PLANT MOLECULAR BIOLOGY

Robert B. Goldberg, Organizer

April 16 — April 23

Plenary Sessions and Workshops

Poster Sessions

Prospects For Genetic Engineering in Higher Plants

1156 PROSPECTS FOR GENETIC ENGINEERING IN HIGHER PLANTS

J. Schell, M. Van Montagu, J.P. Hernalsteens, L. Willmitzer, J. Leemans, H. Joos, L. Otten, H. De Greve, M. Holsters, P. Zambryski, L. Herrera and A. Depicker

Max-Planck-Institut für Züchtungsforschung, Köln, W-Germany
Laboratorium voor Genetika, Rijksuniversiteit Gent, Belgium
Laboratorium voor Genetische Virologie, Vrije Universiteit Brussels, Belgium

Large Plasmids in Agrobacterium tumefaciens (Ti) and A.rhizogenes (Ri) are known to endow these bacteria with the capacity to transfer a defined DNA fragment (T-DNA) into the plant cell nucleus and to covalently integrate this T-DNA segment in chromosomal DNA, thus creating a new locus at a number of possible sites. The mechanism of DNA transfer is still poorly understood. Genetic evidence indicates that a relatively large segment including the so-called vir region and the T-DNA region of Ti-plasmids are transferred. Under normal circumstances only the T-region is inserted in the chromosomal DNA and thus stably maintained. No functions located within the T-region are required for either transfer or integration. Large foreign DNA sequences of 50 kb or more, experimentally inserted within the T-region, are efficiently transferred and integrated in the Plant chromosomal DNA. The plasmid derived T-DNA was shown to consist of a number of well defined transcriptional units transcribed by the host polymerase II and coding for a number of different functions, i.e. enzmyes involved in opine synthesis and functions involved in the inhibition of plant differentiation. Thus separate shoot suppressing and root suppressing functions have been identified. Removal of these tumor controlling genes does not affect DNA transfer or integration. Thus it was possible to design modified Ti-plasmids that can insert foreign genes in plant cells from which normal plants can be regenerated that express the foreign genes and transmit them sexually with normal mendelian seggregation ratios. To be expressed in plants, the coding sequences of foreign genes have to be inserted behind plant promotor sequences. Several such constructed genes have been made and introduced in tobacco plants and their expression has been studied.

Molecular Biology of RI and TI Plasmids and Their Use To Investigate Plant Gene Structure and Function

1157 BIOCHEMICAL-GENETIC ANALYSIS OF TI-PLASMID FUNCTIONS INVOLVED WITH CROWN GALL TUMOR INDUCTION AND MAINTENANCE, Eugene W. Nester, Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195; Milton P. Gordon, Department of Biochemistry, University of Washington, Seattle, WA 98195; Tsune Kosuge, Department of Plant Pathology, University of California, Davis, CA 95616; and Roy O. Morris, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

The tumor inducing (Ti) plasmid of Agrobacterium tumefaciens can be divided into two domains with regards to regions required for tumor induction. These regions include the T-DNA (onc region), the region of DNA integrated into plant nuclear DNA and the virulence (vir) genes, a 35 Md region of DNA which apparently is required for the early steps in tumor induction. The T-DNA can be divided into 3 regions on the basis of mutant analysis. Transposon insertions into one region increase the degree of shoot formation of the induced tumors (tms); insertion into another region increases the degree of root formation (tmr) and insertion into a third region increases the size of the tumor on certain plants. Tumors induced by each of these mutant strains have been analyzed for their level of cytokinins, as well as free auxin. The ratio of cytokinin to auxin was altered significantly in the tumors induced by strains with insertions in various regions of the T-DNA. The highest ratio was observed in tumors induced by bacteria with insertions in the T-DNA which induced excessive shoot formation; the lowest ratios were found in tumors with insertions in the T-DNA which resulted in excessive root formation. Thus we conclude that the cytokinin-auxin balance is regulated by specific loci in the T-DNA. Deletions covering the tms, tmr and tml region resulted in a strain which was avirulent, but still able to integrate the remaining segment of its T-DNA.

In contrast to the effect of insertions in the T-DNA, which altered morphology, insertions in the vir region abolished virulence completely on all plants. The vir region can be divided into 5 major loci; insertions between these loci have no effect on virulence. One of these regions, vir B, can be subdivided into additional transcription units, on the basis of complementation studies. All of these five units, except 1, can be complemented by any of the other mutants as well as by wild type DNA.

1. Garfinkel, David J., Simpson, Robert B., Ream, Lloyd W., White, Frank F., Gordon, Milton, P., and Nester, Eugene W.: Cell 27:143-153 ,1981.
2. Akiyoshi, D. E., Morris, R. O., Hinz, R., Mischke, Barbara Sue, Kosuge, T., Garfinkel, D. J., Gordon, M. P., and Nester, E. W.: Accepted, PNAS.

1158 *AGROBACTERIUM TUMEFACIENS* AND ITS Ti-PLASMID AS TOOLS IN TRANSFORMATION OF PLANT CELLS, R.A. Schilperoort, J. Hille, A. Hoekema, P.J.J. Hooykaas, F.A. Krens, and G.J. Wullems, Department of Biochemistry, State University of Leiden, 2333 AL Leiden, The Netherlands.

Upon induction of the plant tumour, that is called crown gall, *A. tumefaciens* introduces a piece of DNA, originating from a large Tumour-inducing (Ti) plasmid, in the nuclear genome of the plant cell(1). This DNA fragment is called T-DNA and is expressed in RNA and proteins(2). One of the proteins is an enzyme which, dependent on the type of T-DNA present, catalyzes either octopine or nopaline in tumour cells. T-DNA also carries genes for tumour development and maintenance. These genes are called *onc*-genes some of which are shown to disturb the balance of the phytohormones auxin and cytokinin(3). Besides *onc*-genes the Ti-plasmid carries *vir*-genes. These *vir*-genes are located on a *vir*-region at some distance left to the T-region. By genetic complementation it has been demonstrated that *vir*-genes act in *trans* and consequently are expressed in the bacterium(4). Recently we accomplished to separate T-DNA and *vir*-region on different compatible plasmids. Agrobacteria harbouring both types of plasmid havea normal capacity to induce tumours on many plant species. It, moreover, is found that an *A. tumefaciens* with limited host range acquires a broad host range upon the introduction of the plasmid carrying only the octopine T-DNA. We observed that octopine T-DNA in transformants obtained by cocultivation of tobacco leaf protoplasts with *A. tumefaciens* differed largely from octopine T-DNA in transformants isolated after incubation of protoplasts with octopine Ti-plasmid DNA. In the first case a fairly well defined and normal T-DNA is present, whereas in the second case both more extended and scrambled T-DNA is observed in independent tissues (5). We assume that in using the bacteria the activity of *vir*-genes are responsible for the introduction of a distinct T-DNA by a mechanism in which T-DNA border sequences(6) are recognized.

1. Chilton, M.D., Saiky, R.K., Yadav, N., Gordon, M.P. and Quetier, F. (1980). Proc. Nat. Acad. Sci. USA 77:4060-4064.
2. Willmitzer, L., Simons, G. and Schell, J. (1982). The Embo Journal 1:139-146.
3. Ooms, G., Hooykaas, P.J.J., Moolenaar, G. and Schilperoort, R.A. (1981). Gene 14:33-50.
4. Hille, J., Klasen, I. and Schilperoort, R.A. (1982). Plasmid 7:107-118.
5. Krens, F.A., Wullems, G.J. and Schilperoort, R.A. (in press). Proc. NATO, ASI, FEBS Advanced Course 1982, Plenum Press.
6. Simpson, R.B., O'Hara, P.J., Kwok, W., Montoya, A.L., Lichtenstein, C., Gordon, M.P. and Nester, E.W. (1982). Cell 29:1005.

1159 **TI PLASMID AS A VEHICLE FOR GENETIC ENGINEERING OF PLANT CELLS,** **John D. Kemp, Donald J. Merlo, Dennis W. Sutton, Richard F. Barker, Jerry L. Slightom, and Timothy C. Hall**

Agrigenetics Advanced Research Laboratory, 5649 E. Buckeye Road, Madison, WI 53716

The Ti plasmid of Agrobacterium tumefaciens is a useful vehicle for the transfer of foreign genes into plants. Its utility has become apparent for a number of reasons: 1) the T-DNA of the Ti plasmid is stably integrated into the plant genome; 2) transformed plant cells can be regenerated into normal plants when the T-DNA is mutated in various loci; 3) the T-DNA is transferred to the F1 generation of regenerated plants; 4) active T-DNA genes, e.g., nopaline synthase or octopine synthase, are also active in the regenerated plant tissues; 5) foreign DNA can be engineered into T-DNA and remains stable through meiosis; 6) engineered foreign genes are active at the level of transcription; and 7) an engineered T-DNA gene, nopaline synthase, is fully active. Experiments will be described where the coding sequence of phaseolin is ligated to either the nopaline synthase or the octopine synthase promoter region. The ligations were made so that the reading frame of the phaseolin gene matches that of the coding sequence adjacent to the promoters. The utility of T-DNA will be fully realized by the demonstration of protein expression of a foreign gene in regenerated plants.

1160 DISARMED VECTORS FOR PLANT TRANSFORMATION, M.-D. Chilton, A. De Framond, M. C. Byrne, G. Helmer, J. Koplow, W. S. Chilton, Department of Biology, Washington University, St. Louis, MO 63130, USA; Chantal David, Jacques Tempe, Institut de Microbiologie, Faculte de Science, Universite Paris - Sud, Orsay 91405, France.

Regeneration of complete plants from crown gall tumor cells containing T-DNA is rare, and regenerants appear to be mutants that contain large deletions of T-DNA (1,2). Nopaline T-DNA can be disarmed by a single insertion mutation, such that complete tobacco plants regenerate readily from cloned transformed cell lines (3). The regenerated plants were found to contain multiple copies of full length T-DNA and to transmit these to R1 progeny plants (3) We have disarmed T-DNA of pTi T37 more extensively by excision of all T-DNA genes except that encoding nopaline synthase. Such disarmed T-DNA should allow regeneration of all kinds of transformed cell lines. One can view the Ri plasmids of Agrobacterium rhizogenes as a naturally disarmed vector system, for plants regenerate readily from transformed cell lines containing Ri plasmid T-DNA (4). Such regenerants contain opines characteristic of the original transformed colls, and contain T-DNA. Transposon mutagenesis studies and transcript analysis are in progress. These studies should reveal locations of genes in this T-DNA that could be removed in order to produce transformed plant regenerants of more normal morphology.

1. Yang, F. M. & Simpson, R. G. 1981. Proc. Nat. Acad. Sci. USA 78:4151.
2. De Greve, H., Leemans, J., Hernalsteens, J. P., Thia-Toong, L., De Beuckeleer, M., Willmitzer, L., Otten, L., Van Montagu, M. and Schell, J. 1982. Nature, in press.
3. Barton, K. A., Binns, An N., Matzke, A. J. M. & Chilton, M.-D. 1983. In press.
4. Chilton, M.-D., Tepfer, D. A., Petit, A., David, C., Casse-Delbart, F. & Tempe, J. 1982. Nature (Lond.) 295:432.

Molecular Biology of DNA and RNA Plant Viruses

1161 THE RESPONSE OF PLANTS TO *IN VITRO* MODIFICATIONS OF THE CaMV GENOME, Stephen H. Howell and Richard M. Walden, Biology Department C016, University of California San Diego, La Jolla, CA 92093.

Cauliflower mosaic virus (CaMV) DNA can be introduced directly into plants simply by rubbing the viral DNA onto plant leaves. We have modified the CaMV genome *in vitro* and have examined the response of plants to these altered genomes. From the DNA sequence, Franck *et al.* (*Cell 21*, 285, 1980) and Gardner *et al.* (*Nucleic Acids Res 9*, 2871, 1981) demonstrated the CaMV genome contains six major open reading frames (I-VI), a large and a small intergenic region. Frame IV apparently encodes the virus coat protein and frame VI encodes a non-virion protein which is found in the matrix of the inclusion body (Odell and Howell, *Virology 102*, 349, 1980; Covey and Hull, *Virology 111*, 463, 1981). Within the small intergenic region is a promoter site for a 19S RNA that encodes the product of frame VI and within the large intergenic region is a promoter for an RNA that is a transcript of the entire CaMV genome (Dudley *et al.*, *Virology 117*, 19, 1982; Guilley *et al.*, *Cell 30*, 763, 1982).

Howell *et al.* (*Science 208*, 1265, 1980) showed that CaMV DNA could be cloned in infectious form by inserting a bacterial plasmid at a unique restriction site in frame V. The cloned viral DNA was infectious only when excised from the recombinant plasmid by cutting the plasmid at the cloning site. Most *in vitro* modifications (small insertions and deletions) of the cloned CaMV genome destroy the infectivity of the viral DNA (Howell *et al.*, *Nature 293*, 483, 1981). Two sites, one in the large intergenic region and the other in frame II, tolerate small modifications. Gronenborn *et al.* (*Nature 294*, 773, 1981) found that DNA fragments no larger than about 250 bp could be inserted into frame II.

Two defective CaMV genomes (bearing non-overlapping, lethal modifications) can rescue each other when coinoculated onto plants. Rescue occurs by intergenomic recombination as evidenced by the production of hybrid genomes when plants are coinoculated with genomes derived from two different viral isolates (Walden and Howell, *J. Mol. Appl. Gen 1*, 447, 1982). One mechanism which explains the recombination is that separate viral genomes in the inoculum are ligated together to form mixed dimers and a single crossover event resolves the dimers into a single normal monomer.

Plants are infected when inoculated directly with recombinant plasmids containing multimers of the CaMV genome (Lebeurier *et al. Proc. Nat. Acad 79*, 2932, 1982). In the infected plant, normal viral genomes are excised from these plasmids by intragenomic recombination. The recombination is homologous and the frequency depends on the length of the homologous arms that encompass the monomeric viral genome. Such multimers permit the direct transfer of CaMV-containing recombinant plasmids from bacteria to plants.

This work was supported by the NSF and by the USDA/SEA.

1162 THE MINICHROMOSOME OF CAULIFLOWER MOSAIC VIRUS: STRUCTURE AND TRANSCRIPTION, Tom J. Guilfoyle and Neil E. Olszewski, Department of Botany, University of Minnesota, St Paul, MN 55108

Nuclei isolated from turnips which have been infected with cauliflower mosaic virus (CaMV) contain transcriptionally active CaMV DNA complexes that produce viral-specific transcripts in vitro.[1] Viral-specific in vitro transcripts are synthesized in nuclei isolated from both leaves and tubers and account for several percent of total in vitro transcripts. The in vitro transcripts are heterogeneous in size, and the entire viral genome is transcribed in vitro. Only the coding strand of CaMV DNA is transcribed in isolated nuclei, and RNA polymerase II is responsible for the transcription obtained in vitro. Viral-specific in vitro transcription is stimulated by high salt concentrations (i.e., 200-400 mM ammonium sulfate), sarkosyl, heparin, and aurintricarboxylic acid, all of which prevent RNA chain initiation but enhance RNA chain elongation by chromatin-associated RNA polymerase II. We have purified CaMV transcription complexes from isolated nuclei, and the CaMV DNA within these complexes appears to contain a nucleosome structure as determined by micrococcal nuclease digestion.[2] The bulk of the CaMV DNA associated with chromatin proteins is in the form of a covalently closed circle unlike the virion DNA which contains site-specific discontinuities. At least a portion of the CaMV minichromosomes is transcriptionally active, and the characteristics of in vitro transcription with purified minichromosomes are identical to virus-specific transcription obtained with isolated turnip nuclei. We are attempting to determine the types of proteins associated with the CaMV minichromosomes by probing protein blots of CaMV DNA complexes with specific antibodies (i.e., RNA polymerase II and RNA polymerase II subunit, histone, and virus coat protein antibodies). We are also probing isolated CaMV minichromosomes and recombinant plasmids containing CaMV DNA inserts for nuclease sensitive sites.

[1]Guilfoyle, T. J. (1980) Virology 107: 71-80.
[2]Olszewski, N., Hagen, G., and Guilfoyle, T. J. (1982) Cell 29: 395-402.

1163 GENE STRUCTURE AND FUNCTION OF THE RNAs OF CUCUMBER MOSAIC VIRUS, Robert H. Symons, Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5001.

Four major single-strand RNAs are encapsidated in virions of cucumber mosaic virus (CMV). Of these, the three largest, RNAs 1, 2 and 3, are genomic, containing all essential information for virus infection. RNA 3 of the Q-strain of CMV has been sequenced and is 2193 residues long with two major open reading frames. The 5'-cistron encodes a protein (3A) of M_r 36,700, whereas the 3'-cistron is the gene for the M_r 26,200 coat protein which is also contained within RNA 4, the subgenomic mRNA for the coat protein. *In vitro* translation studies using rabbit reticulocyte lysates have shown that the four viral RNAs act as monocistronic messengers. RNAs 1 and 2 encode polypeptides of M_r 95,000 and M_r 110,000, respectively. Nucleotide sequencing studies in progress on RNAs 1 and 2 should give the location of these genes and whether any other reading frames are available; e.g., through unencapsidated subgenomic mRNAs. There is sufficient information on RNA 1 to code for an additional 3'-terminal protein of M_r 35,000; preliminary sequencing data supports this.

In looking for possible functions for the translation products of the CMV RNAs, we are characterizing the RNA-dependent RNA polymerase (RNA replicase) induced in cucumber seedlings by CMV infection. RNA replicase solubilized from a well-washed particulate fraction that can synthesize full-length viral RNAs has been extensively purified and contains several polypeptides absent from similar preparations from uninfected plants and which are of similar size to the three largest viral RNA translation products. However, peptide mapping and other studies have shown that full-length translation products are not present in the highly purified RNA replicase.

Our current model is that the RNA replicase is membrane-bound in CMV-infected cucumber seedlings and consists of a virus-induced host protein of M_r 100,000 as the catalytic subunit together with translation products of CMV RNAs 1, 2 and 3 acting in a replication complex to ensure specificity and control of the whole process. Our solubilization and purification procedures appear to have concentrated the M_r 100,000 catalytic subunit plus other host proteins but eliminated any viral-coded proteins.

1164 CUCUMOVIRUS-ASSOCIATED SATELLITE RNA: A FAMILY OF SMALL VIRUS-DEPENDENT PARASITIC RNAs CAPABLE OF MODIFYING DISEASE EXPRESSION, J.M. Kaper, Plant Virology Laboratory, Plant Protection Institute, ARS, U.S. Dept. of Agriculture, Beltsville, MD 20705

Cucumoviruses contain in addition to their three genomic RNAs, and the fourth subgenomic RNA, a fifth RNA of relatively low molecular weight. This fifth RNA depends upon the viral RNA for its replication, but not vice versa. It has no nucleotide sequence homology with the genomic RNAs, and can therefore be considered a viral "satellite RNA" like the satellite of tobacco necrosis virus described some 20 years ago. The first reported cucumber mosaic virus (CMV)-associated RNA 5 (CARNA 5) is a 335-nucleotide RNA. Its viral parasitic properties are evident when in CMV-infected tobacco plants viral RNA synthesis can be seen to be overtaken and virtually arrested, presumably as a result of the rapidly increasing synthesis of CARNA 5 competing for a limited amount of virus-induced replicase enzyme. In many host plants this leads to a decrease or disappearance of disease symptoms. However, in tomato plants CARNA 5 is responsible for a new lethal disease, tomato necrosis. Peanut stunt virus (PSV), another member of the cucumovirus group, contains a fifth RNA which was designated PARNA 5. PARNA 5 is 30-50 nucleotides longer than CARNA 5. The two molecules have little overall nucleotide sequence homology, although their 3' and 5' ends are quite similar, probably reflecting the similarity in replication strategies of their helper viruses. The large differences in the nucleotide sequences of CARNA 5 and PARNA 5 are reflected in the fact that the replication of these molecules cannot be supported by each other's helper viruses. In contrast, the specificity of the viral helper function is not lost when the nucleotide sequence differences are small. This is the case with the 334-nucleotide CARNA 5 obtained from CMV strain 1, the earliest CMV identified in the USA. The replication of this CARNA 5 from CMV-1 is supported by all other CMV isolates with which it has been tested. However, it is unable to cause the tomato necrosis disease. One or more of the 26 differences that have been identified in the nucleotide sequences of tomato necrotic CARNA 5 and the CARNA 5 from CMV-1 must therefore be responsible for this disease characteristic. Other CARNA 5s of similar length have been obtained by propagation of different CMV strains in different host plants. Thus, in nature there appears to exist a multitude of CARNA 5 nucleotide sequence variants. Their emergence and replication in infections of specific CMV strains in specific host plants seems to be determined by factors that are not yet understood.

Molecular Biology of RNA Viruses and Viroids

1165 TOBACCO MOSAIC VIRUS GENES AND GENE PRODUCTS, Milton Zaitlin, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The recently-published sequence of the complete genome of TMV (1) confirms the probable number of gene products and their location on the RNA, gleaned from many other types of studies during the last decade (2). Basically, TMV RNA (M_r = ~ 2 x 10^6) is the mRNA for at least 4 proteins: The coding sequences for a protein of M_r 126,000 are initiated 69 nucleotides from the 5' end; read-through of the terminating UAG for this protein generates a protein of M_r 183,000. Thus, these proteins share most of their amino acid sequences in common from their amino termini, but the larger protein also has unique sequences. Overlapping the terminal 5 codons of the M_r 183,000 protein there is coding information for a protein of M_r 30,000 (30K protein) and finally, terminating close to the 3' end of the RNA there is an open reading frame for the coat protein. Judging from in vitro translational studies, these latter two genes are "silent" on the genomic RNA but are generated from smaller, 3' coterminal subgenomic mRNAs.

The only protein for which a function is known is the coat protein. It has been postulated that one or both of the two larger proteins are somehow involved in viral replication, but conclusive data are lacking. Evidence will be presented which suggests that the 30K protein is a factor which potentiates the movement of virus (or viral RNA) from cell-to-cell in the host plant (3). This judgement is based on tryptic peptide analysis of the protein products of two strains of TMV, one of which has a temperature-sensitive lesion which precludes viral movement at a restrictive temperature. The only change detectable was a slight modification of the 30K protein.

Finally, data will be presented on a protein (H protein, M_r 26,500) which is a component of TMV virions at about one copy per virion. H protein was originally thought to be of host origin (4), but more recent studies (5) have shown that it contains the complete coat protein sequence plus another polypeptide of either host or viral origin. It is postulated that the additional polypeptide is covalently bound to the coat protein through an unusual isopeptide linkage.

1. Goelet, P. et al. (1982). PNAS (USA) 79, 5818-5822.
2. Hirth, L. and K. E. Richards. (1971). Advances in Virus Research 26, 145-199.
3. Leonard, D. A. and M. Zaitlin. (1982). Virology 117, 416-424.
4. Asselin, A. and M. Zaitlin. (1978). Virology 91, 173-181.
5. Collmer, C. W., V. M. Vogt and M. Zaitlin. (1983). Virology (In press).

1166 COMPLEXITY OF THE RNAs PRESENT IN PREPARATIONS OF BARLEY STRIPE MOSAIC VIRUS, A. O. Jackson*, G. D. Gustafson*, J. E. McFarland* and J. Stanley**, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana* and Department of Virus Research, John Innes Institute, Norwich, England**

Barley stripe mosaic virus (BSMV) is a multicomponent RNA virus with rod-shaped particles (1). Different strains of the virus have a high degree of nucleotide (NT) sequence homology, but they vary in the number of RNAs which can be resolved by gel electrophoresis (2). Northern hybridization of the RNAs of four BSMV strains with recombinant DNA clones containing viral-specific inserts reveals that these strains of BSMV encapasidate at least three distinct high molecular weight RNAs (3). The high molecular weight RNAs of the North Dakota 18 (ND18) and Norwich (NO) strains resolve into three components (RNAs 1, 2 and 3 = about 4200, 3650 and 3250 NT respectively) during gel electrophoresis and each RNA has extensive regions of unique sequence. In contrast, only two components (4200 and 3650 NT) are resolved during gel electrophoresis of the Type strain. The 4200 NT RNA of Type has sequence homology with the RNA 1 of ND18 and NO, but the 3650 NT component of Type consists of two RNA species which coelectrophorese. One of the Type 3650 NT RNAs shares common sequences with RNA 2 of ND18 and NO and the other species has sequence homology with RNA 3. The Argentina mild (AM) strain of BSMV consists of four RNAs of 4200, 3650, 3250 and 2950 NT. The AM 3650 NT component contains a mixture of RNAs with sequences common to both RNA 2 and 3 of ND18 and NO. The AM 3250 and 2950 NT components contain sequences common to RNA 3 of ND18 and NO.

