-DNA could be transferred to the plant cells within hours, but that most of the DNA was rapidly degraded. Both "normal" and rearranged T-DNA sequences were detected at early times after infection. Ti-plasmid sequences mapping far from the T-DNA could occasionally be detected at these early times. We are also investigating a potential T-DNA intermediate found in Agrobacterium after contact with plant cells.

1785 ASPECTS OF Fd-GLUTAMATE SYNTHASE DEFICIENCY IN ARABIDOPSIS THALIANIA, B. Grumbles, F.N. Nargang and C.S. Somerville*, Genetics Department, University of Alberta, Edmonton, Alberta, Canada T6G 2E9 and *Plant Research Lab, Michigan State University, East Lansing, Mi. 48824 USA.

Utilizing a previously described screen for conditional photorespiratory mutants (Somerville and Ogren NATURE 286: 257-259, 1980), we have isolated twenty-six EMS-induced mutants of A. thaliania that are deficient for fd-glutamate synthase. Immunological analysis using an antibody raised against spinach glutamate synthase revealed the presence of CRM in all the mutants. SDS-PAGE of the immunoprecipitates showed the mutant glutamate synthase CRM in each mutant was identical to that of the wild type. However, one mutant was shown to possess a glutamate synthase with a tryptic digest peptide map slightly different from that of wild type.

Revertants to one allele have been obtained by selection for growth in air levels of CO_2 (remaining air). All were found to be second-site revertants. The photosynthetic properties of the revertants are only slightly more efficient than the original mutant.

We are currently examining fd-glutamate synthase at the mRNA level in wild type A. thaliania.

1786 HETEROLOGOUS EXPRESSION OF A SOYBEAN GENE ENCODING A SMALL HEAT SHOCK PROTEIN IN SUNFLOWER TUMORS, William B. Gurley, Eva Czarnecka, Ronald T. Nagao and Joe Key, University of Florida, Gainesville, FL 32611 and University of Georgia, Athens, GA 30602

A 4.25 kb Bgl II fragment containing the soybean heat shock gene Gmhsp17.5-E was inserted into the T-left region of the Agrobacterium plasmid pTi-15955. RNA from primary sunflower tumors incited by the Agrobacterium strain harboring the soybean heat shock gene was analyzed by RNA blot hybridization, and the 5'- and 3'-termini of the heat shock transcript determined by S1 nuclease hybrid protection mapping. The soybean gene was strongly expressed transcriptionally in a thermally inducible manner. The 5'-terminus in sunflower RNA showed a slight ambiguity in start site with the major terminus coincident with the site utilized in soybean. All of the 3'-termini present in soybean RNA are also present in the transformed sunflower RNA. Several minor 3'-termini within the untranslated 3'-tail were unique to heterologous expression in sunflower.

1787 SITE-SPECIFIC MUTAGENESIS OF RIBULOSEBISPHOSPHATE CARBOXYLASE/OXYGENASE Joanna F. Hanks, Mark Estelle, Christopher R. Somerville, and Lee McIntosh, MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Since attempts to identify useful mutants of RuBP carboxylase (Rubisco) by conventional in vivo genetic approaches have met with limited success, we have chosen oligonucleotide-directed in vitro mutagenesis to directly alter the nucleotide sequence of the Rubisco gene. The structurally simple Rubisco from Rhodospirillum rubrum was chosen as a model system for this work (Somerville and Somerville, Nargang et al. 1984) since only one gene, encoding large subunit, was required for expression of a fully active Rubisco in E. coli. Mutagenic oligonucleotides containing one or more mismatches to the wild type nucleotide sequence were used as primers in an in vitro DNA polymerase reaction on a single strand M13 clone containing the second Sal I restriction fragment of the Rubisco gene. The first mutation constructed codes for insertion of an additional lysine codon adjacent to lysine-191, the site of carbamate formation during activation of R. rubrum Rubisco. Although expression of the mutant or wild type gene in E. coli JM105 results in high levels of a protein of identical molecular weight, the mutant lacks carboxylase activity. Subcloning of the mutant Sal I fragment into the wild type gene was complicated by the presence of the additional Sal I site, necessitating construction of a derivative of the wild type clone in which the second fragment had been removed. In order to simplify the construction of further mutations in the activation region of the protein, an oligonucleotide encoding a silent mutation resulting in elimination of the additional Sal I site was employed in a mutagenesis reaction on an M13 mp18 clone containing the entire Rubisco gene.

1788 MOLECULAR GENETIC ANALYSIS OF THE VOLVOX GENOME, Jeffrey F. Harper, Washington University, St. Louis, MO 63130

A linkage map of the Volvox carteri f. nagariensis genome has been constructed with DNA restriction fragment polymorphisms in order to facilitate the identification and molecular analysis of developmentally important genes. The reg locus is of particular interest since it regulates the germ-soma dichotomy, exhibits hypermutability at a particular developmental stage and shows no detectable linkage to other conventional markers.

Polymorphisms are easily detected between two strains (Adm and Ev) using the following repetitive sequence probes: 1) the synthetic polymer poly dG·poly dC; 2) an intron from a Volvox β tubulin gene and; 3) an uncharacterized cDNA. Segregation analysis of 21 polymorphisms currently defines 13 linkage groups (A minimum of 14 groups are expected). However, no polymorphisms have been found that cosegregate with the reg locus.

Repetitive sequences with homology to the poly dG·poly dC (GC sequence) probe have been characterized in order to determine their structure and function. The GC sequences are highly dispersed with an average frequency of 1 per 150 kb of DNA. The GC regions of 3 genomic clones which have been mapped are each closely associated with an S1 nuclease sensitivity site in vitro. The sequence of one GC region contains an unusual stretch of the pentameric repeat (CCCGT). Instability of long stretches of the pentameric repeat may account for some of the GC polymorphisms.

1789 RESTRICTION FRAGMENT POLYMORPHISMS AS GENETIC MARKERS AND MEASURES OF PLANT DIVERSITY, Tim Helentjaris, Mary Slocum, Gretchen King, Alison Shaefer, and Scott Wright, NPI, 417 Wakara Way, Salt Lake City, Utah 84108.

