Program and abstracts of lectures and posters for

The Fifth International Conference on *Arabidopsis* Research

August 19-22, 1993

The Ohio State University
Columbus, Ohio

Organizers:

Roger Hangarter
Randy Scholl
Keith Davis
Ken Feldmann
The Ohio State University Conference Unit

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FIFTH INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH

CONFERENCE PROGRAM

Note: All lectures will be presented in Weigel Hall Auditorium and poster sessions will be in the Holiday Inn on Lane Avenue. Workshop locations are as indicated. There is a campus map included on the last page of this book.

Thursday, August 19

Noon - 8:00 pm Registration - *Holiday Inn on Lane Avenue*
Poster Set-up - *Holiday Inn Ballroom*

1:00 - 5:00 pm Workshops -
Smith Lab, Room 1153. Transposon Tagging - Nina Fedoroff, Carnegie Institution of Washington, Baltimore, Maryland
Hitchcock Hall, Room 31. Plant Metabolism - Robert Last, Boyce Thompson Institute, Ithaca, New York and Brian Keith, University of Chicago, Chicago, Illinois

7:00 - 9:30 pm Keynote Session - *Weigel Hall Auditorium*

7:00 Opening Remarks - Roger Hangarter, Ohio State University and Randy Scholl, Ohio State University, Columbus, Ohio

7:30 *Towards a Complete Set of Genes*, Dr. J. Craig Venter, The Institute for Genomic Research, Gaithersburg, Maryland

8:30 *Title to be announced*, Dr. Sydney Brenner, Medical Research Council, Cambridge, England

9:30 - Midnight Reception/Posters - *Holiday Inn Ballroom*
Computer Demonstrations - *Holiday Inn Buckeye Room, 11th floor*

Friday, August 20

7:00 - Noon Registration - *Weigel Hall*

8:00 - 10:00 am SESSION I: *Weigel Hall*
Growth Regulation - Maarten Koornneef, session chair

8:00 Introduction - Maarten Koornneef, Wageningen Agricultural University, Wageningen, The Netherlands

8:15 *Molecular Characterization of an Auxin-Resistance Gene* - Mark Estelle, Indiana University, Bloomington, Indiana

Friday, August 20 continued

8:45 *Stimulus-response Interactions in Roots* - Kiyotaka Okada, National Institute for Basic Biology, Okazaki, Japan

9:15 *Steps of Abscisic Acid Action* - Erwin Grill, Federal Institute of Technology, Zürich, Switzerland

9:30 *Cloning and Characterization of the etr1 Gene* - Caren Chang, California Institute of Technology, Pasadena, California

9:45 *Mutations at the SPINDLY Locus of Arabidopsis thaliana Alter Gibberellin Signal Transduction* - Steven Jacobsen, University of Minnesota, St. Paul, Minnesota

10:00 - 10:30 am Break

10:30 - 12:30 SESSION II: *Weigel Hall*
Photobiology - Judy Brusslan, session chair

10:30 Introduction - Judy Brusslan, University of California, Los Angeles, California

10:45 *Genetic Analysis of the Plant Photoreceptors Controlling Photomorphogenesis* - Brian Parks, Ohio State University, Columbus, Ohio

11:15 *Genetic Interactions Controlling Arabidopsis Photomorphogenesis* - Joanne Chory, The Salk Institute, San Diego, California

11:45 *Characterization and Molecular Cloning of COP9, a Genetic Locus Involved in Light-regulated Development and Gene Expression in Arabidopsis* - Ning Wei, Yale University, New Haven, Connecticut

12:00 *A Nuclear Encoded Protein of Arabidopsis thaliana Passes Through Microsomes Prior to Uptake in Isolated Chloroplasts* - Ann Van Gysel, Universiteit Gent, Gent, Belgium

12:15 *Overexpression of Mutant phyA Sequences Reveals Novel Features of Phytochrome Function in Arabidopsis* - Margaret Boylan, Plant Gene Expression Center, Albany, California

12:30 - 1:45 pm Lunch on your own

1:45 - 3:45 pm SESSION III: *Weigel Hall*
Metabolism - Chris Somerville, session chair

1:45 Introduction - Chris Somerville, DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan

2:00 *Molecular Genetics of Nitrogen Assimilation into Amino Acids in Arabidopsis thaliana* - Gloria Coruzzi, New York University, New York, New York

2:30 *The Genetics and Biochemistry of Lipid Metabolism in Arabidopsis* - John Browse, Washington State University, Pullman, Washington

3:00 *Isolation of a Mutant Arabidopsis Plant that Lacks N-acetyl Glucosaminyl Transferase I and is Unable to Synthesize Golgi-modified Complex N-linked Glycans* - Antje von Schaewen, University of Osnabrück, Osnabrück, Germany

