C. Benning

THIRD INTERNATIONAL MEETING ON ARABIDOPSIS

APRIL 10-12, 1987

MICHIGAN STATE UNIVERSITY

ABSTRACTS of LECTURES and POSTERS

THIRD INTERNATIONAL ARABIDOPSIS MEETING
DOE PLANT RESEARCH LABORATORY
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#11

RECENT TRENDS IN MAKING USE OF THE AIS-SEED BANK. A.R. Kranz.
Botanical Institute, J.W.Goethe-University, POB 111 932,
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With reference to the trends in Arabidopsis research from the factorial, over mutable and functional to the molecular gene (1 to 4) the seed collection of Arabidopsis Information Service, Frankfurt (FRG) has been used in a new and additional style. In retrospect three periods of handling the gene resources can be distinguished, the sampling, the conservation, and the employment. Recent experience shows that an improved usage of the Arabidopsis gene pool depends on a detailed classical and molecular exploration of this fascinating genome of exceptional suitability (4,5).

In this context an eminent assay of this crucifer species has been discussed (6), the mutation spectrum induced in comparison with the natural genetic variability of populations collected from hundreds of different ecological habitats. Since we are now living in an era of gene transfer between alien DNA, evidently, the additional variability of related crucifer genera should be also enclosed in this study.

References

1. Laibach, F. (1943) Bot. Arch. 44, 30
2. Ivanov, V.I. (1974) Nauka (Moscow) 27, 190 pp.
3. Redei, G.P. (1975) Annual Rev. Genet. 9, 111 (1975)
4. Meyerowitz, E.M., R.E. Pruitt (1985), Science 229, 1214
5. Koornneef, M. et al. (1983) J. of Heredity 74, 265
6. Kranz, A.R. (1984) IAEA Panel Proceed. Ser. STI/PUB 665, 59

#21

Agrobacterium-Mediated Transformation of Intact Tissues of **Arabidopsis thaliana**. K.A. Feldmann, M.D. Marks, M.L. Christianson. Sandoz Crop Protection Corp., 975 California Ave., Palo Alto, CA 94304.

Traditional **Agrobacterium**-mediated transformation systems employ cells or tissues that must pass through a tissue culture phase. For **Nicotiana** this is not a difficult step. For **A. thaliana**, however, this step results in a dramatic loss in morphogenetic potential in transformed cultures, and as a result the transformant is not useful in genetic studies. Even when rapid regeneration schemes (1) are used in conjuction with **Agrobacterium**-mediated transformation, few fertile transformed whole plants result (unpublished data). Additionally, it takes 4-5 months to go from explant to transformed seed with **in vitro** transformation techniques. A procedure that allows the transformation of genetically effective cells (meitoic cells or precursors) of intact tissues would be of great use for **Arabidopsis** transformation.

We have developed a transformation protocol that allows us to infect imbibing seeds of **A. thaliana** (geographic race WS) with **Agrobacterium tumefaciens** (c58C1rif, containing the 3850:1003 Ti plasmid, conferring resistance to kanamycin; 2;3). Treated plants (T1) produced some progeny (T2) that were resistant to kanamycin (>100 mg/l). These kanamycin-resistant plants were allowed to self and the T3 progeny were collected. Germination of the T3 families on medium containing kanamycin and scoring for Kan^R and kan^S seedlings indicated that the majority (57%) of these isolates contained one functional neomycin phosphotransferase gene. Thirty-three percent of the families segregated for 2 to 4 functional inserts. Ten percent of the families did not segregate in a Mendelian fashion; these families fall into two groups: group I families segregate approximately 1R:100S, group II segregate approximately 1R:1S. All kanamycin resistant seedlings are positive for NPT II activity. Southern hybridization analysis demonstrated that even families segregating for a single Mendelian factor conferring kan^R have multiple T-DNA inserts. The hybridization patterns for most of the transformants is quite complex. The hybridization profiles indicate that the inserts occur as single integrates, as well as tandem repeats, and inverted right and left border repeats. Linkage of the inserts will be tested by Southern hybridization analysis.

It is not clear exactly how or when the genetically effective transformation (GET) of germinating seeds occurs. The numerous physical parameters that appear to be important in the transformation of intact tissues will be discussed. The potential of this whole plant transformation system for doing insertional mutagenesis in **Arabidopsis** will be assessed.

1. Feldmann, K., Marks, M. 1986. Plant Science 47:63-69.
2. Velten, J., Schell, J. 1985. Nucleic Acids Research 13:6981-6998.
3. Feldmann, K., Marks, M. 1987. MGG, in press.
4. Stewart, R. Burk, L. 1970. Amer. J. Bot. 57:1010-1016.

#22

PHYSICAL AND GENETIC ANALYSIS OF TRANSFORMED PLANTS. Robert B. Simpson, Barbara Ballo. Gabriel Meeker. Dennis Prosen. The Plant Cell Research Institute. Inc.. 6560 Trinity Court. Dublin. CA 94568.

Using Agrobacteria with a Ti plasmid-derived vector (1) containing a selectable marker for kanamycin resistance. we have transformed tobacco using the leaf disk procedure (2). The transformed plants have been analyzed physically using Southern blot analysis of the transferred DNA (T-DNA) and genetically by following the heritability of resistance to kanamycin in the germination medium.

One to six copies of DNA from the vector were transferred and maintained in the plant genome (3). Approximately 30% of the copies transferred were found to be incomplete or rearranged and in some cases joined as inverted repeats (3). Almost half of the 133 plants analyzed genetically contained more than one kanamycin resistance locus (4). In one plant, kanamycin-resistance traits were at four independent chromosomal positions. although two were genetically linked at about 3 centimorgans (3). We have preliminary evidence (4) that in two of the 133 plants, a recessive mutation is linked to the kanamycin-resistance locus and thus may represent a gene mutated through insertional inactivation by the T-DNA. In one case. the mutation appears to be an embryo lethal while in the other case, the abnormal phenotype is reduced apical dominance.

Encouraged by the finding that two Agrobacteria can transform the same plant cell (5). we have constructed a model genomic library consisting of ten distinguishable inserts in our vector (6). Physical analysis of the transferred DNA in 66 transgenic plants showed that each of the ten library members was stably transferred to at least two plants. and that there may have been some discrimination against longer inserts. Although most plants contained T-DNA from only one of the ten library members. about a quarter of the plants contained T-DNA from two or more different library members. One plant contained T-DNA from at least four different bacteria indicating that multiple bacteria can transform a plant cell. Genetic analysis of 32 plants for the kanamycin-resistance trait showed no correlation between the number of functional loci and the number of different T-DNAs.

1. Simpson. RB. A Spielmann. L Margossian and TD McKnight (1986) Plant Mol Biol 6. 403.
2. Horsch, RB. RT Fraley. SG Rogers, PR Sanders. A Lloyd and N Hoffmann (1984) Science 223.496
3. Spielmann. A and RB Simpson (1986) Mol Gen Genet 205,34.
4. Ballo, B, G Meeker and RB Simpson (1987) unpublished.
5. McKnight. TD. M Lillis and RB Simpson (1987) Plant Mol Biol. in press.
6. Prosen, D and RB Simpson (1987) in preparation.

#23

Transformation of Arabidopsis thaliana var. Columbia via leaf-piece cocultivation with an Agrobacterium tumefaciens strain containing a plasmid encoding for hygromycin resistance. A. Lloyd, Y. Muskopf, A. Barnason, R. Horsch. Monsanto Co.; 700 Chesterfield Village Pkwy; St.Louis, MO 63198.

Transformed fertile Arabidopsis plants are produced using a modification of the leaf disc transformation system (1). The transformation procedure is outlined below and will be discussed in a presentation. We are currently using the published Arabidopsis transformation procedure (2) modified for rooting regenerated shoots. Arabidopsis plants are grown sterilely for 3-4 weeks. Leaf pieces are cut across the blade so as to maximize the cut surface and placed on callus regeneration medium for 2 days. These pieces are placed in 5ml of hormone free liquid MS medium and 1ml of an overnight LB culture of the Agrobacterium containing a hygromycin vector is added. After 1-2 minutes of agitation the excess liquid is blotted from the leaf pieces with sterile filter paper and the pieces are placed on tobacco cell feeder plates (1) containing the callus/regeneration medium. After 2 days these pieces are placed on the same medium, minus the feeder layer, containing 500 ug/ml carbenicillin and 30 ug/ml hygromycin. In 2-3 weeks, uninoculated leaf pieces are killed while hygromycin resistant callus grows from approxiamately 1/3 of the inoculated leaf pieces. When subcultured on selection medium every three weeks, approximately 1/2 of these calli will form shoots within 2-12 months. These shoots are excised and placed on GA medium without hygromycin (0.1mg/l GA3, 0.5 mg/l NAA, 0.1 mg/l BA, otherwise identical to callus/regeneration medium) for 10-20 days. After this, the shoot base is dipped in Rootone, placed in soil, and kept at 100% humidity until visible shoot growth starts (1-3 weeks). The humidity is then gradually reduced. We have transferred 206 putative Arabidopsis transformants to soil using this system with a variety of hygromycin vectors. Only 110 of these have produced seed. Of these 110 plants, 85 produced nopaline while 25 did not. Most of the 25 opine negative plants are non-transformed escapes. Seed from 78 transformants has been screened for inheritance of the T-DNA by germination on selection medium. Twelve of these 78 had no functional T-DNA insert while 66 had 1 or more unlinked functional inserts. In our hands, selection for transformants on G418 using vectors encoding NPTII can be accomplished, but at greatly reduced efficiency.

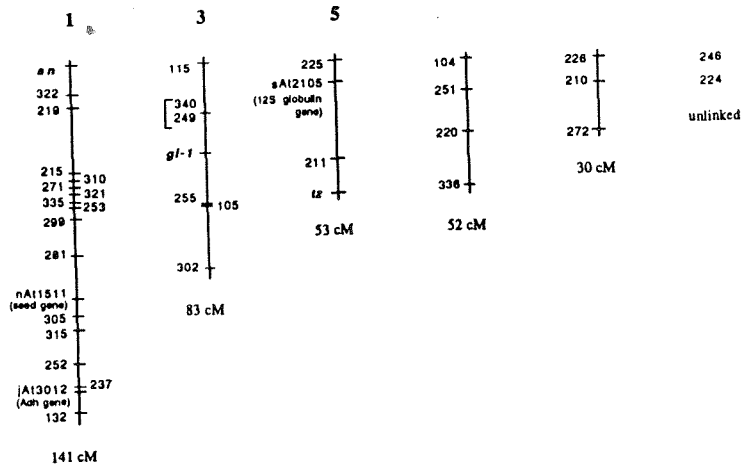
1. Horsch, R.B. et al. (1985) Science 227:1229.
2. Lloyd, A.M. et al. (1986) Science 234:464.

#31

A Restriction Fragment Length Polymorphism Map of the *Arabidopsis* Genome

Chang, C., DeJohn, A. W., Pruitt, R. E., and Meyerowitz, E. M. Division of Biology, Caltech, Pasadena, California 91125

We are constructing a restriction fragment length polymorphism (RFLP) map in *Arabidopsis* to make available a gene cloning method for *Arabidopsis* in which RFLP markers are used as starting points for chromosome walking (as long as the desired gene has a phenotype allowing its map location to be determined). This method should become generally applicable when the map density reaches ~250 RFLP markers so that the average distance between adjacent markers is <300 kb. So far the map contains 33 markers within 5 linkage groups (fig), 3 of which are assigned to chromosomes 1, 3, and 5 based on linkage to mapped visible markers. Chromosomes 2 and 4 are presently being identified. We hope to integrate the RFLP map with the existing visible marker map (1).



To construct this map we examined segregation of RFLP markers in the progeny of a cross between wild-type Neiderzenz and a Columbia strain homozygous for *an*, *gl-1*, and *tz* (on chromosomes 1, 3 and 5 respectively). F1 plants were allowed to self-cross, as were F2 plants. The visible markers were scored in the F3 to reveal the genotype of each F2 parent. Genomic DNA of 106 different F3 pools was digested, blotted, and probed with clones having RFLPs between the two original parents. Using the segregation data we calculated map distances for pairwise combinations of clones. Polymorphic clones were obtained from cloned *Arabidopsis* genes and from random λ clones that had been screened for low copy number. ~40% of the random clones are polymorphic with *Eco*RI, *Xba*I, and/or *Bgl*II.

1. Koornneef, M. *et al.* (1983) *J. Hered.* 74: 265-272.

#32

PROGRESS IN CONSTRUCTING A PHYSICAL MAP OF THE *ARABIDOPSIS THALIANA* GENOME. Brian Hauge, Christian Fritze, Hong-Gil Nam, Kyung-Hee Paek and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114.

Our general interest is in elucidating mechanisms of gene regulatory control in higher plants. We are currently studying several regulated gene systems, namely organ specificity and light regulation of the gene families encoding glutamine synthetase and glyceraldehyde-3-phosphate dehydrogenase and nitrate induction of nitrate reductase. However, some of the most interesting genes should be those involved in early development and embryogenesis. The short life cycle, availability of mapping strains, and the small genome size of *Arabidopsis thaliana*, offer the potential for the identification and cloning of those genes which, based on their mutant phenotype, appear likely to be involved in developmental regulation. Such genes might even be unique to the plant kingdom, potentially reflecting fundamental differences in development between animals and plants. Developmentally regulated genes are traditionally difficult to clone and analyze molecularly and are usually initially defined by mutants and their corresponding phenotypes. Successfull isolation of such genes, will be limited by the availibility of techniques which readily permit the cloning of genes based solely on their mutant phenotype. As a first major step toward cloning such genes we are constructing a complete physical map of the *Arabidopsis thaliana* genome. In all likelihood, total continuity of the map (i.e. 5 linkage groups) will be difficult to achieve. Nevertheless, even at a level of 80 to 90% completion the map should allow immediate access to most regions of the genome which can be genetically identified.

A random shear cosmid library of *Arabidopsis thaliana* DNA (geographical race Columbia), with an average insert size of ~40kb, was constructed using a derivative of the cosmid vector, loristB. The mapping of individual clones is being carried out using the methodology essentially as described by Coulson, A. *et al.* (1). Briefly, DNA is isolated from randomly selected clones, digested with HindIII, the ends labeled using reverse transcriptase, and then digested with Sau3A. The resultant fragments are of suitable size for separation on denaturing polyacrylamide gels. The position of each labeled band is entered into a computer using a digitizer and stored in a growing data base. Overlapping clones ("contigs") are identified using a computer matching program. At present over 6000 clones, corresponding to four genomic equivalents, have been fingerprinted and ~600 have been entered into the computer and analysed. From this limited data base 39 contigs have been identified. To assess the reliability of this approach, early

linkages are being confirmed by conventional restriction analysis and Southern blotting.

To be useful this overlapping cosmid set ("the physical map") must be correlated with the genetic map of *Arabidopsis thaliana*. Unfortunately, very few cDNA and/or genomic probes are available for genes of known genetic map position. To circumvent this problem a RFLP map is being constructed using probes from the same library employed for the physical map. As parental strains we have chosen *Arabidopsis thaliana* Columbia and Landsberg erecta, which display a surprisingly high degree of polymorphism despite the fact that these two geographical races are otherwise thought to be very closely related. Once identified, RFLPs will be mapped against known genetic markers yielding an ordered series of physical markers of known genetic location. We estimate that ~150 polymorphic probes will be required in order to generate a RFLP map with 10cM resolution.

Once completed, the combined RFLP/physical map will allow ready access to any region of the genome of known genetic map location. Clearly, the resolution and completeness of the maps will determine how easily and accurately overlapping cosmids in the vicinity of the mutation can be identified. To determine when the desired gene has been cloned, it will be necessary to transform mutant plants and assay for the gene by functional complementation. Since only a limited number of clones in the vicinity of the gene of interest need be examined, this procedure does not require a high efficiency transformation system as would be essential for "shotgun cloning". Obviously all the molecular tools currently available can be used to investigate the structure and function of the gene product once the appropriate clone has been identified.

References

1. Coulson, A., Sulston, J., Brenner, S., and Karn, J. (1986) Proc. Natl. Acad. Sci. USA 83,7821-7825.

#33

Sreening transgenic Arabidopsis for T-DNA-induced insertional mutants: an initial approach. Y. Muskopf, A. Lloyd, J. Medford, S. Rogers and H. Klee. Monsanto Corporate Research. 700 Chesterfield Vlge. Pkwy. Chesterfield, MO 63198.

Plant genes have been traditionally identified by producing genetic lesions with a variety of mutagens. While this has been a powerful approach, the mutations often provide no clue as to the identity of the gene product. An alternative approach involves gene "tagging": disruption of a genetic element by insertional mutagenesis. This can be accomplished by use of a transposon such as Ac or by transformation with an Agrobacterium T-DNA. The disrupted gene can easily be isolated by cloning the inserted element and the flanking plant DNA. The small genome size and paucity of dispersed repetitive DNA in Arabidopsis (1) make this an ideal organism for a T-DNA gene tagging approach. We have generated a large number of transgenic Arabidopsis plants in the course of developing a reliable transformation system (2). If T-DNA insertion is random, a significant number of the insertions will be into genes. Consequently, we have been screening progeny of transformants for any visible mutations. The progeny of over sixty such plants have been screened for morphological abnormalities. The most interesting mutants are selfed and outcrossed to score for tight linkage of the T-DNA and the mutation. Several interesting mutants have been identified and are currently being characterized. The phenotypic abnormalities of these mutants will be presented.

(1) Meyerowitz, E. and Pruitt, R. (1985) Science 229:1214.
(2) Lloyd, A., et al. (1986) Science 234:464.

#34

SEPARATION, ISOLATION AND CLONING OF VERY LARGE DNA MOLECULES. By: Ronald W. Davis, Doug Vollrath, Gil Chu, Chris Traver and Marge Thomas. Stanford University School of Medicine, Dept. of Biochemistry, Stanford, California 94305.

Very large DNA molecules can be separated by gel electrophoresis using a contour clamped homogeneous electric field and transverse pulses at an angle of 120° We can resolve all of the chromosomes of S. cerevisiae, Candida albicans, S. pombe and most of the chromosomes of Neurospora. We cannot yet resolve DNA molecules much greater than 10 megabases. We observe at least three regions of differing resolution. There is a region in the lower molecular weight range (above 50kb) that almost gives a linear relationship between mobility and molecular weight. Unexpectedly, at higher molecular weight the gels resolve with considerably increased resolution. This high resolution region, however, is only over a fairly narrow molecular weight range. At still higher molecular weights, the gels rapidly lose their resolving power and all DNA molecules run at approximately the same mobility independent of molecular weight. The window of high resolution is a function of pulse time. Therefore, one can place the molecules whose molecular weight is to be determined into this high resolution window simply by selecting the appropriate pulse time. The major limitation to the application of this gel electrophoresis to DNA molecules larger than 10 megabases is that low electric field strengths are required to allow the DNA molecules to enter the gel. For example, at present separation of molecules in the 5 to 10 megabase range requires low temperature, long pulse time (10 minutes) and low voltage gradients for up to one week of electrophoresis. Presumably, larger DNA molecules could be resolved by decreasing the voltage gradients. However, this would require at least one month of electrophoresis.

We have developed a new cloning vector for the cloning of very high molecular weight DNA molecules in yeast. The vector consists of adding two different DNA sequences to the ends of a large linear molecule. The left half vector consists of a yeast telomeric sequence (Ya) and URA3 gene. The right half vector consists of yeast centromere 4, TRP and a telomeric sequence (Yb). After ligation to a large DNA molecule and transformation of yeast, these molecules replicate and segregate as a yeast chromosome. The molecules can be resolved with the above electrophoresis apparatus. They also can be manipulated through the extensively developed yeast genetic techniques, which include deletions, inversions, translocations, gene disruptions and transplacement.

#41

In Search of Transposable Genetic Elements in *Arabidopsis thaliana*.

Kevin G.Mossie and Elliot Meyerowitz. Division of Biology, California Institute of Technology, Pasadena, CA 91125

Motivated by observations that a high proportion of dispersed middle repetitive DNA sequences in eukaryotes have properties of transposable elements (1,2), we have taken a non-genetic approach in screening the *Arabidopsis* genome for transposable elements by initially isolating repetitive sequences (3), analyze these sequences and amount of homologous sequences in different *Arabidopsis* races, and then characterizing isolated genomic clones to determine if these elements have properties characteristic of most transposable elements. We have chosen to study the structural properties of two non-homologous, dispersed repetitive DNA elements in *Arabidopsis*, *Are-1* and *Are-3*.