We have utilized single copy DNA clones to evaluate the diversity available in different crop plants and the practicality of their use in both basic genetic research and applied breeding problems. We have determined that there are large differences in the degree of variability detectable by this technology in different plants. Maize possesses a very high level of variability with virtually every clone able to distinguish many commercial cultivars. Tomatoes on the other hand, appear very conserved and it is quite difficult to detect variability between commercial lines, less difficult between undomesticated isolates. Development of these as genetic markers is possible with linkage demonstrable both to each other and phenotypic traits. The results reflect fundamental differences in plant genome variability and potential mechanisms are discussed.

1790 INTEGRATION AND EXCISION OF TRANSFORMING DNA, Barbara Hohn, Thomas Hohn and Nigel Grimsley, Friedrich Miescher-Institut, Basel, Switzerland

Tandem units of the cauliflowermosaic virus genome were placed within the T-DNA borders of the agrobacterium Ti plasmid. Turnip plants, when wounded and infected with agrobacteria carrying the hybrid plasmid become systemically infected with the virus. Agrobacteria containing the virus tandem DNA on a broad host range plasmid not containing T-DNA borders do not mediate virus infection. The mode of excision of the viral DNA from the integrated form will be discussed as well as the general applicability of the system to deliver infectious DNA.

1791 THE HYPER-VIRULENCE OF AGROBACTERIUM TUMEFACIENS STRAIN A281, Elizabeth E. Hood, Washington University, St. Louis, MO; Mary-Dell Chilton, Ciba-Geigy Corp., RTP, N.C. and Robert T. Fraley, Monsanto Co., St. Louis, MO.

A. tumefaciens strain A281 was constructed by conjugation of the Ti (tumor-inducing) plasmid from Bo542 (the wild type isolate) into A136, a drug-resistant strain of A. tumefaciens which had been cured of its Ti plasmid (Plasmid, 1978, 1:238). Strain A281 makes large, fast-growing tumors (i.e. is hyper-virulent) on the Solanaceous plants tobacco and tomato, and also is virulent on soybean and alfalfa, characteristics not exhibited by the wild type isolate. These tumors contain agropine, leucinopine, and agrocinopine C. The Ti plasmid of strain A281 has been mapped and the T-DNA defined (Bio/Tech. 1984, 2:702). Cosmids containing the T-DNA have been put into "helper strains" (containing Ti plasmids from which the T-DNA has been deleted) and were tested on several plant species. These strains do not exhibit the hyper-virulent response and have an altered host range. The data suggest that the hyper-virulence determinants are not in the T-DNA region. Experiments are in progress to test the hyper-virulence of the remainder of the Ti plasmid of strain A281 (after deletion of its T-DNA) when complemented with T-DNAs from heterologous Ti plasmids.

1792 A SIMPLE AND GENERAL METHOD FOR TRANSFERRING GENES INTO PLANTS, Robert B. Horsch, Robert T. Fraley, Steven G. Rogers, Monsanto Company, St. Louis, MO 63167

Transformed petunia, tobacco and tomato plants have been produced from surface sterilized leaf discs that were inoculated with an *Agrobacterium tumefaciens* strain which contained a modified Ti plasmid (in which the T-DNA tumor genes had been deleted and replaced with a chimeric kanamycin resistance gene). After 2 days of culture, the leaf-discs were transferred to selective medium containing kanamycin. Shoot regeneration occurred rapidly (2-4 weeks) and transformants were identified by their ability to form roots in medium containing kanamycin.

In typical experiment with *Petunia hybrida*, 25 out of 69 independent shoots that grew from discs infected with pTIB6S3SE::pMON200 rooted in the presence of kanamycin within 12 days. Of these 25 plants, 12 produced nopaline while grown in soil and 18 showed kanamycin resistance in the leaf callus assay, although three of these plants were delayed in their growth on kanamycin. Of the six nopaline negative, kanamycin-resistant plants that were obtained, four of these demonstrated induction of nopaline synthesis upon transfer of leaves to culture medium. Despite the frequent occurrence of these different pehnotypes, it should be emphasized that it is a simple matter to identify the desired transformants for studying gene expression, protein function and inheritance of the inserted foreign DNA. For example, in this experiment, 12 out of the 25 plants displayed the expected pattern of gene expression. Similar results were obtained with tobacco and tomato.

1793 RECOGNITION OF FOREIGN TRANSCRIPTIONAL SIGNALS IN PLANT CELLS, Arthur G. Hunt, Nathan M. Chu and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021

We are interested in the ability of plants to recognize transcriptional signals of non-plant origin. To this end, we have introduced chimeric genes containing various introns and polyadenylation signals from animal virus genes into Nicotiana tabacum using Ti-mediated gene transfer techniques. Such experiments have revealed that tobacco cells either do not recognize, or aberrantly recognize, polyadenylation signals from the adenovirus 5 E1A gene and from the SV40 early genes. These experiments suggest that there are substantial differences between the polyadenylation signals that are recognized in plants and animals.

In contrast to these results, we see that the small t intron of the SV40 early genes is properly spliced in tobacco cells. We infer from these results that the mRNA splicing apparatus in plants and animals retain a high degree of functional homology.

We will present these and other results and discuss their implications concerning the expression of foreign genes in plants, as well as the molecular biology of these processes in plants.

1794 ROLE OF T-DNA BORDERS IN CROWN GALL TUMORIGENISIS, George Jen and Mary-Dell Chilton, Department Biology Washington University, St. Louis, MO 63130 and Ciba-Geigy Biotechnology Group, Research Triangle Park, NC

We have examined the role of the T-DNA border sequences in crown gall tumorigenesis using the binary transformation system (deFramond *et al.*, Bio/Tech. 1, 262-269., Hoekema *et al.*, Nature 303, 179-181). Mini-T plasmids containing single right or left T-DNA border segments were transformationally active, as were those that contained both border segments. In contrast, mini-T plasmids without any border segment were inactive. The mini-T plasmids containing only the left border segment were much less active in transformation than those that contained the right or both border segments. A 25 bp direct repeat sequence exists at both ends of the T-DNA, within the border segments. We investigated the possibility that differences in transformation activity between the right and left border segments may be due to the relative positions and/or orientations of their direct repeats to the oncogenes within the T-DNA core. Transformation studies with mini-T plasmids that reversed the positions and orientations of the border segments showed no changes in the relative activities of the right and left border segments. These results indicate that sequence differences between the right and left border segments are responsible for their differences in activity. The implications of these results on the possible mechanisms of T-DNA transmission into plant cells will be discussed.