Virions of BSMV also contain a subgenomic (SG) RNA of about 825 nucleotides with sequence homology to recombinant DNA clones derived from RNA 3 of ND18. Wandering spot analysis of partial P-1 nuclease digests of SG RNA reveals a polyadenylic acid (poly A) rich region at the 3' end. Gel electrophoresis of cDNAs transcribed in the presence of dideoxynucleotides shows that SG RNAs from Type and ND18 have nearly identical sequences for at least 150 nucleotides adjacent to the poly A region. Preparations of SG RNA direct synthesis in vitro of two polypeptides (mr = 21 to 22 x 10^3 in polyacrylamide gels).

1) Jackson, A. O. and Brakke, M. K. 1973. Virology 55, 483-494.
2) Palomar, M. K., Brakke, M. K. and Jackson, A. O. 1977. Virology 77, 471-480.
3) Gustafson, G. D., Milner, J. J. McFarland, J. E., Pedersen, K., Larkins, B. A. and Jackson, A. O. 1982. Virology 120, 182-193.

1167 INVOLVEMENT OF VIRUSES AND VIRUS-LIKE AGENTS WITH THE MALE STERILITY TRAIT OF PLANTS L. K. Grill, S. J. Garger and T. H. Turpen Department of Molecular Biology, Zoecon Corporation, 975 California Avenue Palo Alto, CA 94304

Several viruses are capable of causing male sterility in plants (1), but such viruses are primarily studied only when there are additional disease symptoms present. When maternally inherited male sterility exists without other obvious symptoms, it has not generally been classified as a virus disease. However, there are many reasons to believe that a number of these cytoplasmic male sterile (CMS) plants have a virus as the etiological agent (2-5). One of the more characterized plants with probable viral-induced CMS, is the Vicia faba "447" plant described by Bond, et al (6). In 1974 Edwardson, et al used an electron microscope to describe spherical bodies existing only in the cytoplasm of CMS plant tissues (7). Subsequent studies revealed that the spherical bodies contain a double-stranded RNA which is also only associated with the sterile plants (8-11). Our latest studies provide information suggesting that the CMS in Vicia faba has a viral-like causal agent which may not fit into any existing plant virus class. The existence of such agents may not be limited to the faba bean plant, as we have found similar dsRNA molecules associated with other male sterile plants.

1. Shephard, R.J. (1972) IN "Prin. and Tech. in Plant Virology" pp 267-292, (C.I. Kado and H.O Agrawal, eds.) VanNostrand-Reinhold, Princeton, NJ.
2. Corbett, M.K. and J.R. Edwardson (1964) Nature 201:847-848.
3. Curtis, G.J. (1967) Euphytica 16:419-424.
4. Ohta, Y (1970) Japan. J. Genetics 45:277-283.
5. Atanasoff, D. (1971) Phytopath. Z. 70:306-322.
6. Bond, D.A., J.L. Fyfe and G. Toynbee-Clarke (1966) J. Agric. Sci. 66:359-377.
7. Edwardson, J.R., D.A. Bond and R.G. Christie (1976) Genetics 82:443-449.
8. Grill, L.K. and S.J. Garger (1981) Phytopathology 71:878 (abstr).
9. Grill, L.K. and S.J. Garger (1981) Proc.Natl.Acad.Sci. USA 78:7043-7046.
10. Scalla, R., G. Duc, J. Rigaud, A. Lefebvre and R. Meignoz (1981) Pl. Sci. Letters 22:269-277.
11. Duc, G., R. Scalla, J. Rigaud, A. Lefebvre and R. Meignoz (1981) C. R. Acad. Sc. Paris 292:1227-1230.

Novel Approaches to Genetic Engineering in Plants

1168 EXOGENOUS DNA CAUSED PHENOTYPIC VARIATION IN COTTON, G.Zhou,j. Weng,Shanghai Institute of Biochemistry, Academia Sinica;J.Huang, S.Qian,G.Liu, Institute of Economic Crops,Jiangsu Academy of Agricultural Sciences, China.

The total DNA (fragmented to pieces of 10^6-10^7) of G.barbadense 416 was introduced (1) into the embryos of self pollinated G.hirsutum glandless. Phenotypes of the first generation (D_1) did not show much changes. They behaved mainly as the receptor plant;78% were nonpubescent like the donor and 47% had the color of their anther changed from milk white to light yellow. The second generation showed more obvious variations. Among ninety offsprings which showed changes,two(102-4 and 99-4) changed more markedly.102-4 more resembled the receptor while 99-4 resembled more the donor.Another plant of D_3 which came from the population of the rest 88 D_2 plants was also like the donor. After selectively following these three individual offsprings to the 5th generation, it was found that with the exception of changes in certain minor traits, the 102-4 series was rather stable,while the 99-4 and 65/-6 series varied frantically and continuously.Their donor traits gradually changed at different rates in the population towards receptor traits. For exemple, the corolla color and leaf shape reached 1:1 in donor to receptor ratio in the 5th generation, anther changed color from that of the donor to receptor ratios of 8.8:1(D_4),6.7:1(D_5);petal bottom spots from light red (99-4) to colorless in the ratio of 1:0.5(D_3),1:0.98(D_4),1:1.7(D_5). By actually counting each altered trait in a single offspring, the changes in the offspring of 99-4 and 65/-6 in D_4-D_5 were continuously 100%. Neither receptor nor donor were exactly reformed or segregated in a population of more than 100-240 plants.

The alteration or segregation of the traits in the descendents did not follow Mendel's law. The dominant characters, such as the corolla and anther color, the red spot on the petal bottom, the gossypium gland of the donor etc.,lost sometimes in some of the offspring, will reappear among the next or still next generation of a single plant.The phenomena are much like those that McClintock[2] described in maize only that the above results were observed after the introduction of exogenous DNA.

It seems that exogenous DNA caused rearrangement of the receptor genome and then changed the gene expression. May be the transposable elements or repeated sequences played some important role.

(1) Zhou Guang-Yu, Weng Jian, Huang Jun-Qi, Qian Si-Ying and Liu Qui-Ling. Introduction of exogenous DNA into cottom embryos, Methods in Enzymology, Recombinant DNA Part B, ed. by Ray Wu, Lawrence Grossman and Kivie Moldave, Academic Press. In press.

(2) B. McClintock (1967) Genetic systems regulating gene expression during development, Develop. Biol. 1(supl) 84-112

1169 IN VITRO TRANSFORMATION OF PETUNIA PROTOPLASTS BY A. TUMEFACIENS, Robert T. Fraley and Robert B. Horsch, Monsanto Co., St. Louis, MO 63167

The in vitro transformation method based on the co-cultivation of protoplasts with A. tumefaciens cells, initially developed by Marton, et al. (Nature 277:129, 1979) for tobacco protoplasts, has been improved and extended to use with petunia protoplasts. Key features of the system combine, 1) the use of feeder plates to permit rapid, density-independent growth of protoplast-derived colonies, and 2) effective selection conditions for hormone autotrophy obtained from reconstruction experiments with normal and transformed protoplasts. Using this method we have obtained in vitro transformants at frequencies near 10^{-1} following inoculation of protoplasts with a variety of mutant and wild-type A. tumefaciens strains. Opine analysis and southern hybridization experiments have been used to confirm the transformation results. The chief advantages of this method are the ability to obtain large numbers of independent, axenic, clonal transformants in only 3-4 weeks. In addition, this procedure has been adapted to permit 1) rapid screening (48 hr. after transformation) for opine production by protoplasts, and 2) selection of transformants which contain avirulent T-DNA's.

1170 DNA TRANSFER TO PLANT CELLS USING THE TI-PLASMID VECTOR, Robert B. Simpson, Linda Margossian, Marcella Lillis and Thomas D. McKnight, Molecular Biology Group, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568

Current data suggests that there are sites (termini sequences) at the ends of the T-region portion of the Ti-plasmid which direct the transfer and/or integration of this region in the nuclear plant genome (e.g. 1). We are testing this hypothesis by using the marker exchange technique (2) to insert a small DNA fragment containing a terminus sequence at several locations in and around the T-region. The extent of plasmid DNA transferred to plant cells by Agrobacteria containing these altered Ti-plasmids will be determined.

The marker exchange technique and Ti-plasmid vector are also being used to ascertain whether a series of plant and animal genes can be expressed in plant tumors. Examples include a pea gene encoding the small subunit of ribulose-1, 5-bisphosphate carboxylase (with A.R. Cashmore), the cauliflower mosaic virus genome (from S. Howell), fusions of the promoter region of the soybean small subunit with bacterial genes for resistance to either kanamycin or chloramphenicol (T. D. McKnight thesis, Univ. of Georgia, 1982) and the P-element of Drosophila (from G. Rubin), a transposable element apparently encoding its own transposase.

1. Simpson, R.B., O'Hara, P.J., Kwok, W., Montoya, A.M., Lichtenstein, C., Gordon, M.P. and Nester, E.W. (1982). Cell 29, 1005-1014.
2. Garfinkel, D., Simpson, R.B., Ream, L.W., White, F.F., Gordon, M.P. and Nester, E.W. (1981). Cell 27, 143-153.

Molecular Biology of Host/Pathogen Relationships

1171 VIRULENCE DETERMINANTS IN PLANT-PATHOGEN INTERACTIONS, Tsune Kosuge and Luca Comai, Department of Plant Pathology, University of California, Davis, CA 95616.

Symptoms expressed by diseased plants represent the host responses to processes initiated through invasion by a pathogen. Two terms define these processes: pathogenicity, a qualitative term which defines ability of an organism to cause a disease on its host; and virulence, a quantitative term which defines the severity or amount of disease (or symptoms) caused by the pathogen. Thus virulence of a pathogen is assessed by the intensity of the disease reaction of the plant. By classical genetic approaches, pathogens can be shown to possess one or more virulence genes; however, the products of most such genes have not been identified.

In some cases expression of virulence can be associated with a pathway for synthesis of a secondary product by the pathogen. Therefore, concepts of metabolic regulation can be extended to control of expression of virulence in the pathogen. Since control of metabolism also affects fitness and competitiveness of a microorganism, the molecular basis for expression of virulence and fitness can be related to metabolic regulation in a pathogen.

An example of such a system is Pseudomonas savastanoi which causes tumors on oleander and olive. Virulence of this bacterium for its hosts is conferred by capacity for indoleacetic acid (IAA) synthesis (1). Genes for IAA synthesis are plasmid-borne in oleander isolates of the pathogen. Experiments on cloning of the gene (iaaM) which encodes tryptophan monooxygenase, the first enzyme concerned with conversion of tryptophan to IAA have shown that iaaM is a virulence gene and tryptophan monooxygenase, its product, is essential for virulence in the pathogen. The IAA genes may occur on different versions of the IAA-plasmid (pIAA) among oleander strains; in olive strains, the IAA genes are on the chromosome. Mutants have been isolated in which virulence can be attenuated by a) loss of pIAA from curing, b) inactivation of iaaM through a natural insertion element (IS-PS1), or c) from deletions in the monooxygenase gene. Other factors such as tryptophan pool size also affect virulence since mutants with relaxed control of tryptophan synthesis produce more IAA and therefore tend to be more virulent than parental types. Exceptional capacity for IAA synthesis, however, does not necessarily lead to hypervirulence in nature since fitness of such mutants is reduced. The concepts being developed from this plant-pathogen system may be applicable to other host-pathogen interactions.

Comai, L., and T. Kosuge. 1982. Cloning and characterization of iaaM, a virulence determinant of Pseudomonas savastanoi. J. Bacteriology 149:40-46.

1172 MOLECULAR GENETICS OF FUNGAL SPORE FORMATION, William E. Timberlake, David I. Gwynne, Bruce L. Miller, Karen Miller and Charles R. Zimmermann, Department of Plant Pathology, University of California, Davis, CA 95616

The spread of pathogenic fungi from one plant part, plant or geographical location to another is commonly mediated by their differentiated reproductive cells, the spores. Control of plant diseases often depends on preventing or reducing the production, dissemination or germination of fungal spores through cultural practices, or on inactivating dormant or germinating spores using physical or chemical methods. Even though fungal spores play a central role in the development of plant disease, little is known about the biochemical and genetic processes that operate to control the onset of sporulation, to coordinate the activities of the many genes that are expressed during spore differentiation, to establish and maintain spore dormancy, and to reinitiate somatic growth at an appropriate time. An understanding of these processes could provide important clues to novel methods for controlling the spread of this class of plant pathogens.

In many agriculturally important fungi, the predominant form of reproduction involves the formation of aerially dispersed, asexual spores, called conidia, on specialized reproductive structures, termed conidiophores. We have shown that conidiation in the experimentally advantageous Ascomycete Aspergillus nidulans is characterized by the coordinated expression of numerous stage- and cell-specific genes (1). Many of these genes have been cloned (2) while others have been defined mutationally and mapped on the Aspergillus chromosomes (3). Thus, it is possible to investigate the regulation and functional significance of the many genes whose expression appears to be required for the normal formation and biological activity of fungal spores, using a combination of molecular and genetic techniques.

We have found that the spore-specific genes of Aspergillus often occur in tightly linked clusters (4), an organizational pattern that may be related to the mechanisms controlling their expression. To investigate this possibility, we have characterized the organization and expression of one gene cluster, called SpoC1. The SpoC1 cluster extends over more than 40 kb of DNA and contains at least 13 discrete genes that are expressed preferentially in developing spores. Developmental regulation is very strong for genes near the center of the cluster but weakens near the boundaries. This and other characteristics of the region suggest that a chromatin level control mechanism may regulate gene expression.

1. Timberlake, W.E. (1980) Dev. Biol. 78, 497. 2. Zimmermann, C.R., et al. (1980) Cell 21, 709. 3. Clutterbuck, A.J. (1969) Genetics 63, 317. 4. Orr, W.C. and Timberlake, W.E. (1982) Proc. Natl. Acad. Sci. USA 79, 5976.

1173 ON THE ROLE OF BIOLOGICALLY ACTIVE CARBOHYDRATES IN PLANT-MICROBE INTERACTIONS, Peter Albersheim, Alan C. Darvill, Janice K. Sharp, Keith R. Davis, Eugene A. Nothnagel, Stephen C. Fry, Barbara S. Valent and Michael McNeil, Department of Chemistry, University of Colorado, Boulder, Colorado 80309

Plants utilize a variety of mechanisms to resist disease; complex carbohydrates have been shown to play key regulatory roles in at least some of these mechanisms. One complex carbohydrate possessing such regulatory properties is an oligosaccharide present in the cell walls of some fungi. Nanogram levels of this oligosaccharide will elicit plant cells to accumulate antibiotics called phytoalexins, the synthesis of which appears to be a general defense mechanism against potential pathogens. Structural characterization of this oligosaccharide by h.p.l.c., g.l.c., g.l.c.-m.s., f.a.b.-m.s., and n.m.r. has established that it is a specific beta-linked heptaglucoside. Structurally similar heptaglucosides that are inactive as elicitors have also been characterized. Structure and function relationships of the heptaglucosides will be discussed.

Potential plant pathogens can also elicit the accumulation of phytoalexins by causing the release of an endogenous elicitor that has been shown to be a fragment of a plant cell wall polysaccharide. The endogenous elicitor has been structurally characterized as an alpha-1,4-dodeca-D-galacturonide. This elicitor has been isolated from partially hydrolyzed soybean cell walls and from citrus pectin and characterized by g.l.c., g.l.c.-m.s., f.a.b.-m.s. and n.m.r. The endogenous elicitor can be released during infection by a plant enzyme or by enzymes secreted by the infecting pathogen.

Another important mechanism by which plants resist microbial invasion is the hypersensitive response. This response, which involves the death of the first plant cell(s) contacted by an invading microorganism, inhibits the growth of the invading microorganism. Results will be presented that suggest plant cell wall fragments may be responsible for initiating the hypersensitive response.

These results, together with other observations indicating that oligosaccharides of plant cell wall origin can also function as regulatory molecules in the control of growth and differentiation of plant cells, have led to the hypothesis that such complex carbohydrate regulatory molecules have an important role in normal plant growth and development as well as in plant-microbe interactions.

Supported by grants from the Dept. of Energy (#DE-AC-76ER01426) and The Rockefeller Foundation (#RF 78035).

Molecular Biology of Nitrogen Fixation

1174 REGULATION OF INDUCTION OF NODULINS AND LEGHEMOGLOBINS DURING ROOT NODULE DEVELOPMENT IN SOYBEAN Desh Pal S. Verma, Forrest Fuller, Jong Lee, Shrikant Purohit, and Ben Sutton, Plant Molecular Biology Laboratories, Department of Biology, McGill University, Montreal, Canada H3A 1B1

Nodulins and leghemoglobins are two major groups of host gene products that are found in root nodules of leguminous plants. It has been shown (1) that the induction of leghemoglobin occurs prior to and is independent of the ability of nodules in reducing dinitrogen. Using a kinetically purified cDNA probe for nodule-specific sequences (devoid of leghemoglobin), it was found (2) that the nodule specific sequences are also induced prior to the commencement of nitrogen fixation activity and represent a middle abundant fraction of RNA in nodules, indicating that these sequences may play an important role in the establishment of symbiotic state between the plant and Rhizobium. We have cloned some of these sequences and have shown, by hybrid-released translation followed by immunoprecipitation with antiserum against nodule protein, that they encode for nodule-specific proteins, nodulins (3). Southern-blot hybridizations with genomic DNA from soybean indicated that these sequences are represented in the plant genome as a low copy number in comparison to leghemoglobins which are present as a small family of closely related sequences. Four of the latter genes are found to be linked, in the same orientation, and are flanked by genes that are more abundantly transcribed in root tissues. One of these genes is also transcribed in leaf. The most abundant nodulin sequence represents 6.5% of

the total polysomalmRNA in nodules and encode for a 44,000 MW polypeptide. Other sequences range from 1.1 to 0.6% of the RNA. The level of induction of these sequences in ineffective nodules formed by mutant strains of Rhizobium japonicum, and attempts to induce them without infection by bacteria, will be discussed.

(1) Verma, D.P.S., Haugland, R., Brisson, N., Legocki, R. and Lacroix, L. (1981) Biochem. Biophys. Acta. 653: 98.
(2) Verma, D.P.S., Legocki, R. and Auger, S. (1981) In: Current Perspectives in Nitrogen Fixation, eds. A.H. Gibson and W.E. Newton. Aust. Acad. Sci. p. 205.
(3) Fuller, F., Künstner, P., Nguyen, T. and Verma, D.P.S. (1982) Submitted for publication.

This study was supported by grants from the Natural Sciences and Engineering Council of Canada.

1175 SYMBIOTICALLY IMPORTANT GENES OF RHIZOBIUM MELILOTI

Gary Ditta, David Corbin, Sally Leong, Leslie Barran and Donald R. Helinski, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Symbiotic nitrogen fixation by Rhizobium species involves a complex developmental interaction between plant and bacteria. To investigate the molecular details of this process we have isolated symbiotically essential Rhizobium meliloti DNA sequences from a previously constructed gene bank (1). Of particular interest is a 14-16 kilobase (kb) stretch of DNA, shown by transposon analysis to represent a cluster of nitrogen fixation (nif) genes surrounded on either side by at least 10 kb of symbiotically nonessential DNA. In vivo, these genes are located on a large endogenous plasmid (>500 kb) characteristic of R. meliloti strains and referred to as a megaplasmid (2).

S1 nuclease analysis of nodule RNA has revealed two centrally located promoters which control transcription from the nif region. Promoter 1 (P1) controls the production of a 5.6 kb transcript encoding nitrogenase that is sufficiently abundant to be visualized by Northern analysis. Promoter 2 (P2) is located 1.9 kb upstream from P1 and controls the production of a transcript that is at least 3-4 kb in length and is synthesized in a direction opposite to the nitrogenase transcript.

Another symbiotically essential gene, that encoding δ-aminolevulinic acid synthetase (δ-ALAS), has been isolated from the gene bank (3). δ-ALAS is the first enzyme in the heme biosynthetic pathway. Because bacterial heme is an essential component of both cytochromes and leghemoglobin, mutants defective in δ-ALAS are auxotrophic for δ-aminolevulinic acid and have a symbiotically defective phenotype. In contrast to the nif genes, δ-ALAS is not located on the megaplasmid. Transcriptional regulation of δ-ALAS has been studied both during vegetative growth and during symbiosis.

1. Ditta, G., Stanfield, S., Corbin, D. and Helinski, D.R. (1980) Proc. Natl. Acad. Sci. USA 77, 7347-7351.
2. Rosenberg, C., Boistard, P., Denarie, J. and Casse-Delbart, F. (1981) Mol. Gen. Genet. 184, 326-333.
3. Leong, S., Ditta, G. and Helinski, D.R. (1982) J. Biol. Chem. 257, 8724-8730.

1176 IDENTIFICATION AND CHARACTERIZATION OF RHIZOBIUM MELILOTI SYMBIOTIC GENES

Frederick Ausubel, William Buikema, Venkatesan Sundaresan, Wynne Szeto and Lynn Zimmerman, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Rhizobium meliloti is the nitrogen-fixing symbiont of alfalfa (Medicago sativa). In the symbiosis, nitrogen fixation occurs in specialized differentiated structures called nodules which form on the roots. Major stages in the nodulation process include penetration of root hairs by Rhizobium, formation of a cellulose infection thread which transports the bacteria to the inner cortex of the root, proliferation of root cortical cells and the establishment of a nodule meristem, release of bacteria into nodule cells, and differentiation of bacteria into nitrogen fixing bacteroids. Recently, a cluster of R. meliloti symbiotic genes has been identified, which is located on a large indigenous R. meliloti plasmid (1-3). In particular, our laboratory has identified symbiotic genes which are essential for early (3,4) and late (5) stages in the symbiosis within a 90 kb region of the megaplasmid (3). Late genes include those which code for the polypeptides which comprise nitrogenase (nifH, nifD, nifK) (5). The nifH, nifD and nifK genes are adjacent and form an operon which is transcribed in the direction nifH to nifD to nifK (5). Klebsiella pneumoniae, a free-living nitrogen fixing species, also contains a nifHDK operon (6). In K. pneumoniae, the activity of the nifH operon is positively regulated by the product of the nifA gene (7). By constructing fusions between the K. pneumoniae and R. meliloti nifH promoters and the lacZ gene of Escherichia coli, we have shown that the K. pneumoniae nifA gene product can activate the R. meliloti nifH promoter, with almost

the same efficiency with which it activates the K. pneumoniae nifH promoter. Moreover, by using a site-directed transposon Tn5 mutagenesis procedure (8) we have identified a R. meliloti gene analogous to the K. pneumoniae nifA gene. The R. meliloti "nifA" gene is located approximately 5 kb upstream of the nifHDK operon and is essential for nifHDK expression.

1. Banfalvi, Z., Sakanyan, V., Koncz, C., Kiss, A., Dusha, I and Kondorosi, A. Mol. gen. Genet 184: 318-325 (1981).
2. Rosenberg, C., Boistard, P., Denarie, J. and Casse-Delbart, F. Mol. gen. Genet. 184: 326-333 (1981).
3. Buikema, W., Long, S., Brown, S., van den Bos, R. and Ausubel, F. J. Mol. Appl. Genet., in press (1982).
4. Long, S., Buikema, W. and Ausubel, F. Nature, in press (1982).
5. Ruvkun, G., Sundaresan, V. and Ausubel, F. Cell 29: 551-559 (1982).
6. Ausubel, F., Brown, S., de Bruijn, F., Ow, D., Riedel, G., Ruvkun, G. and Sundaresan, V. In Genetic Engineering (Setlow, J. and Hollaender, A., eds.), Plenum Press, New York, pp. 169-198 (1982).
7. Buchanan-Wollaston, V., Cannon, M., Beynon, J. and Cannon, F. Nature 294: 776-778 (1981).
8. Ruvkun, G. and Ausubel, F. Nature 289: 85-88 (1981).

1177 ANALYSIS OF SYMBIOTIC NITROGEN FIXATION GENES CARRIED BY RHIZOBIUM MELILOTI PLASMID. A. Kondorosi[1,2], É. Kondorosi[1,2], Z. Banfalvi[1], W.J. Broughton[2], C.E. Pankhurst[2], and G.S. Randhawa[1], [1]Biological Research Center, Hungarian Academy of Sciences, Box 521, Szeged H-6701, Hungary and [2]Max-Planck-Institut für Züchtungsforschung, D-5000 Köln,FRG.

Previous genetic studies on mutants of R.meliloti strain 41, defective either in nodulation (Nod^-) or in nitrogen fixation (Fix^-), revealed that some fix genes were located on the bacterial chromosome (1). On the other hand, using various molecular-genetic techniques, some nod, nif, and fix genes were found on a large indigenous plasmid (pRme41b). Plasmid pRme41b was made susceptible to mobilization with the P-1 type plasmid pJB3JI by inserting the mobilization region of RP4 into it. When pRme41b was transferred into nod-nif deletion mutants, both Nod^- and Fix^+ phentypes were restored. The plasmid was introduced in other Rhizobium species and also in Agrobacterium tumefaciens. A.tumefaciens carrying pRme41b formed small,ineffective nodules on M.sativa, indicating that at least the early steps of nodulation are coded by pRme41b (2,3,4).