Friday, August 20 continued

3:15 *Expression Cloning of a Plant Amino Acid Transporter by Functional Complementation of a Yeast Amino Acid Transport Mutant* - Daniel Bush, USDA/ARS, University of Illinois, Urbana, Illinois

3:30 *trp4, a Suppressor of Blue Fluorescence, is an Arabidopsis Anthranilate Synthase Mutation* - Brian Keith, University of Chicago, Chicago, Illinois

3:45 - 4:15 pm Break

4:15 - 6:15 pm SESSION IV: *Weigel Hall*
Stress/Pathogenesis - Brian Staskawicz, session chair

4:15 Introduction - Brian Staskawicz, University of California, Berkeley, California

4:30 *Downy Mildew of Arabidopsis: Dissecting the Genetics of Host Resistance* - Alan Slusarenko, Institute of Plant Biology, Zurich, Switzerland

5:00 *Loci for Disease Resistance and Intercellular Signaling* - Jeff Dangl, Max-Delbrück Lab, Köln, Germany

5:30 *Systemic Acquired Resistance in Arabidopsis* - Terrence Delaney, Ciba Geigy Agricultural Biotechnology, Research Triangle Park, North Carolina

5:45 *Isolation and Characterization of Phytoalexin Deficient Mutants of Arabidopsis thaliana* - Jane Glazebrook, Massachusetts General Hospital, Boston, Massachusetts

6:00 *Characterization of Genes Differentially Expressed in Arabidopsis Roots upon Progressive Drought Stress* - Jérôme Giraudat, Institut des Sciences Végétales, CNRS, France

6:15 - 8:00 pm Dinner on your own

8:00 - Midnight POSTER SESSION - *Holiday Inn*
Computer Demonstrations - *Holiday Inn Buckeye Room, 11th floor*

Saturday, August 21

8:00 - 10:00 am SESSION V: *Weigel Hall*
Cell Differentiation - Robert Goldberg, session chair

8:00 Introduction - Robert Goldberg, University of California, Los Angeles, California

8:15 *Trichome Formation as a Model for Cell Differentiation* - David Marks, University of Nebraska, Lincoln, Nebraska

8:45 *Molecular Genetic Analysis of Tubulin Gene Families in Arabidopsis*- Carolyn Silflow, University of Minnesota, St. Paul, Minnesota

9:15 *Genetic and Molecular Analysis of Root Hair Development in Arabidopsis* - John Schiefelbein, University of Michigan, Ann Arbor, Michigan

Saturday, August 21 continued

9:30 *An Arabidopsis Mutant with Clustered Stomata* - Ming Yang, Ohio State University, Columbus, Ohio

9:45 *An Arabidopsis T-DNA Mutant with Impaired Root Hair Formation* - Claire Grierson, John Innes Institute, Norwich, UK

10:00 - 10:30 am Break

10:30 - 12:30 SESSION VI: *Weigel Hall*
Embryos/Early Development - David Meinke, session chair

10:30 Introduction - David Meinke, Oklahoma State University, Stillwater, Oklahoma

10:45 *Arabidopsis Fusca Mutants: Phenotypes and Cloned Genes* - Linda Castle, Oklahoma State University, Stillwater, Oklahoma

11:15 *Genetic Analysis of Pattern Formation in the Embryo* - Gerd Jürgens, Institut für Genetik, Universität München, München, Germany

11:45 *T-DNA-mediated Promotor Trapping to Identify Embryonic Genes in Arabidopsis* - Keith Lindsey, University of Leicester, Leicester, UK

12:00 *Testa Development in Arabidopsis: Studies with a Seed Shape Mutant* - Karen Léon-Kloosterziel, Wageningen Agricultural University, Wageningen, The Netherlands

12:15 *Mutations that Affect the Switch from Embryonic to Vegetative Development in Arabidopsis thaliana* - Laura Conway, University of Pennsylvania, Philadelphia, Pennsylvania

12:30 - 1:45 pm Lunch on your own

1:45 - 3:45 pm SESSION VII: *Weigel Hall*
Reproductive Development - Renee Sung, session chair

1:45 Introduction - Renee Sung, University of California, Berkeley, California

2:00 *Cellular Interactions in Arabidopsis* - Bob Pruitt, Harvard University, Cambridge, Massachusetts

2:30 *Cell Cycle Regulation during Nicotiana tabacum and Arabidopsis thaliana Pollen Development* - Erwin Heberle-Bors, University of Vienna, Vienna, Austria

3:00 *Molecular-genetic Analysis of Flowering-time in Arabidopsis: Isolation of the CO Locus and Identification of Early-flowering Mutants* - Joanna Putterill, The John Innes Centre, Norwich, UK