1795 GENETIC STABILITY OF GENES INTRODUCED TO PLANTS VIA T-DNA, Richard A. Jorgensen, Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608

Tobacco plants carrying the indoleacetamide hydrolase (iamH) gene were shown to be hypersensitive to naphthaleneacetamide sprayed on leaves. The effectiveness of this hypersensitivity as a selection for loss of the iamH gene is being evaluated both somatically in leaf-derived protoplasts and meiotically in the progeny of transformed plants.

1796 **NUCLEOSOMAL ORGANIZATION OF FOREIGN DNA IN PLANTS, Guenter Kahl, Willi Schaefer and Kurt Weising, Department of Biology, University of Frankfurt/Main (Germany).**

Most of the nuclear DNA in higher plants is organized in nucleosomes. It is unknown,whether foreign DNA introduced into plant cells by natural or artificial engineering and covalently linked to nuclear DNA is organized the same way, not regarding its origin. We have solved this problem using two systems:

1. The T-DNA in tobacco cells, originating from the Ti-plasmid of Agrobacterium tumefaciens.
2. A truncated T-DNA in tobacco cells, in which the oncogenic functions were deleted and replaced by pBR 322 (pGV 3850; kindly supplied by Prof.Schell,Cologne).

Nucleosomal DNA from both systems was isolated and hybridized against nick-translated probes representing

1. eight different T-DNA fragments with differing in vivo transcription rates,
2. sequences at the borders of the truncated T-DNA of the 3850 plant and pBR 322.

These experiments lead to the following conclusions:

1. The different T-DNA sequences as well as the pBR 322 sequences in the nuclear DNA of plant cells are organized in nucleosomes with the same repeat length as the bulk of plant nucleosomes.
2. Actively transcribed sequences (i.e. the nos gene) are organized in nucleosomes with an altered , highly nuclease-sensitive configuration.

1797 T-DNA TRANSFER FROM AGROBACTERIUM TO THE PLANT CELL INVOLVES THE INDUCTION OF CIRCULAR INTERMEDIATES, Zdena Koukolíková, Raymond D. Shillito, Barbara Hohn, Friedrich Miescher-Institut, Basel, Switzerland; Kan Wang, Marc Van Montagu, Patricia Zambryski, Laboratorium voor Genetica, Rijksuniversiteit Gent, Gent, Belgium

Circular T-DNA structures were isolated by cosmid or plasmid rescue. These structures were specifically induced following cocultivation of Agrobacterium with plant cells suggesting they are intermediates in transfer of the T-DNA from Agrobacterium to the plant genome. The junction of the T-DNA circles occurs precisely within the 25-bp terminus sequence known to be important for T-DNA transfer.

1798 ASPECTS OF THE APPLICATION OF IN VITRO (DNA) TRANSFORMATION SYSTEMS IN PLANTS, Frans A. Krens, Rindert Peerbolte, J.Harry C. Hoge, George J. Wullems and Rob A. Schilperoort,State University of Leiden, 2333 AL Leiden,Netherlands.

A reproducible in vitro DNA transformation procedure for plant protoplasts has been developed in our laboratory based on the tumorigenic properties of the Agrobacterium tumefaciens Ti-plasmid. The transferred DNA does not consist of one contiguous stretch of T-DNA analogous to the T-DNA integrated after cocultivation or in vivo wounding. The DNA transformants show phenotypic diversity correlated to the presence of different loci on the transferred DNA. Although the organization and structure of the T-DNA is rather complex, the polyA mRNA fraction obtained from transformed material, shows transcripts which confirm the relation between the foreign, integrated DNA and the observed phenotypes. Cotransformation may occur using this method as is demonstrated by the presence of non-selected sequences derived from parts of the pTi outside the T-region and derived from calf thymus DNA (carrier in the procedure).Segregation in the progeny evidences the independent integration in non- homologous chromosomes. Experiments have implicated that DNA transformation with plants using E. coli derived vectors with genes of interest can be performed and that genes located on different plasmids can be introduced as well. Transformation frequency with a selected gene is rather low, 10^{-4}-10^{-5}, although the frequency of DNA uptake and incorporation in the plant's genome is considerably higher,10^{-2}-10^{-3}.

REFERENCE: Krens, F.A. et al. (1982) Nature 296,72-74.

1799 Cloning of a Gene which encodes both the Ricin A and Ricin B Polypeptides, Beth F. Ladin, Kevin C. Halling, Amy C. Halling, Elizabeth E. Murray, L.L.Houston, Robert F. Weaver, Univ. of Kansas, Lawrence, Kansas 66044.

The toxic plant protein ricin, isolated from the endosperm of Ricinus communis, contains two subunits: the A subunit which contains the toxic activity and the B subunit which binds to galactose containing cell surface receptors. Immunotoxin production using the ricin A polypeptide as the toxin requires a source of ricin A free of ricin B. Existing biochemical procedures are inadequate. Therefore the ability to clone the ricin A gene into a bacterial expression vector may produce that source of ricin A. Messenger RNA isolated from developing castor bean seeds has been used to prepare a cloned cDNA library. cDNA clones specifically encoding the gene/genes for the ricin subunits were identified using an oligonucleotide probe for sequences in ricin B. One 1050bps cDNA clone (pBL-1), identified with this probe, contains an entire sequence for the ricin B polypeptide at its 3' end. No ATG is found at the 5' end of this sequence. Instead a short segment of the sequence encoding the ricin A polypeptide is found, followed by an intragenic sequence directly 5' to the ricin B sequence. Processing to separate polypeptides must occur concommitant with or after the completion of translation. Using the pBL-1 cloned cDNA as a probe, 30 additional cDNA clones were found. One of these clones,pBL-3, which is 2200bps in length, contains the complete ricin A sequence as well as the complete ricin B sequence.
This work has been funded by the Elli Lilly Corporation.