R plasmids carrying segments of the R.meliloti chromosome or plasmids have been isolated using in vivo procedures. Some R primes carrying a minimum of 120 kb piece of pRme41b were shown to carry both nif and nod gene clusters, supporting our earlier suggestions that nod and nif genes are located closely on pRme41b (2). With the help of a R.meliloti gene library, made in cosmid vehicle pJB8, the physical map of this region has been determined. Using these cosmid clones, symbiotic mutants carrying either insertions or deletions in the nod-nif region have been identified. Intra- and interspecies complementation of these mutants suggested the nod-fix-nod-nif-fix gene (set) order. Hybridization of ^{32}P-RNA from vegetatively grown bacteria and from N_2-fixing nodules to R primes and cosmid clones indicated that only a small region including the nif structural genes, was actively and specifically transcribed in the nodules in contrast to hybridizations with vegetatively grown bacterial RNA. Using the nodule RNA probe,further DNA sequences, expressed in the nodules, were fished out from the R.meliloti gene library.

(1) Forrai, T., Vincze, E., Banfalvi, Z., Randhawa, G.S., Kiss, G.B., and Kondorosi, A. (1982) J. Bacteriol. (in press)
(2) Banfalvi, Z., Sakanyan, V., Koncz, C., Kiss, A., Dusha, I., and Kondorosi, A. (1981) Mol. Gen. Genet. 184, 318-325
(3) Török, I. and Kondorosi, A. (1981) Nucl. Acids Res. 9, 5711-5723
(4) Kondorosi, A., Kondorosi, E., Pankhurst, C.E., Broughton, W.J., Banfalvi, Z. (1982) Mol. Gen. Genet. (in press)

Genome Rearrangements

1178 CHROMOSOME AND HETEROCHROMATIN REARRANGEMENTS IN MORPHOGENIC TISSUE CULTURES, Alan R. Gould, Plant Genetics Department, Pfizer Central Research, Groton, CT 06340

Giemsa C-banding studies have suggested that heterochromatin may be preferentially amplified and/or conserved in both normal and tumour-derived plant tissue cultures of Brachycome dichromosomatica (2n=4) and Crepis capillaris (2n=6). Recent cytological investigations on cell cultures of B. dichromosomatica demonstate that ring and multiconstrictional chromosomes can result from breakage-fusion-bridge cycles in suspension cultures. These and other gross karyotypic changes can generate pseudodiploids and increases in the proportion of heterochromatin. Roots regenerated from chromosomally unstable callus contain several different karyotypes in the same meristem. This suggests that these gross rearrangements of the genome do not upset multicellular morphogenic interaction-at least for the process of rhizogenesis. In contrast, long term tissue maintained as differentiated leaf masses is stably diploid. These data have important implications for an understanding of the genetic variability seen in plants regenerated from culture.

Genomic Manipulation of Bacteria to Aid Plant Productivity

1179 ANALYSIS OF MUTANTS OF RHIZOBIUM JAPONICUM ALTERED IN HYDROGEN OXIDATION ACTIVITY AND NITROGEN FIXATION, Maier, R.J., Merberg, D., Moshiri, F. and Stults, L.Q., Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

The presence of an H_2 oxidation system in Rhizobium japonicum is beneficial to nitrogen fixation by soybean plants. A large number of H_2 uptake negative (Hup^-) mutants of R. japonicum have been isolated as described (1). The mutant isolation procedure is based upon the inability of mutants to grow in an H_2 and CO_2-containing atmosphere. The mutants that were Hup^- in free-living culture were tested for H_2 uptake ability symbiotically as bacteroids from soybean root nodules. Of 78 free-living Hup^- mutants tested, most (56 strains) had no or very little H_2 uptake activity as bacteroids. In this survey we also found two mutant strains that produced nodules incapable of nitrogen fixation, in addition to expressing the Hup^- phenotype. One of these mutants (SR143) formed green nodules, and the nodules contained low levels of leghemoglobin. The other Nif^- Hup^- mutant (SR139) produced pink nodules, and the nodule leghemoglobin concentration was similar to wild type nodules. Revertants capable of growth in H_2/CO_2 were easily obtained from both types of Nif^- Hup^- mutants. All revertants tested expressed both H_2 uptake activity and nitrogenase activity, indicating the phenotype of the mutants is due to a single lesion. Strain SR139 also lacked methylene blue dependent H_2 uptake activity. Attempts to complement crude bacteroid extracts of strain SR139 for nitrogenase activity by adding extracts of characterized Azotobacter vinelandii Nif^- mutants were unsuccessful. These results indicate that this mutant (SR139) lacks activity for three iron-sulfur proteins: hydrogenase, and both component proteins of nitrogenase.

We previously reported the isolation of mutants of R. japonicum that were hypersensitive to oxygen mediated repression of the H_2 uptake system (2). We have now isolated mutants that are relieved of O_2 repression. These mutants express H_2 uptake activity when derepressed in 20% partial pressure oxygen, whereas the parent strain has no activity under the same conditions. As bacteroids from soybean nodules, these mutants also have greater (2-3 fold) H_2 uptake activity than the parent strain.

1) Maier, R.J. 1981. J. Bacteriol. 145: 533-540.
2) Maier, R.J. and D.M. Merberg. 1982. J. Bacteriol. 150: 161-167.

1180 NITROGEN FIXING CORN-BACTERIA ASSOCIATION, Winston J. Brill, Stephen W. Ela, Craig R. Lending, Department of Bacteriology, University of Wisconsin, Madison, WI 53706

Through a screening and breeding program, we have been able to derive maize plants that will support bacterial nitrogen fixation on the plant roots. The ability to support such associative nitrogen fixation can be bred into modern midwestern United States cornbelt lines without demonstrating any obvious defect in such plants. With the use of ^{15}N, we demonstrated that the N_2 fixed by the associative bacteria is transferred to the plant. Conditions for immediate and linear nitrogen fixation by the bacteria-plant association have been demonstrated without any requirement for pre-incubation. In order to examine the association in more detail, techniques for demonstrating the relationship of the bacteria to the plant are being utilized. Several approaches are being used to maintain this associative nitrogen fixation under field conditions.

1181 SEQUENCES HOMOLOGOUS TO THE T DNA REGION ARE PRESENT IN BACTERIA WHICH INTERACT WITH PLANTS, A.A. Szalay and R.G. Hadley, Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

DNA sequences homologous to the T DNA region of the octopine-type Ti plasmid from Agrobacterium tumefaciens are present in different Rhizobium species. Plasmid DNA from each of two R. leguminosarum, two R. meliloti, and four slow-growing Rhizobium strains examined contain restriction endonuclease fragments that hybridize with the T DNA region, or with DNA sequences at or near the adjacent Ti plasmid transfer (tra) region. Four different BamHI fragments that contain homology to the T DNA region were cloned from R. leguminosarum 300 plasmid DNA. Cloned fragments of 5.9 kb and 10.3 kb hybridize to each other and are homologous to sequences which map at the right boundary region (EcoRI fragment 24) of the "core" T DNA. Ti plasmid sequences homologous to those present in cloned fragments of 10.9 kb and 2.0 kb map in adjacent fragments near the tra genes, approximately 10 kb to the right of the "core" T DNA.

A large fraction of the bacteria isolated from the inner root surface of rice plants are capable of fixing nitrogen when supplied with low levels of fixed nitrogen. DNA was isolated from 3 of these nitrogen fixing "Pseudomonas-like" strains; H3, KLH76, and LH4. Agarose gel electrophoresis of restriction endonuclease digested plasmid DNA indicates that each of these strains contain 1 or more large plasmids. The nitrogenase structural gene region, nifKDH, from Klebsiella pneumoniae hybridizes to the DNA of each strain and distinct hybridization was observed to the plasmid DNA fraction of KLH76. In addition, each strain contains DNA sequences that are homologous to the T DNA region of the octopine-type Ti plasmid from Agrobacterium tumefaciens.

When plasmid DNA obtained from 6 plant pathogens was hybridized with the T DNA region (described above) intense hybridization was detected. A possible explanation for the presence of the homology to the T DNA region is that these microorganisms contain an important common function for the inteaction with their hosts.

Nuclear Genes: Structure, Function and Expression I

1182 SEED PROTEIN GENE STRUCTURE AND EXPRESSION IN NORMAL AND MUTANT SOYBEAN LINES. Robert B. Goldberg and Diane Jofuku, Department of Biology, UCLA, Los Angeles, CA 90024, Lila O. Vodkin, Seed Research Laboratory, USDA, Beltsville, MD 20705

A number of soybean inbred lines have been identified which are defective in the expression of various seed protein genes. In an attempt to uncover DNA sequences which might be important for the control of seed protein gene expression, we have investigated seed protein gene structure and function is two mutant lines, one which lacks seed lectin (Le^- line) and one which is devoid of Kunitz trypsin inhibitor activity (Kti^- line).

In normal, Le^+Kti^+ lines, lectin and Kunitz trypsin inhibitor gene expression is under strict developmental control (1,2). The primary control level appears to be transcriptional (2). Recent studies using a runoff transcription system with isolated nuclei have indicated, however, that lectin and Kunitz trypsin inhibitor genes are not regulated coordinately in the formal sense (L. Walling and R.B. Goldberg, unpublished data). Both liquid (1,2) and filter hybridization studies showed that lectin and Kunitz trypsin inhibitor proteins are encoded by small gene families containing approximately two and five genes each, respectively. R-loop studies failed to reveal detectable introns in any lectin or Kunitz trypsin inhibitor gene investigated to date.

Mutant Le^- and Kti^- lines were shown to contain the same number of lectin and Kunitz trypsin inhibitor genes as their normal, Le^+ and Kti^+ counterparts. In contrast, Le^- and Kti^- embryos were found to possess reduced amounts of lectin and Kunitz trypsin inhibitor mRNAs respectively. These reduced mRNA levels correlate well with the extent of seed protein reduction in each line. For example, the Le^- line which has no detectable seed lectin, has <0.01% the normal level of lectin mRNA. On the other hand, the Kti^- line, which has significant Kunitz trypsin inhibitor cross-reacting material, has 10% of the Kti^+ Kunitz trypsin inhibitor mRNA level. These data suggest that each mutation is due to either a transcriptional or a post-transcriptional lesion.

A detailed study of lectin gene structure in Le^+ plants showed that one gene encodes seed lectin, while the other is a diverged gene of unknown function. In Le^- and Le^+ plants the latter gene region is identical; however, the Le^- seed lectin gene was shown to contain a 3.4 kb insertion element which resembles a transposable element. Preliminary studies failed to reveal any obvious differences in Kunitz trypsin inhibitor gene structure or organization in Kti^+ and Kti^- plants. Together, these data suggest that the Le^- phenotype is a result of an insertional inactivation of the seed lectin gene, and that a point mutation and/or a small chromosomal alteration may be responsible for the Kti^- mutation.

1. Goldberg et al. (1981), Dev. Biol. 83, 201. 2. Goldberg et al. (1981), Dev. Biol. 83, 218.

1183 DEVELOPMENTAL REGULATION OF TWO CATALASE GENES IN MAIZE, John C. Sorenson, Experimental Agricultural Sciences, The Upjohn Company, Kalamazoo, MI 49001

Maize catalase is a tetrameric enzyme with a subunit molecular weight of 56,000 daltons. There are three catalase structural genes in maize (Cat1, Cat2, and Cat3), which differ in their temporal and spatial expression. In the scutellum of the germinating seedling there is a developmental shift in the expression of catalase loci. Scutella from newly imbibed seeds contain a single catalase isozyme (the Cat1 homotetramer). By the second day of germination expression of the Cat2 locus can be observed. This locus continues to be expressed until the scutellum degenerates. There is a transition period of several days during which both genes are expressed simultaneously. Cat1 expression can no longer be detected in the scutellum after the fourth or fifth day of germination, although de novo synthesis of Cat1 polypeptides can be detected until that time. This differential expression is determined by differing rates of synthesis and degradation of the enzymes and by changing patterns of compartmentation during the developmental period in question. Catalase mRNAs have been isolated by polyribosome immunoprecipitation and have been used to identify cloned catalase gene sequences. These sequences are currently being used to characterize the transcriptional aspects of catalase regulation during early maize development.

1184 HORMONAL REGULATION OF STORAGE PROTEIN GENES IN BRASSICA NAPUS L., Martha L. Crouch and Karen M. Tenbarge, Department of Biology, Indiana University, Bloomington 47405

When immature embryos of Brassica napus are removed from the seed and grown on nutrient medium, they precociously germinate and do not exhibit the dramatic increase in storage protein synthesis associated with embryo maturation. If abscisic acid (ABA) is included in the culture medium, precocious germination is inhibited and the embryos accumulate storage proteins at a rate comparable to the in vivo rate (1). We are studying the regulation of storage protein synthesis at the molecular level, using ABA as a hormonal "switch" to modulate the rate of storage protein accumulation in cultured embryos.

Two storage proteins are synthesized during embryogeny in B. napus, a 12S glycoprotein and a 1.7S basic protein. The 12S "legumin-like" storage protein is composed of several polypeptides between 20,000 and 30,000 daltons. The 1.7S basic proteins are a family with molecular weights between 12,000 and 14,000 daltons. Each 1.7S protein consists of two subunits bound together by disulfide bridges (2).

Using antibodies for the storage proteins, we have shown that the in vitro translation products are much larger than the mature polypeptides. The precursor polypeptide for the 12S protein is 50,000 daltons, which is large enough to encode both the 20,000 and 30,000 dalton polypeptides of the mature protein. This result is not unexpected (3). The in vitro translation product of the 1.7S storage protein mRNA is a 21,000 dalton polypeptide.

The amounts of storage protein precursor polypeptides synthesized in vitro from total RNA are much greater for embryos grown with ABA in the medium. This suggests that regulation is at the level of mRNA accumulation. To study mRNA in more detail, we have constructed cDNA clones from embryo RNA, and have isolated specific storage protein cDNA sequences by differential screening.

The mRNA for the 12S precursor is 850 b, determined from Northern blots. We have sequenced an 800 bp clone (R. Ferl, unpublished data; A. Simon, unpublished data) and have confirmed that both subunits are encoded by one mRNA. The protein sequence will be discussed. We also have a full-length 1750 bp cDNA clone representing the 12S precursor, which has not yet been sequenced. These cDNA clones are being used as probes to determine the timecourse of ABA simulation of storage protein mRNA accumulation in embryos of various ages. Synthesis and turnover rates of these mRNAs are also being studied in the presence and absence of the hormone.

1) Crouch, M.L. and Sussex, I.M., Planta 153:64-74 (1981); 2) Lonnerdal, B. and Janson, J-C., Biochim. Biophys. Acta 278:175-183 (1972); 3) Croy, R.R.D., Lycett, G.W., Gatehouse, J.A., Yarwood, J.N., and Boulter, D., Nature 295:76-79 (1982).

From Test Tube to Farm

1185 SELECTIVE ALLELE INACTIVATION OR LOSS IN CULTURED CELERY CELLS, Thomas J. Orton Department of Vegetable Crops, University of California, Davis 95616.
Isogenic Pgm-2^{FS} plants were synthesized in two different cytoplasmic backgrounds. Callus tissues were initiated from petioles of these plants to study the relationship of chromosomal change to isozyme expression in vitro. Loss of PGM-2^{F} activity was observed in 24% of single cell clones from 12 month-old cultures and in 100% from 30 month-old cultures, and this change was not entirely due to differential selection. The resulting Pgm-2^{S} lines were stable in vitro and expression was identical in regenerates. No Pgm-2^{F} lines have yet been identified. Extensive karyological analyses have shown no correlation between chromosomal constitution and PGM-2 phenotype. SDH-1 phenotype was completely stable, but Sdh-1 is linked with Pgm-2 (39.2 map units). It is hypothized that maternally inherited insertion sequences were responsible for selective inactivation Pgm-2^{F}.

1186 INFECTIVITY STUDIES WITH CLONED VIROID cDNA, Dean E. Cress, Robert A. Owens, U.S. Department of Agriculture, Beltsville, Maryland, 20705
Viroids are infectious agents composed entirely of closed circular single-stranded RNA of low molecular weight ($1.1 \times 1.3 \times 10^5$ daltons). They replicate in a wide variety of plants, and are often associated with specific disease symptoms. Full-length double-stranded cDNA clones of potato spindle tuber viroid (PSTV) were constructed from two partial-length cDNA clones. The nucleotide sequence is identical to that predicted from the primary RNA sequence data of Gross et al. (Nature 273, 203-208 (1978)). Plasmid DNA as well as low molecular weight RNA was isolated from E. coli harboring several different viroid cDNA clones, and inoculated onto tomato seedlings. PSTV disease symptoms and viroid progeny were detected in some cases. Preliminary attempts to employ in vitro mutagenesis to identify regions of the viroid molecule involved in viroid-host interaction will be described.

Nuclear Genes: Structure, Function and Expression II

1187 ZEIN GENE STRUCTURE AND EXPRESSION IN MAIZE, B. A. Larkins, K. Pedersen, M. D. Marks, and D. R. Wilson, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Maize seed storage proteins are a group of alcohol-soluble proteins called zeins. When separated by 2-dimensional gel electrophoresis zeins can be resolved into several major groups of polypeptides with apparent mol wts of 22,000 and 19,000. There are also several minor polypeptides with apparent mol wts of 15,000 and 10,000. During development of normal maize endosperms these proteins are synthesized at 12 days after pollination and their synthesis continues until seed maturity. However, in certain mutant genotypes, such as opaque-2, zein synthesis is delayed by several days and it ceases midway through endosperm development. We have constructed and analyzed zein cDNA clones in order to investigate the structure, organization, and expression of zein genes during development of the normal and mutant genotypes.

Comparison of cDNA clones corresponding to different zein families showed that these sequences are sufficiently different that they do not cross-hybridize (1). Sequences encoding the Mr 22,000, Mr 19,000, and Mr 15,000 zeins can be discriminated at moderate (Tm -35°) criteria, and at more stringent criteria (Tm -20°) subgroups of different Mr 22,000 and Mr 19,000 genes can be distinguished. This has allowed us to determine the level of expression of different gene families during development of the normal and mutant genotypes, and has revealed quantitative and qualitative differences in gene transcription. The change in the levels of gene transcripts is not associated with gross alternations in the organization of the genes as determined by Southern blot hybridization. However, several qualitative differences in gene organization have been observed.

To better understand the factors regulating transcription of these genes we have isolated and characterized several zein genomic clones. Unlike many other eukaryotic genes zeins do not have intervening sequences (2). They nevertheless have typical "CCAT", "TATA", and "AATAAA" sequences as have been found in other eukaryotic genes. One unusual feature of these genes is the presence of a conserved repetitive sequence that encodes a short peptide of approximately 20 amino acids. This repeated peptide appears to play an important role in folding the protein into a rod shaped structure (3).

(1) Marks, M. D. and B. A. Larkins. 1982. J. Biol. Chem. 257, 9976-9983.
(2) Pedersen, K., J. Devereux, D. R. Wilson, E. Sheldon, and B. A. Larkins. 1982. Cell 29, 1015-1026.
(3) Argos, P., K. Pedersen, M. D. Marks, and B. A. Larkins. 1982. J. Biol. Chem. 257, 9984-9990.

1188 THE ORGANIZATION OF THE SOYBEAN LEGHEMOGLOBIN GENES, K. A. Marcker, E. Ø. Jensen, K. Paludan and O. Wiborg, Department of Molecular Biology, University of Aarhus, DK-8000 Århus C, Denmark

Soybean nodules contain at least four different leghemoglobins (Lbs) called Lba, c_1, c_2 and c_3, respectively. The difference among the various Lb species is minor corresponding to 6-10 amino acids only. Southern blotting experiments of EcoR1 digested soybean DNA using a Lb cDNA probe revealed the presence of seven hybridizing fragments. These DNA fragments have all been cloned and further characterized. Thus DNA sequence analysis have been completed on five Lb genes and the sequences corresponding to Lba, Lbc_1, Lbc_2 and Lbc_3 have been determined (1,2,3). The fifth DNA sequence corresponds to a Lb gene with unusual properties. Thus the coding sequence of this gene shows only 85% homology with the four Lb genes, in contrast to the 95% homology found for the other Lb genes. Attempts to identify an mRNA for the unusual Lb gene have failed. Thus it is quite possible that this particular Lb gene is a pseudo gene. In agreement with this hypothesis the 5' flanking sequence of this gene contains mutations in two areas considered to be critical for transcription.

Seven Lb genes are located within a region of approximately 90 kb on the soybean chromosome and within this region the order of the Lb genes has been determined. The Lba, Lbc_1, ψLbc and Lbc_3 genes are very closely linked, the distance between these genes being of the order 2.5-3 kb, while the distance between the remaining Lb genes is considerably more (15-25 kb). Within the Lb gene region several types of repetitive sequences have been located.

Active eukaryotic genes are hypomethylated when compared to the corresponding inactive genes. This is also the case for the Lb genes. Thus the Lb gene region in young nodules is hypomethylated when compared to the corresponding region in seedlings and old nodules. The differences in the methylation pattern detected so far have been allocated to a 5 kb region which is situated between the Lbc_3 and the Lbc_2 gene.

1) J.J. Hyldig-Nielsen, E.Ø. Jensen, K. Paludan, O. Wiborg, R. Garrett, P. Jørgensen and K.A. Marcker. Nucl. Acids Res. 10, 689 (1982).
2) O. Wiborg, J.J. Hyldig-Nielsen, E.Ø. Jensen, K. Paludan and K.A. Marcker. Nucl. Acids Res. 10, 3487 (1982).
3) N. Brisson and D.P.S. Verma. Proc. Natl. Acad. Sci. USA 79, 4055 (1982).

1189 CONTROL OF TUBER PROTEIN SYNTHESIS IN POTATO, William D. Park, David Hannapel, Lisa Lee, Greg Mignery, and Edilson Paiva, Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

Among the major tuber proteins of potato is a family of glycoproteins with apparent molecular weights of approximately 40,000. These proteins have been given the trivial name patatin. Patatin is heterogeneous on both SDS and native gels, but all the isoforms of patatin are immunologically identical and they have homologous NH_2-terminal sequences (1).

Patatin makes up approximately 40% of the total soluble protein in potato tubers regardless of whether they are formed on underground stolons, in tissue culture or from axillary buds. Under normal conditions patatin makes up less than 0.1% of the protein in other tissues, even in uninduced stolon tips (2).

By manipulating source sink relationships, patatin can be induced to accumulate in other tissues (2). This accumulation can occur in any stem or petiole and will occur even under conditions which are normally noninductive for tuberization. Stems and petioles can accumulate large amounts of patatin without any obvious tuber-like swelling. However, in all cases tissues which contain large amounts of patatin also contain the other major tuber proteins as well as large amounts of starch. For example, by removing the axillary buds of single-leaf stem cuttings we have been able to produce morphologically normal leaf petioles which contain as much starch and patatin as mature underground tubers.

This data suggest that the biochemical differentiation of somatic storage tissue in potato can occur in any stem or stem-like tissue and can occur without the cell proliferation and morphological changes normally associated with tuberization. Not only are these two components of the process of tuberization separable, they appear to be regulated very differently.

(1) Park, W.D., C. Blackwood, G. Mignery, M.A. Hermodson, and R.M. Lister. Analysis of the heterogeneity of the Mr 40,000 tuber glycoprotein of potatoes by immunological methods and by NH_2-terminal sequence analysis. Plant Physiol. (in press).

(2) Paiva, E., R.M. Lister, and W.D. Park. The major tuber proteins of potato can be induced to accumulate in stems and petioles. Plant Physiol. (in press).

1190 CONSEQUENCES OF INSERTIONS IN THE Adh1 GENE OF MAIZE, Michael Freeling, Judith N. Strommer, Sarah Hake, Mitrick A. Johns, Mary Alleman and William C. Taylor, Department of Genetics, University of California, Berkeley, CA 94720; Jeffrey Bennetzen and Jean Swanson, International Plant Research Institute, San Carlos, CA 94070.

The genetics and protein-level biology of the alcohol dehydrogenase-1 (Adh1) gene in maize have been reviewed (1). Because Adh1 is one of a small group of genes that elevate mRNA levels during anaerobiosis, cDNA cloning has generated probes for Adh1 (2) as well as for other anaerobic messages. The 11.7 kb BamH1 fragment containing Adh1-S has been cloned; based on reverse transcriptase mapping and S1 protection experiments, Adh1-S has approximately 5 introns, with the 1.65 kb of RNA-coding sequences spread over about 4 kb of chromosome. The definitive structure awaits a complete sequence.

We are studying 5 independent insertions that either reduce or obliterate ADH1 polypeptide synthesis. Three are insertions of the same 1.35 kb element, Mu1, within a large 5' intron of Adh1. Although the exact points of insertion differ, all reduce ADH1 expression to 40% control levels. We suspect that RNA processing is involved. Several derivative alleles that differ in mRNA levels, reversion frequency or morphological phenotype have been studied; we have not yet found evidence for physical movement of Mu1 from its initial point of insertion. A 13 kb genomic clone including Adh1 plus Mu1 has allowed us to evaluate the use of Mu1 as a general tool for cloning maize genes and to describe Mu1 as a sequence. Only part of these results are published (3 and references therein).