Our results indicate that members belonging to the *Are-1* family of disperse middle repetitive DNA elements are long (at least 5 kb), homogeneous, and are present in approximately 25-35 copies per haploid genome in Bensheim and Columbia races. Furthermore, this structural homogeneity of *Are-1* family members is confirmed in similar analyses of eight other *Arabidopsis* races. Analysis of another element, *Are-3*, suggests that while this element is shorter, (ca. 2 kb), and is present in 15-25 copies per haploid genome, members belonging to this family are also homogeneous in different *Arabidopsis* races, although we do observe some degree of restriction site polymorphisms when internal fragments are used as probes, suggesting that some members of the *Are-3* family are amplified as structurally distinct forms. We have been unsuccessful in identifying transcripts specific for both elements in some tissues, and are currently pursuing the possibility that these elements are transcribed in a stage and tissue-specific manner. We are also in the process of determining nucleotide sequence of the termini of different members of these families to determine if these elements have properties of established transposable elements.

References :

1. Finnegan, D., J. (1986). Int. Rev. Cytol. Suppl.17
2. Mossie et al. (1985). J. Mol. Biol. 182, 31-43.
3. Pruitt & Meyerowitz (1986) J. Mol. Biol. 187, 169-183.
4. Boeke et al. (1985). Cell 40, 491-500.

#42

AN ARABIDOPSIS ACETOLACTATE SYNTHASE GENE IN TOBACCO CONFERS RESISTANCE TO SULFONYLUREA HERBICIDES.

G.W. Haughn, C.R. Somerville. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824. J.K. Smith, B.J. Mazur, Central Research and Development Dept., Experimental Station, Du Pont and Co., Wilmington, DE 19898

Two classes of herbicides, the sulfonylureas and the imidazolinones are inhibitors of the branched-chain amino acid biosynthetic enzyme acetolactate synthase(ALS). In an attempt to isolate genetic markers for the gene encoding ALS, we isolated by direct selection(1) *Arabidopsis* mutants resistant to both the sulfonylurea herbicide chlorsulfuron and the imidazolinone herbicide imazapyr. Genetic and biochemical analyses of one chlorsulfuron-resistant line(GH50) have suggested that the mutant has a chlorsulfuron-resistant ALS activity due to a mutation within the ALS structural locus(2; we designate the mutant allele *Csr-1*). To prove this hypothesis and to obtain a selectable marker for molecular-genetic studies, we cloned the ALS gene from line GH50. DNA sequence analysis showed that *Csr-1* differs from the wildtype allele by a single nucleotide substitution. Transgenic tobacco plants carrying *Csr-1* are approximately two orders of magnitude more resistant to chlorsulfuron than wildtype. Preliminary results have indicated that *Csr-1* can function as a selectable marker for plant transformation.

Results on three imazapyr-resistant lines have indicated that resistance is also due to a single dominant nuclear mutation that cosegregates with an imazapyr resistant ALS activity. The imazapyr-resistant line also shows cross resistance to chlorsulfuron suggesting that the two classes of herbicides have similar modes of action. We are currently determining if the mutation(s) causing imazapyr resistance map to the *Csr-1* locus.

1. Haughn, G.W., Somerville, C.R. (1987) American Chemical Society Symposium of 1985, in press.
2. Haughn, G.W., Somerville, C.R. (1986) Mol. Gen. Genet. 204, 430.

#43

CHARACTERIZATION OF UBIQUITIN GENES IN ARABIDOPSIS THALIANA. Thomas J. Burke, Janelle Cortner, and Richard D. Vierstra. Department of Horticulture, 1575 Linden Dr., Univ. of Wisconsin-Madison, Madison, WI 53706

Ubiquitin is a highly-conserved, 76 amino acid protein which covalently attaches to other cellular proteins as a key step in plant protein degradation (1,2). To determine what roles the ubiquitin dependent proteolytic pathway may play in plant development and stress response, a molecular approach is being used to study ubiquitin gene expression. Two clones have been isolated from an Arabidopsis thaliana lambda gt11 genomic library which showed reactivity to both anti-oat and anti-human ubiquitin antibodies. The presence of ubiquitin coding sequences in both clones was confirmed by hybridization to a chicken ubiquitin cDNA clone and to a yeast ubiquitin genomic subclone. Northern blot analysis using RNA from green plants indicates that four abundant transcripts accumulate, ranging in size from 850-1900 bases. Genomic Southern blot analysis is consistent with Arabidopsis containing at least four ubiquitin genes. DNA sequencing shows that the Arabidopsis genes, like those from mammals and yeast, encode a polyubiquitin precursor protein. Studies are now underway to determine if ubiquitin mRNA levels vary during development or in response to stress.

References

1. Vierstra, Richard D., Langan, Susan M., and Haas, Arthur L. (1985) Jour. Biol. Chem. 260, 12015-12021

2. Vierstra, Richard D., Langan, Susan M., and Schaller, G. Eric (1986) Biochem. 25, 3105-3108

#44

CLONING OF A NITRATE-REGULATED GENE FROM ARABIDOPSIS

N. Crawford and R. Davis, Stanford University, Deptartment of Biochemistry, Stanford Medical School, Stanford, CA 94305.

A cDNA clone encoding nitrate reductase from squash (Cucurbita maxima) (1) was used as a heterologous probe to isolate cross-hybridizing cDNA clones from Arabidopsis. One such clone with an insert of 3.5 kbase in length was isolated and characterized. This clone hybridized to an RNA species of 3.5 kbase which was nitrate-regulated. In the leaves this RNA was 15 fold more abundant in plants grown with nitrate compared with plants grown without nitrate (ie. ammonia). In the presence of nitrate, ammonia had very little effect on the level of this RNA. The level of this RNA could be increased ten fold after sixteen hours by the addition of nitrate to ammonia-grown plants. The levels of this RNA correlated well with level of nitrate reductase activity measured in the same tissue. It is likely that this cDNA encodes nitrate reductase. The cDNA also hybridizes to another RNA of 0.8 kbase. We are in the process of determining the origin of this other RNA.

REFERENCES:

1. Crawford, N, Campbell, W. and R. Davis (1986) Proc. Natl. Acad. Sci. USA 83: 8073-8076.

#45

A POTENTIAL VECTOR FOR THE CLONING OF PLANT GENES BY PHENOTYPIC COMPLEMENTATION. N. Olszewski, F. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

We have constructed a binary cosmid vector (pOCA18) for the cloning of plant genes by phenotypic complementation of mutations. This vector contains the right and left border regions from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens and a chimeric kanamycin resistance gene for use in plant cells. Kanamycin resistant shoots have been regenerated from tobacco leaf discs following co-cultivation with A. tumefaciens containing pOCA18. All transgenic plants analyzed contained multiple copies of pOCA18 arranged head to tail in tandem arrays. A genomic library of Arabidopsis thaliana DNA has been constructed in pOCA18. This library is stable in both E. coli and A. tumefaciens. Seven genomic clones were transformed into tobacco by co-cultivating leaf discs with A. tumefaciens containing these genomic clones. In ten out of sixteen transgenic plants analyzed the A. thaliana DNA appears to have been transferred without deletion or rearrangement. The mutant line GH50 (1) was the source of the genomic DNA used in the construction of this library. This line contains a mutation in the structural gene for acetohydroxy acid synthase (AHAS) which results in decreased sensitivity to inhibition by the herbicide chlorsulfuron. We have identified genomic clones containing this gene using an A. thaliana AHAS probe provided by B. Lalonde and G. Fink. When these genomic clones were transformed into tobacco, transgenic plants which exhibit increased resistance to chlorsulfuron were obtained. These transgeneic plants were obtained either by directly selecting for chlorsulfuron resistance, or by first selecting for kanamycin resistance. These plants are resistant to >20-fold higher levels of chlorsulfuron than control plants; indicating that the A. thaliana (AHAS) gene is being expressed.

Reference

1. Haughn, G., Somerville, C. (1986) Mol. Gen. Genet. 204, 430-434.

#46

TUBULIN GENES OF A. THALIANA COLUMBIA: TISSUE-SPECIFIC PATTERNS OF TRANSCRIPT ACCUMULATION. P. Snustad, D. Oppenheimer, S. Ludwig, N. Haas, S. Kopczak, C. Silflow. University of Minnesota, St. Paul, MN 55108.

The genomes of A. thaliana Columbia and Landsberg have been shown to contain at least four α- and seven β-tubulin genes and/or pseudogenes by genomic Southern transfer experiments using homologous 5' and 3' coding sequence subclones as probes. Genomic clones of two α-tubulin genes and five β-tubulin genes and/or pseudogenes have been isolated from a lambda library provided by E. Meyerowitz. The first clones were isolated using heterologous Chlamydomonas reinhardtii α- and β-tubulin coding sequence probes (1,2); subsequent clones were isolated using homologous probes. The complete sequences of one β- and two α-tubulin genes have been determined, and all three have been shown to be transcribed by S1 nuclease protection and RNA blot hybridization experiments.

Noncoding 3' gene-specific hybridization probes have been prepared for all three of the sequenced tubulin genes and have been used to initiate an investigation of the expression of these genes. Two of the three genes analyzed to date produce transcripts that accumulate in a tissue-specific manner. When total RNAs from leaves, roots, and flowers were analyzed by blot hybridization using gene-specific probes, the α3-tubulin gene transcript was present in approximately equal amounts in RNAs isolated from all three tissues. In contrast, the β1 transcript was prevalent in roots, rare in flowers, and barely detectable in leaves. The α1 transcript was relatively abundant in flowers, but rare in roots and leaves. Total RNA was also isolated from flowers at each of five different stages of development: flower buds, unopened flowers with pollen, open flowers, flowers with elongating carpels, and green seed pods. Northern blot analyses of these RNAs using the α1 and α3 gene-specific probes showed that the α3 transcript was present at all five stages of development, but the α1 transcript was only detected in unopened flowers with pollen, open flowers, and flowers with elongating carpels. Possible loading and transfer errors were excluded by stripping the filters and reprobing them with the appropriate coding-sequence probe.

References

1. Youngblom, J., Schloss, J.A., and Silflow, C.D. (1984). Mol. Cell. Biol. 4, 2686-2696.

2. Silflow, C.D., Chisholm, R.L., Conner, T.W., and Ranum, L.P.W. (1985). Mol. Cell. Biol. 5, 2389-2398.

#47

CONSTRUCTION OF A PLANT-TRANSFORMABLE *ARABIDOPSIS THALIANA* GENOMIC LIBRARY IN *AGROBACTERIUM*. Bob Ludwig, Pascal Stein, Neil Olszewski†, and Fred Ausubel†. Dept. of Biology, University of California, Santa Cruz, CA. 95064, and Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA. 02114.†

We have constructed an *Arabidopsis thaliana* genomic library of some 19,000+ entries in *Agrobacterium tumefaciens* using a cosmid T-DNA vector. The *A. tumefaciens* host strain, AGL3, is an attenuated strain C58C1 derivative carrying the pTiBo542 hypervirulence plasmid whose T-DNA has been deleted; it is also RecA$^-$. The T-DNA-derived cosmid vector, pOCA18, is a broad host-range plasmid carrying the *A. tumefaciens nos*::*aphII*::*nos* plant-selectable chimera and polycloning sites within the T-DNA borders, as well as the linked "overdrive" DNA sequence.

A. thaliana genomic DNA from leaf nuclei was purified, partially digested with *Taq*I, and ligated into the pOCA18 *Cla*I site. Ligation mixes were packaged into λ particles in vitro; *A. tumefaciens* λ-receptive strain AGL3 / pTROY41614 was then infected with these phage particles carrying recombinant cosmids. As expected, all *A. tumefaciens* AGL3 / pOCA18 recombinant cosmid transductants tested showed that pTROY41614, a plasmid of shared incompatibility, had been evicted. *A. thaliana* DNA inserts in the library averaged approximately 20 kb in size. Some 19,000+ library entries were picked onto microtiter plates.

This library is currently being tested for representation of *A. thaliana* DNA sequences by shotgun cloning experiments. We intend to make this library available to the *Arabidopsis* research community.

#48

STRUCTURE, ORGANIZATION AND EXPRESSION OF THE GENES ENCODING THE HISTONES H3 AND H4 IN ARABIDOPSIS THALIANA.

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Two histone H3 and two histone H4 genes have been cloned from a λgtWESλ·B Arabidopsis thaliana gene library (1). The two H3 genes encode the same protein but their coding regions show only 85% sequence homology. The 5' and 3'-flanking sequences are completely divergent. The H4 genes on the contrary show extensive sequence homology even in their non-coding regions.

All four genes show the classical histone gene specific consensus sequences upstream of the initiation sites. A conversed octanucleotide 5'-CGCGGATC-3' specific of the plant histone genes is present approximately 200 to 250 nucleotides upstream from the initiation ATG.

The 3'-flanking regions lack the classical histone-gene-specific T-hyphenated dyad symmetry motif responsible in animal genes for the correct 3'-processing of the transcripts. The 3'-end of the histone mRNA is located at 170 to 200 nucleotides after the termination codon, suggesting that the 3'-processing of the transcripts is different in plants that in animals.

Both H3 and H4 genes exist as 5 to 7 copies per haploid genome. The copy number is variable from one race to another. The H3 and H4 genes are not (or poorly) linked in the genomes. Genomic blot hybridized with non-coding 5'-flanking regions show that each gene (except the two cloned H4 genes) is located in a different environment. Comparison with the organization of the histone genes in cereals (2-3) suggests that the very simple genome of A. thaliana could be a simplified picture of the topological arrangement and the evolution of the histone genes in the genomes of higher plants.

References

(1) Chaboute, M.-E., Chaubet, N., Philipps, G., Ehling, M., Gigot, C. (1987) Plant Mol. Biol. 8, 179.
(2) Tabata, T., Fukasawa, M., Iwabuchi, M. (1984) Mol. Gen. Genet. 196, 397.
(3) Chaubet, N., Philipps, G., Chaboute, M.-E., Ehling, M., Gigot, C. (1986) Plant. Mol. Biol. 6, 253.

#49

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

We have been analyzing the purine salvage pathway of *Arabidopsis* to identify genes that might be used as selectable reporter genes in promoter fusion experiments or as transposon traps. Adenine phosphoribosyl transferase (APRT) converts adenine and PRPP to adenosine monophosphate. It is possible to directly select for APRT-deficient mutants by their ability to grow in the presence of toxic analogues of adenine: APRT-deficient mutants cannot salvage exogenous adenine and therefore are not killed by the analogue.

We have isolated APRT-deficient mutants of *Arabidopsis* by germinating the progeny of a mutagenized seed population on a nutrient agar plate supplemented with 0.1 mM 2,6 diaminopurine. Under these conditions, wild type seed does not germinate, and mutants can be recovered at a high frequency. Three independently isolated mutants have been characterized. All three contain allelic nuclear recessive mutations, and all are deficient in APRT activity. The mutants grow more slowly that wild type and are male sterile. Pollen development appears to become aberrant during the meiotic divisions of the pollen mother cell. The size of the adenine nucleotide pools in the mutants and the wild type are being measured to investigate the possibility that a deficiency in these pools may be the basis of the male sterility.

We wish to isolate the *aprt* gene from *Arabidopsis*, and test its utility as a selectable marker in transformation experiments. The available heterologous genes from *Drosophila*, *E. coli*. and mouse did not cross-hybridize to *Arabidopsis* DNA. Therefore the enzyme has been purified to homogeneity from *Brassica juncea* to facilitate the cloning of the plant *aprt* gene.

To test whether the bacterial gene can be used to select against the expression of "promoter" activity in plants, the bacterial gene has been cloned into T-DNA vectors under control of plant expression signals, and transferred into *Arabidopsis* and tobacco. Isozyme analysis of APRT activity indicates that active bacterial APRT is being produced in the transformed callus.

#51

THE DEVELOPMENTAL PROCESS OF SHOOT INITIATION IN VITRO BY **ARABIDOPSIS THALIANA**. D. Warnick, K. Feldmann and M. Christianson. Zoecon Research Institute, 975 California Avenue, Palo Alto, CA 94304

The process of organogenesis from leaf explants *in vitro* has been shown to include three developmetal phases: phase 1, acquisition of competence for induction; phase 2, induction, which is completed when cells or groups of cells are determined or fated for organogenesis; and phase 3, morphological differentiation and growth (1). While many species and cultivars are capable of completing all three phases on a single culture medium, other species, including *Arabidopsis thaliana*, require a series of media with differing phytohormone balances. The classical approach for regenerating shoots from explants of *Arabidopsis* requires the establishment of a callus culture on a callus-inducing medium, CIM, which contains an intermediate auxin:cytokinin ratio. This is followed by a subsequent culture on a shoot-inducing medium, SIM, which contains a low auxin:high cytokinin ratio (2). Unfortunately, this regime requires extensive culture time and often produces only a few shoots (3). A slight modification of this protocol whereby explants receive the minimum, necessary preculture on CIM (about 5 days) to acquire competence before transfer to SIM has been shown to result in a more rapid and efficient regeneration system (4).

We have recently begun to identify and time the points at which two land races, Columbia and WS, become competent for shoot induction and determined for shoot formation using a series of media transfer experiments. A useful and detailed understanding of this presumably gene controlled developmental pathway emerges when explants at known phases are examined for changes in mRNA populations.

References

1. Christianson, M., Warnick, D. (1985) Dev. Bio. **112**, 494.
2. Gresshoff, P., Doy, C. (1972) Aust. J. Biol. Sci. **25**, 259.
3. Negrutiu, M., Jacobs, M., Cachita, D. (1978) Pflanzenphysiol. **86**, 113.
4. Feldmann, K., Marks, M. (1986) Plant Science **47**, 63.

#52

ORGAN-SPECIFIC EXPRESSION OF GLUTAMINE SYNTHETASE GENES IN ARABIDOPSIS THALIANA. T. Kaye Peterman, Klaus Trinks, and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Wellman-11, Boston, MA 02114.

Glutamine synthetase (GS) plays a critical role in the nitrogen metabolism of higher plants. GS is essential for the primary assimilation of ammonia produced by nitrate reduction as well as the reassimilation of ammonia resulting from photorespiration and amino acid degradation. Western blot analysis of *Arabidopsis* leaf and root crude extracts revealed at least two different GS polypeptides; 44 kd and 39 kd GS polypeptides were present in leaves while only a 39 kd GS was detected in roots. In etiolated seedlings only the 39 kd GS was detected and GS activity was only 50% of that of light-grown seedlings. After greening both GS activity and the 44 kd GS polypeptide were detected at levels comparable to those observed in light-grown plants, therefore the 44 kd GS is light-inducible. We are interested in the molecular basis and regulation of this organ-specificity and light-inducibility. A cDNA library was constructed in lambda gt11 from *Arabidopsis* leaf polyA+-RNA. Using GS antibody as probe we isolated cDNA clones which fall into three different classes. Northern blot analysis indicated that two of these are preferentially expressed in roots while one is preferentially expressed in leaves. Northern blots of light- and dark-grown seedlings indicate that the leaf-specific cDNA, gs11, represented a mRNA which is light-inducible. Further characterization of these cDNA clones and the corresponding genomic clones is in progress.

#53

Seed specific gene expression in *Arabidopsis thaliana*. Patty P. Pang, Elliot Meyerowitz. Division of Biology, California Institute of Technology, Pasadena, CA 91125

Four classes of genes expressed specifically in seeds have been isolated from a *Arabidopsis* genomic library using cDNA from 7 - 8 days old embryos. Three of the classes hybridize to abundant RNA species of approximately 1700 nucleotides long, and one to an RNA species approximately 650 nucleotides (1). One of the three large seed specific genes cross-hybridizes to and is 86% homologous to the *B. napus* 12S storage protein gene. DNA sequence of a second large seed specific gene shows a 75% homology to the 12S storage protein genes. Using a ^{32}P-labeled RNA probe synthesized the first classing 12S clone, weak homology was detected to DNA fragments of the other two large seed specfic clones at extremely low hybridization stringency. Our results thus suggest that there are at least three distantly-related 12S seed storage protein genes in *Arabidopsis*. This can explain the high complexity of the 12S seed storage proteins observed in 2D protein gels and that seed protein gene families in plant species can be fairly divergent (2). The three 12S protein genes, however, are single copy in most *A. thaliana* races, while usually in multiple copies in other plants.

The developmental profiles of the seed protein gene messages are very similar. They are expressed in the late stage of embryogenesis. The smaller message, however, appears earlier during development. In *Brassica napus*, the transcription of the 12S cruciferin gene is increased in excised embryos when the plant hormone abscisic acid (ABA) is present (3). I analyzed seed protein messages from *A. thaliana* ABA deficient and insensitive mutants. Results show no significant changes in the level and expression profile of the various messages during embryogenesis.