1800 ANALYSIS OF POLYMORPHISM ASSOCIATED WITH DISEASE RESISTANCE GENES, Benoit S. Landry and Richard W. Michelmore, University of California, Davis, CA 95616.

Many genes for resistance have been characterized at the whole plant level but little is understood about the generation of novel resistance genes. Also, genes for resistance to diverse diseases tend to be clustered in the genome but the evolutionary and functional significance of this arrangement is unknown. We hypothesize that regions of the genome determining resistance are less evolutionarily stable than other regions and that genome rearrangements are important in the generation of new resistance genes in a population.

Resistance in lettuce to downy mildew has been well characterized genetically; major genes for resistance are matched in a clear gene-for-gene relationship with genes for virulence in the pathogen. We are developing a detailed genetic map of lettuce using restriction fragment length polymorphisms. Random fragments of low copy number, genomic sequences are being used as probes. We will compare the frequency of polymorphism in loci which are linked and unlinked to disease resistance genes. This will indicate whether loci which flank resistance genes exhibit enhanced levels of polymorphism and provide clues to the evolution of disease resistance genes.

1801 EXPRESSION OF A GENE ENCODING THE α'-SUBUNIT OF SOYBEAN β-CONGLYCININ UNDER THE TRANSCIPTIONAL CONTROL OF A CaMV PROMOTER. Michael A. Lawton, Donna Shattuck-Eidens, Robert T. Fraley† and Roger N. Beachy Washington University, St. Louis MO 63130 and †Monsanto Corporation, St. Louis MO 63167.

We are studying the expression and stablity of a the α'-subunit of the soybean β-conglycinin storage protein when expressed in petunia plants. The coding region of the gene has been placed under the transcriptional control of a CaMV promoter. The chimeric gene has been introduced into the Ti-plasmid of Agrobacterium tumefaciens and the latter co-cultivated with petunia protoplasts. Both transformed callus and plants regenerated from transformed cells express a polyadenylated mRNA that hybridizes to an α'-gene probe. This mRNA has the same mobility as the authentic mRNA from soybean cotyledons suggesting that the transcript is processed to remove its introns. S1 mapping is being used to confirm that processing is carried out with fidelity. No α'-subunit polypeptide has been detected in callus, leaf or seed tissues using ELISA and western blotting techniques indicating that the protein is either not made in sufficient quantities to be detected by these methods or that it is not stable in petunia cells. We are using hybrid-release translation to assay the translatability of the α'-transcipt and northern analysis to detect this message in the polysomal mRNA pool.

1802

DNA MEDIATED TRANSFORMATION OF CEREAL CELLS, Horst Lörz, Barbara J. Baker, Rüdiger Hain and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D-5000 KOELN 30,Fed.Rep.of Germany

Evidence for direct gene transfer to cultured cells of Triticum monococcum will be presented. Transformation was achieved by using the plasmid pMPI 1103-4. The pBR322 derived plasmid contains a selectable chimeric gene comprising the protein region of the Tn5 aminoglucoside phosphotransferase type II gene (NPT II), the nopaline synthase promotor (pNOS), and the maize controlling element Ac. To induce DNA uptake protoplasts isolated from a cell suspension of T. monococcum were incubated with plasmid DNA (10 µg plasmid DNA with or without 50 µg carrier DNA per 1-2 x 10^6 protoplasts) following the method of Krens et al.(1982). After an initial culture in liquid medium without selection, regenerating and dividing cells are plated 4-7 days after culture initiation into agarose solidified medium containing 100 µg/ml kanamycinsulfate. This level was found to be lethal for non-transformed T. monococcum tissue. Selected colonies were tested for expression of the NPT II gene and the enzyme activity was assayed by the method described by Reiss et al.(1984). No activity was found in untreated callus tissue of T. monococcum. However, NPT II enzyme was found in protoplast derived tissue treated with pMPI 1103-4 DNA. Experiments are in progress to analyse the DNA of transformed tissue. The possibility of direct gene transfer to cereal cells will allow investigations with Gramineae which have so far been restricted to dicotyledonous species.

1803

RESISTANCE TO TABTOXIN AND MSO AS A FUNCTION OF GENE DOSAGE, Elzbieta Tarantowicz-Marek, Robert C. Dickson, University of Kentucky, Lexington, KY 40536-0084

Tabtoxin isolated from Pseudomonas tabacci inhibits glutamine synthetase in infected leaves causing accumulation of NH_3 derived from photorespiration and resulting in chlorosis. A similar pathogenic effect is produced by methionine sulfoximine (MSO) - an irreversible inhibitor of glutamine synthetase. MSO and tabtoxin also inhibit growth of enteric bacteria and yeast.

We constructed an E. coli strain containing multiple copies of the glutamine synthetase gene by transforming strain HB101 (containing wild type GS gene) with Gln A gene cloned in pBR322. We also transformed S. cerevisiae strain SJ21 (containing wild type GS gene) with the GLN1 gene cloned in YEp24. In E. coli transformants glutamyltransferase activity of GS increased 4-fold but the resistance to MSO increased almost 30-fold. In S. cerevisiae the 6-8 fold increase of resistance to MSO was consistent with the increase in enzyme activity.

We isolated from a yeast gene bank other genes conferring MSO resistance when present in multiple copies per cell. Those genes are not the GLN1 gene, since they do not complement a gln1 defect in strain 745-gln1 and do not contain the 0.8Kb Eco R1 fragment that is contained in GLN1. Different pMSO plasmids contain different MSO resistance genes as shown by southern hybridization of Eco R1 fragments to yeast DNA cut with Eco R1.

1804

TOMATO TRANSFORMATION, Sheila McCormick, Monsanto Co., St. Louis, MO. 63167

Transformed tomato plants of several commercial cultivars have been rapidly produced using the leaf disc transformation/regeneration system. The regenerated plants are normal and transmit the foreign DNA in normal Mendelian ratios. Results on gene expression, copy number and genetic mapping of the foreign DNA inserts in tomato will be presented.