Two additional insertions will be discussed: one is a 1.5-2 kb, transcribed (putative) Ds element and the other followed infection with the RNA virus, Barley Stripe Mosaic. All 5 insertions lie within the Adh1 transcriptional unit. We do have other mutant alleles that clearly change the organ-specificity of Adh1 expression; these have not been mapped.

(1) Freeling, M. and J.A. Birchler, 1981. Genetic Engineering, Principles and Methods, Vol. 3. (Setlow, J.K. and A. Hollaender, Eds.). Plenum, NY, USA. pp.223-264.
(2) Gerlach, W.L., A.J. Pryor, E.S. Dennis, R.J. Ferl, M.M. Sachs and W.J. Peacock, 1982. Proc. Natl. Acad. Sci. USA 79:2981-2985.
(3) Strommer, J.N., S. Hake, J. Bennetzen, W.C. Taylor and M. Freeling, 1982. Nature, in press.

Nuclear Genes: Structure, Function and Expression III

1191 MULTIGENE AND NON CODING DNA FAMILIES; STRUCTURE, EVOLUTION AND EXPRESSION, Richard B. Flavell, Plant Breeding Institute, Trumpington, Cambridge, U.K.

Different members of a repeated DNA family often vary in structure and expression. Comparisons between different members of a family, within and between species, illustrate how sequence variation accumulates in the family and how the family has evolved. In the ribosomal RNA gene family of wheat and related cereals, for example, many variants for the non-transcribed spacer are present but genes within the same tandem array are closely related and clearly evolve in concert, not independently. The number of genes in an array varies from plant to plant. Different members of the family have their cytosines methylated in CCGG sequences to different extents. The extent of methylation is genetically controlled. Not all members of the gene family are expressed. Whether a particular gene is expressed depends upon other genes. These and other examples of heterogeneity within families of repeated sequences will be discussed.

1192 GENE EXPRESSION IN RESPONSE TO HEAT SHOCK, Joe L. Key, Fritz Schöffl, Ewa Ceglarz, Ron T. Nagao and C. Y. Lin, Department of Botany, University of Georgia, Athens, Georgia 30602.

When soybean seedlings are subjected to a shift in growth temperature from 30° to about 40° (heat shock or hs), there is a rapid decline in translational activity followed by a gradual increase in polyribosome level. Associated with these changes in translational activity is the loss in capacity to synthesize 30° proteins and the appearance of a new set of proteins (hsp's). This change in pattern of protein synthesis relates to an apparent loss in translational efficiency of most 30° mRNAs coupled with the production and efficient translation of a new set of mRNAs (hs mRNAs).

The hs mRNAs are detected using cloned cDNA probes within 3 to 5 min of the temperature shift and rapidly accumulate, in the case of several of the hs-induced RNAs up to 20,000 or so copies per cell. A high proportion of the 30° mRNAs persist during a 2 hr hs at 40°, eventhough they are weakly translated. The hs mRNAs are detected at about 35°, increase in concentration up to 40°-41°, and accumulate to much lower levels at higher hs temperatures. Upon return to 30°, the hs mRNAs deplete with a ½ time of about 1 hr.

Hybrid selection-translation and 2D gel analysis of the translation products have permitted the identification of some 20 to 25 hsp's relative to their respective cloned cDNAs. There is considerable sequence homology among several identifiable groups of the cloned cDNAs and their respective proteins based on cross hybridization of the different cloned sequences and hybrid selection of mRNAs for several different hsp's by a single cloned cDNA.

Southern hybridization analyses have been performed using the cloned cDNAs as probes; the results are suggestive of hs genes occurring as small families of related sequences down to essentially single copy genes for a given protein, in agreement with hybrid selection-translation data. Genomic clones have been isolated from a λ 1059 library for several of the hs genes and are currently being characterized.

Several lines of evidence suggest that the physiological significance of the hs response relates to the development of thermal protection/tolerance in concert with the accumulation and localization of hsp's during hs. A "breakpoint" temperature (40° for soybean seedlings) hs for 1-2 hr permits hsp and hs mRNA synthesis and accumulation at otherwise non-permissive temperatures (e.g. 45° and 47.5°).

We have made comparative analyses of the hs response in several plant systems (eg., pea, millet, sunflower, cotton, wheat, corn) and all respond similarly to soybean. The temperature (eg. "breakpoint") of the response and the exact pattern of proteins varies among the different plants. Some soybean cloned cDNAs are sufficiently homologous to hs mRNAs of the other plants to give strong and specific hybridization on 'northern' analyses; other cloned cDNAs do not show cross species hybridization.

1193 STRUCTURE AND REGULATION OF EXPRESSION OF α-AMYLASE mRNAs IN BARLEY ALEURONE LAYERS. Peter M. Chandler J.A. Zwar, T.J.V. Higgins J.V. Jacobsen, Division of Plant Industry, CSIRO P.O. Box 1600, Canberra 2601, Australia.

α-amylase is synthesized in the aleurone layer of germinating barley grains in response to gibberellin - a growth regulator originating in the embryo. During embryo growth and development, α-amylase is secreted by the aleurone cells into the endosperm where it assists in hydrolysis of starch reserves, providing carbohydrate for the growing seedling. Our interest lies in the mechanism by which gibberellin stimulates synthesis of α-amylase, and in the inhibitory effect of another growth regulator, abscisic acid (ABA) on this response.

cDNA clones were constructed from the poly A^+ RNA of isolated aleurone layers which had been incubated in 10^{-6} M gibberellic acid (GA_3) for 24 hr. (conditions under which α-amylase synthesis is induced). Clones representing mRNA sequences more abundant in GA_3-treated layers than in control layers were identified by colony hybridization, and amongst these, α-amylase clones were identified by hybrid release translation and immunochromatography.

In "Northern" hybridizations the α-amylase clones defined an mRNA species of approx. 1500 nucleotides. When aleurone layers were incubated in GA_3 (10^{-6}M) the abundance of this mRNA began to increase after 2 to 4 hr, reaching a maximal level of 50 to 100-fold over control layers (relative to ribosomal RNA) after 24 to 30 hr. The presence of ABA (2.5×10^{-5}M) in such incubations prevented the accumulation of α-amylase mRNA. These results are in accord with previous data based on in vitro translation (1) and suggest that the major effect of GA_3 and ABA in regulating synthesis of α-amylase occurs at the level of mRNA abundance.

Northern hybridizations using the α-amylase clones also revealed a high molecular weight RNA species containing sequences homologous to α-amylase mRNA. The possibility that this represents a precursor to the mature mRNA is currently under investigation.

Higgins T.J.V., Jacobsen J.V., Zwar J.A. (1982) Plant Molecular Biology, in press.

Organization and Expression of Mitochondrial Genomes

1194 PLASMID-LIKE DNAS ASSOCIATED WITH THE MAIZE MITOCHONDRIAL GENOME, C. S. Levings, III and R. R. Sederoff, Department of Genetics, North Carolina State University, Raleigh, NC 27650.

Plasmid-like DNAs are associated with fertile and cytoplasmic male sterile maize. The S-1 and S-2 species are found in cms-S while the R-1 and R-2 species are observed in some normal cytoplasms. It appears that S-1 resulted from a recombinational event between R-1 and R-2. Hybridization and nucleotide sequence data are shown to support the proposed recombinational event. The complete nucleotide sequence of S-2 is presented and analyzed for open reading frames, repeats and inverted repeats. S-1 and S-2 contain homologous regions; this homology is compared at the nucleotide sequence level.

The mtDNA of normal (fertile) maize contains integrated sequences which are homologous with the plasmid-like DNA species. Hybridization and sequencing data are shown which suggests that the integrated sequences are similar to R plasmids rather than the S. The R and S group of plasmid-like DNA contain inverted repeats of approximately 200 bp. Sequences homologous with the inverted repeats have been identified in the mtDNA of normal maize and verified by sequencing. The sequence of the inverted repeat has also been found in the mtDNA of other maize cytoplasms.

The cms-S cytoplasm of maize spontaneous reverts to male fertility at a high frequency. Correlated with this phenotypic change are the loss of the plasmid-like DNAs, S-1 and S-2, and rearrangements in the mtDNA which may be due to transpositional events (Levings, et al., 1980). We present hybridization and sequencing studies of these rearrangements.

Levings, C. S., III, B. D. Kim, D. R. Pring, M. F. Conde, R. J. Mans, J. R. Laughnan, and S. J. Gabay-Laughnan. 1980. Cytoplasmic reversion of cms-S in maize: Association with a transpositional event. Science 209:1021-1023.

1195 MITOCHONDRIAL GENES AND THEIR EXPRESSION IN HIGHER PLANTS, C.J. Leaver, E. Hack, A.J. Dawson, P.G. Isaac, Department of Botany, University of Edinburgh, Scotland.

Recent reports that the mitochondrial (mt) genome of higher plants is considerably larger and more variable in size (150-2500 kb)[1] than animal and fungal mt genomes raises several questions: 1. does plant mtDNA contain structural genes not found in other organisms, 2. what is the functional significance, if any, of the wide range in size of plant mtDNAs?

Despite differences in size, animal (16-17 kb) and yeast (68-75 kb) mt genomes both contain essentially the same genes which are organised in a strikingly different manner. These genes code for mt rRNAs and tRNAs and a small number of polypeptides (8-15) vital for the assembly of the functional inner membrane. In yeast these polypeptides are three of the seven subunits (I, II and III) of cytochrome c oxidase, the apocytochrome b subunit of the cytochrome bc_1 complex and subunits 6 and 9 of the ten subunit ATPase complex.

We are currently attempting to identify and map the protein coding genes and assign a functional role to the 20-30 polypeptides synthesised by plant mitochondria. The first approach we have used for the identification of specific genes on plant mtDNA exploits the fact that the sequences of many mt genes have been evolutionarily conserved. Defined yeast and bovine mt gene probes have been hybridised to restriction fragments of maize mtDNA[1]. The genes for subunits I and II of cytochrome oxidase and apocytochrome b have been cloned into bacterial plasmids and sequence analysis has revealed major differences in gene structure and codon usage compared to the homologous genes in other organisms.

The limitations to this approach are the availability of homologous gene probes and the possibility that plant mtDNA encodes additional genes. As an alternative approach to establishing the number and identity of polypeptides synthesised by plant mitochondria and by extrapolation encoded in mtDNA, we are analysing mitochondrial translation products by a variety of techniques, including: 1D and 2D gel electrophoresis, isolation of specific enzyme complexes, immunoprecipitation with antibodies against defined yeast polypeptides and peptide mapping of authentic and labelled mitochondrial polypeptides.[1] To date we have shown that most of the mitochondrial translation products are associated with the energy-transducing inner membrane and have identified cytochrome c oxidase subunits I and II, and the α-subunit and subunit 9 of the ATPase complex. The identification of the α-subunit of the ATPase complex as a mitochondrial translation product contrasts with the situation in other organisms where this polypeptide is encoded in the nuclear genome and synthesised on cytoplasmic ribosomes.

Progress in analysing mutations in the mitochondrial genomes of maize and sorghum which lead to the synthesis of variant polypeptides and the phenotypic expression of cytoplasmic male sterility will also be discussed.

1. C.J. Leaver & M.W. Gray (1982) Ann. Rev. Plant Phys. 33: 373-402.

1196 ORGANIZATION OF THE MITOCHONDRIAL DNA OF MAIZE, David M. Lonsdale, Christiane M.-R. Fauron & Tony P. Hodge, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K.

The organization of the mitochondrial DNA of maize is being studied using recombinant DNA techniques. Mitochondrial genome fragments 35-50 Kb in length, have been cloned into cosmid vectors. Analysis of these clones has allowed 650 Kb of restriction map to be constructed. Like the majority of other mitochondrial genomes, the maize genome has single copy genes for the large and small ribosomal RNAs[1]. In addition it also has a 5S ribosomal RNA gene[2] which is closely linked to the small ribosomal RNA gene and 16 Kb from the large ribosomal RNA gene[1]. Another unusual feature, is that the maize mitochondrial genome contains large repeated sequences[3]. These repeated sequences 10 Kb to 30 Kb in length have a reiteration factor of two. They occur in both inverted and tandem orientations and no evidence for frequent homologous recombination between these repeats has been found.

Restriction enzyme digestion of maize mitochondrial DNA results in a complex profile, some bands of which appear to be present in sub-molar amounts. These sub-molar fragments can in some cases be attributed to contaminating chloroplast and perhaps nuclear satellite sequences. However, sub-molar fragments arising from the mitochondrial genome have been found to be associated with the insertion or deletion of sequences at specific locations on the physical map.

A comparison of the restriction map of the mitochondrial genome from the normal fertile cytoplasm to the restriction maps of the cms-C, cms-S and cms-T genomes has identified regions in which sequence alterations have occurred. In one instance a deletion of 23 Kb has been mapped. One of the major features of these sequence alterations in the male sterile genomes is that they map in similar positions. This suggests that the present day cms cytoplasms arose from a common ancestor. Alternatively they could have arisen from three separate but similar events, either by mutation or from the introduction of a related cytoplasm.

1. Stern, D.B., Dyer, T.A. and Lonsdale, D.M. (1982) Nucl. Acids Res. 10, 3333-3340.
2. Leaver, C.S. and Harmey, M.H. (1976) Biochem. J. 157, 175-177.
3. Lonsdale, D.M., Thompson, R.D. and Hodge, T.P. (1981) Nucl. Acids Res. 9, 3657-3669.

1197 MAIZE MITOCHONDRIAL DNA STRUCTURE AND FUNCTION, Virginia Walbot, K.J. Newton, A. Maloney, P. Bedinger, T. Sandie, Department of Biological Sciences, Stanford University, Stanford, CA 94305. B.S. Masters, D. McCarty, W.W. Hauswirth, Department of Immunology and Medical Microbiology, University of Florida, Gainesville, FL 32610.

Total RNA prepared from mitochondria isolated from etiolated shoots has been analyzed on urea-acrylamide gels. There are approximately 17 large transcripts in addition to the 26S and 18S ribosomal RNAs. These transcripts have been individually eluted from gels and hybridized to members of a genomic library of B37N Bam HI fragments cloned in pBR322 to identify which plasmids contain transcribed sequences. Restriction maps have been prepared for each of the identified transcribed plasmids to localize the transcript and to determine whether any transcripts are clustered. These transcripts have also been hybridized to restricted bovine mtDNA at varying stringencies to determine if there are homologous sequences in maize and cow. At least two of the maize transcripts demonstrate reasonable homology with bovine sequences. Work is in progress to define the function of these two transcripts. In addition to mapping and describing the RNA transcripts present in mitochondria of seedlings, we have also surveyed other tissue types for mitochondrial diversity. RNA samples have been prepared from purified mitochondria, and we have also prepared total mitochondrial protein and ^{35}S-labelled protein from mitochondria incubated in an organelle protein synthesizing system (1). Mitochondrial protein samples have been analyzed from dark grown seedling leaves, green seedling leaves, developing kernels, and unfertilized cobs. We have found both qualitative and quantitative variation in the protein composition and proteins synthesized by the organelles. For seedling studies we have also used a novel control to eliminate the possibility of plastid contamination. Iojap-affected seedlings contain plastids totally lacking in ribosomes, and these plastids neither synthesize RNA nor protein. Consequently, organelle-synthesized proteins and RNA products originate entirely from the mitochondria.

1. Forde, B.G. and C.J. Leaver. 1980. Proc. Natl. Acad. Sci. USA 75: 3841-3845.

Controlling Elements in Higher Plants

1198 CONTROLLING ELEMENTS IN HIGHER PLANTS. P.Starlinger, U.Courage-Tebbe, H.P.Döring, E.Tillmann, E.Weck, W.Werr. Institut für Genetik, Universität Köln, Federal Republic of Germany.

Transposable elements were first discovered in Zea mays by B.McClintock (1951). As gene products are not yet known, the elements must be isolated as DNA present in mutants only at the locus of a known gene that can be isolated by conventional techniques. We have concentrated on transposable element Ds at the gene encoding endosperm sucrose synthase.
Genomic clones have been obtained from two mutants induced by Ds at this locus. Both contain a contiguous segment of DNA, also found in the wild type clone at this location, and adjacent to it another DNA segment not present in the wild type clone. The "foreign" DNAs in the two mutants are similar but not identical in the region adjacent to the breakpoint. The structure of this DNA is being characterized by restriction mapping and DNA sequence analysis. Hybridization of the "foreign" DNA to genomic DNA of various maize lines reveals the presence of up to 40 bands hybridizing to these probes. The DNA sequences hybridizing to the probes are heterogeneous.

In collaboration with Dr.N.Fedoroff, Baltimore, we studied the structure of mutant sh-m5933. The sucrose synthase gene is split by a DNA rearrangement. A DNA segment comprising the 5'-part of the gene, DNA adjacent to it on the 5'-side and "foreign" DNA on the 3'-side is duplicated in the mutant. Only one of the duplicated copies disappears in a revertant (isoated by N.Fedoroff), giving rise to a fragment of the same mobility, as found in wild type DNA.

Geiser M, Weck E, Döring HP, Werr W, Courage-Tebbe U, Tillmann E, Starlinger P (1982) Genomic clones of a wild type allele and a transposable element-induced mutant allele of the sucrose synthase gene of Zea mays L. The EMBO Journal, in press.
Courage-Tebbe U, Döring HP, Geiser M, Starlinger P, Tillmann E, Weck E, Werr W (1982) Two partial copies of heterogeneous transposable element Ds cloned from the shrunken locus in Zea mays L. In: Manipulation and Expression of Genes in Eukaryotes" (P.Nagley, A.W.Linnane, W.J.Peacock, J.A.Pateman, eds.) Academic Press, Sydney, in press.
Fedoroff N, Chaleff D, Courage-Tebbe U, Döring HP, Geiser M, Starlinger P, Tillmann E, Weck E, Werr W (1982) Mutations at the shrunken locus in maize caused by the controlling element Ds- NATO Advanced Study Institutes Series, Genome Organization and Expression in Plants (L.Dure, O.Ciferri), Plenum Press, in press.

TI Plasmids, Viruses and Genetic Engineering

1199 MOLECULAR CHARACTERIZATION OF T-DNA LOCI AFFECTING TUMORIGENESIS, Harry J. Klee, Conrad P. Lichtenstein, Alice Montoya and David J. Garfinkel, University of Washington, Seattle, WA 98195

Mutagenesis has defined three loci in an octopine Ti-plasmid, pTiA6NC, which affect tumor morphology. While a wild-type Ti-plasmid incites an undifferentiated callus growth on most host plants, insertions into one of these loci will result in tumors with a proliferation of roots (tmr), shoots (tms), or an abnormally large, undifferentiated tumor (tml). All three of these genetic loci correspond to the positions of several polyadenylated RNAs suggesting that these loci code for proteins of unknown function. The phenomenon of altered morphologies induced by mutation strongly suggests that these proteins are involved in biosynthesis of phytohormones. Transposon mutagenesis of the Tmr locus has, in fact, been shown to result in tumors with drastically lowered levels of cytokinins. To further characterize these genes and their roles in tumorigenesis, we have begun to determine the nucleotide sequences of each. One of these loci, tmr, has been entirely sequenced. The boundaries as well as the direction of transcription have been determined by S1 nuclease digestion. The gene contains an open reading frame without any introns coding for a 240 amino acid protein. Further experiments which help elucidate the role of the tmr gene product will be presented.

1200 DELETIONS OF THE ENDS OF THE T REGION OF THE A.TUMEFACIENS TUMOR-INDUCING PLASMID, Lloyd W. Ream, Milton P. Gordon and Eugene W. Nester, University of Washington, Seattle, Washington 98195

During crown gall tumor induction by Agrobacterium tumefaciens a specific portion of the tumor-inducing (Ti) plasmid, called the T-DNA, integrates into plant nuclear DNA. The T-DNA contains tumor morphology genes required for tumor growth. Similar sequences which occur at the ends of the T region of several different Ti plasmids may play a role in T-DNA integration. Therefore, we generated Ti plasmid deletions of either end of the T region without affecting the tumor morphology genes. Deletions of the right end of the T region abolished virulence, but deletions of the left end had no apparent effect on virulence. A secondary integration sequence in the Ti plasmid may promote T-DNA integration in strains with deletions of the left end of the T region. Alternatively, the left end of the T region may not play a role in tumorigenesis.

1201 PROPERTIES OF *AGROBACTERIUM* STRAINS ISOLATED FROM QUEBEC, Carole Beaulieu, Louis J. Coulombe and Patrice Dion, Laval University, Quebec, P.Q., G1K 7P4

Three different selective media were used in an attempt to obtain oncogenic isolates of *Agrobacterium tumefaciens* from soil and from diseased apple, vine and other fruit crops. Of 215 isolates, 8% were found to utilize an opine, but only 0.5% were oncogenic. A second series of experiments involved replica plating of colonies from selective media onto minimal medium with an opine as the sole nitrogen source. This method allowed the rapid isolation of 533 opine-utilizing strains. Octopine and nopaline-utilizing strains were obtained in roughly equivalent numbers. Biotype 2 and 3 strains were isolated from apple and plum and biotype 3 strains were isolated from vine. Most of the strains were resistant to *Agrobacterium radiobacter* K 84. Surprisingly, most of the strains (98%) were still found to be avirulent on Kalanchoe, tomato and sunflower. In particular, oncogenicity could be demonstrated for none of the strains isolated from apple. The possibilities that some of the strains are attenuated or have a narrow host range, or that the capacity to form tumors is an unstable characteristic in these strains were considered and for this reason the biological and molecular characterization of the plasmids was undertaken.

1202 COMPARISON OF PLASMID ONCOGENES FROM LIMITED AND WIDE HOST RANGE STRAINS OF AGROBACTERIUM TUMEFACIENS. William R. Krul, Brenda A. Lowe and Stephen L. Dellaporta*, University of Rhode Island, Kingston, R.I. 02881 and Cold Spring Harbor Lab*, Cold Spring Harbor, N.Y. 11724. The tumor inducing DNA (T-DNA) isolated from wide host range strains (WHR) of Agrobacterium displays little or no homology to plasmids isolated from limited host range strains (LHR). That nonhomologous sequences of DNA are capable of producing a similar phenotype poses interesting questions concerning the nature of oncogenes. Some Vitis species and their interspecific hybrids form tumors in response to both WHR and LHR strains of the bacterium. We have identified LHR and WHR plasmid sequences in grape tumor DNA. Cosmid clones of LHR-pTi63Tr were constructed to identify LHR T-DNA sequences and to examine the fine structure differences between WHR and LHR T-DNA in grape tumor cells. These differences will be discussed.

1203 THE BOUNDARIES OF THE ONCOGENIC T-DNA IN PLANT TUMORS INDUCED BY AGROBACTERIUM TUMEFACIENS, Marcelle Holsters, Patricia Zambryski, Raimundo Villarroel, and Ann Depicker, Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium)

The oncogenic T-DNA of the Ti plasmids of A. tumefaciens is integrated in the genome of transformed plant cells. Both from nopaline-type and from octopine-type tumor lines genomic clones containing T-DNA boundaries were isolated, either in a phage vector [1, 2] or in a cosmid vector [3, 4]. The DNA sequence of the T-DNA ends was compared to the sequence of the corresponding region of the Ti plasmid. The T-DNA ends are fairly precise, more so to the right than to the left, however. Both in nopaline and in octopine Ti plasmids a sequence of 25 bp is present as a direct repeat at or nearby the ends of the T-DNA. This sequence therefore might be involved in the integration mechanism. In both types of tumor lines the T-DNA can be arranged as a tandem repeat. Most often the T-DNA was found inserted in repetitive plant DNA sequences.

References

1. Zambryski, P., Holsters, M., Kruger, K., Depicker, A., Schell, J., Van Montagu, M., and Goodman, H.M. (1980). Science 209, 1385-1391.
2. Zambryski, P., Depicker, A., Kruger, K., and Goodman, H. (1982). J. Mol. Appl. Genet. 1, 361-370.
3. Holsters, M., Villarroel, R., Van Montagu, M., and Schell, J. (1982). Mol. Gen. Genet. 185, 283-289.
4. Holsters, M., Villarroel, R., De Greve, H., Gielen, J., Van Montagu, M., and Schell, J. An analysis of the boundaries of the octopine TL-DNA of A. tumefaciens. In preparation.