In order to analyze the 5' region required for the expression of the 12S genes, gene fusions of the *A. thaliana Adh* gene to the promoters of the 12S genes have been reintroduced into *A. thaliana* using a binary vector *Agrobacterium* transformation system (4). A *Adh* protein fusion construction to one of the 12S promoters will also be introduced into *A. thaliana* so that one can select for trans-acting mutations that do not allow expression of the seed storage proteins.

References:

1. R.E. Pruitt and E.M. Meyerowitz. In preparation.
2. C. Domoney *et al.* Plant Molecular Biology. **7**:467-474.
3. A.J. DeLisle and M.L. Crouch. Molecular and Cellular Biology. Submitted.
4. A.M. Lloyd *et al.* Science **234**:464-234.

#54

THE CHALCONE SYNTHASE GENE OF ARABIDOPSIS THALIANA IS INDUCED BY HIGH LIGHT INTENSITY. R. Feinbaum, F. Ausubel. Department of Molecular Biology, Mass. General Hospital, Boston, MA 02114.

Chalcone synthase (CHS), the first enzyme unique to flavonoid biosynthesis, is highly regulated both during development and in response to environmental stimuli. In several plant systems, the regulation of chalcone synthase activity has been well studied and it has been established that the induction of chalcone synthase activity by specific stimuli (u.v. light and pathogen attack) is due to an increased rate of transcription of the chalcone synthase gene (1,2). We have isolated several CHS genomic clones from an Arabidopsis lambda EMBL4 library (obtained from E. Meyerowitz) by cross hybridization with a parsley cDNA clone (3). Restriction mapping data of these genomic clones together with southern blot analysis suggest that CHS is a single copy gene in Arabidopsis. We have sequenced a 3.8kb HindIII partial fragment which contains the entire Arabidopsis CHS coding region as well as both 5' and 3' flanking sequence. The Arabidopsis gene appears to contain a single intron and is 90% homologous to the parsley CHS cDNA clone at the amino acid level. We have established environmental conditions (increased light intensity) which lead to an increase in CHS enzyme activity and a concomitant accumulation of purple pigments in the leaves and stems of mature Arabidopsis plants, providing an endogenous screenable marker for CHS induction. Further, we have shown by northern blot analysis that high light intensity induces an accumulation of CHS mRNA which at least in part is due to an increased rate of transcription of the chalcone synthase gene as demonstrated by nuclear run-on experiments. Thus, the chalcone synthase gene of Arabidopsis is transcriptionally regulated in response to a specific envrionmental stimulus and may be a useful model for elucidating some of the mechanisms by which plants regulate gene expression in response to their environment.

References

1. Chappell, J., Hahlbrock, K. (1984) Nature 311:76
2. Lawton, M.A., Lamb, C.J. (1987) Mol. Cell. Biol. 7:335
3. Kreuzaler, F., Rugg, H., Fautz, E., Kuhn, D.N., Hahlbrock, K. (1983) Proc. Natl. Acad. Sci. 80:2591

#55

GENETIC ANALYSIS OF A PHYTOCHROME TRANSDUCTION PATHWAY MEDIATING LIGHT-REGULATED TRANSCRIPTION IN ARABIDOPSIS. George A. Karlin-Neumann and Elaine M. Tobin. Dept. of Biology, UCLA, Los Angeles, Calif., 90024.

We are interested in performing a genetic and molecular analysis of the phytochrome transduction pathway by which red light regulates transcription of some nuclear genes. Such a response has been found in at least eight plant species, including both monocots and dicots (1), and is therefore probably a general phenomenon in higher plants. To identify relevant mutants more easily, we plan first to introduce a suicide gene under a phytochrome-regulated promoter into a plant whose seeds will be used for subsequent mutagenesis and screening. Therefore, we have investigated phytochrome-regulated gene expression in etiolated seedlings of Arabidopsis thaliana since it is both amenable to transformation by Agrobacterium and especially well-suited to later molecular genetic analysis. We have found that the light-harvesting chlorophyll a/b-protein (LHCP) mRNA levels are highly and rapidly inducible by light and appear to be phytochrome-regulated in Arabidopsis. Furthermore, we have determined that mRNA from one of the three LHCP genes of Arabidopsis (2), AB140, accumulates more readily than the others in response to red light. We are now preparing gene fusions between this presumptive phytochrome-responsive promoter and a suicide gene, for subsequent transformation into Arabidopsis. Preliminary results suggest that α-naphthaleneacetamide (NAM, an auxin analogue precursor) will function suitably as a suicide substrate in Arabidopsis, and we have therefore chosen the tms2 gene (which converts NAM to the auxin analogue, NAA, or α-naphthaleneacetic acid) as the suicide gene for these fusion constructs. Finally, we have been successful in transforming markers into Arabidopsis and regenerating fertile plants following the methods of Lloyd et al. (3).

References

1. Silverthorne, J. and Tobin, E.M. (1987) BioEssays. In press.

2. Leutwiler, L.S., Meyerowitz, E.M. and Tobin, E.M. (1986) Nucleic Acids Res., 14, 4051-4064.

3. Lloyd, A.M., Barnason, A.R., Rogers, S.G., Byrne, M.C., Fraley, R.T. and Horsch, R.B. (1986) Science, 234, 464-466.

#56

GENETIC ANALYSIS OF PHOTORECEPTOR ACTION PATHWAYS. J. Chory and F. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA. 02114.

The photoconversion of photoreceptors by light induces the diverse morphogenic responses which result in greening. During this process, many nuclear and chloroplast-encoded gene products become induced. The factors which might be needed to affect transcription of these genes after photoreceptor excitation are not known. We are currently pursuing a dual approach for identifying the factors involved in transducing the developmental signals which trigger greening.

The first approach is directed at the identification of regulatory gene(s) which affect the transcription of nuclear-encoded photosynthesis genes, specifically the cab genes. The strategy involves fusing a cab promoter to a selectable marker and transferring this chimeric construction back into A. thaliana to find mutants which aberrantly express the marker gene. The three cab genes from A. thaliana have been cloned and sequenced by Leutwiler et al. (1). We have constructed a transcriptional fusion between the promoter for one of these genes and the hygromycin phosphotransferase structural gene. Our experimental results indicate that this construction confers resistance to hygromycin and is regulated by light in tobacco transformants. We are currently trying to transform A. thaliana by leaf disc transformation and by seed cocultivation protocols.

The second approach involves the direct selection for plants with a predicted mutant photoreceptor phenotype. Mutants which do not show the classical phytochrome-mediated hypocotyl elongation inhibition response due to light have been isolated by Koornneef et al (2). They fall into 5 complementation groups, two of which appear to be mutations which affect the level of phytochrome. We are currently analyzing these, as well as looking for new long hypocotyl mutations. Other strategies for mutants in this pathway are also being pursued. We are screening for mutations in the blue-light photoreceptor pathway by looking for plants which are phototropic-minus (3). We have obtained five such mutants. Together, these mutants should yield the tools to study the red and blue light mediated responses which affect photosynthesis gene expression, and to look at the interactions, if any, between the two pathways of signal transduction in response to light.

References:

1. Leutwiler, L.S., et al. 1986. Nuc. Acids Res. 14:147.
2. Koornneef, M., et al. 1980. Z. Pflanzen. 100:147.
3. Steinitz, B., et al. 1985. Plant Physiol. 77:248.

#61

Expression of prokaryotic genes in Arabidopsis. Rédei, G.P., Csaba Koncz and Jeff Schell. University of Missouri, 117 Curtis Hall, Columbia, MO 65211, Max-Planck-Institut, Köln 30, D-5000.

The original goal of the experiment was to develop efficient ways for tagging and isolation of known genes. For this purpose we have developed a system of transformation utilizing binary vectors of Agrobacterium tumefaciens. So far six different vectors were employed; five of them were free of the oncogenes of the Ti plasmid. pPVC311 contained genes 5,2,1,4 of the T-DNA, kanamycin resistance, and the octopine synthase gene. Vector pPCV 310 contained Km^r with the nos promoter and a functional octopine synthase gene. Vectors pPCV 601 and 631 also were equipped with the NPHT II gene but lack the promoter. The former one included a complete ocs gene, the latter did not have ocs but to the nos promoter Hyg^r gene was added. Plasmid pPCV501 had the Hyg^r gene with the nos promoter and the octopine synthase gene, and pPCV730 had the Hyg^r gene hooked to the T_R promoter as well in opposite orientation the gene for Km^r attached to the nos promoter. Thus, transformant with the last one are selectable for resistance to both aminoglycoside and aminocyclitol antibiotics. For infection, bacterial cultures of approximately 0.8 OD were used. Plants of Columbia wild type as well as various single and multiple mutants were grown on E medium in test tubes for 3 to 6 weeks and either leaves or stem segments were infected. Selection of growing calli was carried out on media containing 12 to 22 microgram/mL hygromycin B and 100 microgram/mL kanamycin sulfate, respectively. Agrobacterium was removed from the cultures by the use of 500 microgram/mL cefotaxime. For tissue culture either Murashige & Skoog or RIV mineral solutions were used with organic supplements and the medium was solidified with the gellan gum Gel Grow. The latter medium was substantially more favorable for the rapid regeneration of leaves and shoots; within 9 to 12 days after initiation of the culture shoots appeared on non-infected leaves. The success of transformation varied greatly. Albeit transgenic calli were obtained with all six vectors, the frequency with the promoter-less Km^r gene was only about 3% whereas with some other vectors up to 90% of the infected explants developed resistant calli. The success of transformation was confirmed by the prolonged growth on media containing the antibiotics, the capability of the tissues to synthesize octopine, the presence of neomycinphosphotransferase activity in the transgenic material, and by Southern hybridization to T-DNA borders. Flowering plant progenies were obtained after infection with vectors pPCV 310, 501 and 730. Seeds were secured on several plants and the genetic analysis of the progeny is in progress.

#62

A FLUCTUATION TEST FOR THE DETECTION OF LOW FREQUENCY GENETIC ALTERATIONS IN ARABIDOPSIS. Rédei, G.P. and Csaba Koncz. University of Missouri, 117 Curtis Hall, Columbia MO 65211, Max-Planck-Institut, Kõln 30, D-5000.

The fluctuation test (1) initiated modern bacterial genetics and it became applicable to genetic studies of unicellular higher organisms as well as to mammalian cell genetics. In recent years we have adopted the principles of this procedure to Arabidopsis mutation studies. The fluctuation test reveals whether a particular genetic alteration is recovered because of selection of preexisting variation in the population or the hereditary alteration arises as a direct consequence of a particular experimental treatment. Thus, by its use, we can distinguish between low levels of spontaneous mutation, chance contamination and newly arisen hereditary changes. The principles permit the identification of weak environmental mutagens, the work of transposable elements, and can assist in estimating the frequency of transformation by the Feldmann and Marks (2) procedure. By this method of transformation insertions of foreign DNA can occur for an unknown period of time during the experiment but it is conceivable that bacteria escaping the washing of the infected seed may survive and insert at very low frequency T-DNA not just into the experimental material but also into the cultures which would be later used as seed donors to infections. The experimental layout requires the planting of treated and untreated seeds in separate small but many containers. From each container the seed is harvested in separate bulks from which representative samples are withdrawn for screening of selective markers or any other genes. In case the mutations are supposed to occur at the dry seed stage, it may be sufficient, for maximum efficiency, to test samples as small as twice the number of plants per container. In case of the use of the Feldmann & Marks procedure or transposable elements such as Ac, a larger sample must be tested from each bulked progeny because of the occurrence of late insertions. The distribution of the genetic changes in both series can then be fitted to the Poisson series or to the expectations on the basis of the negative binomial distribution. The fitting to the latter is more justified because the final distribution is determined by more than a single random event. The difference between control and treated series can be ascertained by the use of the log-likelihood ratio and chi square. This procedure permits the detection of differences even when the frequency of alterations is extremely low. Furthermore, the frequency of mutation per genome or plant can be determined. This fluctuation test has been tried earlier with the weak mutagen, ascorbate, and it proved to be very useful. Currently it is being tested by the seed infection method (2).

References

Luria, S.E. & Delbrück, M. (1943) Genetics 28:491
Feldmann, K.A. & Marks, M.D. (1987) Molec. Gen. Genet. in press

#63

SELECTION OF BIOCHEMICAL MUTANTS IN ARABIDOPSIS: AN EVALUATION. M. JACOBS. Lab. of Plant Genetics, V.U.B., 65 Paardenstraat, B1640 St.-Genesius-Rode, Belgium.

One of the merits of the in vitro culture methodology has been to offer the prospect of applying to plants techniques developed in microbial genetics; in particular methods for selecting biochemical mutants were success-fully applied. However, the life cycle characteristics of *Arabidopsis* allow to apply mutagenesis-selection schemes on large M_2 populations of seeds, as illustrated by the obtention of nitrate-reductase deficient mutants (1), thiamine-less mutants (2,3), defective mutants in the phosphorespiratory pathway (4) or in starch and lipid metabolism (5) and in abcissic acid and gibberellin metabolism (6). Such mutants represent valuable tools to study gene expression and to isolate key genes governing important metabolic functions.

This review concerns the induction and study of various classes of mutants, characterized by their resistance to amino acid analogues (s-aminoethylcysteine, ethionine, hydroxyproline, 2-azetydilic acid) or inhibitory amino acid combinations (lysine + threonine), or a deficiency for specific enzymes as alcohol dehydrogenase or acid phosphatase or showing polymorphism for isozyme systems.

The suitability of *Arabidopsis* as a model system for determining the consequences of modifying metabolic pathways by genetic means will be underlined.

References.

(1) Oostindier-Braaksma, F.J. and Feenstra, W.J. (1973). Mutation Res. *19* : 175.
(2) Feenstra, W.J. (1966). Genetica *35* : 259.
(3) Li, S. and Redei, G. (1960). Bioch. Genet. *3* : 163.
(4) Somerville, C.R. and Ogren, W.L. (1982). Trends Bioch. Sci. *7* : 171.
(5) Somerville, C.R. *et al.* (1985) in Plant Genetics, Alan K. Liss Ed. , N.Y. pp. 651-660.
(6) Koornneef, M. *et al.* (1985) in Plant Genetics, Alan K. Liss Ed., N.Y., pp. 103-114.

#64

DEVELOPMENTAL AND MOLECULAR GENETICS OF EMBRYOGENESIS IN ARABIDOPSIS THALIANA. D.W. Meinke. Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

The purpose of my research over the past several years has been to study the genetic control of embryo development in higher plants (1) through the isolation and characterization of embryo-lethal mutants. I have chosen to work with Arabidopsis thaliana as a model system because it offers the best combination of genetic, developmental, and molecular characteristics for this type of study. Over 30 recessive embryo-lethal mutants of Arabidopsis with a wide range of lethal phases have been isolated in my laboratory following EMS seed mutagenesis (2). Unusual mutant phenotypes include the presence of abnormal suspensors (3), fused and distorted cotyledons, reduced hypocotyls, and arrested embryos without distinct cotyledons or hypocotyl tissue. Analysis of these mutants has included a determination of: (a) segregation ratios and lethal phases at several temperatures; (b) patterns of abnormal development; (c) gametophytic expression of mutant genes; (d) response of mutant embryos in culture; (e) development of homozygous mutant plants in culture; (f) formation of protein bodies in mutant embryos; (g) accumulation of seed storage proteins in mutant embryos (4); (h) allelism tests and linkage studies; and (i) analysis of double mutants. Other projects in the laboratory include the regeneration of wild-type Arabidopsis plants from cultured cotyledon segments, biochemical analysis of a biotin-requiring embryonic lethal of Arabidopsis, and tagging of mutant genes following transformation with Agrobacterium tumefaciens. Future projects may include the analysis of endogenous hormone levels in a rootless embryonic lethal of Arabidopsis, the isolation of temperature-sensitive embryonic lethals, and the isolation of mutant genes through chromosome walking with RFLP maps.

(1) Meinke, D.W. (1986) Embryo-lethal mutants and the study of plant embryo development. Oxford Surveys of Plant Molecular and Cell Biology 3: 122-165.

(2) Meinke, D.W. (1985) Embryo-lethal mutants of Arabidopsis thaliana: Analysis of mutants with a wide range of lethal phases. Theor. Appl. Genet. 69: 543-552.

(3) Marsden, M.P.F., Meinke, D.W. (1985) Abnormal development of the suspensor in an embryo-lethal mutant of Arabidopsis thaliana. Amer. J. Bot. 72: 1801-1812.

(4) Heath, J.D., Weldon, R., Monnot, C., Meinke, D.W. (1986) Analysis of storage proteins in normal and aborted seeds from embryo-lethal mutants of Arabidopsis thaliana. Planta 169: 304-312.

#65

GENETIC ANALYSIS OF LIPID BIOSYNTHESIS AND FUNCTION IN ARABIDOPSIS. John Browse[1,2], Ljerka Kunst[2], Peter McCourt[2] and Chris Somerville[2]. [1]Plant Physiology Division, DSIR, Palmerston North, New Zealand; [2]DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

We are investigating the relationship between the fatty acid composition of membrane lipids and their biological functions by the analysis of a series of mutants of Arabidopsis with specific alterations in leaf lipid composition. Mutations in at least five loci (see Table below) were identified by direct measurement of the fatty acid compositions of leaves from individual plants in a mutagenized population.

The mutants have been useful for investigating the mechanisms which regulate fatty acid biosynthesis. Analyses of the fatty acid desaturation (fad) mutants have indicated that the enzymes controlled by fadA and fadB appear to show strict substrate specificity. However, the enzymes controlled by fadC and fadD desaturate chloroplast fatty acids with little or no specificity for the length of the acyl chain (16- or 18-carbon) its point of attachment to the glycerol or for the lipid headgroup (1).

The mutants are also very useful for probing the relationship between chloroplast fatty acid composition and the biophysics and physiology of photosynthesis. Thus, the properties of the fadA mutant (2,3) were inconsistent with previous suggestions that trans-16:1 had some vital function in photosynthesis. Analysis of the fadD mutant (4) demonstrated that the high levels of trienoic fatty acids found in chloroplasts are not essential for normal plant development and growth. The most pronounced difference between this mutant and the wild-type was a 45% reduction in the cross sectional area of the chloroplasts and an increase in the number of chloroplasts per cell. We infer that the high trienoic acid content of thylakoid membranes may be related to a role in chloroplast morphogenesis rather than directly to photosynthesis.

Gene Symbol	Enzyme Deficiency
fadA	16:0-PG desaturase (producing trans 16:1)
fadB	16:0-MGD desaturase (producing cis 16:1)
fadC	16:1/18:1 desaturase
fadD	16:2/18:2 desaturase
act1	acyl-ACP:glycerol-3-P acyltransferase

1. Browse J, McCourt P, Somerville CR (1986) Plant Physiol 81:859
2. Browse JA, McCourt P, Somerville CR (1985) Science 227:763
3. McCourt P, Browse J, Watson J, Arntzen CJ,Somerville CR (1985) Plant Physiol 78:853
4. McCourt P, Kunst L, Browse J, Somerville CR (1987) Plant Physiol (in press)

#71

COLD ADAPTATION IN ARABIDOPSIS THALIANA. Sarah J. Gilmour, Michael F. Thomashow. Dept. of Crop and Soil Sciences, Mich. State Univ., E. Lansing, MI 48824.

Many plant species can cold harden, becoming more resistant to freezing conditions when they are first exposed to low non-freezing temperatures. Numerous biochemical changes have been associated with this cold adaptation process--including changes in protein content, lipid composition and membrane structure (1). Recently, it has been shown in spinach (2) and rapeseed (3) that cold temperatures induce changes in translatable mRNA populations. It has been suggested that some of these changes are responsible for the cold hardening response. We are interested in using Arabidopsis thaliana as a model to study the cold adaptation process. Our first goals, therefore, have been to determine whether A. thaliana can cold harden, and whether low temperatures induce changes in gene expression. Our initial experiments indicate that both of these changes do indeed occur. Using a freeze-tolerance test, we found that plants that were grown at 24°C and then exposed to low temperatures (4°C) for 10 to 14 days could survive lower freezing temperatures than plants grown continuously at 24°C. To study changes in gene expression in response to cold, plants grown at 24°C were given a 3-day treatment at 4°C, after which poly(A)$^+$ RNA was extracted and translated in vitro using a rabbit reticulocyte system. Two dimensional gels of the in vitro translation products indicated a number of changes (approximately 5 to 10) in translatable mRNAs between cold treated and control plants; both "induction" and "repression" were observed. Experiments are now in progress to isolate cDNA copies of the cold responsive genes. Our future goals are to determine how temperature regulates the expression of these genes, and what roles, if any, the gene products have in the cold adaptation process.