1805

RECOMBINATION AND GENE CONVERSION IN CAULIFLOWER MOSAIC VIRUS DNA, Ulrich Melcher and R. C. Essenberg, Oklahoma State University, Stillwater, OK 74078

Mutant cauliflower mosaic virus (CaMV) DNAs recombine with other CaMV mutant DNAs at high frequency. When mutant DNAs from different isolates of CaMV are inoculated on leaves of turnip plants, diseased plants contain viral DNA originating from both isolates. These were recognized by restriction site polymorphisms. The progeny DNA from individual plants was heterogeneous at a diagnostic EcoRI site and the ratio of alleles varied from plant to plant. Cloned representatives of these progeny, mapped by restriction, contain regions of mixed alleles that must have arisen by repair of heteroduplex mismatches during recombination between duplex DNAs. Junctions between parental sequences were found at three map locations.

In plants, non-infectious DNAs contributed sequence information to wild type DNAs. The degree of this apparent gene conversion depended on the extent of sequence homology between mutant and wild type DNAs. A diagnostic EcoRI site in the infectious UM130 was in most plants entirely converted by non-infectious UM24 mutant DNA. Partial conversion of CM4-184 by a non-infectious mutant of pLW303X DNA(containing DNA of a closely related but not identical isolate) was seen. No conversion was seen when the two DNAs came from entirely different isolates (eg. NY8153 and CabbS). Interference of wild type DNA infection by mutant DNAs also occurred and can be interpreted as being due to gene conversion. Supported by the Herman Frasch Foundation and the Okla. Ag. Expt. Sta.

1806 CHLOROPLAST psbA GENE-ENCODED HERBICIDE RESISTANCE IS RECESSIVE IN CHLAMYDOMONAS. Laurens J. Mets, University of Chicago, Chicago, Il. 60637

Previous studies have shown that chloroplast encoded mutations conferring resistance to the photosystem II-inhibiting herbicides atrazine, diuron, and bromacil are associated with cpDNA sequence alterations in the psbA gene. Since these changes produce amino acid substitutions in the highly conserved 32 kilodalton protein product of psbA, it has been accepted that they are the determinants of resistance. We have now used deletion mapping in Chlamydomonas to prove that the genetic determinants of resistance, as expected, lie within the portion of psbA carrying the sequence alterations.

Because the genetic determinant is known, herbicide resistance has attracted attention as a selectable marker which might be useful in chloroplast transformation experiments. We have now obtained direct evidence confirming our previous inference that in Chlamydomonas (which has a single chloroplast), herbicide sensitivity is dominant over resistance. Thus, selection for herbicide resistance coferred by psbA will not aid in the establishment of transforming DNA in heteroplastomic chloroplasts.

The protein product of psbA shows a characteristic light-dependent turnover which can be blocked by herbicide. The dominance of sensitivity over resistance is consistent with a model for turnover control in which individual proteins are sensitized for degradation by engaging in electron transport. It is not consistent with models in which electron transport activates a diffusible degradation activity.

1807 TRANSFORMATION OF ALFALFA PROTOPLASTS BY INTRANUCLEAR MICROINJECTION OF Ti PLASMIDS Brian Miki[1], Terry Reich[2], and V. N. Iyer[2], [1]Ottawa Research Station, Agriculture Canada, Ottawa, Ontario, K1A 0C6 and [2]Biology Dept., Carleton University, Ottawa Ontario K1S 5B6 Canada

Transformation of alfalfa protoplasts following intranuclear microinjection of pTi C58 was revealed by detection of nopaline and nopaline synthase activity in about 30% of the recovered colonies. Protoplasts were derived from cell suspension lines and allowed to recover in culture for 24 hours. The nucleus was stained with specific non-toxic fluorescent dyes such as Hoescht 33258 or DAPI. Immobilization on glass coverslips was achieved with poly-L-lysine. Following microinjection, protoplasts were left, undisturbed for 1–2 days then individually transferred to feeder layers using the double filter paper technique, which yielded plating efficiencies as high as 10–20% with alfalfa protoplasts. From the first two experiments completed, 36 cloned lines were recovered and analyzed for nopaline and nopaline synthase activity. Of these, 10 were clearly positive. The nature of the integrated T-DNA has not yet been examined.

1808 AMINO ACID ANALOGUE RESISTANT MUTANTS IN BRASSICA SPECIES, Stephen J. Molnar and John L. Grainger, Agriculture Canada, Ottawa Research Station, C.E.F., Ottawa, K1A 0C6, Canada

The objective of this program is to obtain nuclear, chloroplast and mitochondrial genetic markers in Brassica species to facilitate protoplast fusion and the transfer of cytoplasmic genetic traits. The isolation and preliminary characterization of four 5-methyl-tryptophan (5 MT) resistant variant lines in suspension cultures of B. nigra will be reported.

Brassica nigra suspension cultures proved unusually sensitive to 5 MT as their growth was inhibited by less than 0.2 µg/ml. The variants were recovered from suspension cultures exposed to 3.0, 6.0, 6.0 and 9.0 µg/ml of 5 MT, respectively. The variant frequency in the parental culture is estimated to have been 1.25×10^{-7}. The resistance was stable in the absence of selective pressure. The behavior of the variant(s) is consistent with that expected from variants which over-produce free tryptophan. Studies to test this hypothesis are in progress.

1809 STATE OF CYTOSINE METHYLATION OF THE 5' END OF THE MAIZE ALCOHOL DEHYDROGENASE GENE, Harry Nick, Barbara Bowen, Robert Ferl*, Walter Gilbert, Biogen Res. Corp. Cambridge, Ma., *Dept. of Botany, Univ. of Florida, Gainesville, Florida.