1204 USE OF T-DNA AS A VECTOR FOR TRANSFER OF THE E. COLI chlM GENE TO NITRATE REDUCTASE MINUS PLANTS, Thianda Manzara and Paul F. Lurquin, Washington State University, Pullman, WA 99164-4350

The A. tumefaciens Ti plasmid can be used as a vector for introducing foreign genes into plant cells through crown gall transformation. Selectable markers are required to assess the validity of pTi as a vector for plant genetic engineering. We have chosen N. tabacum and N. plumbaginifolia nitrate reductase mutants deficient in functional molybdenum cofactor (MoCo) as recipients for the E. coli chlM gene. This gene has been shown to be involved in MoCo production; its product appears to complement corresponding plant mutants and has been identified in minicell and maxicell systems. We have cloned the chlM gene into several sites of the Bam HI fragment #8 of the pTiA6 by marker exchange. This involved construction of intermediate vectors in which a plasmid carrying the chlM gene was inserted into cloned T-DNA (Bam HI #8) carried by a broad host range plasmid. The intermediate vectors were introduced by transformation into A. tumefaciens strain A136 harboring pTiA6. Subsequent introduction of an incompatible plasmid by conjugational transfer allowed for selection of clones in which the chlM gene was transferred to pTiA6. Several of these clones have retained tumorigenicity as tested on N. tabacum SR1 plants and carrot disks. The induced tumors appear to be morphologically identical to those incited by A136 pTiA6. Insertion of chlM under the control of an SV40 early promoter into pTiA6 has also been studied. The introduction of recombinant pTiA6 into mutant plants and cells will be discussed.

1205 THE T-REGION OF TI-PLASMIDS EXPRESSES FOUR PROTEINS IN BACTERIA, Joachim Schröder, Werner Klipp, Annette Hillebrand and Gudrun Schröder, Max-Planck-Institut für Züchtungsforschung, Abt. Schell, D-5000 Köln 30

Expression studies in E. coli minicells and with cell-free systems from E. coli and Agrobacterium tumefaciens show that the T-regions both of octopine and nopaline plasmids contain a set of four genes which is expressed in bacteria into protein. The genes are localized in the center part of the T-region which is responsible for inducing hormone-independent growth in plant cells, and genetic evidence indicates that at least one of the genes plays a role in the production of transzeatin in Agrobacteria. This suggests that the proteins are functional in the bacteria, and the possibility will be discussed that the gene products participate in mediating hormone-effects which maintain and stimulate wound-induced cell divisions in infected plants.
The regions expressed in bacteria are transcribed in transformed plant cells after transfer of the T-region, but the protein products are not known. Comparison of the coding regions shows that three of the bacterial proteins correlate with three plant transcripts, suggesting that the same genes are expressed. The fourth bacterial protein has no corresponding transcript in plants. This and other results suggest that continued expression of this gene and possibly of other genes as well is not necessary in plant cells for maintenance of hormone-independent growth of crown gall cells.

1206 ANALYSIS OF IN VITRO MUTANTS OF CAULIFLOWER MOSAIC VIRUS, L. Dixon, J. Penswick, I. König, M. Pietrzak and Th. Hohn, Friedrich Miescher-Institute, CH-4002, Basel, Switzerland

Mutations in the cauliflower mosaic virus genome were generated in vitro by insertion of short DNA fragments containing SmaI restriction enzyme sites. Mutants in reading frames II, VII and in the large intergenic region were viable causing normal symptons after the usual incubation period on plants. Those in reading frames I, III, IV, V and VI were either lethal or development of symptons was substantially retarded. Pairs of insertion mutants were used to delete the segment of DNA between the SmaI restriction sites producing deletion mutants which were viable only if reading frames II, VII or certain parts of the intergenic region was affected. Homologous or heterologous DNA fragments were inserted into viable mutants, results concerning stability of inserts will be presented. In one case a short DNA fragment containing pregap region β3 was reintroduced into a viable mutant from which a larger segment containing the β3 gap region and most of reading frame II had been deleted. After infection of plants progeny viral DNA contained the gap if the fragment was introduced in the forward but not in the reverse orientation.

1207 TOBACCO TUMOR VIRUS. Arun Misra; Dept. Microbiology,Dental School, Washington University; and HLA Lab., American Red Cross; St.Louis, MO. 63108 USA.

Characterization and properties of the newly discovered virus (TTV,Gliem and Nienhaus 1973) will be discussed. Special mention will be made of the behavi our of the virus in tissue culture; changed IAA metabolism of the host due to virus infection; effects of antibiotics on the replication of the virus etc.

Comparisons will be made with the tumors from WTV of leguminous plants and the crown-gall bacterium and its plasmid. Relationship between viruses and plasmids will be pointed out.

Plant tumors from Corynebactrium will also be mentioned.

1208 STRUCTURE AND REASSEMBLY OF TURNIP CRINKLE VIRUS (TCV), Peter G. Stockley, Peter K. Sorger, Akiko Maeda and Stephen C. Harrison, Harvard University, Cambridge, MA 02138

The structure of TCV coat protein has been determined by high resolution x-ray diffraction (Hogle and Harrison, unpublished). The virus can be reassembled in vitro making it an ideal system in which to study the structural aspects of viral assembly and disassembly. The TCV RNA genome has been cloned and extensive regions have been sequenced. An in vitro reconstitution system has been developed and several defined states (other than native and compact) have been identified and characterized. The putative N-terminal RNA binding domain has been purified and is being crystallized and its structure has been investigated by circular dichroism. Its interaction with genomic RNA has also been studied. The results of these experiments will be discussed in terms of general mechanisms for assembly/disassembly in the simple plant virus.

1209 MULTIPLE RNA SPECIES RELATED TO TURNIP CRINKLE VIRUS SATELLITE RNA, Susan B. Altenbach and Stephen H. Howell, University of California, San Diego, La Jolla, CA 92093

Natural isolates of turnip crinkle virus (TCV) contain a satellite RNA of about 500 bases. The satellite RNA is not required for TCV infection; however, it is efficiently replicated and extensively accumulated in turnip plants infected with TCV. The appearance of the satellite RNA in TCV-infected turnip plants is associated with an increase in the severity of systemic symptoms. The satellite RNA and the TCV genomic RNA share little sequence homology as determined by T1 RNase oligonucleotide mapping and RNA-copy DNA hybridization analysis.

Recently, we have cloned a partial DNA copy of the satellite RNA in E. coli. Using the cloned DNA as a hybridization probe, we have found multiple small RNA species which are related to the satellite in both infected plants and virions. Experiments are underway to determine whether these additional RNA species represent other satellite RNAs or multiple forms of the 500 base satellite RNA which may play an important role in satellite RNA replication.

In addition, sequence information on the satellite RNA will be presented.

This work is supported by grants from NSF and USDA/SEA.

1210 STRUCTURE AND EXPRESSION OF THE CAULIFLOWER MOSAIC VIRUS GENOME, David J. Lyttle, Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078

Cauliflower mosaic virus is one of the few plant viruses which contain DNA rather than RNA. The virus genome is approximately 8000 nucleotide pairs and contains eight open reading frames. The products of two cistrons, IV and VI, have been identified as CaMV coat protein and a 61K inclusion body protein, respectively. In addition to the coding regions a large intergenic region of about 700 bp. has been identified in CaMV DNA. This region contains one of the single-stranded interruptions that occur in the CaMV genome and may serve as the site of initiation of DNA replication.

We have constructed plasmids which contain the intergenic region and plasmids from which the region has been deleted. Studies in progress are aimed at determining whether plasmids containing this intergenic region can complement plasmids lacking it and give productive infection. Further modification of this region by in vitro mutagenesis is planned to determine whether sequences in this region are critical for the replication and expression of the CaMV genome.

1211 DRUG RESISTANT PLANT CELL LINES AFTER TREATMENT OF PROTOPLASTS WITH HYBRID DNA, Th. Hohn, G. Lazar, B. Hohn, H. Shinshi, J. Paszkowski, R. Shillito and I. Potrykus. Friedrich Miescher-Institut, CH-4002, Basel, Switzerland.

Plant protoplasts of carrot and tobacco were treated with plasmid hybrids containing the coding sequences of bacterial methotrexate resistant dihydrofolate reductase or bacterial neomycin phosphotransferase (from Tn5 or Tn903). Protoplasts were cultured in liquid medium for one to two weeks and thereafter plated onto agarplates containing methotrexate or the neomycin analogue G418, respectively. Resistant Calli were obtained in each experiment, at frequencies at least 10 fold higher than controls. Most calli retained their resistance when grown for three months in absence of the selective marker. Southern hybridisation experiments were hampered by the polyploid nature of the cells but preliminary findings suggest that the DNA marker may be present in the transformed cells in a rearranged form.

1212 EXPRESSION OF BACTERIAL GENES IN HIGHER PLANTS, Michael Bevan, Plant Breeding Institute, Cambridge, U.K., Richard B. Flavell, Plant Breeding Institute, Cambridge, U.K. and Mary-Dell Chilton, Washington University, St. Louis, Missouri, U.S.A.
We have used the promotor region of the nopaline synthase gene from the Ti plasmid pTiT37 and the promotor region of the major 35S RNA transcript from cauliflower mosaic virus, both coupled to polyadenylation signal sequences of the nopaline synthase gene, to direct the synthesis of polyadenylated transcripts complementary to the structural genes for a bacterial dihydrofolate reductase gene, and a bacterial gene for neomycin phosphotransferase. Care was taken in the construction of these chimaeric genes that the Aug initiation codon of the bacterial gene was the first initiation codon on the mRNA. The chimaeric genes have been introduced into petunia and tobacco cells using routine methods involving a virulent form of pTiT37. We will present data on the correct initiation of transcription of these genes from the two different promotors, and we hope to have evidence of activity of these two enzymes, and evidence that these activities confer resistance to methotrexate and g418.

1213 GENETICALLY ENGINEERED GLYPHOSATE TOLERANCE IN ESCHERICHIA COLI, Stephen G. Rogers, Corporate Research Laboratories, Monsanto Co. St. Louis, MO 63167

Recent biochemical analysis by Amrhein and colleagues (B.B.R.C. 94:1207-1212, 1980) suggests that the predominant cellular target of the herbicide glyphosate is the enzyme 5-enolpyruvyl-shikimate-3-phosphoric acid synthase (EPSP synthase). As a means of biologically testing this finding, we cloned a segment of DNA that encodes this enzyme from Escherichia coli. Clones carrying the gene for EPSP synthase were identified by genetic complementation. Cells that contain a multi-copy plasmid carrying the EPSP synthase gene and over-produce the enzyme 5 to 17-fold exhibit at least an eight-fold increased tolerance to glyphosate. These experiments provide direct biological evidence that EPSP synthase is a major site of glyphosate action in E. coli and that, in an amplified form, it can serve as a selectable glyphosate resistance marker.

1214 FUSION OF A PLANT PROMOTER TO GENES FOR RESISTANCE TO KANAMYCIN AND CHLORAMPHENICOL, Thomas D. McKnight, Sandy Berry-Lowe*, Richard B. Meagher*, and Robert B. Simpson, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568 and *University of Georgia, Athens, GA 30602.
We have characterized the 5' end of a soybean gene encoding the small subunit of ribulose bisphosphate carboxylase. S1 nuclease mapping showed that the mRNA for this small subunit gene started at a typical mRNA initiation site. Nucleotide sequences homologous to the TATA box and - 80 site of other eukaryotic RNA polymerase II promoters were located at appropriate distances upstream from the mRNA start site.
A Hind III site 10bp before the first transcribed nucleotide provided a convenient way to separate the 5' flanking region of this gene from its transcribed portion. The coding regions of bacterial genes for resistance to tetracycline, kanamycin, and chloramphenicol have been fused to the small subunit promoter at this Hind III site. Sequences at or near the plant promoter bind E. coli RNA polymerase in vitro. The plant DNA allows a low level of expression of the drug resistance genes in E. coli. The kanamycin and chloramphenicol resistance genes fused to the plant promoter are being tested for expression in higher plants where they should confer a dominant, selectable phenotype on transformed cells. We are using the Ti plasmids of Agrobacterium tumefaciens as a vector to introduce these hybrid genes into plants.

1215 THE EXPRESSION OF PLANT DNA SEQUENCES IN YEAST, J. Lee Compton, Marc R. Krauss, William H. R. Langridge and Aladar A. Szalay, Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853.
The yeast Saccharomyces cerevisiae is being used to screen cloning vectors for future use in higher plants and to identify plant promoter sequences from genomic libraries. Hybrid genes consisting of the promoter region of the Cauliflower Mosaic Virus P66 gene and either the neomycin resistance gene from the bacterial transposon Tn5 or the distal portion of the ß-galactosidase gene are being constructed and screened for activity in yeast. Genomic libraries of tobacco and carrot have been constructed in vectors that contain a ß-galactosidase gene lacking a promoter. These libraries are being screened for plant DNA sequences that function as promoters in yeast.

1216 INTRODUCTION OF A ZEIN GENE INTO TOBACCO AND SUNFLOWER CELLS, Peter B. Goldsbrough, Brian A. Larkins and Stanton B. Gelvin, Purdue University, West Lafayette, IN 47907
We are interested in understanding the mechanisms that control the synthesis of zein proteins. As a first step in this we have introduced a gene coding for zein into the genomes of tobacco and sunflower cells, using the Ti plasmid as the vector. A genomic DNA sequence which codes for a 19,000 M.W. zein protein has been isolated for Z. mays. This fragment contains both 5' and 3' flanking sequences. Using the technique of marker exchange between plasmids containing homologous DNA sequences, this gene has been introduced into the T-DNA of the A6 Ti plasmid of A. tumefaciens at a number of specific sites. These sites have been chosen primarily because of the availability of suitable restriction enzyme sites. However they cover regions of the T-DNA which are necessary for tumorigenesis (T_L), and those which are not but which are frequently found in the DNA of transformed cells (T_R). The latter are of particular interest because this region is highly transcribed in most tumor lines in which it is present. A. tumefaciens strains harboring these newly constructed Ti plasmids have been used to incite tumors on both tobacco and sunflower plants. These tumors have been propagated in culture and are now being analyzed for the presence of the zein gene, and its expression into RNA and protein.

1217 PHYSICAL MAP OF THE A. RHIZOGENES VIRULENCE PLASMID $pR_i8196TC$, Jane Koplow and Mary-Dell Chilton, Washington University, St. Louis, Mo. 63130
Agrobacterium rhizogenes strain 8196, which induces hairy root disease on dicotyledenous plants, carries three plasmids. The intermediate-sized plasmid, in a transconjugant strain, C58Cl(pR_i8196), is responsible for the virulence properties. As with the virulent plasmids of the closely related species A. tumafaciens, a portion of the A. rhizogenes virulence plasmid, designated T-DNA, is transfered to the host plant genome by an as yet unknown mechanism. A physical map of the $pR_i8196TC$ virulence plasmid was determined by walking through a Bam Hl clone bank using colony filter hybridization to determine overlaps and Southern blot analysis to sort out contiguous fragments. The physical map will be correlated with the genetic map and functional properties of plasmid $pR_i8196TC$.

1218 INTRODUCTION OF SELECTABLE MARKERS INTO CORN CELLS BY PROTOPLAST FUSION, Kathryn Kolacz, Maureen Gilmore-Hebert, INGENE, 1701 Colorado Avenue, Santa Monica, California 90404

In order to introduce DNA into plant cells, it is necessary to have a selectable marker. There are two drugs which may be useful for this purpose, the kanamycin derivative G418 or the HPGRT inhibitor mycophenolic acid. Sensitivity studies on corn protoplasts have shown both drugs to be cytotoxic. Cell death occurs within 7-8 days of treatment with 50mg/ml of G418, 5-6 days with 25 mg/ml of mycophenolic acid.

Transient drug resistance to mycophenolic acid has been conferred on corn cells by the introduction of Ecogpt gene using protoplast fusion. Similar studies are in progress for the G418 marker.

Nitrogen Fixation, Stress, and Host/Pathogen Relationships

1219 PLANT GENES EXPRESSED SPECIFICALLY IN NITROGEN FIXING NODULES OF SOYBEAN, Forrest H. Fuller, Peter W. Kunstner, Truyen Nguyen and Desh Pal S. Verma, McGill University, Montreal, Quebec, Canada H3A 1B1.

In order to identify and study plant genes and their products which are involved in symbiotic nitrogen fixation, we have prepared a cDNA library using poly(A) RNA isolated from active soybean nodules. A total of 5700 clones were screened and about 800 were found to contain sequences also present in root tissue while 2100 others encoded nodule specific sequences as judged by hybridization to cDNA probes. More than half of the nodule specific clones could be accounted for by 4 sequence species. Leghemoglobin coding sequences were found in 860 clones while unidentified species nodA, NodB and NodC correspond to about 350, 55 and 60 clones respectively. These later three species composed about 6%, 1% and 1% of nodule poly(A) RNAs and were found to code for polypeptides of molecular weights 44 kd, 27 kd, and 24 kd respectively. All three polypeptides reacted specifically with antiserum prepared against total nodule proteins. These sequences were shown to be encoded by the plant nuclear genome and could not be detected in root RNA. Several other clones have subsequently been shown to code for nodule proteins. The cDNA clones have been used as probes to isolate corresponding genomic clones and restriction and sequence analyses are currently in progress.

1220 THE CLONING OF THE GLUTAMINE SYNTHETASE GENE (*glnA*) FROM *Rhizobium japonicum*, Todd A Carlson, Mary Lou Guerinot, and Barry K. Chelm, DOE Plant Research Laboratory, Michigan State University, East Lansing MI., 48824.

During the process of legume root nodulation, bacteria of the genus *Rhizobia* differentiate to form bacteroids, the intracellular organelles of nitrogen fixation. This differentiation is such that the metabolism of the bacteroid is specifically adapted to function as a nitrogen fixing symbiont. During symbiosis, atmospheric nitrogen is reduced to ammonia by the bacteroid specific enzyme nitrogenase. In contrast, the primary enzyme of ammonia assimilation, glutamine synthetase, is less active in the bacteroid. Thus, the nitrogen metabolism of the bacteroid has been adapted to make it an ammonia exporting organelle. We have cloned the structural gene of glutamine synthetase, *glnA*, from *Rhizobium japonicum* as a first step in characterizing the genetic regulation of nitrogen metabolism during soybean root nodulation.

We constructed a *E. coli* phage lambda library with DNA from *R. japonicum* cv. 110. A single recombinant phage, λRjglnA, was isolated by lytic complementation of glutamine auxotrophy in *E. coli* ET8051 (*glnA*$^-$). We confirmed the presence of *glnA* by heterologous hybridization of the *E. coli glnA* gene to restriction fragments of λRjglnA DNA by the Southern technique. Restriction fragments from the insert region of λRjglnA that hybridized to the *E. coli glnA* probe have been subcloned into the pBR322 plasmid vector. Using subclones as hybridization probes, we have rescreened the lambda phage library in order to isolate additional flanking regions of the *R. japonicum* genome. The flanking regions are currently being analyzed and plasmid subclones are being tested for complementation ability.

1221 *Rhizobium* uses asymbiotic N_2 Fixation to promote Growth by a Mechanism of Ammonium Crossfeeding. Robert A. LUDWIG. Dept. of Biology, University of California, Santa Cruz, CA. 95064.

Rhizobium has been viewed to primarily fix N_2 during root nodule symbiosis, however some strains do so as free-living soil microbes. Such strains have defied attempts to demonstrate N_2-fixation-dependent growth, and thus the role of asymbiotic N_2 fixation has remained unclear. It will be demonstrated that N_2 fixation occurs when both growing and non-growing Rhizobium cells are present in microaerobic cultures. The pleiotropic Nif$^-$ strain RC3312 exhibits no such non-growing cells under these conditions. In Nif-dependent growth conditions, RC3312 Nif$^-$ can have its own growth selectively promoted by N_2 fixation (NH_4^+-export) when co-cultured with Nif$^+$ strains. It is proposed that asymbiotic *Rhizobium* N_2 fixation operates by the following mechanism: certain cells stop growin, become specialized for N_2 fixation, export NH_4^+ so produced, and crossfeed other N-limited cells that remain vegetatively growing. Thus asymbiotic N_2-fixing rhizobia are formally analogous to symbiotic "bacteroids" in legume root nodules. The implications of these studies will be discussed.

1222 MOLECULAR CHARACTERIZATION OF THE SYMBIOTIC GENES OF RHIZOBIUM TRIFOLII, John M. Watson, Peter R. Schofield, Michael A. Djordjevic, Barry G. Rolfe and John Shine, Centre for Recombinant DNA Research and Genetics Department, RSBS, Australian National University, Canberra, A.C.T. 2601, Australia.

Bacteria of the genus Rhizobium interact symbiotically with plants to form root nodules in which atmospheric nitrogen is fixed. For fast-growing Rhizobium species, this interaction is highly specific: R. trifolii only nodulates clovers (Trifolium).

Transposon Tn5 mutagenesis of R. trifolii ANU843 yielded a mutant (ANU851) which is defective in nodule induction. The region of DNA carrying Tn5 in ANU851 was cloned and used to identify the corresponding cloned wild-type sequence. Restriction enzyme mapping of this region, which is located on a large (180kb) Sym plasmid, revealed that it is located 30kb from the NifHD structural genes. A recombinant plasmid (pRt032), carrying a 14kb HindIII fragment from the nodulation region, was found to restore nodulation capacity to a Sym plasmid-cured Nod^- strain (ANU845). Transfer of pRt032 to Agrobacterium tumefaciens strain A136 and to the fast-growing Cowpea Rhizobium strain ANU240 conferred on these strains the ability to nodulate clover plants. The ANU240/pRt032 derivative also retained its normal host range for tropical legumes. Tn5 mutagenesis and deletion analysis of pRt032 have revealed the detailed arrangement of the genetic determinants of nodulation in R. trifolii ANU843.

1223 GENETIC CHARACTERIZATION OF SYMBIOTIC GENES IN THE SLOW-GROWING BROAD HOST RANGE COWPEA RHIZOBIUM STRAIN IRc78, Mittur N. Jagadish, Ray G. Hadley, Adrienne Koermendy, and Aladar A. Szalay, Boyce Thompson Institute for Plant Research, Cornell University, Tower Road, Ithaca, New York 14853.

Three IRc78 DNA fragments of sizes 21, 9.6 and 5.1 kb containing nif K, D and H hybridizing regions respectively have been cloned into pSUP201, a mobilization vector.

Transposon Tn5, has been used for site-specific mutagenesis of nif hybridizing and flanking regions to define functional domains in these three DNA fragments.

A "suicide vector" method has been successfully used in IRc78 for substitution of Tn5 mutagenised fragments to wild type fragments.

Cowpea plant tests with IRc78::Tn5 mutants have shown that Tn5 insertion in nifK hybridizing region and in regions outside of nifK destroy the ability to fix nitrogen.

Work is in progress to identify the regions essential for an effective symbiosis between IRc78 and its host plants.

1224 Nitrogen regulation in Klebsiella pneumoniae: The nifA gene can substitute for the general nitrogen regulatory gene glnG (ntrC). David W. Ow and Frederick M. Ausubel, Mass. General Hospital, Boston, MA

Nitrogen fixation in K. pneumoniae requires the expression of at least 15 nif genes that are regulated by the products of the nifLA operon. The nifA gene product is required for activation of all nif operons except its own while the nifL gene product mediates oxygen and NH_4^+ repression. Recent studies have shown that nitrogen regulation in enteric bacteria is centrally controlled. The positive regulators of nitrogen assimilatory pathways (such as those involved in the transport and catabolism of amino acids) are products of genes glnG (ntrC) and glnF (ntrA). Negative regulation also involves the glnG product and, in addition, the products of genes glnL (ntrB) and glnB. Our study shows that the response of the nif pathway to nitrogen regulation occurs at the nifLA promoter. In addition, we have found that the nifA product can substitute for the glnG product in the activation of its own promoter, the glutamine synthetase (glnA) promoter, and genes involved in arginine, histidine and proline degradation. nifA-mediated gene activation also requires the glnF product. These and other observations suggest that nifA and glnG may be ancestrally related. Since glutamine synthetase plays a major role in NH_4^+ assimilation, nifA-mediated activation of both the glnA and nifLA promoters may serve to ensure efficient assimilation as well as fixation of nitrogen.

1225 EARLY NODULATION GENES OF RHIZOBIUM MELILOTI, Thomas Egelhoff, Diana Beebe, Thomas Jacobs, Sharon Long, Stanford University, Stanford CA. 94305

Bacterial genes for symbiotic function can be identified by analyzing mutants defective in symbiosis. We are studying two mutants of Rhizobium meliloti defective in root hair curling (Hac⁻), an early stage of infection. Plasmid pRmSL26 contains a 19 kb fragment from R. meliloti in pLAFR1, and can restore function to the two Hac⁻ mutants. The DNA region bearing the Hac gene(s) maps to the R. meliloti megaplasmid, within 20 kb of the nitrogenase genes(1). We have subcloned the EcoRI fragments of the insert into pBR325. The lesions in the original mutants are located in an 8.7 kb EcoRI fragment. The region has been mapped with enzymes EcoRI, HindIII, Pst 1, BamH1, and BglII. When the 8.7 kb fragment alone is re-cloned into pRK290 and conjugated into the Hac⁻ mutants, they are restored to Nod⁺ phenotype. We are also constructing, by in vitro techniques, deletion mutants of the 8.7 kb region. The cloned 8.7 kb fragment has been forced back into the R. meliloti genome (2) to test the symbiotic phenotype of insertions at various points. An important question regarding nodulation genes is whether they are expressed by free-living bacteria or require particular inductive conditions. This has been tested by hybridization of RNA from free-living R. meliloti cells, from nitrogen-starved cells, and from bacteroids, to cloned fragments from this nodulation region. Preliminary results indicate that genes in this region are expressed in the latter two conditions but not in free-living bacteria.