3:15 *The Role of CLV1 in Floral and Apical Meristem Development* - Steven Clark, California Institute of Technology, Pasadena, California

3:30 *The TOUSLED Gene Encodes a Novel Protein Kinase in Arabidopsis* - Judith Roe, University of California, Berkeley, California

3:45 - 4:15 pm Break

Saturday, August 21 continued

4:15 - 6:15 pm SESSION VIII: *Weigel Hall*
Vegetative Development - George Rédei, session chair

4:15 Introduction - George Rédei, University of Missouri, Columbia, Missouri

4:30 *Patterns and Signals in the Vegetative Shoot Apex* - June Medford, Pennsylvania State University, University Park, Pennsylvania

5:00 *Epidermal Differentiation in the Arabidopsis Root* - Liam Dolan, John Innes Institute, Norwich, UK

5:30 *The Genetic Control of Chloroplast Development in Arabidopsis* - Kevin Pyke, University of York, Heslington, York, UK

5:45 *A Novel 154kd Protein Encoded by sabre Affects Cell Elongation in Arabidopsis thaliana* - Roger Aeschbacher, New York University, New York, New York

6:00 *amp1 - A Mutant with High Cytokinin Levels and Altered Embryonic Pattern, Faster Vegetative Growth, Constitutive Photomorphogenesis, and Precocious Flowering* - Abdul Chaudhury, CSIRO and Australian National University, Canberra, Australia

6:15 pm Conference Dinner - Drake Union

Sunday, August 22

8:00 - 10:00 am SESSION IX: *Weigel Hall*
Gene Regulation - Elizabeth Dennis, session chair

8:00 Introduction - Elizabeth Dennis, CSIRO Division of Plant Industry, Canberra, Australia

8:15 *Mechanisms of mRNA Instability in Plants* - Pam Green, DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan

8:45 *Mobile Elements and Inducible Genes in the Nitrate Assimilation Pathway of Arabidopsis* - Nigel Crawford, University of California, San Diego, California

9:15 *Molecular Characterization of the TCH4 Gene of Arabidopsis: Genomic Organization, Sequence Similarity to meri-5 and gusA-Fusion Expression Patterns* - Wei Xu, Rice University, Houston, Texas

9:30 *Circadian Regulation of cab2 Gene Expression in Arabidopsis thaliana* - Isabelle Carré, University of Virginia, Charlottesville, Virginia

9:45 *Pleitropic Effects of a T-DNA Tagged Gene Encoding a Regulatory Protein with beta-transducin Repeats* - Csaba Koncz, Max-Planck Institute, Köln, Germany and Biological Research Center of Hungarian Academy, Hungary

10:00 - 10:30 am Break

Sunday, August 22 continued

10:30 - 12:30 SESSION X: *Weigel Hall*
New Technologies - Ken Feldmann, session chair

10:30 Introduction - Ken Feldmann, University of Arizona, Tuscon, Arizona

10:45 *Progress Toward a Complete Map of the Arabidopsis Genome* - Joe Ecker, University of Pennsylvania, Philadelphia, Pennsylvania

11:15 *Progress in Transposon Tagging and Physical Mapping* - Caroline Dean, John Innes Institute, Norwich, UK

11:45 *Isolation of a MALE STERILITY Gene using a Two Element En-I Transposon Tagging System* - Mark Aarts, Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands

12:00 *Large Scale Sequencing of Arabidopsis thaliana var. Columbia cDNAs and Generation of Expressed Sequence Tags* - Thomas Newman, DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan

12:15 *Spectrum of Insertional Mutagenesis in Arabidopsis using Agrobacterium-mediated Zygotic Embryo Transformation Methodology* - R. S. Sangwan, Université de Picardie Jules Verne, France

12:45 pm First buses depart for Port Columbus Airport from Weigel Hall

1:30 - 4:30 pm Workshop - *1153 Smith Lab*
Genome Research - Tony Schaeffner, University of Munich, Germany

1:45 pm Buses depart for Port Columbus Airport from Holiday Inn

5:30 pm Last buses depart for Port Columbus Airport from Holiday Inn

WORKSHOP AGENDAS

1. **PLANT METABOLISM WORKSHOP** - Robert Last, Boyce Thompson Institute, Brian Keith, University of Chicago, Organizers.