The maize alcohol dehydrogenase enzymes are specified by two unlinked genes. We have analysed the state of cytosine methylation of the 5' flanking region of the maize Adh1 gene in leaves by genomic sequencing. 5-methylcytosine in plants is detected in more than 30% of the cytosine residues, preferentially in the dinucleotide CG and in the trinucleotide CXG. Our results reveal that in leaves, a non-expressing tissue, all of the potential sites for methylation within 250 bp surrounding the start of the gene are unmethylated. A transition in the state of methylation occurs 800 bps 5', where the cytosine residues in each of three CAG trinucleotides is methylated. The next potential methylation site , a CG dinucleotide , is 100 bps 3' and is unmethylated. Most importantly we have demonstrated the applicability of genomic sequencing for the detection of maize genomic sequences, which will also allow for th the study of in vivo protein-nucleic acid interactions involved in gene regulation.

1810 HAIRY ROOT: T-DNA, T-DNA TRANSCRIPTS AND PROGENY, R.Peerbolte, J.H.C. Hoge, G.J. Wullems and R.A. Schilperoort, State university Leiden, The Netherlands

Three tissues transformed with Agrobacterium rhizogenes LBA9402(pRi1855) were studied: Daucus carota AKR828, Nicotiana plumbaginifolia RNP1, and N. tabacum HR-SR1-9402. The T-DNA contents of the former two root cultures were determined by Southern blotting: AKR828 contains TL-DNA only and RNP1 contains both TL- and TR-DNA. By Northern blotting about 10 TL- and 4 TR-transcripts were detected in RNP1 roots. Polarity and location of these transcripts have been established. From HR-SR1-9402 and RNP1 transformed plants were regenerated and crossed with wild type plants as male parents. The HR-SR1-9402 progeny showed segregation of the hairy root features 'wrinkled leaves' (WRL) and 'mannopine/agropine synthesis' (MAS/AGS), whereas these features were linked in the RNP1 progeny. These findings together with the fact that AKR828 is $MAS^{-}AGS^{-}$ and lacks TR-DNA, indicate that WRL and MAS/AGS are located on different T-DNA segements. Currently this is being verified with T-DNA analysis of progeny plants. Progeny derived from tobacco hairy root male and crown gall female parents, show that the crown gall trait 'no roots' dominates the hairy root feature 'excessive roots'.

1811 T-DNA BORDER SEQUENCES REQUIRED FOR CROWN GALL TUMORIGENESIS, Lloyd Walter Ream and Ernest G. Peralta, Department of Biology, Indiana University, Bloomington, IN 47405, USA.

Similar 23 bp direct repeat sequences occur at the ends of three different T-DNAs. To test the role of these sequences in crown gall tumorigenesis, we constructed deletion mutants in pTiA6NC. This plasmid has two adjacent but non-continuous T-DNAs: T_L, the left T-DNA, contains all the genes necessary for tumor maintenance; and T_R, the right T-DNA, plays no apparent role in tumorigenesis. Deletions of the T_L left border did not affect virulence. Two different T-DNAs in tumors induced by a left border deletion mutant both ended within the same 354 bp segment inside the T-region. Two sequences within this segment share 60% homology with the conserved bases of the border repeats and may substitute for the deleted left border. A deletion of the three border repeats to the right of T_L severely attenuated virulence. We introduced restriction fragments containing the T_L right border into this right border deletion mutant. A 1435 bp fragment containing the T_L right border in its wild-type (direct repeat) orientation restored virulence fully. A 311 bp fragment containing the T_L right border inserted in the wild-type orientation restored virulence substantially, but in the opposite orientation this fragment enhanced virulence only slightly.

1812 MITOCHONDRIAL DNA STRUCTURES IN MALE-STERILE MAIZE AND ITS FERTILE REVERTANTS. C.L. Schardl & D.M. Lonsdale, Plant Breeding Institute, Cambridge CB2 2LQ, England. D.R. Pring, USDA-ARS, University of Florida, Gainesville, FA 32610.

The S-type male-sterile cytoplasms of Zea mays are characterised by the presence of the linear mitochondrial episomes S1 and S2 with molecular sizes of 6397 bp and 5454 bp respectively. These episomes have identical pairs of 208 bp inverted repeats (IR's) at their termini. Sequences homologous to the IR's were identified at two loci on the mitochondrial chromosomes. Sequence data and restriction endonuclease analysis demonstrated that these sequences recombine with the IR's of S1 and S2, resulting in linearisation of the mitochondrial chromosomes.

The M825 nuclear genome promotes frequent spontaneous reversion of S cytoplasms resulting in male-fertile types. The fertile revertants lost S1 and S2 as free episomes and lacked the linear mitochondrial chromosomes characteristic of the male-sterile parents. In addition, they contained episomal sequences integrated into the mitochondrial chromosomes. The integrated S1 sequences were virtually complete. However, the majority of the IR sequence was always deleted from one end of integrated S2. This deletion apparently eliminated transcription of an S2-encoded gene, suggesting involvement of this gene in male-sterility conferred by the S cytoplasms.

1813 USE OF NUCLEAR ENCODED SEQUENCES TO DIRECT TRANSPORT OF A FOREIGN PROTEIN INTO CHLOROPLASTS, Peter H. Schreier, Marcel Kuntz, Susanne Lipphardt, Hans J. Bohnert, Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, W. Germany, Bayer AG, Leverkusen, University of Arizona, Tucson, USA

Gene fusions between the 5' coding region of the small subunit of Rubisco and the coding region of neomycin phosphotransferase II have been transferred into the plant nucleus. The gene product of the fusion is transported to the chloroplasts and processed. Mutants are under construction to detect the signals which are responsible for transport and processing.

1814 MOLECULAR GENETICS OF PLANT-PARASITE INCOMPATIBILITY, Brian J. Staskawicz, Carolyn Napoli, Doug Dahlbeck and Jean Swanson, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Classical genetic analyses of plant-pathogen interactions have laid the foundation for providing a strategy for identifying and cloning genes from both plants and pathogens that are involved in recognitional specificity and the expression of disease resistance. This is based on the assumption that the events leading to the induction and expression of disease resistance in higher plants are dependent on the specific genotype of both the host and the pathogen.

We have recently cloned and partially characterized two race-specific incompatibility genes from Pseudomonas syringae pv. glycinea. Clones have been detected from genomic libraries of race 6 and race 0 that convert virulent races to avirulence only on specific cultivars of soybean. The regions of activity have been analyzed by restriction mapping, subcloning, Tn5 mutagenesis, deletion analysis and nucleotide sequencing. We are currently attempting to determine the primary gene products of these genes by employing various plasmid expression vectors and studying the regulation of these genes by transcriptional and translational fusions to β-galactosidase.