(1) Long et al. Nature 298:485-488 (1982)
(2) Ruvkun and Ausubel. Nature 289:85-88 (1981)

1226 CLONING OF THE GLUTAMINE SYNTHETASE I GENE FROM RHIZOBIUM MELILOTI, Michael Kahn and John Somerville, Washington State University, Pullman, WA 99164

A gene bank of Rhizobium meliloti strain 104A14 has been constructed using the plasmid pRK248-bacteriophage P4 cosmid pMK318. Cosmids that contain the structural gene for glutamine synthetase I (GSI) have been isolated from this gene bank by selecting for recombinants that permit ET8051, an E. coli mutant deleted for gln A, to grow in the absence of glutamine. One of the original cosmids, pJS36, contains an insert of 13 kb and grows very poorly in the absence of glutamine. When a 3.8 kb Hind III fragment derived from this DNA is cloned into the Hind III site of pACYC177 rapid cell growth is observed in when the insert is in one orientation (pJS44) but not the other (pJS45). GS activity in ET8051(pJS44) is heat stable. pJS36 will hybridize with pUC346, a clone carrying the glutamine synthetase structural gene from E. coli. 104A14 (pJS36) has higher levels of both GSI and GSII activities than 104A14 when the cells are grown on Yeast-mannitol broth. In both strains GSII activity is repressed in the presence of ammonia.

1227 THE STRUCTURE OF THE ROOT HAIR WALL IN THE ACTINORHIZAL HOST, ALNUS RUBRA BONG., Alison M. Berry,[1] John G. Torrey,[2] and Margaret E. McCully,[1] [1]Carleton University, Ottawa, Ont., CAN K1S 5B6; [2]Harvard Forest, Harvard University, Petersham MA 01366.

In the early stages of establishment of an actinorhizal dinitrogen-fixing association between Frankia sp. and Alnus rubra, the bacterially-induced deformation of root hairs appears to be one necessary precondition for successful root-hair infection by Frankia (Knowlton et al. 1980). Root hair deformation occurs as a result of new wall deposition at the tips of elongating hairs, as observed using time-lapse interference microscopy. Histochemical changes have been noted in the walls of such deformed hairs as a consequence of inoculating axenically-grown Alnus seedlings with Frankia sp. Ar13 or with Pseudomonas cepacia 85 (Berry and Torrey in press). These observations are correlated with ultrastructural evidence in a further study of root hair wall formation in this system.

References:
Berry A.M. and J.G. Torrey. (in press). Root hair deformation in the infection process of Alnus rubra Bong. Can. J. Bot.
Knowlton S., A. Berry and J.G. Torrey. 1980. Evidence that associated soil bacteria may influence root hair infection of actinorhizal plants by Frankia. Can. J. Microbiol. 26: 971-977.

1228 A R. MELILOTI LOCUS ESSENTIAL FOR THE PRODUCTION OF NITROGENASE POLYPEPTIDES, Wynne Szeto, J. Lynn Zimmerman, D. Ow & Fred Ausubel, The Biological Laboratories, Harvard University, Cambridge, MA 02138, USA.

By site-specific mutagenesis (using transposon Tn5) of cloned R. meliloti megaplasmid DNA, several gene regions essential for symbiotic nitrogen fixation have been identified (Buikema et al.,in preparation). As an initial approach to understanding the actual functions of these loci during symbiosis, a series of Rhizobium strains carrying Tn5 insertions in different symbiotic regions were used to induce ineffective nodules in alfalfa. Bacteria-derived and plant-derived proteins were purified from these nodules and compared with those obtained from nodules induced by wild-type Rhizobium. Using this strategy, the DNA region marked by the Tn5 insertion carried in strains 1352 and 1354 was found to be essential for the production of nitrogenase polypeptides. Restriction mapping and Southern blot analyses demonstrated that this locus maps about 6 kilobases to the right of the nitrogenase (nif) promoter. That this region is distinct from the nif operon was further coroborated by genetic complementation analyses. Some properties of strains 1352 and 1354 which may contribute to our understanding of the functions of this nif "regulatory" locus will be discussed.

1229 TEMPERATURE-SENSITIVE NODULATION IN THE PEA/RHIZOBIUM , T.A. Lie and Noud van Swaay, Laboratory of microbiology,Agricultural University,Wageningen, The Netherlands.

Several pea lines require a short period of exposure to a higher temperature than the normal temperature for plant growth. This characteristic is heritable and due to a simple dominant host gene. A temperature-sensitive step is detected which is restricted to the second and/or third day after inoculation with Rhizobium. A search was made for specific proteins in the infected roots,which were exposed to the higher root temperature.

1230 Potential Use of Some Seed Proteins as Factors in Host/Pathogen-Predator Relationships. D.E. Foard, L. L. Murdock, and P. E. Dunn Purdue University, W. Lafayette, IN 47907

Seeds contain a variety of proteins that may confer resistance to pathogens or insect predators by inhibiting their digestive or other enzymes. An example is the Bowman-Birk inhibitor (BBI) which is active against some serine proteinases. If a specific interaction of BBI and the proteinases of a predator or pathogen could be established, one might investigate whether an increase in the concentration of BBI in the vulnerable seed would confer resistance. Preliminary data from 75 strains of soybean (Glycine max) shows the BBI to be present in all and to vary from about 2 to 6% of total extractable seed protein. The BBI gene exists in a single copy per haploid genome in the cultivars Provar and Tracy. Were BBI shown to confer resistance through inhibition of specific enzymes, one might in the future attempt to introduce additional copies of the gene into the soybean genome. If these introduced genes were expressed, they would confer resistance to races of pathogens/predators that had overcome the normal level of BBI via producing an excess of proteinase. The small size of the BBI gene and the single copy per genome provide simple and potentially favorable features to exploit in genetic engineering experiments

1231 REGULATION OF PLANT DEFENSE MECHANISMS, R. J. Kaufman, S. J. Wratten and T. L. Graham, Monsanto Agricultural Products Co., St. Louis, MO 63167

Chemical pesticides such as herbicides, fertilizers, PGRs, insecticides and fungicides have played a vital role in increasing the productivity of American agriculture. However, long term disease and insect control have been extremely difficult to achieve, particularly at non-epidemic, yield-robbing levels. The concept of cross protection (immunization) is well known in physiological plant pathology and recent advances in plant biochemistry have shed light on the molecular mode of stimulation of plant defense mechanisms, particularly phytoalexins and their elicitors. For instance, previous work by Albersheim indicated that carbohydrates play a crucial role in the elicitation of plant defensive mechanisms. Recent work in our laboratory has suggested that both biotic and abiotic soybean phytoalexin elicitors may trigger, in addition to the classical phytoalexins, other apparently metabolically related but non-phytoalexin and systemic disease defense mechanisms. This led to our suggestion of a metabolite shunting model for soybean in which metabolites loaded into a central phenyl propanoid pool could be simultaneously but differentially shunted into the pterocarpan phytoalexins and also into other components (e.g., lignin, tannins) potentially involved in induced disease resistance. Obviously the examination of this hypothesis requires a matrix of new techniques not only for the non-wounding treatment of whole potted plants with molecular elicitors but also for sophisticated and broad metabolite profiling. In this paper we report the development of such techniques for elicitor treatment, extraction and HPLC profiling of plant secondary products as well as the implications of these results in terms of gene regulation. These techniques have been extended to other crops.

1232 RESEARCH PROGRAM, J. P. Helgeson, USDA, ARS and Departments of Plant Pathology and Botany, University of Wisconsin-Madison, Madison, WI 53706

We are using callus, suspension and anther cultures as well as protoplasts of tobacco, potato and various Solanum species as tools for studies on the following: A. Molecular aspects of disease resistance in plants: As our model we are using tobacco tissue cultures and Phytophthora parasitica var. nicotianae. With this system we are: 1) searching for the means by which pathogens are recognized by host cells and how pathogens suppress responses of the host or avoid recognition, 2) studying the nature and control of the hypersensitive response, a common host response to pathogens, and 3) determining the chemical structures, biological activities and rates of production of phytoalexins produced in response to stresses such as pathogen invasion. Additional studies will focus on the utilization of host cell responses for screening for altered cells. B. Genetic variation in progeny from cultured cells: Using protoplasts from potato and other Solanum species we are examining variation in progeny from protoplasts and attempting to obtain new variation by interspecific fusions. Experiments involving mutations and insertion of specific DNA sequences are also planned. C. Differentiation of new plants from isolated plant cells: We are using protoplasts from potato and other Solanum species in an attempt to improve the percentage of cells that differentiate into new plants. Investigations on metabolic and phytohormone changes are underway.

1233 HOST-PATHOGEN COMPATIBILITY BETWEEN PLANTS AND LIMITED AND WIDE HOST RANGE STRAINS OF AGROBACTERIUM TUMEFACIENS, Brenda A. Lowe[1], William R. Krul[1], and Stephen L. Dellaporta[2] 1. Plant Science Dept. Univ. of Rhode Island, Kingston, RI 02881 2. Box 100 Cold Spring Harbor Lab, Cold Spring Harbor, NY 11724.

Host-pathogen compatibility is dependent on genes in both host and pathogen. In crown gall disease, compatibility between plant and Agrobacterium tumefaciens is partially determined by Ti plasmid sequences (Thomashow 1980, Nature 283:794). The Ti plasmids of wide host range (WHR) strains cause tumors in a wide variety of dicot genera, whereas Ti plasmids of limited host range (LHR) strains narrow compatible hosts to only a few genera. The role of the plant genome in host compatibility is not known.

To study the genetics of this interaction, species and interspecific hybrids of Vitis and Nicotiana were screened for tumor formation by LHR and WHR strains of A. tumefaciens. Compatible interactions were detected by hormone autonomy, opine biosynthesis, and the presence of T-DNA in host tumor cells. Tumors were incited by LHR and WHR strains in hybrids of resistant and susceptible species. We suggest that plant compatibility genes are passed to progeny as dominant factors.

1234 ACCUMULATION OF MESSENGER RNAs ASSOCIATED WITH BIOTIC AND ABIOTIC INDUCTION OF DISEASE RESISTANCE PROTEINS IN PLANTS, L. A. Hadwiger, W. Wagoner, D. C. Loschke, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164, and Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611

Messenger RNAs coding for phenylalanine ammonia lyase and 20 other major disease-resistance-response proteins accumulate in pea endocarp tissue following treatment with $CdCl_2$ ($5x10^{-4}$), UV light (260nm), actinomycin D (10 µg/ml), psoralen compounds (activated with 365 nm UV), chitosan (1 mg/ml) or Fusarium solani macroconidia. High M.W. RNA isolated from pea tissue following each treatment was translated in a rabbit reticulocyte in vitro protein-synthesizing system. Labeled PAL was recovered with anti-PAL antiserum purified with electrophoresis. These induction treatments yielded increases in PAL mRNA activity 6-21 times those of the control. PAL and all other labeled peptides were also separated 2-dimensionally and autoradiographed. The 2-D patterns induced by most of the DNA-specific compounds were closely related to the biotic inducers, chitosan and F. solani. The $CdCl_2$, quinacrine and heatshock treatments were radically different. Thus, the regulation of certain translatable mRNAs in peas associated with resistance proteins is sensitive to the influence of multiple DNA-influencing treatments.

1235 Heat shock genes in soybean: Molecular studies on a subset of genes for low molecular weight hs-proteins. Fritz Schöffl and Joe L. Key. Universität Bielefeld, Biology VI, D-4800 Bielefeld 1, FRG. University of Georgia, Athens, Georgia 30602, USA. We are investigating the structure and regulation of stress genes in soybean, which are expressed only under elevated temperature conditions. We report here the isolation and characterization of a subset of genes for low molecular weight hs-proteins. When seedlings are transferred from 28° to 40°C heat shock response is elicited. This is characterized by the synthesis of a new set of proteins (hs-proteins) and by cessation of normal protein synthesis (Key et al., 1981, PNAS 78, 3526-30). At the level of polyA$^+$mRNA a new highly abundant RNA component appears which corresponds with a group of hs-proteins in the low MW-range of 15-18 KD (Schöffl and Key, 1982, J. Mol. Appl. Genet. 1, 301-314). The classification of these proteins into several sub-classes (based on hybrid selected translations) defines a complex sequence relationship for class I proteins/genes. Partial sequence homology is not only indicated for the 13 members of class I but also for temperature induced polyA$^+$RNA of other plant systems. Class I genomic DNA clones isolated from a soybean DNA library hybrid select the mRNAs for the same set of 13 hs-proteins as do individual cDNA clones of class I. Temperature dependent release of hybrid selected RNAs at 50, 60, 70, and 85°C results in a more specific correlation between cDNA and its corresponding protein. Mapping of the structural regions on genomic DNA indicates some clustering of class I genes. DNA sequence analyses are expected to result in more detailed information about both, particularly the common features and individual characteristics of class I genes and their coordinately regulated expression.

1236 A COMPARATIVE STUDY OF HEAT SHOCK AND OTHER ENVIRONMENTAL STRESSES IN SOYBEAN SEEDLINGS, E. Ceglarz, L. Edelman, F. Schöffl, and J. L. Key, Botany Department, University of Georgia, Athens, GA 30602.

The heat shock response has been characterized in intact soybean seedlings, Glycine max variety Wayne. Whether a similar response is elicited by different stresses has been evaluated. Plants were incubated for 2 hrs in medium containing a variety of chemical compounds; these agents included plant growth regulators, uncouplers of oxidative phosphorylation, heavy metals, osmotica, N_2 gas, and amino acid analogues. As determined by Northern blot hybridization with heat shock specific cDNA clones the response of the plants to these conditions is to synthesize a set of new mRNAs that are not present or present in low amounts in untreated non-induced plants. Of the agents studied, arsenite more closely mimics heat shock than the others.

1237 REGULATION OF PROTEIN AND mRNA SYNTHESIS DURING HEAT SHOCK IN DEVELOPING SOYBEAN SEEDS, Joseph P. Mascarenhas, Mitchell Altschuler, Department of Biological Sciences, State University of New York at Albany, Albany, N.Y. 12222.

The translation of storage protein mRNAs in the developing seed appears to be insensitive to inhibition by a heat shock unlike the situation for most proteins in seedlings or cultured cells (Altschuler and Mascarenhas 1982 Plant Mol. Biol. 1:103-115). This property of the storage protein mRNAs has been further characterized. Protein synthesis has been studied by labeling with ^{35}S-methionine and SDS gel electrophoresis after immunoprecipitation with antisera to 7S and 11S proteins. When mid-maturation seeds are given a rapid temperature shock from 25°C to various higher temperatures, they continue to synthesize storage proteins up to 49°C. There is a greater amount of storage protein synthesis than heat shock protein (hsp) synthesis at the higher temperatures. The optimum shock temperature for HSP synthesis in seeds is 40°C. Synthesis of hsp's decreases thereafter but continues, although in very low amounts even at 49°C. In seedlings, on the other hand, a shock to 45°C results in an almost complete inhibition of all protein synthesis.

The presence and synthesis of mRNAs representative of the different mRNA classes in the seed following a heat shock have been studied by the use of several cloned probes -- those to hsp's, to prevalent embryo specific mRNAs and to actin. These results will be presented in relation to the unique transcriptional and translational metabolism of the developing seed.

1238 **DEFINING STRESS INDUCED GENES IN THE TOMATO**, Bruce R. Thomas, Danny C. Alexander, Lisa Staraci, Bill Williams, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568.

Tomato and its wild relatives collected from stressful habitats provide a valuable system for study of the molecular biology of plant stress responses. Definition of the minimum lethal dose for various stresses provides a means to identify both adaptive responses within a particular variety, and differences in levels of tolerance between varieties. We have determined minimum lethal doses from dose response curves of both plants and cell cultures exposed to heat shock, salinity, PEG-induced osmotic stress, anaerobiosis, and chilling. mRNA has been isolated from plants and cell cultures subjected to adaptive stress treatments. In vivo translation patterns identify newly induced mRNA species, which have been fractionated on agarose-urea gels for subsequent cDNA cloning.

1239 THE ANAEROBIC GENES OF MAIZE, Sarah Hake, Dawn Banasiak, William C. Taylor and Michael Freeling, Genetics Dept., University of Calif., Berkeley, Calif., 94720

Anaerobically induced roots of maize synthesize a strikingly different profile of proteins as compared to the proteins of the aerobic controls. Anaerobic incorporation of labeled amino acids is restricted to a few proteins: one is ADH1 (Sachs, Freeling and Okimoto, 1978). A long range goal is to understand how the anaerobic genes of maize are coordinately regulated. A cDNA library was prepared from mRNA of anaerobically induced roots and screened with aerobic and anaerobic mRNA. In addition to a cDNA clone to Adh1, four other clones were found that hybridize with more intensity to 24 hour anaerobic RNA than to aerobic RNA. The size messages represented by the 4 other clones are 3600 bp, 1550 bp, 1500 bp and 1200 bp. Three of the clones, like ADH1, are strongly induced by anaerobiosis (50-fold induction); the fourth is present aerobically and induced 4-fold by anaerobiosis. In two cases, the anaerobic cDNA probes hybridize to several genomic restriction fragments, reminiscent of the results expected with a multi-gene family. This is particularly interesting because Adh1 is a single copy gene (Strommer, et al., 1982).

Strommer, J. N., S. Hake, J. Bennetzen, W.C. Taylor and M. Freeling. accepted for publication. Nature 1982

Sachs, M.M., M. Freeling and R. Okimoto. Cell 20:761-767. 1980

Nuclear Genes

1240 CHARACTERIZATION OF LIGHT REGULATED NUCLEAR GENES IN LEMNA GIBBA, Charles F. Wimpee and Elaine M. Tobin, Biology Dept., UCLA, Los Angeles, CA 90024

The nuclear genome of the aquatic monocot Lemna gibba contains approximately 12 copies of the gene encoding the small subunit (SSU) of ribulose 1,5-bisphosphate carboxylase. Overlapping genomic clones from Lemna containing two SSU genes on a single 6.7 kilobase Hind III fragment have been isolated and mapped. The genes are separated by approximately 2600 base pairs and are in divergent orientation. However, neither gene shows any homology with the 3' untranslated region of a full length SSU cDNA clone constructed by Dr. Willem Stiekema in our laboratory. The genes either have different 3' untranslated regions, or they are truncated and are, therefore, inactive. The genes have been individually subcloned and are presently being mapped in more detail in order to determine the full extent of the coding regions.

Genomic clones representing several other members of the SSU gene family as well as members of the chlorophyll a/b binding protein gene family are being characterized.

1241 mRNA SWITCHING DURING LEAF DEVELOPMENT, Lino L. Fragoso, and William C. Taylor, Department of Genetics, UC Berkeley, Berkeley, CA 94720

We have been working with a multigene family that is developmentally regulated in the maize leaf. After four days of germination we find a 1.5 kb mRNA whose concentration becomes nil after the eighth day. During the sixth day of development we detect a related mRNA species (2.1 kb). From the sixth day on the 2.1 kb mRNA increases in concentration until it is the predominant form. Upon exposure to light, the 2.1 kb mRNA increases two to three fold; the 1.5 kb mRNA, on the other hand, is unaffected by light. We have identified the 1.5 kb messenger product as a 32 kilodalton polypeptide. Work is in progress to establish the identity of the gene .

1242 NUCLEAR GENE FAMILIES ENCODING CHLOROPLAST PROTEINS IN PEA, Michael P. Timko and Anthony R. Cashmore, Rockefeller University, New York, NY 10021

The small subunit of the chloroplast enzyme, ribulose-1,5-bisphosphate carboxylase, and the constituent polypeptides of the light-harvesting chlorophyll a/b-protein complex are major products of nuclear gene expression in pea leaves. Each of these polypeptides is encoded by a small family of genes. When cloned small subunit cDNA (pSS15) is hybridized to Eco R1 restricted pea DNA, five complementary fragments are observed. Two of these Eco R1 restriction fragments (3.3 and 8.0 kb) have been isolated by cloning, and are seen to correspond to duplicated small subunit genes separated by 10 kb of DNA. These genes have been sequenced and shown to contain two intervening sequences. The first exon of these genes encodes 59 amino acids, 57 of which correspond to the transit sequence of the small subunit precursor. The polypeptides encoded by these two genes are identical except for a single amino acid substitution within the transit sequence of the small subunit precursor. We have also isolated from pea DNA an 8.0 kb Eco R1 fragment which hybridized to a cDNA sequence (pAB96) encoding the major chlorophyll a/b-binding polypeptide. Sequence studies have confirmed that the 8.0 kb Eco R1 fragment encodes a chlorophyll a/b-binding polypeptide, although this sequence is significantly diverged from the cDNA sequenced.

1243 cDNA CLONING OF PHYTOCHROME AND OTHER RED-LIGHT REGULATED SEQUENCES. Howard P. Hershey, James T. Colbert, and Peter H. Quail, Department of Botany, University of Wisconsin, Madison, WI 53706.

Phytochrome is a regulatory photoreceptor that plays a pivotal role in plant development. The molecule is a chromoprotein consisting of a chromophore linked to a 124 kdalton polypeptide. The mRNA coding for this polypeptide is a very low abundance message in Avena (<0.01% of the translatable poly(A) RNA). The cDNA cloning of phytochrome was undertaken by construction of a cDNA library from mRNA enriched > 200-fold for phytochrome. Screening of this library using a synthetic oligonucleotide wobble mixture, whose sequence was deduced from amino acid sequence data, has yielded a cDNA clone tentatively identified as phytochrome specific. This clone is being further characterized to positively confirm its identity. By further synthetic oligonucleotide screening of the library we have discovered cDNA clones representing two other mRNAs under red light control as determined by northern blot analysis. These two mRNAs, 3 kb and 5.2 kb respectively, decline to undetectable levels within 3 hours following a saturating dose of red light. These two mRNAs interestingly also share some region(s) of sequence homology. The cDNA clones for these two mRNAs are being further characterized in order: (1) to determine the products they code for; (2) to find and determine the potential significance of the common sequence(s) shared by these mRNAs; and (3) to determine the relationship between these products and phytochrome action.

1244 NUCLEOTIDE SEQUENCE RELATIONSHIPS BETWEEN mRNAs FOR PEA VICILIN POLYPEPTIDES. Peter M. Chandler, Z. Ariffin, T.J.V. Higgins and D. Spencer, CSIRO, Division of Plant Industry, PO Box 1600, Canberra 2601 Australia.

Several cDNA clones coding for the Mr 50,000 polypeptides of pea vicilin have been sequenced to investigate the relatedness between different mRNAs and polypeptides in this multi-gene family. Sixteen vicilin "50K" clones were sorted into three distinct hybridization families and a representative clone of each family studied. The insert in one clone (pPS 15-84) codes for 14 5'untranslated nucleotides, a presumed signal peptide of 22 amino acids, 432 amino acids corresponding to the mature polypeptide, and 97 3'untranslated nucleotides preceding the poly(A) tail. The other two clones have been studied in less detail, but sequence comparisons can be made between all three clones at the 5' and 3'ends of the mRNAs, as well as on either side of a common XbaI site towards the centre of the coding region. Within the translated regions of the clones the overall degree of nucleotide sequence divergence between any two clones averages 16% (range 14-19%). The corresponding amino acid sequence divergence between any two polypeptides averages 18% (range 13-24%) in the internal region of the polypeptide (i.e. surrounding the XbaI site) and 28% (range 22-33%) in the C-terminal region. There was variation in the position of the translation stop codon leading to differences in polypeptide length of up to 17 amino acids. Nucleotide sequence divergence in the 3'untranslated regions of the mRNAs is only slightly greater than in the translated regions (18-23% compared with 14-19%). The length of this region is variable however, being 97, 138 and 224 nucleotides in the three clones.

1245 ISOLATION AND CHARACTERIZATION OF HIGH SULFUR PROTEIN MESSENGER RNAs FROM DEVELOPING BRAZIL NUTS, Samuel S. M. Sun, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568

A 2S albumin protein fraction in Brazil nuts (Bertholletia excelsa) was reported by Joule and Huang (Amer. J. Bot. 68:44, 1981) and confirmed by us to contain an exceptionally high quantity of sulfur amino acids, methionine (19%) and cysteine (8%). Purified high S protein fraction contains several polypeptide components of similar molecular weights (10,000 daltons) but with charge heterogeneity. Peptide mapping of these proteins after proteolytic and chemical cleavage shows that they are highly homologous. The synthesis and accumulation of these high S protein components were found to occur during the later stages of fruit development. Messenger RNAs have been isolated from the developing Brazil nut kernels, and translated in a wheat germ extract. A number of discrete polypeptides were synthesized, and bands at molecular weights corresponding to the high S protein components were evident among the translation products separated by SDS-PAGE. Current work involves further characterization of these translation products and the identification of the high S protein mRNAs for future cloning experiments.