References

1. Levitt, J. (1980) Responses of Plants to Environmental Stresses, 2nd ed., Vol. I, Academic Press

2. Guy, C.L., Niemi, K.J., Brambl, R. (1985) Proc. Natl. Acad. Sci. USA 82:3673-3677

3. Meza-Basso, L., Alberdi, M., Raynal, M., Ferrero-Cadinanos, M-L., Delseny, M. (1986) Plant Physiol. 82:733-738

#72

FROST TOLERANCE AND COLD ACCLIMATION IN ARABIDOPSIS THALIANA. E. Tapio Palva, Pekka Heino, Viola Lång, Kerstin Nordin and Björn Welin. Department of Molecular Genetics, Swedish University of Agricultural Sciences, Box 7003, S-750 07 Uppsala, Sweden.

Arabidopsis thaliana is capable to cold acclimate; exposure to low but non-freezing temperatures increased the frost tolerance of A. thaliana plantlets rapidly from -3°C to about -10°C. This cold acclimation treatment was correlated with the induction of a set of specific proteins as visualized by ^{35}S-methionine labelling of plantlets and analysis by 1- and 2-dimensional SDS-PAGE. The synthesis of these proteins was induced early (12 h) during the acclimation treatment and was continuous.

The induction of these cold acclimation related proteins appeared to take place at the gene expression level, as evidenced by analysis of in vitro translation products from isolated mRNA.

The same proteins were also induced by exposing the plants to exogenous abscisic acid (ABA) at room temperature, a treatment that in addition led to increased frost tolerance. The central role of ABA in cold acclimation was assessed by analysis of ABA-deficient mutants (1); these mutants were impaired in cold acclimation but could be complemented by addition of exogenous ABA.

In order to facilitate identification of other factors (than ABA) affecting frost tolerance in A. thaliana, mutants with altered frost tolerance characteristics were isolated. Preliminary results obtained with these mutants will be discussed.

References

1. Koornneef, M., Reuling, G., Karssen, C.M. (1984) Physiol. Plant. 61, 377-383.

#73

MUTANTS OF ARABIDOPSIS ISOLATED BY USING AN ATMOSPHERE ENRICHED WITH CO_2 AS A PERMISSIVE OR A NONPERMISSIVE CONDITION FOR GROWTH. N.N. Artus, C.R. Somerville. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Somerville and Ogren (1) isolated a series of mutants of Arabidopsis with defects in photorespiratory metabolism by utilizing 1% CO_2 as a permissive condition for growth and ambient CO_2 as a nonpermissive condition. This was possible since the flow of carbon through the photorespiratory pathway is regulated by the atmospheric ratio of CO_2 to O_2. Theoretically, it should have been possible to isolate several other kinds of mutants in photorespiration, with lesions either in enzymes of the pathway, or in proteins responsible for the transport of metabolites across organelle membranes. We rationalized that these mutants were missed in the original screen either because their phenotype in air was more subtle or more severe than the mutants that were recovered. However, the screening of an additional 13,000 M2 plants for photorespiratory mutants with "subtle" phenotypes and of 2,500 M3 lines for mutants with "severe" phenotypes did not result in any new photorespiratory mutants. Thus, it is possible that there are more than one gene or more than one function for several of the photorespiratory proteins, or, it is possible that the EMS mutagenesis is biased against certain genes for reasons that are not understood.
In the course of screening for new photorespiratory mutants, a few unusual mutants were isolated. One of these mutants requires 2% CO_2 for normal growth and displays severe photooxidation in air. The biochemical basis for this mutant phenotype is not known, but photorespiration and photosynthetic CO_2 fixation are not affected (2). Three other mutants belonging to separate complementation groups were isolated which grow normally at ambient CO_2 but turn yellow in elevated CO_2. Though there may be several plausible explanations for this phenotype, two possibilities are especially intriguing to us. High CO_2 may be detrimental to one or more of these mutants if the lesion imposes a need for photorespiration. Alternatively, if any of these mutants are defective in their ability to regulate the cytoplasmic pH, the observed phenotype may be a result of acidosis caused by high CO_2. We are currently investigating these possibilities.

References

1. Somerville, C.R., Ogren, C.R. (1982) Trends Biochem. Sci. 7, 171-174

2. Artus, N.N., Somerville, C.R. Abstracts of these proceedings

#74

IDENTIFICATION OF RUBISCO ACTIVASE cDNA CLONES FROM ARABIDOPSIS, SPINACH AND BARLEY. J. M. Werneke[a], R. E. Zielinski[b], and W. L. Ogren[a,b,c]. Departments of [a]Agronomy and [b]Plant Biology, University of Illinois and [c] U.S. Department of Agriculture, Agricultural Research Service, 1102 S. Goodwin Avenue, Urbana, IL 61801

An Arabidopsis thaliana mutant requiring a high CO_2 concentration for growth was found to be incapable of activating rubisco upon illumination (1). The mutant was subsequently shown to be completely deficient in a protein, rubisco activase, which mediates the rubisco activation process in vivo (2,3). In order to characterize the rubisco activase gene and determine the specific nature of the mutation, cDNA libraries were constructed from spinach, barley, and Arabidopsis leaf polyA mRNA. Clones expressing activase fusion proteins were detected immunoligcally. A 1.9 kb spinach activase cDNA was sequenced and found to contain the entire gene, including the transit peptide. Purified spinach activase protein was partially sequenced, and 15 of 17 amino acids matched with the predicted sequence. From the predicted amino acid sequence, the consensus nucleotide binding sequence: G--G-GKS was identified, consistent with an ATP requirement in the rubisco activase reaction. The identity of the barley and Arabidopsis cDNAs was confirmed by hybridization to the spinach gene on southern blots. All three cDNAs cross hybridize to 2.2 kb mRNAs. In northern blots of polyA mRNA from the mutant probed with either spinach or Arabidopsis cDNA, bands were seen at 2.0 and 2.4 kb. This suggests that the lesion in the Arabidopsis rubisco activation mutant is the result of aberrant mRNA processing.

References

1. Somerville, C. R., Portis, A. R. Jr., and Ogren, W. L. (1982) Plant Physiol. 70, 381-387.

2. Salvucci, M. E., Portis, A. R. Jr., and Ogren, W. L. (1985) Photosynthesis Res. 7, 193-201.

3. Portis, A. R. Jr., Salvucci, M. E., and Ogren, W. L. (1986) Plant Physiol. 82, 967-971.

#75

AUXIN RESISTANT MUTANTS OF *ARABIDOPSIS* WITH AN ALTERED MORPHOLOGY. Mark Estelle* and Chris Somerville. MSU-DOE Plant Research Lab, Michigan State University, East Lansing MI 48824 and *Dept. of Biology, Indiana University, Bloomington IN. 47405.

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

1. Estelle, M. and C. R. Somerville (1987) Auxin Resistant Mutants of *Arabidopsis* with and altered morphology. Mol. Gen. Genet. in press

#76

A BIOTIN-REQUIRING EMBRYO-LETHAL MUTANT OF ARABIDOPSIS THALIANA. J. Shellhammer, R. Dinkins, T. Schneider, K. Robinson, D. Meinke. Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

122G-E is a recessive embryo-lethal mutant of *Arabidopsis thaliana* that was originally isolated following EMS seed mutagenesis. Arrested embryos range from the globular to mature cotyledon stages of development, are very pale green in color, and have distorted cotyledons with a variety of abnormal shapes. Aborted seeds are pale green but similar in size and shape to mature wild-type seeds. Mutant embryos were originally found to produce extensive callus and homozygous mutant plants only when cultured on an enriched nutrient medium supplemented with amino acids, nucleosides, and vitamins (1). We now believe that the specific nutrient required for growth is biotin. Mutant seedlings initiated in culture develop normally for several weeks when transferred to pots without added nutrients, but eventually turn pale at the onset of flowering and fail to produce seeds or viable pollen (1). Heterozygous seeds produced by homozygous mutant plants following crosses with wild-type pollen are greener than the surrounding silique and germinate to produce plants with 25% aborted seeds. Arrested embryos can be rescued on a variety of basal culture media supplemented only with phytohormones and 5 uM biotin. Heterozygous plants grown in pots watered daily with 500 uM biotin produce siliques with 100% phenotypically normal seeds. It therefore appears that mutant embryos can be rescued *in situ* by exogenous biotin transported to the developing seed. The observed biotin requirement of 122GE mutant embryos may be caused by a defect in the biosynthesis, utilization, or intracellular transport of biotin. Very little is known about the biosynthesis of biotin in higher plants. Four biotin precursors have been identified in bacteria through analysis of biotin auxotrophs. The first and last precursors are pimelic acid and desthiobiotin respectively. We have found that 122GE mutant embryos are rescued by 5 uM desthiobiotin but not by 5 uM pimelic acid. We have been unable to obtain the other chemical intermediates, but we are in the process of synthesizing the known precursor to desthiobiotin in prokaryotes. We are also establishing both microbiological and streptavidin assays to determine the amount of biotin present in mutant and wild-type embryos. These studies should provide further information on the biochemical basis of abnormal development in mutant 122GE, the biosynthesis and transport of biotin in plants, and the role of biotin in plant embryo development.

(1) Baus, A.D., Franzmann, L., Meinke, D.W. (1986) Growth *in vitro* of arrested embryos from lethal mutants of *Arabidopsis thaliana*. Theor. Appl. Genet. 72: 577-586.

#77

CHARACTERIZATION OF AN ETHYLENE-RESISTANT MUTANT IN ARABIDOPSIS THALIANA. A.B. Bleecker, M. Estelle, C.R. Somerville and H. Kende. MSU-DOE Plant Research Laboratory, East Lansing, Michigan, 48824, USA

Application of ethylene to higher plants results in a number of well recognized physiological and biochemical responses, including breaking of seed dormancy, inhibition of both shoot and root elongation, accelerated leaf senescence, and alterations in the rate of ethylene biosynthesis. The molecular mechanisms by which ethylene elicits these responses have not been elucidated. To address this problem, we have isolated a mutant (designated Er-1) from Arabidopsis which is resistant to ethylene-induced inhibition of hypocotyl elongation. While the growth of etiolated hypocotyls in wild type plants was inhibited by >60% with 1 ul/l ethylene, the Er-1 mutant showed no inhibition of growth even at 100 ul/l ethylene. Root growth was also unaffected by ethylene in the mutant. The M_2 generation of the Er-1 mutant was crossed to the wild type and the progeny was allowed to self cross to form the F_2 population. Seedlings of the F_2 population segregated 3:1 as resistant to ethylene indicating that the Er-1 mutation is dominant over the wild type allelle. The mutation affects a number of different biochemical and physiological responses to ethylene, including effects on cell elongation, seed germination, accelerated leaf senescence and regulation of ethylene synthesis. These results indicate that: a) The various responses to ethylene listed above share a common element in their signal transduction pathways. b) The lesion in ethylene resistant plants must occur relatively early in the pathways since the ultimate biochemical bases for the various responses are presumably very different. c) The apparent elimination of a number of recognized responses to ethylene does not drastically alter growth and development of Arabidopsis plants.

#78

A STARCHLESS MUTANT OF ARABIDOPSIS RESPONDS GEOTROPICALLY. Timothy Caspar[1], Chris Somerville[1], and Barbara G. Pickard[2]. [1]DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824; [2]Department of Biology, Washington University, St. Louis, MO 63130

Since the turn of the century, it has been widely accepted that the reception phase of geotropism in higher plants requires statoliths. These statoliths have been thought to be starch-filled plastids, relatively dense organelles that often settle to the bottom of certain cells in geotropically sensitive tissues. We have tested this theory using a previously described (1,2) mutant of Arabidopsis thaliana which, due to a deficiency of the plastid isozyme of phosphoglucomutase, is unable to synthesize leaf starch.

We have shown by light and electron microscopy that seedlings of the mutant completely lack starch, whereas the wild type (WT) contains large quantities of starch in the root cap and the hypocotyl starch sheath. As in many other higher plants, the starch-filled plastids in these tissues in the WT sediment in response to gravity. However, in the mutant, the starchless plastids do not sediment. Although plastids in the mutant lack the ability to respond to gravity, the mutant is geotropically competent. The rates of geotropic hypocotyl curvature in light-grown seedlings of the WT and mutant are indistinguishable. In roots, the rate of geotropism in the mutant is only 70% that of the WT, however other responses such as elongation growth and electrotropism (which do not require gravity sensing but may utilize the same response mechanisms as root geotropism) are also similarly reduced in the mutant. We conclude that in both the hypocotyls and roots, gravity sensing in the mutant and WT are comparable and the reduction in root geotropism is due to an affect on the response rather than the perception phase of geotropism.

These results demonstrate that starch-filled plastids are not required for geotropism in Arabidopsis. Furthermore, starch-filled plastids probably have no role whatsoever in gravity perception because their elimination has no affect on gravity perception per se.

References:

1. Caspar T, Huber SC, Somerville CR (1985) Plant Physiol. 79:11.
2. Caspar T, Lin T-P, Preiss J, Somerville CR; Abstract #144, these proceedings.

#79

MUTANTS OF ARABIDOPSIS THALIANA WITH ALTERED PHOTOTROPISM AND/OR ALTERED GEOTROPISM.

K. L. Poff, T. Best, M. Gregg and Z. Ren; MSU-DOE Plant Research Laboratory; Michigan State University; East Lansing, Michigan 48824 USA.

A screening procedure has been developed and used for the isolation of 35 strains of Arabidopsis with altered phototropism. A second procedure has been developed and used for the isolation of 105 strains of Arabidopsis with altered shoot geotropism. Most of the photo-minus strains also exhibit impaired shoot geotropism, although two strains are photo-minus but wild type for shoot geotropism. Such data indicate a model in which shoot geotropism and shoot phototropism share a common "downstream" pathway, but have separate "upstream" pathways. Based on this model, only those strains which are photo-minus and geo-plus are potential candidates for alterations in the photoreceptor pigment regulating phototropism. The fluence response relationship has been measured for The Estland wild-type parent, and for one isolation strain, zr-8. The fluence response curve for this strain exhibits a shift in threshold toward higher fluence, in response either to single flashes of light or to multiple flashes separated by 15-min dark intervals. Based on these data, the alteration in zr-8 is at or close to the photoreceptor pigment.

This work was supported by the US Department of Energy (DE-AC02-76ER0-1338), and the US Aeronautics and Space Administration (NAGW-882).

#101

SELECTION OF MUTANTS RESISTANT TO PROLINE ANALOGUES: ISOLATION AND PRELIMINARY CHARACTERIZATION.

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Proline accumulates in plants in response to a variety of stresses (1). Such accumulation has been considered by some authors as an adaptive response and the beneficial effect against stress was attributed to proline acting as a compatible osmotic solute, as a stabilizing or solubilizing factor for proteins (2). Another interpretation consists in considering proline accumulation as due to protein degradation and inhibition of proline catabolism caused by the stress shock.

Our approach to try to define the role of proline in stress consists in isolating mutants which overproduce proline under non-stressed conditions. Such mutants are also useful tools for studying proline metabolism, still poorly understood in plants.

The rationale for selecting such mutants is based on their expected resistance to proline analogues which normally inhibit growth by feedback inhibition of defined enzymes of the proline pathway or by being incorporated into proteins instead of the natural amino acid. Two proline analogues, azetidine-2-carboxylic acid (AZC) and trans-4-hydroxyproline were tested for their growth inhibition and its relief by proline. Both analogues were used to select resistant types using M2 Arabidopsis thaliana plantlets obtained from mutagenized seeds (3).

The resistants obtained are presently characterized biochemically and genetically. One Arabidopsis mutant synthetises 3 X more proline than a control line under non stressed conditions.

References:

1. Stewart, C., Larher, . (1980). In: Biochemistry of Plants, Vol.5, pp.609-635.
2. Schubert, B., Tschesche, H. (1978). Biochemica and Biophysica Acta 541, 270-277.
3. Verbruggen, N., Jacobs, M. (1986). Arabidopsis Information Service, in press.

#102

Photosynthetic CO_2 Exchange, Electron Transport Rates and RuBP Carboxylase Activity in Glutamate Synthase Deficient *Arabidopsis* Stressed by Growth in Normal Air.

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An investigation into the loss of viability incurred when ferredoxin-dependent glutamate synthase deficient *Arabidopsis*(1) plants were shifted from a conditional CO_2-enriched atmosphere (continuous illumination, 290 umoles photons m^{-2} s^{-1}; 70% RH; 0.8% CO_2) to normal air was done. On 0 d mutant plants at the rossette stage of development had CO_2 exchange rates in 2% O_2 and 349 ppm CO_2 of 6.15 umoles CO_2 mg protein-1 hr-1. By 2 d this rate was 5.33. *In vitro* electron transport from H_2O to ferricyanide decreased from 215 umoles mg^{-1} chl-1 hr-1 to 171 by 2 d. The initial RuBP carboxylase activity declined from 88 nmol CO_2 mg^{-1} protein min^{-1} on 0 d to a value of 64 by the 2 d of the experiment. Other leaf properties such as the levels of chlorophyll and soluble protein were little changed over the first 2 d, but thereafter decreased steadily. Chlorophyll levels by 6 d were less than 50% of 0 d values. As a control, the wild-type strain's physiological properties showed minor changes over the course of the investigation. Phenotypically, on 9 d mature leaves were yellow; and young, developing leaves green. By 10 to 12 d, mutant plants had negative CO_2 exchange rates and were incapable of flowering. It is proposed that the low initial activity for RuBP carboxylase either increased the susceptibility of mutant plants to photoinhibition (2) or, else, the low RuBP carboxylase activity was a result of a deficient and unknown component of the chloroplast which directly affected many other chloroplast functions.

1. Somerville, C.S., Ogren, W.L. (1980) Nature (London) 286, 257-259
2. Bjorkman, O. (1981) in: Physiological Plant Ecology, vol. 12B, pp. 47-107, eds. Lange, O.L. et al., Springer Verlag.

#103

USING ANALOGUE RESISTANCE TO SELECT FOR *ARABIDOPSIS THALIANA* MUTANTS WITH ALTERED PHOSPHATE, POTASSIUM OR SODIUM TRANSPORT.

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We are attempting to identify *Arabidopsis thaliana* mutants with altered phosphorous, potassium or sodium transport. A simple selection was used based on resistance to transport inhibitors that act as analogues of the ions.

The phosphate analogue, vanadate, was used for these experiments. Similar studies with *Neurospora* yielded vanadate transport mutants with an altered high affinity phosphate uptake system that was induced during phosphate starvation (1). Preliminary tests were conducted to optimize conditions for *Arabidopsis* selections. Phosphate starvation did not result in deficiency symptoms unless agar was replaced with agarose. 384,000 M2 seeds (0.6% albino rate) were selected for growth in the presence of 1.5 mM vanadate in the absence of phosphate, and 51 survivors were obtained. Under similar conditions, 32,000 wild type seeds produced no survivors. Vandadate-resistant M2 mutants are being retested in the M3 generation.

Potassium and/or sodium analogue selections required potassium deficiency at germination. Potassium deficiency symptoms again appeared only when agar was replaced with agarose. In *Nitella flexilis*, alkali ions are known to be transported through the potassium channel are as follows: $K+ > Rb+ > Na+ > Li+ > Cs+$(2). Fresh weight, dry weight and visual symptoms of toxicity with various concentrations of these analgoues in the presence and absence of potassium, were evaluated at 14 days. Based on these results, 30mM LiCl and 5 mM CsCl were chosen to select for analogue resistance in the absence of potassium. 112,000 M2 seeds selected for Cs resistance yielded 7 survivors; similar selection with 32,000 wild-type seeds yielded none. 160,000 M2 seeds selected for Li resistance yielded 10 survivors; similar selection with 32,000 also yielded none. Cs and Li-resistant M2 mutants are being retested in the M3 generation.

References

1. Bowman, B., Allen, K., Slayman, C. (1983) Journal of Bacteriology 153(1), 292-296

2. Cram, W., Janacek, K., Rybova, R., Sigler, K. (ed) (1984) Membrane transport in plants. John Wiley and Sons. 234

#104

Molecular Genetic Analysis of Amino Acid and Protein Biosynthesis in *Arabidopsis thaliana*. Robert L. Last and Gerald R. Fink. Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.

Our major focus is the elucidation of regulatory mechanisms in amino acid biosynthesis in *Arabidopsis*. To this end we have isolated a large number of mutants resistant to 25-250μM concentrations of the leucine analogue 5,5,5-trifluoro-DL-leucine. All five mutants analyzed in detail show dominant resistance. The TFL^R is likely due to feedback resistant α-isopropylmalate synthase, the enzyme catalyzing the committing step in leucine biosynthesis. The dominant resistance alleles should be useful selectable markers in genetic and transformation experiments. We are also testing progeny of M2 plants isolated as resistant to the tryptophan analogue 5-methylanthranilate. Several classes of resistant mutants are expected including those producing feedback resistant anthranilate synthase, the first committed step in tryptophan biosynthesis. To complement these genetic approaches we are attempting to clone the biosynthetic genes likely to be altered in these inhibitor resistant mutants.