1815 STUDIES ON A SYSTEM TO ISOLATE FATTY ACID MUTANTS FROM SOYBEAN CELLS, William B. Terzaghi and Karl G. Lark, University of Utah, SLC, UT 84112.

Plant fatty acid biosynthesis (FAB) is well characterized biochemically, but its genetics are poorly understood. FAB thus seems promising to study using somatic cell genetics, and I am looking for FAB- cultured soybean cells. Plant cells normally make all their own fatty acids, so it was unclear whether FAB- cells may be rescued by furnishing fatty acids in their medium. I tested this by, 1) treating cells with a specific inhibitor of FAB plus fatty acids, 2) incorporating exogenous fatty acids into membrane lipids, and 3) measuring fatty acid synthetic rates in the presence of exogenous fatty acids. Cells treated with a lethal concentration of inhibitor grow if fatty acids are supplied; large quantities of these fatty acids are incorporated into membrane lipids. Membrane lipids of cells grown in 17:0 (a fatty acid not made by soybeans) became 40% 17:0, even in the absence of inhibitor. Cells grown in fatty acids depress FAB by 95% but grow as rapidly as untreated cells. These results suggest that soybean cells can do without FAB if fatty acids are supplied; it thus seems feasible to isolate FAB- mutants. I am screening for colonies which die in the absence of exogenous fatty acids following mutagenesis; positives will be tested for fatty acid synthesis. Mutagenesis and screening techniques will be described.

1816 SELECTION-EXPRESSION PLASMID VECTORS FOR USE IN PLANTS; Jeff Velten, Lorri Velten and Jeff Schell, Max Planck Institut, Koeln W. Germany

New plasmid vectors for expressing foreign genes in plants have been developed using a unique dual promoter fragment isolated from the TR DNA of octopine-type plasmids. One promoter has been fused to the neomycin phosphotransferase gene of Tn5, providing a selecable marker for transformation, while the second promoter of the dual promoter fragment remains free to express any gene of interest. Several versions of the selection-expression vector have been produced by the introduction of polylinker DNA fragments (containing numerous useful cloning sites) and a plant-active transcript polyadenylation signal (isolated from the T-DNA) downstream from the second TR promoter. The vectors have been tested by the insertion and expression of the chloramphenicol acetyltransferase gene. We are currently using the new vectors to introduce and express several genes in plants.

1817 GENE MOVEMENT AND GENETIC MANIPULATION, Kamla K. Pandey, Genetics Unit, Grasslands Division, D.S.I.R., Palmerston North, New Zealand. While certain basic properties of life processes are shared by all life forms each major group has its own limiting factors which exclude many features possessed by others. Certain manipulatable mechanisms in each form of life are therefore inherent and specific to these forms. Plants are distinguishable from animals by the totipotency of their cells, open form of growth, versatile sexual reproduction and multiple forms of vegetative reproduction. Genetic behaviour observed in plants can be appropriately viewed only in the context of their organisational and physiological infrastructure. There have been certain genetic observations in plants that are so divorced from the usual experience of plant geneticists that they have for a long time remained the domain of only a few scientists. Barbara McClintock's "mobile genes" suffered from this fate until linked with observations made in prokaryotes. But her observations were uniquely basic in that they transcended the Kingdoms. Another observation in plants, "flax genotrophs"[1,2], which had certain additional, psychological barrier to cross, has also now been recognised as a true phenomenon, although still far from being understood. A third long-standing phenomenon, "graft-transformation"[3], which is even more heavily tainted by dogmatic considerations, although partially brought into the Mendelian fold lately by certain geneticists in Japan, is still regarded equivocally. These three observations had their origins in the premolecular biology era. Recently, two additional phenomena have come to light - "egg-transformation"[4], and "counterfeit hybrids"[5]. All these five phenomena have one feature in common - unorthodox gene movement. Since the present intense interest in "genetic engineering" is concerned basically with a similar problem, i.e. experimental movement of specific genes between organisms, a better understanding of these phenomena may open new possibilities for plant improvement. Mechanisms of gene movement throwing light on some of these phenomena will be discussed.

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Promising Genetic Systems

1818 TRANSCRIPTIONAL REGULATION OF MAIZE ZEIN GENES. B.A. Larkins, R.S. Boston, P.B. Goldsbrough, and M.D. Marks, Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

The zein genes of maize encode the seed storage proteins, which constitute about 50% of the endosperm protein. Zein genes are expressed approximately 12 days after pollination, and their transcripts become highly abundant during early stages or endosperm development. Our recent studies have shown that while zein proteins are encoded by multiple copies of these genes, certain of the genes are expressed at higher levels than others. In general there is a good correlation between gene number and the relative level of gene transcripts, however certain low copy genes are represented by high levels of transcripts.

To better understand the transcriptional regulation of these genes we have used two approaches. One is based on their transcription in an in vitro system derived from HeLa cell nuclei. The other is based on their expression when transferred to sunflower tissue using the Ti plasmid of Agrobacterium tumefaciens.

In general, we have found that the 5' ends of in vitro transcripts are the same as the 5' ends of RNAs isolated from endosperm tissue as judged by S1 nuclease mapping and primer extension mapping. We have been able to transcribe genes corresponding to both the Mr 15,000 and Mr 19,000 genes in this system, and the relative level of transcripts produced in vitro agrees well with the levels produced in vivo.

We have obtained low levels of expression of zein genes in callus tissues of transformed sunflower plants. Although the level of transcripts is quite low in most transformed tissues, it appears to represent 1 to 10% of the level of octopine synthetase expression.

We have been unable to detect zein proteins in these tissues, but this is most likely a reflection of the low levels of zein mRNAs. The mRNAs have precisely the same 5' and 3' ends as mRNAs from endosperm tissue and are polyadenylated. These results indicate that similar transcriptional regulatory signals are being recognized in both plant tissues.