1246 EXPRESSION OF ZEIN mRNA DURING MAIZE ENDOSPERM DEVELOPMENT, M. David Marks and Brian A. Larkins, Purdue University, West Lafayette, IN 47907

In order to determine the relative levels of specific maize zein mRNAs transcribed during maize endosperm development, we have synthesized a number of full-length or near full-length zein cDNA clones using mRNA purified from developing endosperms. Many of these clones have been characterized using a mRNA hybrid selection procedure, partial or complete DNA sequencing, restriction enzyme analysis and cross-hybridization studies. On the basis of mRNA hybrid selection, most of the clones were found to be homologous to mRNA directing the synthesis of either 19 Kd or 22 Kd zein polypeptides; however, a few of the clones were homologous to mRNA directing the synthesis of the 15 Kd zein polypeptide. The sequence analysis and restriction enzyme mapping experiments have shown that the 19 Kd and 22 Kd zein clones can each be subdivided into several distinct groups. Most of the distinctions among clones revealed by the mapping and sequence analysis could also be made by cross-hybridization experiments. Using labeled cDNA inserts as probes in a sensitive dot hybridization procedure, it has been shown that clones sharing up to 91% of their sequences could be distinguished from one another.

Using a procedure simular to the dot hybridization procedure used in the cross-hybridization studies, we have measured the relative levels of several specific zein mRNA using poly A RNA isolated from normal and mutant endosperms at several different stages in development. In these experiments labeled cDNA probes (synthesized using the poly A RNA) were hybridized under stringent conditions to filters containing dots of representative cDNA clones. The results of these experiments will be presented.

1247 **UREASE IN THE DEVELOPING WATERMELON SEED,** Ann E. Blechl, Yair M. Heimer and Philip Filner, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568

The goal of this work is to determine the molecular basis of developmental and genetic increases in urease in plants. To obtain the cloned urease gene required for such investigations, we are taking advantage of the high concentrations of urease in the seeds of watermelon, Citrullus lanatus. Urease increased 10,000-fold during the course of development of watermelon seeds and ultimately accounted for 5% of the soluble protein in the mature seed. This level is comparable to that of urease in the seeds of jack bean, the classic source of the enzyme.

Urease was purified to near homogeneity from mature watermelon seeds. The native enzyme was smaller than urease purified from jack bean seeds (Sigma). Watermelon urease appeared to be composed of 90,000, 70,000 and 20,000 dalton polypeptides while jack bean urease contained only 90,000 dalton subunits. Limited proteolysis with V8 protease of the 90,000 and 70,000 dalton polypeptides of watermelon urease yielded markedly similar fragment patterns which differed from that of the 90,000 polypeptide of the jack bean urease.

RNA was isolated from developing watermelon seeds in which the urease activity was approximately 10% of its final maximum value. Translation of this RNA in the wheat germ system produced a 90,000 dalton polypeptide which could be immunoprecipitated by antiserum raised in rabbits against the purified native watermelon urease. We are preparing cDNA clones from this RNA and plan to identify those clones which contain the coding regions of watermelon urease.

1248 ANALYSIS OF A MUTANT LACKING THE α' SUBUNIT OF THE SOYBEAN 7S SEED STORAGE PROTEIN
Roger N. Beachy, Jeffrey J. Doyle, and Beth F. Ladin. Plant Biology Program, Washington University, St. Louis, MO 63130. The trimeric 7S seed storage proteins of soybean (Glycine max) are comprised of various combinations of three primary subunits (α', α, and β). The differential temporal and spatial regulation of the expression of members of the multigene family that encodes these subunits has been demonstrated. Recently Kitamura and Kaizuma identified a naturally occurring soybean cultivar, Keburi, that lacks the α' subunit, but contains α and β subunits (Japan J. Breed. 31:353-359, 1981). We have determined that: (1) α' subunits are not present in either the cotyledons or the embryonic axis of Keburi at any stage of embryogenesis; (2) Keburi embryos do not contain α' subunit mRNA; and (3) the EcoRI restriction fragment that contains the α' gene in a standard cultivar, Provar, (the 12.5 kb fragment described in Schuler et al., Nucleic Acids Research, in press) is absent in DNA isolated from Keburi. The latter results were from experiments in which blots of genomic DNAs were hybridized with a cloned α' cDNA, and with unique sequence DNA adjacent to the α' gene. New bands of hybridization were identified in DNA isolated from Keburi that are not present in DNA from Provar. The analysis of protein extracts on 2-D gels revealed an increase in the number of β subunits in Keburi when compared to Provar. Studies are continuing in order to determine the molecular basis for the change in protein content in Keburi, and to use this cultivar in studies relating to the structure and function of the soybean 7S storage proteins.

1249 SUBUNITS OF SOYBEAN GLYCININ AND THE GENES CONTROLLING THEIR SYNTHESIS, N. C. Nielsen and V. H. Thanh, USDA-ARS and Agronomy Dept., Purdue University, West Lafayette, IN, 47907.

Our laboratory is interested in the structure of glycinin subunits and the genes which direct their synthesis. We have approached this problem by first obtaining the complete amino acid sequence of a representative subunit, A_2B_{1a}, and then comparing that with the one deduced from the coding region of its gene. Three genomic clones bearing G-2 Eco R1 fragments (4.1 kb) were examined and shown to have A_2B_{1a} genetic information. Two clones, λ Ala and λ A28-5 have incomplete coding regions which extend from midway through the acidic component to the 3'-end of the gene. The third clone, recently sent to us by Bob Goldberg's laboratory, seems to contain an entire A_2B_{1a} gene. The coding sequence is interrupted by three introns. Two are within the region encoding the acidic component and one is within the region encoding the basic polypeptide component. The gene has an 18 amino acid signal sequence with a typically high leucine content. Potential regulatory regions that are typical of eukaryotic genes have been located in the 5' nontranscribed portion of the gene and polyadenylation sites are encoded downstream from chain termination codon.

1250 STRUCTURE AND EXPRESSION OF PHASEOLUS VULGARIS LECTIN GENES, Leslie M. Hoffman, Agrigenetics Advanced Research Laboratory, 5649 E. Buckeye Rd., Madison, WI 53716. Lectins belong to one of two groups of major seed glycoproteins in Phaseolus vulgaris, the French bean. Using poly(A) RNA from bean cotyledons a cDNA library was constructed which was screened with pea lectin cDNA to yield a clone, pPVL134, found to encode a 244 amino acid peptide. By comparison with the N-terminal sequence of phytohemagglutinin, a red kidney bean lectin, pPVL134 codes for an entire mature lectin peptide and 22 residues of a signal peptide. The 5' region of pPVL134 has 4 ATG codons, the 3'-most of which is contained in the sequence ATCATGG, conforming with the most favored consensus site for translation initiation (Kozak, 1980, Nucl. Acids Res. 9, 5233).

The ontogeny of lectin gene expression in several plant tissues was examined by Northern blot hybridization with the cloned cDNA. 1.1 kb lectin transcripts accumulate rapidly from the 10 mm to the 16 mm stages of cotyledon development, then sharply decrease in levels to the 20 mm stage. Leaves from plants bearing pods contain lectin transcripts, but none are observed in primary leaves or roots.

From a λ1059 library of P. vulgaris DNA, five types of lectin clones were selected using pPVL134 cDNA as a probe. One clone, λA9, has been shown by DNA sequence analysis to correspond to the lectin cDNA and to contain no intervening sequences. The mRNA coding region of the gene is flanked by very AT-rich regions.

Attempts to express the lectin gene in E. coli using prokaryotic promoters will be described. We wish to test the ability of bacterially synthesized, nonglycosylated plant lectin to retain its activity as an agglutinin or a mitogen.

1251 SYNTHESIS AND PROCESSING OF N-LINKED SUGAR SIDE CHAIN OF GLYCOPROTEINS IN PLANTS. Hidetaka Hori and Alan D. Elbein. The University of Texas Health Science Center at San Antonio, San Antonio TX 78284.

N-linked glycoproteins appear to be involved in recognition phenomena in all eukaryotic cells including plants. During the past several years, considerable progress has been made in the understanding of protein glycosylation in virus system and in animal cells. Plant cells also contain N-linked glycoproteins bu the mechanism of biosynthesis of these molecules is not as well understood as that of animal systems. However, some of these glycoproteins, as for example the lectins, may be involved in important plant-microbe interactions.

It is known that plant system also synthesize various lipid-linked oligosaccharide intermediates. We have been studying the mechanism of biosynthesis and the structure of these various oligosaccharide-lipid using cultured soybean cells and mung bean particulate enzyme system. In soybean cells incubated with [2-^{3}H]mannose or[2-^{3}H]glucose the major lipid-linked oligosaccharide was characterized as a $Glc_3Man_9GlcNAc_2$-PP-dolichol, similar or identical to that seen in animal cells. In the mung bean particulate enzyme, a number of lipid-linked oligosaccharides were formed from GDP-[^{14}C]mannose. After purification of the oligosaccharides 5 different oligosaccharides were characterized by enzymatic methods, as well as acetolysis and methylation. The structure of these oligosaccharides were as follows: Manβ-GlcNAc$_2$; Manα1-3 Manβ-GlcNAc$_2$; Manα1-2Manα1-3ManβGlcNAc$_2$; Manα1-2Manα1-2Manα1-3(Manα1-6)Manβ-GlcNAc$_2$ and Manα1-2Manα1-2Manα1-3(Manα1-3[Manα1-6]Manα1-6)Manβ-GlcNAc$_2$. The synthesis of these oligosaccharides in soybean cells was greatly reduced when cells were incubated in tunicamycin. Additionally, the oligosaccharide undergoes further processing following the transfer to proteins.

1252 BIOCHEMISTRY AND GENETIC REGULATION STUDIES OF LECTINS IN LEGUMINOSE PLANTS, Jeannine Horowitz, Univ. Simón Bolívar, Dept. Biología Celular, Caracas, Venezuela.

Lectins or Phytoheamagglutinins (PHA) are proteins that have a number of subunits which contain specific sites for sugar residues. Due to the interactions with these residues, they agglutinate red blood cells and have also leucoagglutinating and mitogenic activities. They constitute an important protein species of leguminose plants. The amount and activities of lectins in different varieties and species of Phaseolus and Vigna is inder study as well as the determination of the amount of similarities between these two species and the heterogeneity of the subunit pattern. In order to study the genetic regulation of PHA total messenger RNA has been isolated and cDNA obtained for cloning purposes into E. coli. This way it is hoped that a probe may be obtained for hybridization experiments to determine gene copy number in dry seeds of different varieties and the changing patterns in gene number or rate of transcription during seed development which is when the protein is synthesized.

1253 POLLEN-STIGMA INTERACTIONS IN PLANTS: POSSIBLE ROLE OF S-ALLELE ASSOCIATED ANTIGENS IN NICOTIANA ALATA, Marilyn A. Anderson, Rosslyn M. Hoggart and Adrienne E. Clarke, Plant Cell Biology Research, School of Botany, University of Melbourne, Parkville, Vic. 3052 Australia

The interacting partners during fertilization in higher plants are pollen grains which bear the male gametes and the female pistil. If mating is compatible , the pollen hydrates and germinates to produce a pollen tube which grows through the pistil to the embryo sac where the sperm are released. In many plant families inbreeding is prevented by rejection of the pollen tube after it has grown some distance down the style. This rejection is controlled by products of the S-gene which has multiple alleles, S1, S2, S3, S4 etc. The molecular basis of the rejection of the pollen tube is poorly understood.

We are investigating the control of self compatibility in Nicotiana alata by attempting to isolate and identify the S-gene-products. Homozygous plants S_2S_2 and S_3S_3 have been obtained by bud pollination of heterozygous plants S_1S_3 and S_2S_3. Specific antisera have been raised against stylar extracts, and using a Western blotting procedure, proteins corresponding to each S-genotype have been identified. As a second approach we are isolating mRNA from developing styles both before and after S-gene expression occurs. In vitro translation techniques will be used in an attempt to identify the S-gene product.

1254 ABA CONTROLLED EXPRESSION OF EMBRYO-SPECIFIC GENES DURING WHEAT GRAIN DEVELOPMENT, Ralph S. Quatrano, John D. Williamson, Barbara L. Ballo and Martha T. Hamblin, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.
Abscisic acid (ABA) at 10^{-6} to 10^{-4} M prevents 10-15 day old wheat (Triticum aestivum, L.) embryos from precocious germination, but promotes normal embryogenesis (e.g. scutellum differentiation and increases in fresh and dry weight) when cultured in vitro. We have identified gene products of embryos in culture that are characteristic of the embryogenic (+ABA) and germination (-ABA) pathways. In ABA(+) embryos, a set of acid-soluble proteins is newly synthesized which is embryo and stage-specific and normally made in vivo in 30-40 day old embryos. The acid-soluble proteins are comprised, at least in part, of scutellum-specific storage proteins located in membrane enclosed bodies and the lectin wheat germ agglutinin. In ABA (-) embryos, these proteins are not synthesized but another set of proteins, including the small subunit of RuBP carboxylase, is accumulated when the embryos precociously germinate into seedlings. These germination-specific proteins are not synthesized in ABA (+) embryos. cDNA to Poly A RNA isolated from ABA(+) and ABA(-) embryos was used to construct libraries from which germination and embryogenic specific clones were isolated. These cDNAs were used to isolate mRNAs for the gene products unique to each pathway and to determine the level(s) of genetic control associated with the regulation of these gene sets. In the case of RuBP carboxylase, the ABA inhibition appears to be at the level of transcription as determined by cell-free translation and hybridization studies.

1255 STRUCTURE AND REGULATION OF α-AMYLASE GENES IN WHEAT, David Baulcombe and Colin Lazarus, Plant Breeding Institute, Cambridge, UK CB2 2LQ
The production of α-amylases and other hydrolytic enzymes in aleurone cells of germinating wheat is dependent on gibberellic acid released from the embryo. For α-amylase and the products of at least six other mRNA species which we have cloned as cDNA, this regulation is mediated by increased mRNA levels. In order to understand the mechanism and integration of this regulation, the structure and expression levels of the genes involved are being investigated.

Analysis of α-amylase cDNA clones by cross hybridisation, by restriction enzyme mapping and by sequencing indicates that the wheat genome contains multiple, polymorphic α-amylase genes. The differences between α-amylase mRNA polymorphs is greatest in the non-coding regions, but also involves sequences within the protein coding sequence. α-amylase mRNA polymorphs are precisely co-regulated during development. Other mRNA sequences which are induced by GA_3 in germinating grains show distinct differences from α-amylase in the temporal pattern of expression during germination, and also in leaf tissue. The data are consistent with a cascade model in which expression of different GA_3 regulated genes is controlled by multiple mechanisms acting in concert.

1256 CLONING AND CHARACTERIZATION OF A FULL LENGTH cDNA FOR BARLEY α-AMYLASE, John C. Rogers, Washington University School of Medicine, St. Louis, MO 63110.
CDNA clones for barley α-amylase were obtained: Poly A(+) RNA from gibberellic acid stimulated aleurone cells was copied by reverse transcriptase, tailed with dC's, and inserted into the dG-tailed Pst-I site of pBR322 by standard methods. Transformants carrying cDNA sequences representing abundant mRNA's in stimulated aleurone cells were identified by comparing hybridization to colony replicas on nitrocellulose membranes, using cDNA probes from stimulated cells and from unstimulated cells in parallel sets. Plasmid DNA's from positive clones were labeled by nick translation and hybridized to northern blots of stimulated aleurone cell poly A(+) RNA electrophoresed through formaldehyde gels. Clones hybridizing to a 1550 bp size class, the most abundant specie present, were assumed to represent α-amylase sequences. This was confirmed when RNA that was selected by filter hybridization to the putative α-amylase sequences directed synthesis of immunoprecipitatable α-amylase in a wheat germ system. An apparently full length cDNA clone has sequenced by the Maxam-Gilbert technique. The 5' half has been sequenced on both strands, while portions of the 3' half have sequence from only one strand; thus the codon assignments are tenative. The clone is 1588 bp long and contains an open reading frame for 438 amino acids. 96 bases preceed a sequence coding for MGKNGSLCCFSLLLLLLLAGFASGHQVLFQGFN...; underlined are 8 consecutive hydrophobic amino acids, a feature that would be consistent with an N-terminal secretory piece. The nucleotide sequence AATAAA, thought to represent a polyadenylation signal, occurs 26 bp from the 3' poly A tract. The isolation of other partial length α-amylase cDNA clones with different 3' restriction sites indicates that more than one gene exists.

1257 HORMONAL EFFECTS ON α-AMYLASE mRNA LEVELS IN BARLEY ALEURONES USING A CLONED cDNA PROBE, S. Muthukrishnan, G. R. Chandra, George P. Albaugh and Abhaya Dandekar, Department of Biochemistry, Kansas State University, Manhattan, KS 66506, Seed Research Laboratory, USDA, Beltsville, MD 20705, and the National Institutes of Health, Bethesda, MD 20205.

A cDNA prepared from purified α-amylase mRNA has been characterized by restriction endonuclease digestion and positive selection of α-amylase mRNA followed by in vitro translation and immunoprecipitation [Muthukrishnan, Chandra and Maxwell (1983) J. Biol. Chem. In Press]. Nucleotide sequence of this clone is presented. Using this cloned DNA as probe, changes in levels of α-amylase mRNA in barley aleurones have been studied as functions of concentration of gibberellic acid, and presence or absence of abscisic acid, cycloheximide and amino acid analogs. Data are presented on the accumulation as well as turnover of α-amylase mRNA in aleurones. Our results indicate that α-amylase mRNA is synthesized de novo in aleurones in response to gibberellic acid. However, this induction requires one or more newly synthesized proteins.

Analysis of Southern blots of barley DNA digested with restriction enzymes using the α-amylase cDNA probe shows the presence of a family of genes for α-amylase. Variations in the pattern of methylation of α-amylase genes are observed when DNA's from embryo, aleurones and leaves are compared.

1258 **A PLANT HORMONE BINDING PROTEIN IN SEVERAL CEREALS,** J. Eugenè Fox and Eileen Gregerson, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568.

A protein which binds plant hormones of the cytokinin type with relatively high affinity and specificity has been isolated from wheat embryos. This protein, designated CBF-1, has a molecular weight of about 155,000 and migrates on SDS-polyacrylamide gels as four unlike subunits (57,000, 53,000, 39,000 and 24,000 Daltons, respectively). Photoaffinity labeling studies indicate that each of the four subunits participates in the domain of the cytokinin binding site. We present evidence of cytokinin binding moieties in several related cereals, including barley, rye, oats and rice, which are very similar to the wheat CBF-1 in structure, binding constants and immunological properties. In addition, work is described which indicates possible roles for these proteins as hormone receptors controlling events in plant growth.

1259 AUXIN-REGULATION OF GENE EXPRESSION: cDNA CLONES AS PROBES IN UNDERSTANDING THE MOLECULAR BASIS OF AUXIN REGULATED GROWTH IN THE SOYBEAN HYPOCOTYL, John C. Walker, Ron T. Nagao and Joe L. Key, Departments of Botany and Biochemistry, University of Georgia, Athens, GA 30602.

We have reported the synthesis and isolation of cDNA clones to two auxin-responsive mRNAs from the elongating region of the soybean hypocotyl (Walker and Key, Proc. Natl. Acad. Sci., in press). RNA blot analyses have shown these mRNAs are most abundant in the elongating zones of the soybean hypocotyl; the level of these mRNAs rapidly declines in excised elongating sections incubated without auxin but is maintained in tissue incubated with auxin; the addition of auxin to excised tissue previously depleted of auxin results in a rapid accumulation of these two mRNAs. These results are the first direct demonstration of a rapid alteration in the level of specific mRNAs in tissue undergoing auxin-induced cell elongation.

To further characterize these auxin-responsive mRNAs and the genes encoding them, as well as those previously isolated in our laboratory (Baulcombe and Key, 1980, J. Biol. Chem. 255, 8907-8913), we have used the cDNA clones as probes to isolate genomic clones from a soybean genomic library. These genomic clones are currently being mapped and subcloned in preparation for sequence analysis.

1260 INTERRELATIONSHIP OF GENE EXPRESSION, POLYSOME PREVALENCE, AND RESPIRATION OF CARROTS AND AVOCADOS EXPOSED TO ETHYLENE AND/OR CYANIDE, Mark L. Tucker and George G. Laties, Dept. of Biology, University of California, Los Angeles, CA 90024

Christoffersen and Laties showed that in vitro translation products of poly $(A)^+$ mRNA from ripe (climacteric) avocado differed from those from unripe (preclimacteric) avocado [Planta (1982) 155:52-57]. Furthermore, carrots exposed to ethylene for 24 hours showed an increase in polysome prevalence, and a change in the in vitro translation products of poly $(A)^+$ mRNA prepared therefrom [PNAS (1982) 79:4060-4063]. This work has been pursued further in order to better define the interrelationship of gene expression, polysome prevalence, and respiration in response to ethylene. Results show that in carrot the increase in polysome prevalence precedes by several hours the accumulation of new, abundant class, poly $(A)^+$ mRNA's as determined by in vitro translation. In avocado, polysome prevalence attains a maximum early in the respiratory climacteric and declines to preclimacteric levels at the peak of respiration. Changes in gene expression in both avocado and carrot, as determined by in vitro translation, correspond more closely with the rise in respiration than with the prevalence of polysomes. In addition, cyanide, which mimics ethylene in inducing the rise in respiration observed in carrots, and the ripening of avocado, causes an increase in the prevalence of polysomes in both tissues. The in vitro translation products of poly $(A)^+$ mRNA from carrots exposed to cyanide for 24 hours differ from those arising from translation of poly $(A)^+$ mRNA from ethylene treated roots. However, when avocados are treated with cyanide and/or ethylene the changes in gene expression, as determined by in vitro translation, are qualitatively similar.

1261 *ETHYLENE REGULATION OF GENE EXPRESSION IN CARROTS*, Scott E. Nichols[1] and George G. Laties[2], [1]Department of Chemistry and Biochemistry, [2]Department of Biology and Molecular Biology Institute, U.C.L.A., Los Angeles, CA 90024

Carrot roots respond to the presence of exogenously supplied ethylene with the initiation of a set of developmental responses. Many phenomena attend the progression of this response. Most notable is an increase in macromolecular synthesis and the rate of respiration. Recently demonstrated is the existence of a genetic component of this program of metabolic development. Christoffersen and Laties (PNAS 79(13), 4060-4065 (1982)) showed that two dimensional electrophoretic gels of the in vitro translation products of polysomal polyadenylated RNA isolated from 24 hour ethylene treated carrots contained many peptides not evinced by corresponding control tissue.

To study these messages on an individual basis, we constructed cDNA clones containing inserts corresponding to ethylene evoked messages. These recombinant plasmids were used to ascertain the change in the cytosolic prevalence of ethylene elicited messages. The kinetics of accumulation were also determined and we find that the onset of accumulation occurs in a non-concerted manner.

To answer the question of whether ethylene exerts its effect on gene transcription (as opposed to processing, transport or turnover) we are currently comparing synthesis of individual transcripts from run-off transcriptions in nuclei isolated from both ethylene treated and control carrots.

1262 ROBERTSON'S MUTATOR TRANSPOSABLE ELEMENTS OF MAIZE, Judith Strommer, Jeffrey Bennetzen, Mary Alleman and Michael Freeling, University of California, Berkeley California 94720 and International Plant Research Institute, San Carlos, CA 94070

Robertson's mutator, an insertional mutagen of maize, generates unstable mutations at a high frequency and fails to segregate in Mendelian fashion. Electrophoretic DNA blots probed with mutator-transposed sequences demonstrate that (1) mutator lines carry 20-100 copies of the 1.4 kb transposed element while non-mutator lines carry few or none; some members of the Zea genus carry related sequences; (3) copy number of the element varies from plant to plant in a manner inconsisten with Mendelian segregation patterns; and (4) stability or instability at a given allele does not correlate with the element's copy number or with the presence of a minor species of related sequence, 300 bp longer than the major species. The DNA sequence of the element has been determined (see Bennetzen and Strommer).

1263 COINDUCTION OF SYNTHESIS OF TWO METALLOTHIONEIN-LIKE, CADMIUM BINDING PROTEINS IN CADMIUM RESISTANT SUSPENSION CELL CULTURES OF DATURA INNOXIA, Paul J. Jackson, E. Jill Roth, and Peter R. McClure, Los Alamos National Laboratory, Los Alamos, New Mexico 87545.