We have isolated cDNA and genomic clones homologous to the yeast ribosomal protein gene *CYH2*. Comparison of the sequence of one of the cDNAs to the yeast gene shows remarkable conservation of amino acid sequence in the N-terminal 60 amino acids of the protein. Mutations in the yeast *CYH2* gene cause recessive resistance to the cytoplasmic translational elongation inhibitor cycloheximide. A strain of *Arabidopsis* that is resistant to micromolar levels of cycloheximide has been isolated. This resistance trait was passed on to the M3 generation and is currently in outcrosses for further genetic analysis. The DNA sequence of the ribosomal protein gene from the cycloheximide resistant plant will be determined, and compared to that of wild-type to see whether the resistance trait is the result of a missense mutation.

#105

IN VITRO MORPHOGENESIS OF ARRESTED EMBRYOS FROM LETHAL MUTANTS OF ARABIDOPSIS THALIANA. L. Franzmann, D.W. Meinke. Department of Botany and Microbiology, Oklahoma State University, Stillwater OK 74078.

Embryo-lethal mutants of Arabidopsis thaliana have been shown previously to contain arrested embryos that differ in size, color, lethal phase, pattern of abnormal development, response in culture, and accumulation of seed storage proteins (1,2). In our original study on the growth of mutant embryos in culture (3), we identified a putative auxotrophic mutant that developed into normal plants only when placed on an enriched medium, and a fused cotyledon mutant that developed into rootless plants, but arrested embryos from most other mutants failed to respond in culture. We have now found a much higher frequency of in vitro morphogenesis with arrested embryos cultured on a shoot regeneration medium containing MS salts with 1 mg/l BAP and 0.1 mg/l NAA. Most of our mutants with lethal phases extending from the globular to cotyledon stages of development have now produced roots and shoots on this medium. Many of the resulting plants have altered leaf morphology, trichomes, pigmentation, and inflorescences. Other plants are surprisingly normal. Two homozygous mutant plants (63A-1A and 109F-1C) have been shown to produce siliques containing 100% aborted seeds with the expected mutant phenotype following self-pollination and 100% normal seeds following pollination with wild-type pollen. These results provide further evidence that lethal mutations may disrupt embryogenesis but not other stages of the life cycle. We have recently discovered that the putative auxotroph described in our original study requires biotin. It therefore appears that a defect in biotin biosynthesis or utilization may be responsible for embryonic lethality in mutant 122G-E.

(1) Meinke, D.W. (1986) Embryo-lethal mutants and the study of plant embryo development. Oxford Surveys of Plant Molecular and Cell Biology 3: 122-165.

(2) Meinke, D.W. (1985) Embryo-lethal mutants of Arabidopsis thaliana: Analysis of mutants with a wide range of lethal phases. Theor. Appl. Genet. 69: 543-552.

(3) Baus, A.D., Franzmann, L., Meinke, D.W. (1986) Growth in vitro of arrested embryos from lethal mutants of Arabidopsis thaliana. Theor. Appl. Genet. 72: 577-586.

#106

Genetic Analysis Of Arginine Metabolism In Arabidopsis thaliana. P. McCourt and G.R. Fink. Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.

Arginine biosynthesis and metabolism has been the focus of study in a number of model genetic systems such as E.coli, yeast and Neurospora due to the fact that this amino acid is both made and degraded via a number of pathways within the same cell. Plants also contain both anabolic and catabolic pathways for arginine and we are interested in characterizing these pathways in Arabidopsis at both the genetic and molecular level.

As a first approach to isolating mutants with altered arginine metabolism we have selected Arabidopsis plants resistant to various arginine analogues. Four homoarginine resistant mutants and two canavinine resistant mutants were recovered from approximatly 40,000 M2 plants. The observation that a number of these mutants are not cross reactive to the analogues suggests they are not simply defective in transport of arginine. Work is in progress to determine the biochemical lesions in these mutants.

A number of ways are being explored to isolate mutants with alterations in arginine degradative pathways. Arginase, an enzyme involved in the urea cycle, converts arginine to ornithine and urea. A sensitive enzyme assay for arginase has been developed which can be carried out on single Arabidopsis leaves. This method should allow the direct screening for mutants with altered levels of arginase activity.

As a second approach to isolating arginase mutants, conditions are being tested which will allow wild type Arabidopsis to grow on arginine as a sole nitrogen source. These conditions should allow the screening of plants which cannot utilize arginine but can grow on alternate nitrogen sources. A subset of these mutants should be defective in arginase enzyme activity.

#107

VARIEGATED MUTANTS OF ARABIDOPSIS. J. M. Martinez-Zapater, S. Somerville, C. R. Somerville. DOE Plant Research Laboratory, Michigan State University, East Lansing MI48824.

Nuclear encoded variegation in plants is a frequent phenomena (1). In variegated plants adjacent cells in the same tissue show a different phenotype that can be the result of physiological or genotypic differences between them. When the different kinds of tissue in the variegated plant differ genotypically, variegation is due to genetic instability of the nuclear genome. This instability is frequently the result of transposable element induced mutations (2).

As an approach to search for the presence of transposable elements in Arabidopsis thaliana we have initiated the characterization of variegated plants which were identified in M2 populations resulting from EMS mutagenesis or in the progeny of plants regenerated from tissue culture. We are currently analyzing four variegated mutants at both the phenotypic and genetic levels.

All the mutations are nuclear encoded and behave as recessives. Two of them, var-3 and var-4, were produced by EMS mutagenesis, and in both cases variegated plants are light-sensitive and bleach under high light intensity. The other two mutations, var-C and var-256, segregated in the offspring of plants regenerated from tissue culture. Variegation in var-C is only restricted to the leaves and is temperature sensitive. When plants are grown at temperatures below 22^{0}C they do not show any variegation. The pattern of variegation in var-256 is more complex than in the other var mutants in that the amount of variegation ranges from 0 to about 100% chlorosis.

References

1. Kirk, J.T.O., Tilney-Bassett, R.A.E. (1978) "The plastids: Their Chemistry, Structure, Growth and Inheritance", 2nd Ed. Elsevier, Amsterdam.
2. Nevers, P., Shepherd, N.S., Saedler, H. (1986) Adv. Bot. Res. 12, 103

#108

CHARACTERIZATION OF THE ARABIDOPSIS ADH GENE AND ANALYSIS OF EMS INDUCED ADH - NULL MUTANTS. R. Dolferus, M. Jacobs. Laboratory of Plant Genetics, Free University of Brussels, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium.

Arabidopsis alcohol dehydrogenase (ADH, E.C.1.1.1.1) is encoded by one single gene, from which three different naturally occuring alleles are known, called S, F, and A (1). The enzyme has been purified and a crude antiserum has been prepared against it. ADH exists as a dimer with a molecular weight of 87.000 daltons.

The enzyme is supposed to play an important role for the survival of the plant under anaerobic stress conditions (2). In Arabidopsis we demonstrated that ADH activity is markedly increased during callus induction. At the plant level, activity rapidly declines during the germination process and is no more represented in mature plants, except for some specific cell types around the vascular bundles and in pollen (3). During anaerobic circumstances it was also shown that Arabidopsis ADH is one of the few proteins which are actively synthesized.

The high degree of homology which was observed between the maize and the Arabidopsis ADH system, indicates that ADH is a highly conserved plant protein. This was also confirmed at the nucleotide level, using cloned maize ADH_1 cDNA as a probe. This observation was exploited to isolate the Arabidopsis ADH gene (4).Several ADH null mutants were isolated using ethyl methane sulfonate (EMS) as a mutagen on M_1 seeds, and allylalcohol selection on M_2 germinating seeds. Mutants were obtained at a frequency of 9.10^{-5}; 11 ADH mutants were selected for the race Be-0 and 21 for the race Ts-1. Mutants R001 to R011 (Be-0 background) were further characterized at the genetical and biochemical level. Some of these mutants were shown to contain an inactive protein, still capable of forming active heterodimers with the wild type enzyme in heterozygotes.The mutants were subdivided into three classes on the basis of the formation of cross reacting material (CRM) using anti-ADH antibodies. Using rocket-immunoelectrophoresis and western blotting techniques, we found that some of the mutants did not produce any ADH like polypeptides at all (CRM^-), some of them produced inactive ADH enzyme at the normal level of expression (CRM^+), and the third class producing ADH CRM at a reduced level. This observation was also confirmed using northern blot hybridization. These mutants are presently analyzed at the DNA level by restriction mapping and some of the mutated genes are isolated in order to map the mutations.

References:

1. Dolferus, R., Jacobs, M.(1984). Biochemical Genetics 22, 817.
2. Sachs, M.M., Freeling, M. and Okimoto, R. (1980). Cell 20,761.
3. Dolferus, R., Marbaix, G. and Jacobs, M. (1985). Molec. Gen. Genet. 199, 256.
4. Chang, C., Meyerowitz, E.M. (1986). P.N.A.S. 83, 1408.

#109

Analysis of Histidine Biosynthesis in Arabidopsis thaliana. Beth A. Lalonde and Gerald R. Fink Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142.

We are interested in studying the regulation of gene expression in Arabidopsis. In particular, we are interested in amino acid biosynthetic pathways and are isolating mutants and cloning relevant genes in an attempt to elucidate mechanisms involved in the regulation of these genes.

We have isolated mutants in histidine biosynthesis by selecting for resistance to the histidine analog, triazole alanine. This compound exerts its effect in yeast and bacteria by false-feedback inhibiting the first enzyme of histidine biosynthesis, ATP PR-transferase, resulting in starvation for histidine. Although none of the Arabidopsis mutants have been extensively characterized, it is likely that at least some of them will have lost feedback inhibition of this first enzyme. Another class of triazole alanine resistant mutants isolated in yeast exhibits constituitive histidine biosynthesis. If Arabidopsis histidine biosynthesis is similarly regulated we might expect to find constituitive Arabidopsis mutants.

We have attempted to clone the Arabidopsis histidinol dehydrogenase gene by hybridization with the corresponding yeast gene (HIS4). A cross-hybridizing Arabidopsis clone has been identified and a portion of it is being sequenced in an attempt to demonstrate amino acid homology with the highly conserved HIS4C region of the yeast gene. This clone will be used as a probe to study regulation of gene expression; to see if there is tissue specific expression and to address the question of whether there is general control of amino acid biosynthesis in plants as there is in yeast.

#110

EMS INDUCED MUTANTS OF *ARABIDOPSIS THALIANA* RESISTANT TO ISOXABEN Dale R. Heim and Ignacio M. Larrinua Lilly Research Laboratories Lilly Corporate Center Indianapolis Indiana 46240

One million seeds derived from EMS treated plants were screened for resistance to isoxaben (N-[3-(1-Ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6, dimethoxybenzamide) by germinating them in concentrations of this herbicide which prevented control plants from advancing to the four leaf stage. A number of putative resistant plants were recovered and allowed to self-pollinate. Seeds from these surviving plants were harvested and tested for resistance to various concentrations ofthe herbicide. All progeny tested were resistant to at least some level of the agent, demonstrating that resistance was a heritable trait.

These mutants appear to fall into three distinct classes: low (~2-3X more resistant than wild type), medium (~30X wild type level) and high (>300X wild type). The high level mutants may be completely resistant to the primary mode of action of this herbicide. At the macroscopic level the susceptibility of the sensitive plants manifests itself as a total lack of root hairs and root elongation together with a swelling of the root tip. However with the high level resistance mutants, we have found no level of the herbicide which impacts this site significantly. They, however, succumb to a secondary mode of action which appears to be approximatedly two orders of magnitude less sensitive than the primary site. This manifests itself as a "burning" of the leaf after the plants have emerged and advanced to the four leaf stage.

Crosses of all three classes of mutants with wild type plants as both the male and female parent demonstrate that in all three classes of mutants, the resistance marker segregates as a Mendelian inherited dominant marker. Plants recovered from these tests for all resistance classes have been allowed to self-pollinate to recover the parental phenotypes. These experiments as well as the mapping of these marker(s) to particular chromosomes are in progress. These should tell us if all of the mutant classes are allelic.

#111

AN UNUSUAL MUTANT OF ARABIDOPSIS THAT DISPLAYS PHOTOOXIDATION IN AIR BUT NOT 2% CO_2. N.N. Artus, C.R. Somerville MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

A new mutant of Arabidopsis which requires a high concentration of atmospheric CO_2 for growth has been isolated. It is apparent from photosynthetic CO_2 exchange and the $^{14}CO_2$ labeling pattern that, unlike previous mutants of this kind, the new mutant is not defective in photosythetic carbon metabolism. The mutant bleaches and develops necrotic lesions when transferred from high CO_2 to air. Since this phenotype requires illumination, we attribute the damage to photooxidation which is believed to be mediated by reactive oxygen species. Therefore, we examined the possibility that the mutant is defective in its ability to detoxify reactive oxygen. The activities of the superoxide and hydrogen peroxide scavenger enzymes, superoxide dismutase and catalase, and the antioxidant regenerating enzymes dehydroascorbate reductase and glutathione reductase were comparable to the activities in wild type. Likewise, the concentration of the antioxidant ascorbic acid was normal in the mutant while the concentrations of the antioxidant glutathione and the singlet oxygen quencher -tocopherol were 1.5 and 2 times the wild type levels respectively. Furthermore, the four major carotenoids were present in similar proportions as in the wild type. Thus, the mutant appears to be fully equiped to detoxify reactive oxygen. We considered the possibility that the mutation is associated with a CO_2-dependent process other than photosynthetic-CO_2 fixation. Since a high concentration of CO_2 will inhibit ethylene action, we tested the hypothesis that the mutant is hypersensitive to ethylene. We also examined the bicarbonate requirement of PSII electron transport. None of these tests suggested a biochemical basis for the lesion. Thus, the properties of the mutant suggest the possible existence of a previously unidentified role for CO_2 in a light dependent process.

#112

A CHILLING-SENSITIVE MUTANT OF ARABIDOPSIS. Suzanne Hugly, Peter McCourt, and Chris Somerville. DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Arabidopsis thaliana is a chilling-resistant species that can survive prolonged exposure to temperatures as low as 2°C. To define genes which condition chilling-resistance, we have isolated single nuclear mutations which convert Arabidopsis into a chilling-sensitive phenotype. The mutants were isolated by screening three week old EMS mutagenised M2 seedlings (grown at 22°C) for plants that became chlorotic following exposure to chilling conditions (13°C). One such mutant line (designated PM11), was identified because it showed signs of chlorosis after a 3 day cold treatment at 13°C, but regained normal pigmentation and resumed growth following a shift back to 22°C. This mutant can not be rescued following cold treatment of 4 or more days.

One characteristic physiological response associated with chilling damage in chilling-sensitive species is cold induced electrolyte leakage (1). We measured electrolyte leakage in PM11 and observed that it leaks ions following exposure to chilling conditions. The electrolyte leakage appears to be maximal approximately three days following the initiation of treatment and is coincident with the onset of chlorosis. Thus, we believe that the mutant represents an applicable example of a bonafide chilling-sensitive phenotype.

As a first step in identifying the biochemical defect responsible for chilling-sensitivity in PM11, we examined the pattern of incorporation of ^{14}C into all of the small molecules in wild-type and mutant plants that were grown in the presence of $^{14}CO_2$ in the light for several days at either normal or chilling temperature. The only difference observed lies within the lipid fractions. Analysis of the lipids by thin layer chromatography indicates that following a 2 day cold treatment of 3 week old seedlings, the lipid composition of PM11 is distinct from that of wild-type plants. This change was not apparent in either line when maintained at 22°C. Thus, our results suggest that the chilling-sensitive phenotype of PM11 coincides with a chilling-dependent change in lipid metabolism.

References

1. Lyons, J.M. (1973). Ann. Rev. Plant Physiol. 24, 445

#113

Aspartate-derived Amino Acid Biosynthesis in *Arabidopsis thaliana*: A Molecular Genetic Analysis. R. Marc Learned and Gerald Fink. Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.

The amino acids lysine, threonine and methionine comprise a family of amino acids that are derived from aspartate, and whose biosynthetic pathway has been subjected to studies in both microorganisms and higher plants. Because the pathway is branched, the balanced production of these amino acids requires a complex, coordinated regulatory mechanism. We are interested in studying both the genetic and molecular basis of this regulation in *Arabidopsis thaliana.*

Biochemically defined mutants have proven to be extremely useful in studies of metabolism and regulation. Therefore, as a first step , we are attempting to isolate mutant strains of *Arabidopsis* with altered metabolism in the aspartate pathway. The selection of such mutants is based on the ability to inhibit plant growth by supplying amino acids or amino acid analogues in the culture medium. In our initial studies, 100,000 seeds were screened for resistance to either lysine + threonine, the lysine analogue S-2-aminoethyl cysteine, or the methionine analogue ethionine. In each case, resistant plants have been isolated. Biochemical and genetic characterization of these mutants is currently underway.

In order to pursue a molecular genetic approach to the study of amino acid biosynthesis, we are attempting to clone the genes affected in these mutants. Because of its important role in regulating production of all the amino acids derived from aspartate, we have concentrated on isolating the gene for aspartate kinase. Although it is still difficult to clone genes directly from plant sources, it is straightforward to isolate specific genes in yeast by complementation. Therefore, we have cloned the aspartate kinase gene from *S. cerevisiae* in order to use the yeast gene as a hybridization probe to screen an *Arabidopsis* library. By using the heterologous yeast probe, we hope to identify and isolate the aspartate kinase gene from *Arabidopsis.* Moreover, this technique may prove to be useful for isolating genes that encode other enzymes in the pathway, such as homoserine dehydrogenase or cystathionine γ- synthase.

With the development of these tools, we hope to exploit the advantages of *Arabidopsis* as an experimental system to study amino acid regulation in a higher plant.

#114

RADIATION GENETICS WITH HEAVY IONS IN ARABIDOPSIS. A.R. Kranz, U. Bork. Botanical Institute, J.W.Goethe-University, POB 111 932 D-6000 Frankfurt am Main 11

With the recently improved availability of heavy ions (HZE) produced by powerful accelerators (BEVALAC, Berkeley, UNILAC, Darmstadt) (1) and in space (flight missions SL-1, LDEF) (2) still open problems of radiation genetics, e.g. the target range of particles and dose calculations, can be solved. Seeds of Arabidopsis, an early successful subject of investigations (3) with sparsely ionizing radiation (x, γ, n), proved to be an excellent tool for studies with the much more densely ionizing HZE-radiation (4,5).

Considering the HZE-fluence (p/cm^2), energy (MeV/u) and order rsp. mass number (A, Z) as well as the number and diameter of genetic cells (GECN) of the seed embryo with its cell elements (nucleus, genome, chromosome) the effective target range for lethality in M_1 and M_2 is estimated for the first time. Evidence for positive, particular correlations between the physical parameter mentioned, the cellular target and the genetic effect on lethality is presented (6,7). With further improvement of this technique more data on the cytogenetic and molecular significance of HZE-targets will be obtained.

References

1. Kranz, A.R. et al. (1986) Adv. in Space Res. in press
2. Kranz, A.R. (1986) Adv. in Space Res. in press
3. Redei, G.P. (1969) Bibliogr. Genet. 20, 151 pp.
4. Fujii, T.M. et al. (1966) Radiation Bot. 6, 297
5. Bork, U., Kranz, A.R. (1985) GSI Sci. Rep. 1984, 241
6. Bork, U., Kranz, A.R. (1987) GSI Sci. Rep. 1986 in press
7. Kranz, A.R. (1987) Arabid.Inf.Serv. 23 in press

#115

CLONING OF PLAMSA MEMBRANE H+-ATPase FROM ARABIDOPSIS THALIANA. Terry K. Surowy and Michael R. Sussman. Department of Horticulture, University of Wisconsin, Madison, WI 53706.

We are cloning and sequencing the structural gene(s) for the plasma membrane H+-ATPase of higher plants. Specific polyclonal antibodies against the plasma membrane H+-ATPase Mr=100 000 polypeptide have been generated using denatured enzyme isolated from oat root cells. These antibodies have been shown to cross-react with purified, active H+-ATPase (particle size 2000 KDa) when oat root plamsa membrane proteins were solubilized and separated on a glycerol gradient. The antibody preparation is highly specific and can detect nanogram quantities of the Mr=100 000 H+-ATPase polypeptide. Plasma membrane H+-ATPases from several other plant species, both monocot and dicot, were shown to react with the antibody using Western blots, suggesting homology between these enzymes.