DNA Expression Systems

1819 TRANSCRIPTIONAL REGULATION OF MAIZE ZEIN GENES. B.A. Larkins, R.S. Boston, P.B. Goldsbrough, and M.D. Marks, Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

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1820 DIRECT GENE TRANSFER FROM E.COLI PLASMID pUC8 TO NICOTIANA TABACUM

Ingo Potrykus, Jerszy Paskowski, Raymond D. Shillito, Michael W. Saul, Thomas Hohn, Barbara Hohn; Friedrich Miescher-Institut, PO Box 2543, CH-4002 Basel, Switzerland

The aim of our studies was to test whether it would be possible to directly transform plants by incubating protoplasts with a selectable gene. Neomycin phosphotransferase (NPH(3')II) from E. coli confers resistance to kanamycin. We constructed a selectable hybrid gene by combining the protein coding region of NPH(3')II with 5' and 3' expression signals from Cauliflower Mosaic Virus gene VI. The gene was introduced into freshly isolated tobacco (N.tabacum Petit Havana SR1) leaf protoplasts by a variety of treatments, including PEG. An absolutely tight selection system was developed by subjecting the developing protoplasts in a "bead type culture" (1) to 50 mg/l kanamycin. Resistant colonies were subcultured on 75 mg/l kanamycin. Shoots and roots were induced with standard methods. Control shoots did not root, bleached and died. Reinitiation of cell cultures from all plant organs and protoplast culture leaves demonstrated that the resistance phenotype was maintained during plant development and without selection pressure. Anther culture analysis revealed a segregation ratio of 1:1 resistant:sensitive haploids on 200 mg/l kanamycin. Genetic crossing analysis of large populations of F_1 and F_2 seedlings provided definite proof for the presence in the transformed clones analysed so far of one dominant Mendel factor for resistance to kanamycin. The biological and genetic data provide evidence for uptake of the NPH(3')II gene into protoplasts, its stable integration into chromosomal DNA, its developmentally independent expression, and its sexual transmission as a stable dominant gene.
Southern blot analysis was done with a nick-translated HindIII fragment representing the protein-coding region of the NPH(3')II gene: Hybridization to undigested DNA from undifferentiated cell cultures and from leaf tissue confirmed tight association to chromosomal DNA. Hybridization to DNA restricted with BstII (which does not cut within pABDI) revealed a transformant-specific pattern which was maintained in the sexual offsprings. Hybridization to DNA restricted with EcoRV (which cuts out the NPH(3')II gene) demonstrated the presence of the expected 1,2 kb fragment of the undisturbed gene. There was strict correlation between kanamycin resistance and the physical presence of the gene both in the original transformants as in their sexual offsprings.
Enzyme assay: Kanamycin-dependent phosphorylation by neomycinphosphotransferase was assayed according to Reiss (2). The test was positive exclusively with tissues from pABDI-treated and kanamycin-resistant clones. It was independent of the developmental state of the material and it was strictly correlated with the presence of the functional copy of the gene, both in the original transformants as in their sexual offsprings.
(1) Shillito et al., Plant Cell Reports 2,244(1983); (2) Reiss et al., Gene, in press.

1821 STUDIES ON THE EXPRESSION IN PLANTS OF FOREIGN GENES INTRODUCED USING MODIFIED TI PLASMID VECTORS, Robert B. Simpson, Marcella Lillis, Linda Margossian, Thomas D. McKnight, Elias Shahin, Albert Spielmann and Mayer Yashar, ARCO Plant Cell Research Institute, 6560 Trinity Ct., Dublin, CA 94568 USA

Agrobacterium is a natural vector which can transfer a portion of the resident Ti plasmid to plant cells. The transferred portion, or T-DNA, is stably maintained in plant chromosomal DNA and its expression alters the phytohormone balance of the cell. This results in a cellular overgrowth or tumor on the plant (reviewed in 1-3). By modifying the Ti plasmid, we have eliminated the T-DNA oncogenes (with their adverse effect on normal development) while retaining the efficient DNA transfer ability of the system. The characteristics of this system will be compared to other gene transfer options currently available for plants.

Using such Ti plasmid vectors, we are modifying genes, transferring them to plants and studying their expression. This is a valuable approach to the identification of signals encoded in the DNA which regulate the expression of these genes. Our progress with genes such as the soybean small subunit of ribulose-1, 5-bisphosphate carboxylase, soybean glycinin, soybean leghemoglobin, and Robertson's mutator DNA Mu1 from maize will be discussed.

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1822 Molecular Biology of the Heat Shock Response in Plants. Joe L. Key*, Ron Nagao, Bill Gurley**, Ewa Czarnecka, Janice Kimple and Elizabeth Verling. Botany Department, University of Georgia, Athens, GA 30601. *Currently, Agrigenetics Corporation, Boulder, CO; **Cell and Microbiology Department, University of Florida, Gainesville, FL.
We have characterized in depth the production of heat shock (hs) mRNAs, and their respective proteins, using cloned cDNAs under a wide range of hs conditions. Their synthesis and stability have been evaluated. The 20 most abundant hs mRNAs accumulate up to 20,000 copies per cell within 1-2 hr. at the "optimum" hs T^{o} (40^{o} for soybean) and stabilize at that level for 4 hrs, after which time they gradually deplete during continuous hs. There is an "autoregulatory" response which comes into play based apparently upon the level of accumulated hs proteins which is to some extent temperature dependent. A return of the tissue to normal growing temperature results in a decay of hs mRNA with a $^{1}/_{2}$time of about 1hr. We have sequenced several hs genes, analyzed potential regulatory sequences, and deduced the amino acid sequence and hydropathic properties of the proteins. The 5' "regulatory" sequences have high homology with the Drosophila "hs consensus" regulatory region. There is little linear amino acid sequence homology of these genes with the genes for the low molecular weight hsp's of Drosophila except for an Asp-Gly-Val-Leu-Thr which is totally conserved in the most hydraphobic region of the soybean and Drosophila hsp's and in bovine α crystallines. One of the soybean hs genes has been expressed in non-homologous tissue using a T-DNA vector for transformation. Details of this system will be presented in poster form.