Cadmium, a group IIB heavy metal and a major contaminant of coal and oil shale, is toxic to both plants and animals. The mechanism of toxicity is probably related to the binding of Cd to enzymes which normally require zinc as a factor for activity. Cells of Datura innoxia have been selected for their ability to grow rapidly in the presence of normally lethal levels of Cd. Lines capable of growth on Cd concentrations 10, 20, 40, 50, 75, and 100 times higher than the normally lethal concentration have been selected and isolated. Resistance is not the result of exclusion of the toxic ion from the cells. In response to a challenge of Cd ion, resistant cells begin de novo synthesis, within one hour, of two small molecular weight, cysteine rich metallothionein-like proteins. Both proteins have a high binding affinity for Cd ions, and neither protein is detectible in Cd-sensitive cell cultures of Datura grown in either the presence or absence of Cd. These results suggest that the mechanism of Cd resistance is related to the over-production, as compared to sensitive cells, of the two metallothionein-like Cd binding proteins. Such over-production, in turn, suggests either a change in the regulatory apparatus governing the synthesis of the two proteins, or an increase in the copy number of each gene coding for the two proteins. Coinduction of the two genes suggests similar or identical regulatory sites for the two genes. The latter implies close genetic linkage. We are currently investigating these different possibilities. This work supported by the USDOE.

1264 VARIABLE DNA SEQUENCES IN FLAX, Christopher A. Cullis, Peter B. Goldsbrough and Linda Charlton, 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. The genotrophs can differ from one another and the original line in a number of characteristics, including plant weight and height, total nuclear DNA amount, the number of copies of the rRNA and 5S RNA genesand the isozyme band patterns of peroxidase and acid phosphatase. The nuclear DNA amount, as measured by Feulgen staining, can differ between genotrophs by up to 20%. The determination of the Cot curves,of the total DNA extracted from various genotrophs, both by the homologous reassociation reaction and by the heterologous drive-trace reactions has indicated that the sequences which have varied in the genotrophs span a wide range of copy numbers.A number of specific sequences have been characterised in greater detail. These include the genes coding for the rRNA. These genes are arranged in tandem arrays of a repeating length of 8.6 kilobases and are very homogeneous, with no variation in either length or restriction sites having been found either within or between genotrophs. The copy number of this sequence varies more than three-fold between genotrophs.The 5S RNA genes vary more than two-fold and in those genotrophs with a low copy number a particular subset of the genes appear to have been lost. A cloned fragment which hybridises to poly-A^+ RNA (pCL 51) shows differences in the pattern of hybridisation to restriction digests of DNAs from different genotrophs. It has a number of characteristicswhich suggest it may me a mobile element and it is presently being sequenced.

1265 NITRATE REDUCTASE FROM PETUNIA, Roger C. Wiegand, Molecular Biology, Corporate Research Laboratories, Monsanto Company, St. Louis, MO 63167

The gene for nitrate reductase (NR) has good potential for use as a dominant selectable marker in plant cell transformation experiments. Because chlorate is toxic to cells expressing NR, deficient mutants are readily isolated in most plants. Transformants can then be selected by their ability to grow on nitrate as a sole nitrogen source. Experiments are therefore underway to clone the NR gene from petunia. To provide sequence information for the synthesis of synthetic DNA probes and antibodies for hybrid selected translation experiments the protein is being purified and characterized.

Nitrate reductase can be extensively purified from petunia using blue sepharose chromatography and metal chelate chromatography (Redinbaugh et al, (1982) Plant Phys. 69, 116). NR elutes from the blue column with NADH but not with NADPH. The enzyme will not use NADPH as a cofactor. Chlorate is a competitive inhibitor of the action of the enzyme on nitrate. NADH is oxidized by the enzyme in the presence of chlorate. It is likely that chlorate is also reduced by NR, perhaps explaining why chlorate is toxic only in NR positive cells. Enzyme activity is induced about 100X on addition of nitrate to cells growing on a reduced nitrogen source. Experiments are underway to determine whether this induction occurs at the level of transcription.

This and other experiments are directed toward understanding the control of transcription in plant cells. Mapping and sequencing of the NR promoter region and promoter regions of other genes as they are isolated, coupled with construction of variants, will help to define the important sequences.

1266 CALMODULIN-AND CALMODULIN-LIKE mRNAs IN PLANTS, Raymond E. Zielinski, Laboratory of Cellular and Molecular Physiology, Howard Hughes Medical Institute and Department of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Calmodulin (CaM) is an intracellular calcium receptor found in all eukaryotes examined. The primary sequence of the protein is highly conserved, showing only 10 changes between spinach and bovine brain out of 148 residues. CaM also shares regions of homology with several other Ca^{2+}-modulated proteins, which suggests that it is a member of a multigene family. Poly A^+ mRNA fractions prepared from barley, spinach, pea, corn and Chlamydomonas reinhardtii contain two to six mRNAs that hybridize with an eel CaM cDNA probe (Munjaal et al., 1981. PNAS 78, 2330) in Northern blot analyses. The higher plants all show two prominent mRNAs homologous with the eel cDNA probe. One mRNA in barley (~900 nucleotides long) encodes a polypeptide with a mass of about 16 Kdaltons, and probable represents authentic CaM mRNA. A second, equally abundant, mRNA (~2000 nucleotides long) encodes a polypeptide with a mass of 36 to 40 Kdaltons. The identities of the 2000-nucleotide mRNA and the minor mRNA species that hybridize with CaM cDNA are not yet known. Since nuclear RNA represents <<5% of the poly A^+ RNA, the minor species are probably not CaM mRNA precursors. There are no apparent qualitative changes in CaM-like mRNAs during light-induced development in barley. We are currently cloning cDNAs made from sucrose gradient-enriched fractions containing the CaM and CaM-like mRNAs described in order to establish their relationship.

Supported by NSF Grant PCM 8242875 and NIH Grant GM 30861.

1267 COORDINATELY REGULATED α_1 and β_2 TUBULIN GENES OF CHLAMYDOMONAS EXHIBIT AREAS OF SEQUENCE HOMOLOGY IN THEIR 5' FLANKING REGIONS. Donald P. Weeks, Karen J. Brunke, Barry U. Buchbinder, E. J. Sternberg and James G. Anthony, Institute for Cancer Research, Phila., Pa. 19111 and Zoecon Corp., 975 California Ave., Palo Alto, Ca. 94304.
When tubulin is required by the cell during division or during the regeneration of flagella, the unicellular alga, Chlamydomonas reinhardi, responds with a rapid and coordinate increase in α- and β-tubulin production. An increase and decrease in the synthesis of four tubulin mRNAs (two α- and two β-tubulin mRNAs) closely parallels the increase and decrease in tubulin production. Since Chlamydomonas appears to have only four single-copy tubulin genes (two for α- and two for β-tubulin) that are not closely linked, we have begun analysis of the 5' regions of clones of these genes to determine if there are shared regions of homology which might account for the observed coordination in the synthesis of the four tubulin mRNAs. Our preliminary analysis of the α_1 and β_2 tubulin genes has revealed regions of extensive homology. One region situated between the cap site and TATAAA box contains a sequence in which 9 out of 10 nucleotides are matched in the two genes. Another particularly interesting match occurs in an area of dyad symmetry just upstream from the TATAAA box. Such regions in similar positions in other eukaryotic genes have been implicated as potential regulatory regions. Transformation experiments are presently underway in which modified tubulin genes are introduced back into Chlamydomonas to determine which regions of the genes are important to transcription and to the coordinate regulation of gene expression.

1268 REGULATION OF AROMATIC AMINO ACID BIOSYNTHESIS IN POTATO (SOLANUM TUBEROSUM), Jose E. Pinto, JoAnn A. Suzich, William D. Park and Klaus M. Herrmann, Plant Physiology Program, Purdue University, West Lafayette, IN 47907
3-Deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase, the first enzyme of the shikimate pathway, was demonstrated in potato tubers. The enzyme catalyzes the conversion of phosphoenolpyruvate and erythrose 4-phosphate to DAHP and inorganic phosphate. DAHP was identified by absorption spectroscopy of its periodate degraded thiobarbiturate adduct. The enzyme was purified from potato tubers several thousand fold by ammonium sulfate precipitation followed by chromatography on phosphocellulose and hydroxylapatite. Potato tubers contain a high molecular weight inhibitor of the enzyme that is removed by ammonium sulfate fractionation. The enzyme is rapidly inactivated upon storage in the absence of SH-group protecting agents indicating the requirement of one or more free cysteinyl residues for activity. Dithiothreitol and β-mercaptoethanol, but not cysteine, protect against inactivation. The enzyme is stabilized by phosphoenolpyruvate; the pH optimum for the activity is 7.0. In contrast to bacterial DAHP synthases, the enzyme from potato tubers is not inhibited by any aromatic amino acid but activated by tryptophan. Kinetic analysis of the tryptophan activation indicates that the potato enzyme is a hysteretic protein and tryptophan affects formation of active oligomers as shown previously for the enzyme from carrot root (Daucus carota). (Supported by Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior - CAPES - of the Brazilian Government).

1269 DEVELOPMENT OF CHICKPEA PLANTLETS FROM GROWING CALLI AND CELLS IN SUSPENSION, S.Riazuddin and T.Husnain, NIAB.Faisalabad, Pakistan.

Apical meristem and nodal portions of chickpea (Cicer arietnum L.) have been successfully cultured in vitro and conditions have been established which can be used to develop calli or induce root and shoot formation. A modification of Murashige & Skoog medium*containing $1x10^{-6}$ M naphthalene acetic acid and 5×10^{-7}M benzyl amino purine has been used to induce root development in growing calli. Effects of different combinations of various auxins and cytokinins on the induction of shoot formation in calli and development of plantlets from cells in suspension are being investigated.

*Murashige, T. & Skoog, F.(1962) Physiol.Pl.15,473-97.

1270 THE GLYCOSYLATED SEED STORAGE PROTEIN GENES OF GLYCINE MAX AND PHASEOLUS VULGARIS, Mary A. Schuler[1], Jeffrey J. Doyle[1], Roger N. Beachy[1], Jerry L. Slightom[2], and Timothy C. Hall[2], [1]Plant Biology Program, Department of Biology, Washington University, St. Louis, MO 63130; [2]Agrigenetics Advanced Research Laboratory, Madison, Wisconsin 53716.

We have compared the complete nucleotide and derived amino acid sequences of the phaseolin seed storage protein gene of Phaseolus vulgaris (Sun et al., Nature 289, 37-41, 1981; Slightom and Hall, manuscript submitted) and a conglycinin storage gene of Glycine max (Schuler et al., Nucleic Acids Research, in press, 1982). Although these proteins are not antigenically related to one another, the architecture of the genes is similar throughout the entire length of the genes. Intervening sequences interrupt the same amino acid positions in both genes. In the genes that were represented in this comparison, approximately 70% of the nucleotides in the coding and intervening sequences are identical excluding the insertions and deletions that have occurred throughout the gene sequences. The nucleotides of the 5' flanking region, the signal peptide and the 3' noncoding regions are also highly conserved. The nucleotide mismatches found in the coding sequences are distributed throughout the three codon positions with little bias towards the third codon position. In addition to the single nucleotide differences, many insertions or deletions, ranging from three or twenty-seven nucleotides up to 396 nucleotides in length, occur in the coding region and are responsible for the molecular weight differences of the conglycinin α'-subunit and the phaseolin subunit. In spite of the fact that the primary amino acid sequences of these vicilin subunits differ by more than 40%, the predicted secondary structures of the two proteins are nearly identical.

Organelle Genomes

1271 The replicative origins of chloroplast DNA in Chlamydomonas reinhardi. Madeline Wu and Jody M. Waddell. University of Maryland Baltimore County, Catonsville, Md. 21228.

Liquid culture of C. reinhardi strain $CW15^{+}$ was grown in an environmental chamber providing 12 hr. light-12 hr dark regimen. Induction synchrony of chloroplast (Ct) DNA synthesis was achieved by a short incubation with 5-fluorodeoxyuridine (FUdR), an effective inhibitor which selectively affects Ct DNA synthesis (Wurtz et al. 1977). After the onset of a light period, the effect of FUdR was reversed by the addition of exogenous thymidine. The replicating structures were stabilized by either glyoxal treatment after DNA isolation or by in vivo crosslinking of DNA with trioxsalen. To localize the origin of replication, the DNA was digested with restriction enyzme EcoRI, BamHI and Bgl II. The restriction maps of those enzymes have been established (Rochaix, 1978). Fragments containing replicative loops or forks were observed and recorded under a transmission electron microscope. After measuring and analyzing hundreds of those fragments, we concluded that replication started at 2 unique positions in the chloroplast genome. Those origins are 8kb apart. One origin was 16kb distal from the 16s rRNA gene. The initial replication is asymmetrical.

Ref. Wurtz, E.A., Boynton, J.E. and Gillham, N.W. (1977) Proc. Natl. Acad. Sci. USA 74: 4552-4556.
Rochaix, J.D. (1978) J. Mol. Biol. 126: 597-617.

1272 CLONE LIBRARY OF SOYBEAN CHLOROPLAST GENOME, G. Singh[1], Neil A. Straus[2] J.P. Williams[2] and D.T.N. Pillay[1]. [1]Department of Biology, University of Windsor, ON N9B 3P4, [2]Department of Botany, University of Toronto, Toronto, ON M5S 1A1

The entire soybean chloroplast DNA except the 23.2 kilobase (Kb) Sac I fragment has been cloned. Pvu II and Sac I restriction fragments encompassing the total genome were selected to establish the clone bank. In all, eight blunt end Pvu II fragments (app. 53 Kb) were inserted in pBR322 and a set of seven Sac I (about 82 Kb) were cloned in 2.2 Kb pDPL14. In both cases the DNA fragments were inserted outside the respective drug markers. Work is in progress to clone the remaining 23.2 Kb Sac I fragment.

1273 REGULATED EXPRESSION OF A MUSTARD CHLOROPLAST GENE IN VIVO AND IN VITRO, Gerhard Link, Rockefeller University, New York, NY 10021 (on leave of absence from the Biol. Instit. II, University of Freiburg, D-7800 Freiburg, W. Germany).

Mustard (Sinapis alba L.) plastid gene expression has been studied both in vivo and in vitro. Gel transfer hybridization experiments were carried out, using cloned chloroplast genes and total organellar RNA from various phases during plastid development. These studies have allowed to define classes of plastid genes in terms of their mode of expression, i.e., constitutive, inducible, and repressed genes.

Efforts were made towards a better understanding of the mechanism(s) involved in developmentally regulated plastid gene expression. Cloned DNA fragments, containing the 5'portion of the gene for the 32 - 35-kdal photosystem II polypeptide (PII), were used as template in a run-off transcription system obtained from plastid lysates. In this system, faithful initiation of transcription was demonstrated to occur, and essential regions in the 5'upstream sequence of the cloned DNA were located. Furthermore, the transcriptional properties of lysates obtained either etioplasts or from chloroplasts were compared in an attempt to characterize the factor(s) responsible for differential expression of this plastid gene.

1274 STRUCTURAL DIVERSITY OF CHLOROPLAST GAPDH FROM ANGIOSPERMS, Rüdiger Cerff, Institut für Botanik, Universität Hannover, Herrenhäuser Str. 2, D-3000 Hannover 21, FRG.

In a previous electrophoretic survey (1), including 13 different angiosperm species, it was demonstrated that cytosolic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a very conservative protein, while the chloroplast enzyme seems to be one of the most variable dehydrogenases so far reported. The present investigation (2) shows that this variability of the native chloroplast dehydrogenase is paralleled by a remarkable interspecific heterogeneity of the enzyme with respect to size and number of subunits and their corresponding precursors synthesized in vitro. The transit peptides of these precursors are especially variable. Among 12 different angiosperm species they vary between 4000 and 12000 daltons and seem to fall into three major size classes.
These results contradict the Gottlieb hypothesis (3), which proposes that chloroplast enzymes in general may be less variable than their cytosolic counterparts. Together with structural data published previously (4) they seem to point to a rather distant evolutionary relationship between the two dehydrogenases, as suggested by the endosymbiotic theory of chloroplast evolution.

References: (1) Cerff, R. (1982) Eur. J. Biochem. 126, 513-515
(2) Cerff, R. & Kloppstech, K. (1982) Proc. Natl. Acad. Sci. USA, in press.
(3) Gottlieb, L.D. (1982) Science 216, 373-380.
(4) Cerff, R. & Chambers, S.E. (1979) J. Biol. Chem. 254, 6094-6098.

1275 IN VITRO SYNTHESIS AND ASSEMBLY OF THE TWO MINOR CHLOROPHYLL a COMPLEXES BY ISOLATED CHLOROPLASTS FROM ACETABULARIA Beverley R. Green, Botany Dept., University of British Columbia, Vancouver, B.C., Canada, V6T 2B1.

The two minor chlorophyll a complexes which are part of the Photosystem II reaction centre core, are synthesized by isolated chloroplasts fed radioactive amino acids in the light. This involves not only synthesis of the apo-proteins on chloroplast ribosomes, but also the correct assembly with chlorophyll in the isolated chloroplast, in the absence of any cytoplasmic contribution.

In contrast, the minor chlorophyll a+b complex "CP 29" and its dimers are not synthesized by isolated chloroplasts. In this they resemble the major Light-Harvesting Chlorophyll a+b complex. This suggests that light-harvesting complexes, which are not essential to the activity of either Photosystem can be under nuclear control and subject to the fine-tuning of environmental influences, whereas the reaction centre polypeptides, cytochromes, and the 32-34 kd protein(which may carry the secondary electron acceptor of PS II)are chloroplast gene products and synthesized under tighter control of that genome.

1276 CHLOROPLAST DNA AND CYTOPLASMIC MALE STERILITY, Li Jigeng and Liu Yinong, Institute of Genetics, Academia Sinica, Beijing, China.

Studies on the chloroplast DNA (ct DNA) of male sterile lines and their respective maintainers of maize, wheat and rape indicate that

1) Heat denaturation of ctDNA from a male sterile line in maize yielded a profile with three melting regions. This base sequence heterogeneity was not observed for the ctDNA of its maintainer.

2) Maize ctDNA was digested with EcoR1 and BamH1 restriction endonucleases, wheat and rape ctDNA with EcoR1 only. No significant differences were observed in the comparison of the fragment patterns from the sterile lines and their maintainers with the exception of the sterile line in rape which lacked one fragment present in its maintainer.

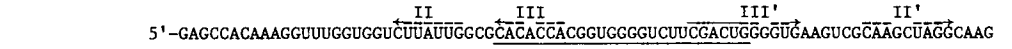
3) Two dimensional electrophoresis using a gradient of denaturing agents revealed several differences in number and relative positions of the separated restriction endonuclease fragments between the sterile lines and their respective maintainers in all three tested crops. This raises the possibility that changes in ctDNA may be involved in CMS.

1277 DNA SEQUENCE HOMOLOGIES BETWEEN MITOCHONDRIAL AND CHLOROPLAST GENOMES, David B. Stern, Jeffrey D. Palmer and David M. Lonsdale*, Carnegie Institute of Washington, Stanford, CA 94305 and *Plant Breeding Institute, Cambridge CB2 2LQ, England

We have been investigating the sequence organization of the corn mitochondrial (mt) genome, and have shown that the mt rRNAs are each encoded by a single gene (1). Also, we have identified a 12Kb sequence in the corn mt genome which is essentially identical to a portion of the corn chloroplast (ct) inverted repeat (2). This discovery raises several questions: 1) Are other corn ctDNA sequences present in the corn mtDNA, and do such homologies exist between the ct and mt genomes of other plant taxa? 2) How often do these events occur, and what is the mechanism? 3) Is there any function attached to the presence of ctDNA sequences in the mitochondrion, as may be suggested by our finding that the 12Kb sequence is altered in the mtDNA of male-sterile lines of corn (2)? Current results include the following: 1) Significant DNA sequence homology exists between a number of different cloned mung bean or spinach ctDNA restriction fragments and mtDNAs from Atriplex, spinach, pea, mung bean, wheat and corn. The degree and extent of homology appear to vary widely. 2) DNA sequences of the borders of the ctDNA inverted repeat and RuBP carboxylase LS gene homologous regions of corn mtDNA indicate that ct sequences within the mt genome can be highly conserved at the nucleotide sequence level. 3) Clone banks of several of these mtDNAs are being used to determine the physical organization of the mt genome, in order to elucidate the precise nature of the evolutionary mechanisms - including intergenomic sequence transfer - responsible for variation in mt genome size and structure.

1. Stern, D.B., Dyer, T.A. and Lonsdale, D.M. Nucl. Acids Res. 11:3333-3337 (1982).
2. Stern, D.B. and Lonsdale, D.M. Nature 299:698-702 (1982).

1278 PARTIAL NUCLEOTIDE SEQUENCE OF MAIZE MITOCHONDRIAL 5S rRNA REVEALS AN UNUSUAL FEATURE, Lilian M. Hsu and Jan Kan Chan, Mount Holyoke College, South Hadley, MA 01075. Mitochondrial (mt) 5S rRNA of maize strain B37N, recovered from the total mt RNAs by agarose gel electrophoresis, was found to be of sufficient purity for RNA sequencing analysis. Using the enzymatic sequencing method, 80 nucleotides from the 5'-end has been determined as follows:



The partial sequence can be folded into the standard 5S rRNA structure, yielding helices II and III (dashed overlines). Two features in the large loop enclosed by helix III are noted. (1) The proposed tRNA TψCG-complementary sequence in this RNA is CGACU (overlined), unlike CGAAC for all prokaryotic 5S rRNAs, but more similar to CGACC for wheat germ mt 5S rRNA. (2) A stretch of G-residues replaces the pyrimidine-rich sequence in the highly conserved region of all sequenced 5S rRNAs spanning the 5'-arm of helix III and the entire large loop (underlined). Sequencing of the 3'-end of this molecule is in progress. Results of Southern hybridization analysis indicated that this RNA is encoded once by the maize mitochondrial genome, as found previously (Stern, Dyer and Lonsdale, (1982) Nucleic Acids Res. 10, 3333-3340). Furthermore, the 5S rRNA gene region is not altered in the various cytoplasmic male sterile strains of maize.

1279 A Split Genome in Mitochondria of *Oenothera*.

A.Brennicke and P.Blanz

Institut für Biologie I, University of Tübingen, Auf der Morgenstelle 1, D-7400 Tübingen, Federal Republic of Germany

The mitochondrial genome in *Oenothera berteriana* is divided into several minichromosome-like circular DNA molecules. These closed circular molecules can be dissolved into different size classes in agarose gels. Each of the size classes consists of distinct sequences, which can be shown by restriction analysis of isolated circular DNA populations. Each size class so far investigated generates a unique set of restriction fragments. Several transcripts have been found that hybridize to different size classes indicating a distribution of the total mitochondrial information in this higher plant over different circular molecules.

1280 STRUCTURAL REARRANGEMENTS OF THE MAIZE MITOCHONDRIAL DNA BETWEEN THE NORMAL AND MALE STERILE LINES, Christiane M.-R. Fauron, David M. Lonsdale and Tony P. Hodge, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England. Comparative studies between the restriction maps of the mitochondrial genomes from the maize cytoplasmic male sterile cms-C, cms-S, cms-T to the normal fertile type have identified regions where sequence alterations have occurred. These alterations, although different between the three male sterile mitochondrial genomes, are located at similar positions when compared to the physical map of the normal mitochondrial DNA. They involve the unique sequences as well as the repeated sequences. Rearrangements or deletions within homologous repeating units are not necessarily the same. Evidence has been obtained that sequences which are contiguous in the normal type mtDNA are no longer adjacent in cms-T, implying a different genomic organization perhaps brought about by genetic recombination.

1281 GENE CONTROL OF PHYCOBILISOME PHOTOSYNTHETIC ADAPTATION. A. Lönneborg, S.R. Kalla, J.-E. Hällgren, G. Öquist and P. Gustafsson. Deps. of Microbiology and Plant Physiology, Umeå University, Umeå, Sweden.
Cyanobacteria (blue-green algae) are prokaryotes but their mode of photosynthesis resembles, except in the light harvesting system, that of higher plants. One major component in the light harvesting system in cyanobacteria is the phycobilisome, a macromolecular aggregate of water-soluble chromoproteins. The phycobiliproteins in the cyanobacteria A. Nidulans, which we have chosen for our studies, are C-phycocyanin and allophycocyanin.
We are interested in the light induced regulation of the phycobilisome as well as its biogenesis. Regulation of the composition can occur both as a variation in size and as the number of light antennas. To study the dynamic changes of the phycobilisome during adaptation we perform shifts between different light conditions. Another area of interest is to use hybrid-DNA technology to get more insight into the adaptation process at the genetic level. We have constructed a gene bank of A. nidulans DNA in the vector pBR322 in E. coli. By using a synthetic oligonucleotide complementory to the amino acid sequence of one phycocyanine subunit we have localized the corresponding gene on the chromosome of the bacteria. We are preparing to use isolated genes to study genome organization and structure and also as probes for m-RNA-production during adaptation. Another interesting aspect is to use isolated genes to study evolutionary aspects of the photosynthetic apparatus.