A λgt11 expression library of Arabidopsis thaliana genomic DNA was screened using the H+-ATPase polyclonal antibody. An immunopositive clone, λat-2, was identified and purified to homogeneity. The clone contains a 6.9 Kb insert which has several internal EcoR1 sites. Insert EcoR1 fragments, of sizes 2.7Kb, 2.5Kb, 1.3Kb, 0.25Kb and 0.23Kb, were cloned into plasmid and M13 vectors for further characterization and sequencing. A detailed restriction map of the λgt11 insert has been constructed. When plasmid subclones were used to probe Northern blots of poly A+ RNA isolated from A. thaliana plants, a combined 2.5Kb/1.3Kb probe hybridized to a poly A+ RNA species of 4.3Kb. This is identical to the size of the poly A+ RNA species which encodes the recently cloned and closely related plasma membrane Mr=100 000 H+-ATPase from the mycelial fungus Neurospora crassa. The 2.5Kb and 1.3Kb insert fragments from the A. thaliana clone are currently being sequenced using M13 and plasmid Sanger dideoxy sequencing techniques. As immunoreactive gt11 clones are generally not full-length, we are also using the 2.5Kb and 1.3Kb fragment subclones to probe a 'full-length' EMBL4 library of A. thaliana genomic DNA. In order to confirm their identity, positive clones isolated from this library will be screened on Southern blots with two oligonucleotide probes. One of these is homologous to the coding region for the known phosphorylated active site of the plasma membrane H+-ATPase; the other is based on the sequence of another extremely conserved area in the plasma membrane ATPases.

#116

MOVEMENT OF THE CHLOROPLAST TUFA GENE TO THE NUCLEUS.

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We are using Arabidopsis thaliana as a convenient system to study the apparent intracellular transfer of the chloroplast tufA gene. TufA is a chloroplast gene in the green alga Chlamydomonas reinhardtii (1), but is absent from the chloroplast chromosome of even the most primitive land plants (2). This suggests a chloroplast to nucleus (or vice versa) gene transfer sometime during green algal/land plant evolution analagous to the organelle to nucleus gene transfers postulated to have occurred during the endosymbiotic establishment of mitochondria and chloroplasts (3).

Using the Chlamydomonas gene as a probe, we have detected the presence of a tufA gene in Southern blots of nuclear DNA from Arabidopsis thaliana. We have isolated two overlapping clones from a library of the this species (4) using the same probe and have sequenced approximately 50% of the portion hybridizing to our probe. The fragment appears to contain a bona fide tufA gene being 95% identical to the Chlamydomonas gene at the amino acid level and 77% at the nucleotide level. Complete sequence data should confirm if the gene is a potentially functional gene, i.e. if it is an open reading frame, has the necessary processing signals and has a 5' transit peptide sequence to direct the chloroplast import of its gene product.

To determine the direction of tufA transfer we have begun Southern blot analysis of chloroplast DNAs from other algal genera. Preliminary results indicate that tufA is present in the more distantly related (relative to land plants) genera, but absent in a genus considered very closely related to land plants, Coleochaete (5). TufA thus appears to be an example of chloroplast to nucleus gene transfer. Further work will include studies of the gene's expression and intracellular localization of its gene product. Additional algal studies using tufA may be useful in defining the ancestry of the land plants.

1. Watson JC & SJ Surzycki. 1982. PNAS 79:2264.
2. Ohyama K, et al. 1986. Nature 322:572
3. Wallace, DC. 1982. Microbiol. Rev., 46:572.
4. Chang, C & M Meyerowitz. 1986. PNAS 83:1408.
5. Graham LE. 1985. Amer. Sci. 73:178.

#117

Genetic transformation of *Arabidopsis*—Complementation of an alcohol dehydrogenase null mutant with the *Arabidopsis Adh* gene

Chang, C. and Meyerowitz, E. M. Division of Biology, Caltech, Pasadena, California 91125

Using *Agrobacterium*-mediated transformation we have genetically complemented an *Arabidopsis* alcohol dehydrogenase (ADH) null mutant with a fragment of DNA containing the *Arabidopsis Adh* gene. The transformation procedure we followed was that of Lloyd *et al.* (1) in which transgenic plants are regenerated from leaf-derived callus. Leaf pieces of strain R002 (an ADH null mutant provided by M. Jacobs) were infected with *Agrobacterium* carrying the binary transformation vector pMON410 (provided by Monsanto Company) into which we had cloned the genomic *Adh* gene (2) (a 9.4 kilobase DNA fragment). pMON410 allows selection of transformants on hygromycin B. ~40% of leaf explants gave rise to callus on medium containing hygromycin B, while < 1% of mock-infected explants gave rise to callus on this medium. By 10 weeks after infection, ~40% of the calli had produced shoots. Excised shoots were placed for 1-2 weeks on rooting medium, then transferred to soil. Mature seed was obtained from regenerated plants as early as 12 weeks after infection. We presently have nine independent regenerated lines that appear to be transformed based on genetic criteria. Progeny resulting from self-fertilization in the regenerated plants were tested for resistance to high levels of hygromycin B. In addition, they were stained for root-specific ADH activity which is easily detected in the wild-type but not detected in R002 plants. Progeny from five of the lines display a 3:1 ratio of both hygromycin B resistance and root-specific ADH activity, indicating the presence of a single active transformed copy; the remaining four lines have greater than 3:1 ratios. Progeny from three lines were also exposed to allyl alcohol. Three-fourths of each line are killed by levels that are toxic to the wild-type but non-toxic to R002 plants. (Thus, allyl alcohol can be used to select against ADH positive transformants.) We have examined one line by blot hybridizations; results indicate a single integration site for the binary plasmid which is consistent with the observed 3:1 progeny ratio in this line for hygromycin B resistance, ADH activity, and allyl alcohol sensitivity. Third generation plants from this same line continue to show inheritance consistent with a single active locus.

1. Lloyd *et al.* (1986) *Science* 234: 464-466.
2. Chang, C. and Meyerowitz, E. M. (1986) *Proc. Natl. Acad. Sci. USA* 83: 1408-1412.

#118

GENOMIC CLONES OF ARGININE DECARBOXYLASE FROM ARABIDOPSIS

Cynthia Collins and Russell Malmberg, University of Georgia, Department of Botany, Athens, GA 30602.

Changes in the levels of polyamines and in the activities of their biosynthetic enzymes have been correlated with a variety of developmental processes in higher plants (1). Plants have two differentially regulated pathways that are known to lead to the formation of putrescine. One pathway derives putrescine from arginine through agmatine via arginine decarboxylase (ADC). A partial cDNA has been isolated by differential hybridization that is believed to encode ADC (2). This 500 bp oat cDNA, AD5, hybridizes to a transcript whose abundance is regulated by polyamines and by pH and potassium stress in the same manner as is ADC activity, and whose organ specific abundance pattern also correlates with the organ specific activity pattern of the enzyme. In addition, the major hybrid select translation product for AD5 has the same molecular weight as does ADC.

Initial Southern blot analysis of tobacco genomic DNA probed with AD5 suggested that ADC is encoded by a large multigene family. Subsequent Southern blot analysis of Arabidopsis Landsburg erecta genomic DNA showed a simple hybridization pattern for several restriction enzymes suggesting that ADC is present as a lower copy number gene in Arabidopsis. An Arabidopsis Landsburg erecta EMBL4 genomic library (3) was screened with AD5, and ten clones were picked for characterization. These clones can be divided into two classes on the basis of crosshybridization. This suggests that the Arabidopsis genome contains at least two genes for ADC. Two AD5 homologous EcoRI fragments can be detected by Southern blot analysis of Landsburg erecta strain genomic DNA, but these two fragments have not yet been indisputably correlated with the two classes of clones isolated from the library. Genomic reconstruction experiments indicate that one AD5 homolgous EcoRI fragment has an approximate copy number of ten. Since this copy number is estimated on the basis of a single genomic restriction fragment, this fragment may be indicative of a tandemly repeated gene. The second AD5 homolgous EcoRI fragment appears to be present at a lower copy number. The emphasis of current research is to determine whether more than two classes of clones can be isolated from the genomic library, to determine the copy number for each class of clone, and to map the transcription unit. We also hope to test if ADC shows similar multiple regulation in Arabidopsis as it does in tobacco and oats.

(1) Galston,A.W. 1983. BioScience 33:382-388
(2) Hiatt,A.C. and Malmberg,R.L., unpublished results
(3) Chang,C. and Meyerowitz,E.M. 1986. PNAS USA 83:1408-1412

#119

AN APPROACH FOR CLONING TELOMERIC SEQUENCES FROM ARABIDOPSIS

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

Telomeres are the structures that form the ends of linear eukaryotic chromosomes. These chromosomal elements facilitate the complete replication of the chromosome ends and prohibit the excessive recombinogenic properties of unmodified DNA ends [1]. Unfortunately, detailed knowledge of telomere structure and function is limited to a handful of lower eukaryotic systems where telomeric DNA sequences have been cloned. We have designed a strategy for cloning telomeric sequences from organisms with larger chromosomes and have applied this strategy to Arabidopsis thaliana.

Our cloning strategy involves a two step enrichment for clones with telomeric inserts. First, two independent genomic libraries were constructed by an 'endcloning' technique similar to that used in the isolation of Trypanosome telomeres [2]. The basic protocol involved blunt-end ligation of a M13 cloning vector to end-repaired, undigested, high molecular weight nuclear DNA, followed by an appropriate restriction digestion, and subsequent ligation to form recombinant circular molecules. The resulting 'endlibraries' should contain inserts corresponding to sequences which lie adjacent to DNA ends; consequently, telomeric sequence inserts should be well represented in the endlibraries. In order to achieve a secondary enrichment for telomeric clones, well represented clones (i.e. clones with inserts that are present in both of the endlibraries) were chosen. To accomplish this, single-stranded plasmids representing both endlibraries were allowed to anneal en masse and plasmids which crosshybridize were purified by hydroxylapatite chromotography. Presently, we are analyzing these clones, using Southern blotting techniques, to determine if they hybridize to genomic sequences that are preferentially sensitive to exonucleolytic digestion, a property characteristic of telomeric sequences.

References

1. Blackburn, E.H., and Szostak, J.W. (1984). Ann Rev Biochem. 53: 163-194.
2. Van der Ploeg, L.H.T., Liu, A.Y.C., and Borst, P. (1984). Cell. 36:459-468.

#120

CHARACTERIZATION OF A GENOMIC FRAGMENT OF Arabidopsis thaliana WITH HOMOLOGY TO A HMG-CoA REDUCTASE cDNA CLONE FROM HAMSTER. C. Caelles, A. Ferrer, F.G. Hegardt and A. Boronat. Unit of Biochemistry. School of Pharmacy, University of Barcelona. 08028-Barcelona. Spain.

The enzyme HMG-CoA reductase catalyzes the synthesis of mevalonate, the specific precursor of isoprenoid compounds functional in many different plant biochemical processes. HMG-CoA reductase is the main rate limiting step in cholesterol biosynthesis in mammals, and its properties and regulation mechanisms have been well characterized. Nevertheless, very little is known about the enzyme and its gene in plants.

In a first approach to identify genomic sequences homologous to a HMG-CoA reductase gene in Arabidopsis thaliana, we used a hamster cDNA clone (1) as a probe in genomic blot analysis. A 2.9 kb XbaI fragment containing most of the translated region for HMG-CoA reductase was labelled by nick translation and hybridized to A. thaliana genomic DNA digested with different restriction enzymes. After a long autoradiographic exposure a faint band corresponding to a 12 kb BamHI fragment was detected. A recombinant lambda Charon 35 clone containing the 12 kb BamHI fragment was isolated from a partial genomic library. After a restriction map of the cloned fragment was established, a 2.7 kb XbaI-BamHI subfragment was identified to be responsible for the hybridization to the hamster HMG-CoA reductase gene. When the 2.7 kb XbaI-BamHI genomic fragment was hybridized to restriction fragments generated from the hamster 2.9 kb XbaI cDNA fragment, the hybridization was specifically restricted to a 646 bp PstI fragment. It is interesting to point out that this fragment, which has been suggested to contain the coding region for the active site of the enzyme, is also homologous to the yeast HMG-CoA reductase genes (2). Experiments concerning the identification of a transcription product for the cloned fragment and the sequencing of the regions of homology are under progress.

1. Chin, D.J. et al. (1984) Nature 308: 613-617.
2. Basson, M.E. at al. (1986) Proc. Natl. Acad. Sci. U.S.A 83: 5563-5567.

#121

In Situ HYBRIDIZATION IN ARABIDOPSIS. John L. Bowman, Elliot Meyerowitz. California Institute of Technology, Pasadena, CA 91125.

A valuable technique to help elucidate spatial and temporal gene expression is *in situ* hybridization to RNA present in tissue. A series of experiments was performed to optimize the procedure of *in situ* hybridization in *Arabidopsis thaliana*.

So far the only labeled probe used was a 12S seed storage protein gene, which is expressed in developing embryos; therefore seeds are the only organs tested thus far. It is reasonable to assume these procedures would apply to other tissues since the fixation worked as well on inflorescences and stems.

Three hour fixations done in a 1% glutaraldehyde solution containing 10 mM phosphate pH 7.4, 100 mM NaCl solution gave much better signals than those done in an ethanol fixative. The tissues were paraffin embedded because it is difficult to obtain good cryostat sections of many plant tissues.

Tritium labeled SP6-promoted RNA probes were used in all experiments. The RNA probes yield better results than nick-translated DNA probes because they are single standed, because RNA-RNA hybrids have higher melting temperatures than RNA-DNA hybrids, and using the sense strand provides a control for non-specific hybridization.

A mild protease treatment (1μg/ml Proteinase K @37^{o}C for 30 minutes) was found much better than extensive protease treatments (.8mg/ml) to make the RNA of the sections accessible to probe. Probe concentrations of 1μg/ml were sufficient for the maximum signal to noise ratio observed, however lower concentrations may be equally effective. Hybridizations performed at 45^{o}C with washing at 42^{o}C yielded better signal to noise ratios than other temperature conditions tested.

#122

RECOMBINATION IN *ARABIDOPSIS THALIANA*. Abdul M. Chaudhury, and Ethan R. Signer. Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139.

In *Arabidopsis thaliana*, as in other organisms, breakage and rejoining of DNA molecules *in vivo* is likely to be involved in processes as disparate as crossing-over in meiosis, repair of damage to native DNA, and integration of foreign DNA. In higher eukaryotes such as plants, however, in contrast to prokaryotes and lower eukaryotes such as fungi, little is known about the mechanism of such processes at the molecular level. We are investigating these issues by defining the basis for homologous genetic recombination in *A. thaliana*. Several experimental approaches will be presented.

#123

TRANSFORMATION OF ARABIDOPSIS THALIANA WITH AGROBACTERIUM TUMEFACIENS STRAINS CONTAINING DIFFERENT CHIMERIC GENE CONSTRUCTS CODING FOR KANAMYCIN OR HYGROMYCIN RESISTANCE.

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Transformed plants of Arabidopsis thaliana have been obtained using Agrobacterium tumefaciens strains containing three different chimeric gene constructs (pTR-NPT-II, pssuA-NPT-II, and pTR-Hph). The transformants were obtained using a leaf disc infection method, principally based on the method described previously (1). Depending upon the chimeric constructs and the infection conditions used, the transformation efficiency varies from 5% to 50%. In contrast to some previous reports, we were able to establish a highly efficient selection procedure on kanamycin-containing medium. In parallel studies, we have established conditions for selecting for transformation using G418 and hygromycin. Transformation has been confirmed by assaying for neomycin phosphotransferase activity. Southern-blot analysis of transformed plants is currently underway. The transformation method will be optimized in order to be useful for insertion mutagenesis studies of the Arabidopsis genome.

Reference

1. Lloyd, A.M., Barnason, A.R., Rogers, S.G., Byrne, M.C., Fraley, R.T., Horsch, R.B. (1986) Science 234, 464-466.

#124

GENETIC VARIABILITY AMONG SIXTEEN GEOGRAPHICAL RACES OF ARABIDOPSIS THALIANA AS REVEALED BY RESTRICTION FRAGMENT-LENGTH POLYMORPHISMS. D. F. Voytas and F. M. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

DNA from a collection of sixteen geographical races of Arabidopsis thaliana is being used to screen for restriction fragment-length polymorphisms. Of particular interest are restriction fragment-length changes due to DNA insertion. Such polymorphisms have been shown to arise by DNA transposition in a number of organisms (e.g. yeast [1], nematodes [2]), and have led to the isolation of active transposable element families.

Large cosmid clones (~20 kb) of the race Columbia are being used as hybridization probes to Southern filters containing restriction digests of DNA from each of the races. To date, approximately 150 kb of single copy sequences have been analyzed. Despite the high level of phenotypic variation among the races, very few polymorphisms have been identified which are due to simple restriction site changes. The heterozygosity between strains (number of polymorphic nucleotides per thousand) has been estimated to be 0.001 [3]. This value, which is comparable to humans (0.003 [4]) and significantly lower than maize (0.05 [4]), suggests a high degree of sequence uniformity between the geographical races.

Two insertional polymorphisms have also been identified: a 0.5 kb insertion common to two races, and a 2.5 kb insertion found only in one race. In the latter case, Southern hybridizations using a border fragment which encompasses part of the insertion reveal that this sequence varies in copy number among the races. The mechanism by which these insertions have arisen (e.g. transposition, recombination) is currently being tested.

References

1. Cameron, J., Loh, E., Davis, R. (1979). Cell. 16,739-751.
2. Emmons, S., Yesner, L., Ruan, K., Katzenberg, D. (1983). Cell. 32,55-65.
3. Engels, W. (1981). Proc. Natl. Acad. Sci. USA. 78,6329-6333.
4. Evola, S., Burr, F., Burr, B. (1986). Theor. Appl. Genet. 71,765-771.

#125

ANALYSIS OF STEM AND/OR CALLUS-SPECIFIC ARABIDOPSIS GENES. J. Peleman, C. Simoens, Th. Alliotte, M. Van Montagu, D. Inzé. Laboratorium voor Genetica, Rijksuniversiteit Gent. K.L. Ledeganckstraat 35, B-9000 Gent (Belgium).

By differential screening of a genomic library of Arabidopsis thaliana (1) we were able to identify a number of genes which are specifically expressed in stem and/or in callus tissue. The organ-specific expression of these genes was confirmed by Northern hybridizations. One gene, 9A1, showed a 50- to 100-fold stronger expression in stem and callus tissue as compared to the expression in leaf, inflorescence, and seed pod tissues. Another gene, 6A8, showed callus specific expression. For both genes complementary cDNA clones were isolated from an Arabidopsis cDNA library. The genomic and cDNA clones of the 9A1 gene have been completely sequenced. The gene encodes a transcript of approximately 1300 nucleotides, has no introns, and contains one open-reading frame of 1182 nucleotides. The upstream region is characterized by a direct repeat of 20 nucleotides, which might be involved in gene regulation. There are most likely three copies of the 9A1 gene in the Arabidopsis genome; the other copies have been isolated by screening a genomic λ library of Arabidopsis.

Currently, we are analyzing the expression of chimeric genes consisting of the promoter sequence of the 9A1 gene and the coding sequence of the npt-II gene.

Reference

1. Inzé, D., Simoens, C., Valvekens, D., Peleman, J., Van Montagu, M. (1987) In Abstracts of the 3rd International Meeting on Arabidopsis (this volume).

#126

FLORAL DEVELOPMENT IN ARABIDOPSIS THALIANA: A COMPARISON OF THE LANDSBERG ERECTA WILD TYPE AND THE PISTILLATA MUTANT. J. P. Hill[1], E. M. Lord[1], E. M. Meyerowitz[2]. [1]Dept. of Botany & Plant Sci., Univ. of California, Riverside, CA 92521; [2]Division of Biology, California Institute of Technology, Pasadena, CA 91125

Flowering studies in A. thaliana have focused on the physiological and structural changes associated with the transition to flowering (1,2,3). Vaughn (3) described the early anatomical aspects of floral organ initiation in a wild-type Arabidopsis in 1955. However, research on the developmental reproductive morphology of floral organs from primordial inception to anthesis has not been reported for either a wild-type strain or any of the floral mutants known to occur (4). Here, we describe the patterns of floral development in the landsberg erecta (l.e.) wild type and the pistillata (pi) mutant.

Normal development in A. thaliana is very similar to the pattern described for Brassica napus (5). Flowers are initiated in a phyllotactic spiral and are bilaterally symmetric. The abaxial sepal is initiated first, followed by the two lateral sepals, and finally the adaxial one. Four small petal primordia appear simultaneously, alternate to the sepals. Petal enlargement lags behind stamen development until just prior to anthesis, when they rapidly expand. Four long stamens are initiated next, positioned in pairs in front of the adaxial and abaxial sepals and acropetal to the petals. The two short stamens arise opposite the lateral sepals. The remaining floral apex gives rise to the gynoecium. The gynoecium has an elongate invagination in which the developing septum can be seen. Throughout floral bud development, the height of the gynoecium is slightly above the level of the long stamens, with its tip appressed to the inside of the sepals. Stamen elongation at anthesis brings the anthers into proximity with the papillate stigma and self-pollination occurs.

The pi mutant is indistinguishable from l.e. until the time of petal initiation. Four primordia arise in the positions of the petals and give rise to organs anatomically and morphologically like sepals. No petals or stamens were observed. Gynoecium development is subsequently disrupted, with a wide variation in phenotypic expression occurring. Abnormalities include various degrees of carpel doubling and the production of naked ovules. The mutant is not completely male sterile, however, with some seed set occurring in the middle flowers of the inflorescence. This heteroblastic developmental variation is currently under investigation in the F_1 generation.

1. Miksche, J.P., Brown, J.A.M. (1965) Amer. J. Bot. 52:533-37.
2. Napp-Zinn, K. (1985) CRC Handbook of Flowering, pp. 432-503.
3. Vaughn, J.G. (1955) J. Linn. Soc. (London) 55:279-300.
4. Meyerowitz, E.M., Pruitt, R.E. (1984) (Request from authors).
5. Polowick, P.L., Sawhney, V.K. (1986) Amer. J. Bot. 73:254-63.

#127

INTRODUCTION OF MAIZE TRANSPOSABLE ELEMENTS INTO ARABIDOPSIS. Hong Zhang, Jose Martinez-Zapater, Randy Scholl and Chris Somerville. DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824; Department of Genetics, Ohio State University, Columbus, OH.

The maize transposable element Mul was introduced into Arabidopsis by first inserting a restriction fragment carrying Mul into the plasmid pMON200 then selecting for integration of this plasmid into the T-DNA region of the non-oncogenic Ti plasmid pGV3850. Callus tissue of Arabidopsis was incubated with Agrobacterium carrying pGV3850::Mul and transformed tissue was selected by growth on 25 ug/ml G418. A fertile plant containing intact Mul DNA was regenerated and amplified during four generations. Southern analysis of transformed tissue indicated that Mul DNA remained entirely within a segment of T-DNA during three sexual generations. The results of a search for spontaneous mutations in approximately 30,000 Mul-containing seedlings suggests that the presence of Mul did not cause an increase in the spontaneous mutation frequency. Thus, it appears that Mul will not be a useful transposable element in Arabidopsis (1).

The maize element Ac was inserted into the binary vector pBIN19 and the resulting plasmid pBIN19::Ac was transformed into Agrobacterium LBA4404. This strain was then used to infect approximately 20,000 germinating seeds of the WS race of Arabidopsis according to the protocol of Feldman and collaborators (2). From approximately 300,000 "T2" progeny of the treated seedlings, about 30 appeared to be significantly more resistant than wild type to growth on 100 ug/ml kanamycin. However, only 5 seedlings have survived after transplantation from the selective medium to potting mixture. Additional analyses will be required to determine if these are genuine transformants.

References

1. Zhang, H. and Somerville, C.R. (1987) Plant Sci Lett, in press
2. Feldman, K. et al, Abstracts of these proceedings

#128

EFFECTS OF L-AZETIDINE-2-CARBOXYLIC ACID, HYDROXY-D-PROLINE AND THIOPROLINE ON SEED GERMINATION IN ARABIDOPSIS THALIANA.

K. Estill, F. Lehle. Department of Plant Sciences, University of Arizona, Tucson, AZ 85721.

Accumulation of intracellular proline in higher plants is thought to confer adaptive advantage during salt stress. Since proline accumulates in Arabidopsis thaliana in response to salt stress we are using this model system to test this hypothesis. In a preliminary experiment, free proline levels were quantified in rosette leaf tissue of mature Arabidopsis plants challenged with a sudden salt stress (5 mM NaCl). Free proline content, determined on the basis of fluorescent derivatives separated by reverse-phase HPLC (1,2), increased 79% after 26 h following imposition of salt stress.

Proline analogs, previously used to select proline over-producing mutants in both prokaryotic and eukaryotic cell cultures (3,4), inhibit germination in Arabidopsis. Germination studies with Arabidopsis were conducted on agar containing various concentrations of L-azetidine-2-carboxylic acid (A2C), hydroxy-D-proline (HYP) and thioproline (THP) with or without inorganic nutrients. On both media, thioproline completely inhibited germination at concentrations greater than 2 mM. In contrast, A2C was more toxic in the presence of supplemental nutrients than on water agar alone. Germination was completely inhibited at concentrations of 1.25 and 4 mM A2C on the nutrient and water agar, respectively. At lower A2C concentrations in water agar, cotyledons of inhibited seedlings were considerably darker in color than the controls. This effect was less obvious on nutrient agar. The toxicity of HYP differed depending on the medium. On water agar, seeds germinated at concentrations up to 16 mM HYP, but subsequently died at all concentrations above 4 mM. On nutrient agar, germination was largely unaffected at all concentrations up to 5 mM HYP, where seedlings appeared healthy except that their hypocotyls were substantially elongated relative to the controls.

References

1. Einarsson, S. (1985) J. Chromatography 348:213-220.
2. Betner, I., Fold, P. (1986) Chromatographia 22:381-387.
3. Csonka, L.N. (1981) Mol. Gen. Genet. 182:82-86.
4. Wasmuth, J.J., Caskey, C.T. (1976) Cell 8:71-77.

#129

HIGHLY EFFICIENT REGENERATION OF PLANTS FROM ROOT EXPLANTS OF ARABIDOPSIS THALIANA.

D. Valvekens, M. Van Lijsebettens, M. Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium).

Leaf, stem, and root explants of Arabidopsis thaliana were exposed to 8 different callus-inducing media (CIM) for 7, 10, and 14 days. Subsequently, these explants were transferred to 3 different shoot-inducing media (SIM). After various periods of shoot induction, the number of shoots were scored for each explant. Root explants gave the best regeneration compared to leaf and stem pieces.

When 2,4-D is used as an auxin in the CIM, roots start thickening and turn into white callus over their entire length. Upon transfer to shoot inducing media, this callus turns green and starts to form numerous shoots over the entire surface. On the contrary, leaf and stem pieces gave rise to only a few, if any, shoots on the same combination of media. Root pieces were also exposed to CIM containing other auxins and some were directly put on a SIM. In these cases shoots also regenerated, but only from the proximal part of the root. Because of their high regenerative potential we are now trying to set up a transformation procedure for A. thaliana roots.

#130

Rec-LIKE PROTEIN IN *ARABIDOPSIS THALIANA*. Mary F. Lopez, and Ethan R. Signer. Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139.

Enzymes that catalyze steps in homologous recombination have been isolated from the bacterium *Escherichia coli* (RecA) and the fungus *Ustilago maydis* (Recl). These proteins catalyze a variety of reactions including annealing of homologous DNA strands, displacement of homologous strands from DNA duplexes, binding to left-handed Z-DNA duplexes, and DNA-dependent ATPase. Using the procedures developed for *U. maydis*, we are purifying what appears to be a similar activity from *A. thaliana* that evidently cross-reacts with antiserum to the *U. maydis* enzyme. Properties of this activity will be described.

#131

ARABIDOPSIS AS A HOST FOR CAULIFLOWER MOSAIC VIRUS. U. Melcher. Oklahoma State University, Department of Biochemistry, Stillwater OK 74078-0454.

How viruses cause disease in plants can be investigated using *Arabidopsis thaliana* and cauliflower mosaic virus (CaMV)(1). I compared the patterns of symptoms caused by several isolates of CaMV in *A. thaliana*, strain Columbia, to provide a foundation for studies of the effects of viral infection on host gene expression. Columbia plants were resistant to infection by the cabbage S isolate of CaMV. All infectious isolates tested induced at least limited chlorosis on cauline leaves and resulted in silique and seed abnormalities. Three characters scored (severity of chlorosis, tip-to-base polarity of chlorosis, stunting of rosette leaves) were isolate-specific. The isolates that caused stunting in *A. thaliana* were not the same isolates that caused stunting in turnip plants, suggesting specific isolate-host interactions. Shoots of CaMV-infected *A. thaliana* often were sectored with respect to chlorosis. Siliques, leaves, and the adjacent stem sector on one side of these shoots were chlorotic while those on the other side were not. The sectoring may be due to events occurring early during development of the shoot. (Research supported in part by the Herman Frasch Foundatiuon and the Okla. Ag. Expt. Sta.)

References:

1. Balazs, E., Lebeurier, G. (1981) Arabidopsis Information Service **18**, 130-134.

#132

ISOLATION OF ORGAN-SPECIFIC GENES OF ARABIDOPSIS THALIANA.

D. Inzé, C. Simoens, D. Valvekens, J. Peleman, M. Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium).

The small size and simple organization of Arabidopsis thaliana (1) enabled us to isolate organ-specific genes by differential screening of a genomic library.

Replicas of 1145 randomly chosen genomic clones, representing 10% to 20% of the total Arabidopsis genome (2), were first hybridized to ^{32}P-labelled probes of chloroplast DNA, 18S/26S ribosomal DNA, total genomic DNA, and non-polyadenylated total RNA. In this way, clones containing repetitive sequences could be identified. Some of them were characterized in detail. Subsequently, we hybridized the 1145 cosmid clones with cDNA probes of poly(A)$^+$ mRNA of leaves, stems, seed pods, inflorescences, callus tissue, plants grown in a 16 hours light/8 hours dark cyclus, and of plants which were transferred for three additional days in the dark. Clones that were candidates to contain organ-specific genes were digested with different restriction enzymes, blotted to nylon filters, and rehybridized with the different cDNA probes. A detailed analysis of the results led to the identification of a large variety of organ-specific Arabidopsis genes. Furthermore, we could isolate genes which are highly and constitutively expressed in all tested organs. The organ-specific character of a number of genes has been confirmed by Northern analysis. Currently, we are focusing on stem- and callus-specific genes (3), and on constitutively and highly expressed genes.

References

1. Leutwiler, L.S., Hough-Evans, B.R., Meyerowitz, E.M. (1984) Mol. Gen. Genet. 194, 15-23.
2. Simoens, C., Alliotte, T., Mendel, R., Müller, A., Schiemann, J., Van Lijsebettens, M., Schell, J., Van Montagu, M., Inzé, D. (1986) Nucl. Acids Res. 14, 8073-8090.
3. Peleman, J., Simoens, C., Alliotte, Th., Van Montagu, M., Inzé, D. (1987) In Abstracts of the 3rd International Meeting on Arabidopsis (this volume).

#133

A NEW METHOD FOR THE HYDROPONIC CULTURE OF ARABIDOPSIS

Hilman C. Ratsch and Ronald E. Jones, Corvallis Environmental Research Laboratory, Environmental Protection Agency, Corvallis, OR 97333.

A hydroponic method has been developed to culture large, uniform populations of Arabidopsis plants for maximum seed production. The method combines the principles of the flowing solution culture with known techniques used in Arabidopsis culture. A hydroponic tray constructed from PVC strips is fitted with inlet and outlet ports to accomodate nutrient solution circulated from a reservoir through a manifold to the tray and back to the reservoir by a magnetic drive pump. Two polyurethane foam strips are placed in the tray and positioned to allow laminar flow on both sides of the foam. The lid of the tray (PVC sheet) is drilled with 2 mm diameter holes in parallel rows positioned above the foam strips. The lid is placed on top of the foam strips in the tray and individual seed are planted in each hole and rest on the surface of the foam.

Plants grown in the hydroponic system in a growth chamber with light flux of 250-300 uE sec-1 m-2 at 25 C day and 18 C night temperature produce 10-11 g. of mature seed with a 5-10 % coefficient of variation between trays.

The method was developed to uniformly treat large numbers of plants with toxic chemicals in a screening bioassay. The technique should be readily applicable for studies requiring uniform and large numbers of plants.

References

1. Clement, C.R., Hopper, M.J., Canaway, R.J., and Jones, L.H.P. (1974) A System for Measuring the Uptake of Ions by Plants from Flowing Solutions of Controlled Composition. Jour. of Exper. Bot. vol 25, 81.

2. Asher, C.J., Ozanne, P.G., Loneragan, J.F. (1965) A Method for Controlling the Ionic Environment of Plant Roots. Soil Science vol 100, 149.

3. Ratsch, H.C., Johndro, D.J., and McFarlane, J.C. (1986) Growth Inhibition and Morphological Effects of Several Chemicals in Arabidopsis thaliana (L.) Heynh. Environ. Tox. and Chem. vol 5, 55.

#134

PROTEIN BODIES AS DEVELOPMENTAL MARKERS IN ARRESTED EMBRYOS FROM LETHAL MUTANTS OF ARABIDOPSIS THALIANA. D. Patton, D.W. Meinke. Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

Arabidopsis thaliana (Cruciferae) has been described previously as a model system for basic research in plant developmental and molecular genetics. Embryo development in Arabidopsis has been examined in part through the isolation and characterization of embryo-lethal mutants (1). These recessive lethals have been shown to differ with respect to the color of arrested embryos and aborted seeds, the expression of mutant alleles prior to fertilization, the response of mutant embryos in culture, and the accumulation of seed storage proteins (2,3). The purpose of the present study was to determine whether mutant embryos continue to differentiate at the cellular level during their period of arrested development. Protein bodies were chosen as markers of development because they normally appear only during the final stages of embryonic maturation. Wild-type and arrested embryos from mutants 115D-4A, 112A-2A, 130B-A2, and 114D-1A were compared. Protein bodies were found in both the hypocotyl and cotyledons of wild-type embryos and began to fill with storage protein during the curled cotyledon stage of development. 115D-4A mutant embryos lacked a defined hypocotyl and cotyledons, accumulated at most only trace amounts of storage protein, and did not form mature protein bodies. 112A-2A mutant embryos contained fused cotyledons, developed into rootless plants when grown in culture, contained numerous protein bodies, and accumulated normal levels of storage protein. The formation of protein bodies in 130B-A2 mutant embryos appeared to proceed normally in the hypocotyl but was delayed in the cotyledons. Immature protein bodies were also found in albino mature embryos from mutant 114D-1A. Mutant embryos therefore differ not only in their pattern of morphogenesis but also in the extent of cellular differentiation.

(1) Meinke, D.W. (1986) Embryo-lethal mutants and the study of plant embryo development. Oxford Surveys of Plant Molecular and Cell Biology 3: 122-165.

(2) Heath, J.D., Weldon, R., Monnot, C., Meinke, D.W. (1986) Analysis of storage proteins in normal and aborted seeds from embryo-lethal mutants of Arabidopsis thaliana. Planta 169: 304-312.

(3) Meinke, D., Franzmann, L., Baus, A., Patton, D., Weldon, R., Heath, J.D., Monnot, C. (1985) Embryo-lethal mutants of Arabidopsis thaliana. In: Freeling, M. (ed.), Plant Genetics, UCLA Symposia on Molecular and Cellular Biology, Vol. 35, pp. 129-146. Alan Liss, New York.

#135

GENETIC VARIATION IN CALLUS CULTURE OF ARABIDOPSIS THALIANA(L)(HEYNH). M.D. GAJ, M. MALUSZYNSKI*. Department of Genetics, Silesian University, Jagiellonska 28, Katowice, Poland. *Joint FAO/IAEA Division, International Atomic Energy Agency, P.O.Box 100, Vienna, Austria.

SCOWCROFT (1984) has discussed some examples of mechanisms which can generate so-called "somaclonal variation". A. thaliana, as it was suggested by NEGRUTIU et al. (1975), is an especially suitable plant for such model experiments on plant cell genetics, considering its easy regeneration from somatic callus. Leaves of hybrid plants **+++/aspyer** (**Wilna** x mutant aspyer) were used as explants. Callus was obtained on PG_2 medium and transferred onto PG_3 medium on which plant shoots began to regenerate. Tests, of recessive embryo-lethal mutations[6](3) and frequency of chlorophyll mutations, were used to determine the level of genetic variability generated during the callus culture. The frequency of lethal embryos in callus-derived shoots was much higher compared to the control (plants from seeds) and increased with the age of callus culture. Similarly, embryos with chlorophyll mutations were only found in siliques of plants regenerated after 13 to 16 weeks. The frequency of calli which have given shoots with chlorophyll mutations was rather high and reached 3.1 percent. The analysis of genetic markers segregation was carried out on the F_2 progeny of 22 callus-derived shoots. The progeny of 16 analysed plants (only one shoot per callus was considered) indicated a segregation of 3:1 for each of the investigated markers. The analysis of segregation in progenies of six other plants indicated an important deviation from the expected ratio. Observed ratios of 35:1 and 21:1 were typical for segregation of tetraploid forms with duplex genotype. The cytogenetic analysis of callus-derived plants confirmed the number of chromosomes 2n=4x=20. All tetraploid, callus-derived plants did not express any morphological changes. Their fertility as well as vitality were the same as in diploid forms. Similar tetraploid plants of **Arabidopsis thaliana** were described by BOUHARMONT (1965) after treatment of seedlings by colchicine. Presented results indicated that somaclonal variation was generated in described in-vitro culture conditions. Callus-derived plants of **Arabidopsis thaliana** carry a high frequency of point mutation and changes in the number of chromosomes. In another paper we showed that fast-neutron irradiation may significantly increase the level of genetic changes in callus cells (5).

References

1. SCROWCROFT, W.R. (1984) IBPGR Report, Rome, 1-41
2. NEGRUTIU, J., BEEFTINK, F., JACOBS, M. (1975) Pl.Sc.Letters 5, 293-304
3. MÜLLER, A.J. (1963) Biol.Zentralbl. 82, 133-163
4. BOUHARMONT, J. (1965) Arabid.Res. 31-36
5. GAJ, M.D., MALUSZYNSKI, M. (1986) IAEA 698, 147-153

#136

HIGH-FREQUENCY PLANT REGENERATION FROM CULTURED COTYLEDON SEGMENTS OF ARABIDOPSIS THALIANA. D. Patton, D.W. Meinke. Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078.

Transformation of Arabidopsis thaliana leaf segments with Agrobacterium tumefaciens strains carrying resistance to hygromycin has recently been reported by Lloyd et al. (1). Regeneration of transformed plants was observed at a moderate frequency on a standard petunia regeneration (PR) medium containing MS salts with 1 mg/l BAP and 0.1 mg/l NAA. Improved regeneration of wild-type plants from leaf explants has also been reported in other laboratories (2). We have found that the regeneration frequency of wild-type strain "Columbia" plants not exposed to Agrobacterium can be increased dramatically by using immature cotyledons as an explant. Our decision to use immature embryos was based on studies with other plants that demonstrated a high morphogenetic potential of embryonic tissues in culture. Our analysis of embryo-lethal mutants of Arabidopsis had also shown that embryos could be readily manipulated in culture despite their small size. We have now demonstrated that isolated cotyledon segments at a mature green stage of embryo development expand rapidly on a PR medium and begin to produce numerous shoots after 2-3 weeks in culture. Approximately 40% of the cotyledon segments eventually produce shoots. Regeneration occurs in the absence of the original shoot apical meristem. Hypocotyl segments from these embryos produce root hairs and dense callus in culture but only rarely develop shoots. Hygromycin, kanamycin, and G-418, antibiotics commonly used to select for transformants following infection with Agrobacterium, all inhibit cotyledon expansion and regeneration in culture. Kanamycin is much less effective at controlling the growth of leaf segments in culture. Carbenicillin, one of the antibiotics most often used to inhibit bacterial growth following transformation, is unfortunately also toxic to cotyledon segments in culture. We are therefore examining two other antibiotics, vancomycin and cefotaxime, to determine whether they might be suitable for use with cotyledon cultures. Our results indicate that cotyledon cultures of Arabidopsis may serve not only as a source of regenerated plants for studies of somaclonal variation, but also as a potentially valuable source of transformed plants.

(1) Lloyd, A.M., Barnason, A.R., Rogers, S.G., Byrne, M.C., Fraley, R.T., Horsch, R.B. (1986) Transformation of Arabidopsis thaliana with Agrobacterium tumefaciens. Science 234: 464-466.

(2) Feldmann, K.A., Marks, M.D. (1986) Rapid and efficient regeneration of plants from explants of Arabidopsis thaliana. Plant Science 47: 63-69.

#137

TISSUE, CELL, AND PROTOPLAST CULTURES OF ARABIDOPSIS. Karen Ford, Elizabeth Sowell, and Charles Sumpter. Biology Department, College of Charleston, Charleston, South Carolina 29424.

We are interested in developing an efficient, relatively simple method for generating large numbers of transformed Arabidopsis thaliana plants. One approach utilizes roots that are maintained by continuous shaking in liquid medium. These roots are wounded by crushing, then cocultivated with Agrobacterium tumefaciens harboring pMON406, a modified Ti-plasmid. Hygromycin-resistant cells are selected immediately following cocultivation. Calli derived from these transformed cells also synthesize nopaline. We are currently optimizing this transformation protocol.

We have established stable A. thaliana cell suspension cultures from cultured roots. Protoplasts are isolated from these cultures. These protoplasts will divide in appropriate medium. We are currently investigating ways to encourage continued division in these protoplast-derived cells. The most recent methods and results from both of these projects will be presented.

#138

ANALYSIS OF THE MECHANISM OF ABSCISIC ACID ACTION USING ABA-INSENSITIVE MUTANTS OF ARABIDOPSIS. R. Finkelstein, C. Somerville. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824

Abscisic acid has been implicated in regulating many aspects of plant growth and development, including physiological adaptation to a variety of environmental stresses and developmental arrest of both seeds and vegetative buds. Although ABA- and stress-induction of many responses have been well-characterized, neither the relationship between the ABA and stress effects nor the molecular mechanism of ABA action are well understood. We are using ABA-insensitive and ABA-deficient mutants of Arabidopsis (1,2) to determine whether several ABA- or osmotic stress-inducible responses are mediated by ABA or if the stress and ABA effects are correlated, but parallel responses. Because initial characterization of the insensitive mutants suggested that one locus affecting sensitivity (abi-3) might be seed-specific (1), we are testing responses observed in both vegetative growth and seed development: proline accumulation in leaves and storage protein mRNA accumulation in embryos. Measurements of proline accumulated in detached leaves showed that ABA-induction of this response decreased in only two of the three classes of insensitive mutants, consistent with the previously observed developmental specificity of these mutations. Northern blot analysis of RNA from cultured wild type embryos, using a cDNA for the highly homologous Brassica napus cruciferin gene (3) as a probe, shows that ABA stimulates accumulation of this mRNA. However, both ABA-deficient and -insensitive mutants contain normal levels of this transcript during embryogenesis demonstrating that ABA is not required for accumulation of this mRNA. We are also analyzing ABA metabolism and uptake to determine whether these are altered in the insensitive mutants. Eventually we hope to clone these genes, using either a transposon-tagging system we are currently building (4) or chromosome walking.

References

1. Koornneef, M., Reuling, G., Karssen, C.M. (1984) Physiol. Plant. 61:377
2. Koornneef, M., Jorna, M.L., Brinkhorst-van der Swan, D.L.C., Karssen, C.M. (1982) Theor. Appl. Genet. 61:385
3. Simon, A.E., Tenbarge, K.M., Scofield, S.R., Finkelstein, R.R., Crouch, M.L. (1985) Plant Molecular Biology 5:191
4. Martinez-Zapater, J.M., Finkelstein, R., Somerville, C.R. (1987) UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 62, in press

#139

USE OF ARABIDOPSIS AS A MODEL SYSTEM TO STUDY PLANT/VIRUS INTERACTIONS. Srinivasa Ramachandra, Steve Lommel, Frank White. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Disease plays an important role in the limitation of crop production, yet little is known about plant genes which trigger responses to attack by pathogens. We propose to study the plant genes involved in plant/virus interactions as a prelude to understanding the mechanisms of disease resistance. Arabidopsis, due to its small genome size and short generation time, is well suited for both molecular and classical genetic studies and is a potential system for analyzing plant/virus interactions at the molecular level.

Our goal is to characterize genetically host responses that are elicited by a single virus. Various plant viruses are known to infect Arabidopsis, which include turnip yellow mosaic virus (TYMV), tomato black ringspot virus, and cabbage black ring virus (1) and cauliflower mosaic virus (2). In addition we have shown that turnip crinkle virus (TCV) and TUMV infect Arabidopsis. Our initial results indicate that a single isolate of TYMV differentially infects five different accessions of Arabidopsis. TYMV caused local lesions on inoculated leaves of Landsberg erecta (L.e.), Nd-0 and En-2 accessions and systemic yellow mosaic symptoms on Columbia (F_2). No symptoms were observed on Fi-3. Once discrete and reproducible accession responses are defined, we propose to determine the mode of inheritance of host genes involved in eliciting the response to virus infection, use mutagenesis to screen for mutants that have altered viral infection responses, and use recombinant DNA techniques to characterize these genes.

References

1. Sosnova, V., and Polak, Z. (1975). Susceptibility of Arabidopsis thaliana to infection with some plant viruses. 17:156-158.

2. Balazs, E., and Lebeurier, G. (1981). Arabidopsis Newsletter. 130-134.

#140

THE EVOLUTION OF THE E. COLI MODEL SYSTEM TO STUDY PROLINE OVERPRODUCTION AND OSMOTIC STRESS TOLERANCE IN ARABIDOPSIS THALIANA. Abhaya M. Dandekar*, Sandra L. Uratsu and Hui-Hwa Chiang, University of California, Department of Pomology, Davis, CA 95616, USA.

The accumulation of proline in response to environmental stress is a ubiquitous response in nature and has been correlated with osmotic stress tolerance in a wide array of plants, bacteria, algae and crustaceans (1). The biochemical or molecular basis of this response in nature is unknown. One of the major hypothesis proposes that proline could be involved in osmotic adjustment by replacing water molecules in stressed cells. Studies on the biosynthesis (1-3) and uptake (4) of proline in bacteria, support the hypothesis that proline may function by osmotic adjustment of the cytoplasm. Genetic analysis has shown that a class of mutations that lead to proline over production are simultaneously tolerant to osmotic stress (2,3). We now have evidence that a single base pair change in the feed-back inhibition site of the first enzyme in the proline biosynthetic pathway leads to a protein that is >650 fold more insensitive as compared to the wild type (5). This highly insensitive form of the enzyme is responsible for the synthesis of higher levels of proline that in turn enable the microorganism to withstand higher osmotic pressure (5).
We would like to extend these observations to study proline overproduction and its relation to osmotic stress tolerance in Arabidopsis. We propose to isolate mutants that overproduce proline by virtue of their resistance to the toxic proline analogue L-azetidine 2-carboxylic acid (azetidine). We have found that germinating seeds of Arabidopsis are highly sensitive to azetidine. The inhibition of germination was found to be reversible with proline. Mutagenesis was carried out in a 0.3% (v/v) solution of EMS. We shall present results on the kinetics of germination in the presence of azetidine or salt and report on the progress we have made in the isolation of mutants.

References

1. Le Rudulier, D., Strom, A.R., Dandekar, A.M., Smith, L.T. & Valentine, R.C. (1984). Science 224, 1064-1068.
2. Csonka, L.N. (1981). Mol. Gen. Genet. 182, 82-86.
3. Jakowec, M.W., Smith, L.T. & Dandekar, A.M. (1985). Appl. Environ. Microbiol. 50, 441-446.
4. Csonka, L.N. (1982) J. Bacteriol. 151, 1433-1443.
5. Dandekar, A.M. and Uratsu, S.L. (1987). Submitted.

#141

POLAR AUXIN TRANSPORT IN ISOLATED INFLORESCENCE STEM SECTIONS OF ARABIDOPSIS THALIANA. C. Benning, M. Estelle*. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824 USA. Dep. of Biology, Indiana University, Bloomington, IN 47405 USA.

Although the physiology of auxin transport has been described in vivo (1) and in vitro (2), the proteins involved have been difficult to characterize biochemically. We hope to exploit the potential for molecular genetics in Arabidopsis as an alternative approach to the analysis of auxin transport proteins. As a first step, we established an in vivo auxin transport assay for Arabidopsis, employing isolated stem sections and modifying the agar block technique first described by Went (3). We were able to demonstrate that the transport of radioactive labeled auxin is strictly polar. The velocity of transport is comparable to that determined by Hertel and Leopold for corn coleoptiles (4). Well known inhibitors of polar auxin transport such as the synthetic auxin 2,4-dichloro-phenoxyacetic acid (2,4-D) and the phytotropin 1-N-naphthylphthalamic acid (NPA) inhibit the polar auxin transport in Arabidopsis as well as in corn coleoptiles.

NPA is thought to act on the efflux carrier located at the basal end of transporting cells. Arabidopsis seedlings plated on agar containing NPA at a sublethal concentration and kept in the dark (for three days) are disoriented (ageotropic) and have short hypocotyls and roots. Control plants on NPA-free agar are geotropic and have long roots and hypocotyls. This clearly defined phenotype may be used to identify specific mutants defective in the NPA receptor protein.

References:

1. Goldsmith, M.H.M., (1977) An. Rev. Pl. Physiol. 28 439-478.

2. Hertel, R., Lomax, T.L., Briggs, W.R, (1983) Planta 157 193-201.

3. Went, F.W. Jahr Wiss. Bot. (1928) 76 528-557.

4. Hertel, R., Leopold, A.C. (1963) Planta 59 535-562.

5. Sussman, M.R., Goldsmith M.H.M., (1981) Planta 151 15-20.

#142

Examination of thiamin phosphate pyrophosphorylase activity for the development of a gene transfer system. Y. Komeda, M. Tanaka, T. Nishimune. the Univ. of Tokyo, Mol. Genet. Res. Lab., Hongo, Bunkyo-ku, Tokyo 113 and Osaka Pref. Inst. of Pub. Health, Higashinari-ku, Osaka 537, Japan.

In Arabidopsis thaliana, some mutants were isolated that required thiamin for their growth. The analysis of the th-1 mutants is not only interesting for the study of biosynthetic pathway of plants, but also is important for the application to the marker of gene transfer. We have examined the activity of the thiamine phosphate pyrophophorylase in Columbia wild and its th-1 mutant.

Plant materials, grown in synthetic media, were freeze-dry and were powdered by coffee mill and mortar-pestle. The powder was disolved in 0.2M phosphate buffer(pH7) and extracted with Polytron. The extract was centrifuged and the supernatant was used as crude enzyme preparation. The examination of the enzymatic activity was performed with fluorescent HPLC.

A th-1 mutant and wild type plant grown in $1X10^{-7}$M thiamin were used for the examination of the production of thiamine and thiamine phosphate from the substrates, thiazole phosphate and OMP pyrophosphate. While the wild type strain formed the products, a th-1 mutant did not. The thiamin itself was produced from the thiamine phosphate by the extracts of both wild type and a th-1 mutant.

Accordingly, the th-1 mutant was concluded to be defective for the activity of the thiamin phosphate pyrophosphorylase. We are trying to use the th-1 mutant for the host of gene transfer experiment with the E. coli thiB gene.

#143

A SINGLE GENE MUTATION AFFECTS THE PARTITIONING OF FATTY ACIDS BETWEEN THE TWO PATHWAYS OF LIPID BIOSYNTHESIS. Ljerka Kunst[1], John Browse[2] and Chris Somerville[1]. [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824; [2]Plant Physiology Division, DSIR, Palmerston North, New Zealand

Glycerolipid synthesis in leaves involves two discrete pathways operating simultaneously in two different cell compartments. Fatty acids synthesized de novo in the chloroplast may enter either the prokaryotic pathway in the chloroplast envelope, or be exported to the endoplasmic reticulum to enter the eukaryotic pathway. In Arabidopsis thaliana (L.) Heynh. chloroplast membrane lipids are derived in approximately equal amounts from the two biosynthetic routes (1).

We have isolated a series of mutants with alterations in leaf fatty acid composition (2). One of the mutant lines, designated JB255, specifically lacks hexadecatrienoic acid (16:3) due to a mutation at a single nuclear locus. This acyl group occurs only on the sn-2 position of monogalactosyldiacylglycerol (MGD) made in the chloroplast envelope, and its absence points to a lesion in the prokaryotic pathway. To test this hypothesis we have assayed chloroplast enzymes involved in MGD synthesis. The mutant lacks the activity of the soluble acyl transferase, which suggests a mutation in the structural locus (act1) for this enzyme. Therefore the mutant described here has only the eukaryotic pathway functional in the synthesis of MGD. A lack of accumulation of precursors within leaves, and an increase in the eukaryotic fatty acids indicate that the leaf cells have a compensatory mechanism which causes a redirection of fatty acids towards cytoplasmic sites of lipid synthesis.

We have begun to investigate the effects of the loss of prokaryotic MGD on membrane structure and function. The most pronounced change in the mutant is a significant increase in the cross sectional area of chloroplasts, as well as the reorganization of chloroplast ultrastructure. However, the general appearance of JB255 mutant is similar to the wild type, and its growth characteristics and the chlorophyll concentration are only subtly affected by the mutation.

References:

1. Browse JA, Warwick N, Somerville CR, Slack CR (1986) Biochem. J. 235:25.
2. Browse JA, Kunst L, McCourt P, Somerville CR, Abstract #65, these proceedings.

#144

THE ISOLATION AND CHARACTERIZATION OF MUTANTS OF ARABIDOPSIS WITH ALTERATIONS IN STARCH METABOLISM. Timothy Caspar[1], Tsan-Piao Lin[2], Jack Preiss[2], and Chris Somerville[1]. [1]DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824; [2]Department of Biochemistry, Michigan State University, East Lansing, MI 48824

In order to help determine the mechanisms and regulation of starch biosynthesis and degradation, and to investigate the role of starch in various physiological processes, we have isolated and initiated the characterization of mutants of Arabidopsis with alterations in starch metabolism. These mutants were isolated by screening leaves from a mutagenized population for the presence of starch using a semi-quantitative iodine starch stain.

Starchless or starch deficient mutants were identified by sampling leaves taken from plants growing in conditions which favor starch accumulation in the wild type (WT) (e.g. high light or elevated atmospheric CO_2 concentrations). The starchless phenotypes are caused by nuclear, recessive mutations affecting 2 of the 4 steps unique to starch biosynthesis: phosphoglucomutase (PGM) and ADP-glucose pyrophosphorylase (PYRO). The PGM mutants, which have been the most extensively characterized (1), alter their photosynthetic carbon partitioning such that soluble sugars (synthesized in the cytosol) replace the starch (made inside the chloroplast) in the WT. Since these mutants eliminate starch throughout the plant, they have been useful in evaluating the role of starch in geotropic gravity sensing (2) and may be similarly useful for analogous studies concerning other roles of starch. Both the PGM and PYRO mutants are phenotypically indistinguishable from the WT when grown in continuous light demonstrating that in these conditions starch is not required for any essential physiological or metabolic processes. The mutation which affects PYRO may identify a regulatory locus since plants heterozygous for this mutation (a null allele) contain PYRO specific activities equal to the WT.

Mutations which cause the over-accumulation of starch have also been identified by sampling leaves from plants after an extended dark period. We are currently characterizing these mutations to determine whether they cause defects in starch degradation or alterations in photosynthetic carbon partitioning between sucrose and starch.

References:

1. Caspar T, Huber SC, Somerville CR (1985) Plant Physiol. 79:11.
2. Caspar T, Somerville CR, Pickard BG Abstract #78, these proceedings.

#145

MOLECULAR GENETICS OF RIBOSOMAL PROTEIN AND TRIHOCHECENE RESISTANCE IN ARABIDOPSIS THALIANA. R. L. Scholl[1], H. Zhang[2] and Y. Kim[1]. [1]Genetics Dept., Ohio State Univ., Columbus, OH 43210, and [2]MSU-DOE Plant Research Laboratory, MSU, East Lansing MI 48824-1312.

Molecular analysis of the expression of Arabidopsis ribosomal genes has been initiated. These genes, although not well studied in plants are highly regulated in other organisms. In yeast a mutant form of the gene for the ribosomal protein, L-3, confers resistance to the fungal toxins termed 12-13 epoxytrichothecenes. Trichothecenes are produced by a variety of fungi which attack crop plants. Hence trichothecene resistant mutants are being sought in Arabidopsis and will be characterized physiologically and genetically.

A yeast clone (pTCM3.2) carrying the coding region for the L3 protein (1) was been utilized as a probe in Southern hybridization experiments with an Arabidopsis genomic DNA. Two Arabidopsis restriction fragments hybridized to this probe, and a lambda genomic library was screened by plaque hybridization with the probe. One hybridizing clone was identified. The hybridizing region has been subcloned restriction mapped and is currently being sequenced. Results of these experiments will be presented.

The genetics of toxin resistance has so far focused on development of variability, utilizing ethyl methane sulfonate mutagenesis. M_2 populations derived from EMS-treated M_1 seeds are being germinated in the presence to trichothecenes, and any resulting resistant genotypes will be characterized.

References

1. Freid, H. M., Warner, J. R. (1982) Nucl. Acids Res. 10:3133.

#146

SUBCELLULAR LOCALIZATION AND CHARACTERIZATION OF STARCH DEGRADATIVE ENZYMES OF ARABIDOPSIS LEAF. Tsan-Piao Lin, Steven Spilatro, Jack Preiss. Michigan State Univ., Dept. of Biochem., E. Lansing, MI 48824-1319.

Starch degradative enzymes of Arabidopsis leaf tissue were isolated and partially purified. Enzyme localization studies indicate that endoamylase, phosphorylase and D-enzyme (transglycosylase) occur in the chloroplasts. Endoamylases are only found in the chloroplast and migrated with an Rf of 0.44 on a 7% electrophoretic gel. D-enzyme was successfully separated from most of the amylase activities by using an FPLC-Mono Q column. End-product analysis showed the accumulation of glucose and maltopentaose from maltotriose within 10 min of reaction. Several other maltodextrins were also observed with longer incubation times, although maltose was never produced. Glucose is the major product throughout the course of reaction. Results also indicate that glucose is a receptor for maltodextrin but in low efficiency. The high activity units of D-enzyme compared to the amylolytic activity in the chloroplast suggests that transglycosylation probably has an important role during starch degradation.

Extrachloroplastic amylases have an Rf of 0.55 on the 7% electrophoretic gel, and constitutes 90% of the total leaf amylolytic activity. The results of substrate specificity studies, action pattern and viscometric analyses indicate that the extrachloroplastic amylases are exolytic.

#148

THE HSP70 GENE FAMILY FROM ARABIDOPSIS. C. Wu, T. Caspar, J. Browse, C.R. Somerville. DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

When plants grown at the normal growth temperature of about 22°C are shifted to 37°C for several hours, the major proteins labelled during a short pulse with ^{35}S-methionine are heat shock proteins (HSPs). One of the most intensely labeled proteins has an apparent molecular weight of 76 kd and is, therefore, thought to be homologous to the HSP70 proteins observed in other species. In support of this, antibodies directed against Drosophila HSP70 cross react with the Arabidopsis HSP76 protein on Western blots (results courtesy of S. Lundquist). This protein is strongly labelled following heat shock but is not readily apparent among the products of in vivo labelling at 22°C. However, the immunoblotting experiments also revealed that the HSP76 protein is present in similar amounts (ca. 4 fold-difference) in both heat shocked and non heat-shocked tissue. In order to investigate the basis for this effect we cloned the three members of the Arabidopsis HSP70 gene family by using the Drosophila HSP70 gene as a heterologous probe. Two of the genes are closely linked (ca. 1 kb apart) in a direct orientation. The relative position of the other gene in the genome was not established. The DNA sequence of the 5' regions of the three genes was determined. All three genes appear to have an intron near the 5' end of the coding sequence at precisely the same position as in the HSC70 (cognate) genes from Drosophila. Gene-specific oligonucleotides complementary to 5' untranslated regions of the predicted mRNA were used to establish that one of the genes is not expressed. One of the genes is constitutively expressed but the amount of mRNA is increased about 4-fold following heat shock. This gene has the HSE consensus sequence found upstream of the TATA box in HSP70 genes from other organisms. Preliminary results suggest that the third gene is not expressed. Our current interpretation of these observations is that the primary mode of regulation of the HSP70 genes in Arabidopsis is by preferential translation of the HSP70 mRNA at heat shock temperature. Thus it appears that all the members of the Arabidopsis HSP70 family are analogous in both structure and mode of regulation of gene expression to the HSC70 genes found in other organisms.

#149

PHYSIOLOGICAL CHARACTERIZATION OF MUTANTS OF ARABIDOPSIS WITH ALTERED GEOTROPISM

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Of the strains of Arabidopsis thaliana which we have identified with altered geotropism, one phenotype appears particularly interesting. One isolation strain, mg-72 has been chosen for more intensive characterization. The seedling shoots in this strain grow almost completely non-oriented with respect to the gravitational stimulus in darkness. Phototropism to unilateral blue light is indistinguishable from that of the Estland wild-type parent. The geotropic response by the roots is also indistinguishable from that of the wild-type parent. These data are consistent with an alteration in the geotropism pathway of mg-72 before the confluence of this pathway with that for phototropism. Moreover, the shoot geotropism pathway has at least one mutable element which is not required for root geotropism.

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