Thursday August 19, 1993, 1:00-5:00 PM
Hitchcock Hall, Room 031

1:00 - 1:10	Introductions - Robert Last, Boyce Thompson Institute Brian Keith, University of Chicago
1:10 - 1:25	*Arabidopsis Dissimilates L-Arginine and L-Citruline for Use as N-Source* - Robert Ludwig, University of California, Santa Cruz
1:25 - 1:40	*Analysis of the Structure and Expression of Genes Encoding E2s from Arabidopsis thaliana* - Sharon Thoma, University of Wisconsin
1:40 - 1:55	*Isolation of Purine Biosynthetic Genes of Arabidopsis thaliana* - Julie Senecoff, University of Georgia.
1:55 - 2:10	*Isoleucine-Insensitive Mutants of Arabidopsis* - George Mourad, University of Saskatchewan.
2:10 - 2:25	*The Tryptophan Pathway: A Paradigm for the Genetics of Amino Acid Biosynthesis in Plants* - Alan Rose, Boyce Thompson Institute at Cornell.
2:25 - 2:40	*A Tryptophan Auxotroph Reveals Two Auxin Biosynthetic Pathways in Arabidopsis thaliana* - Jennifer Normanly, Whitehead Institute for Biomedical Research.
2:40 - 2:55	*Analysis of Protein-Protein Interactions Between the First Two Enzymes of Flavonoid Biosynthesis* - Brenda Shirley, Virginia Polytechnic Institute
2:55 - 3:10	*Genetic Approaches to Flavonoid Research in Arabidopsis thaliana* - John Sheahan, Boyce Thompson Institute at Cornell
3:10 - 3:30	Break
3:30 - 3:45	*Regulation of HMG CoA Reductase Gene Expression by Environmental and Developmental Cues* - R. Marc Learned, University of California, Davis
3:45 - 4:00	*Genetic Dissection of Carotenoid and Chlorophyll Synthesis in Arabidopsis thaliana* - Dean DellaPenna, University of Arizona
4:00 - 4:15	*Genetic Analysis of Starch Synthesis and Accumulation* - Tim Caspar, DuPont
4:15 - 4:30	*Cell Wall Mutants of Arabidopsis* - Wolf-Dieter Reiter, MSU-DOE Plant Research Lab, Michigan State University
4:30 - 4:45	*Genetics of Iron Uptake in Arabidopsis thaliana* - Jenny Saleeba, Dartmouth College
4:45 - 5:00	Concluding Remarks and Discussion

2. TRANSPOSON TAGGING WORKSHOP - Nina Fedoroff, Carnegie Institution of Washington, Organizer.

Thursday August 19, 1993, 1:00-5:00 PM
Smith Laboratory, Room 1153

The tentative participant/speaker list includes:

Thomas Altmann
Barbara Baker
Anthony Bleeker
George Coupeland
Caroline Dean
John Draper
Joseph Ecker
Nina Fedoroff
Jonathan Jones
Andy Pereira
Venkatesan Sundaresan
Robert Whittier

3. GENOME RESEARCH WORKSHOP - Tony Schaeffner, University of Munich, Organizer

Sunday August 22, 1993, 1:30-4:30 PM
Smith Laboratory, Room 1153

- Comprehensive overview of state of the art
- Short statements (5-10 min) to various problems, techniques, advances
- Considerable part of time for open discussion at each topic

Physical map, contigs

Patrick Dunn	Ordering of YACs by anchoring
Howard Goodman	(tentative) Progress on physical map
Renatta Schmidt	Contigs on chromosome 4: conclusions and consequences
Marc Zabeau	Physical mapping using AFLP
Discussion	

ESTs, sequenced genes

Sequencing:	
Herman Höfte	Current status of French EST effort
Thomas Newman	(will be present for discussion)
Functions:	
Kenneth Feldmann	Sequenced genes vs.T-DNA lines
Bernd Reiss	New approaches towards gene targeting?
Discussion	

GENOME RESEARCH WORKSHOP continued

Mutation-based genome research

Igor Vizir	Saturating the top arm of chromosome 5with mutations
Bian Osborne	Cre-lox induced deletions, inversions,..
David Meinke	Saturating the map with embryonic mutations
Discussion	

Molecular markers

Tony Schaeffner	Arabidopsis RFLP mapping set (ARMS)
Jane Glazebrook	Co-dominant amplified polymorphic sequences (CAPS)
Daphne Preuss	PCR based markers
Pablo Scolnik	New approaches to RAPDs
Discussion	

The endgame, the last steps

Fine-mapping strategies:	
Joanna Putterill	Flanking emb for selection of recombinants
Robert Whittier	Thermal assymetric interlaced (TAIL) PCR for walking
Jeffrey Leung	Lessons from position cloning of abi3
Complementation:	
Robert Kuhn/ Robert Ludwig	Transformation competent cosmid library
Transformation:	
Annette Vergunst	Factors influencing transformation efficiency
Discussion	

Abstracts for Session I: **Growth Regulation**

Session Chair: Maarten Koornneef

Friday, August 20

8:00 am - 10:00 am

Weigel Hall Auditorium

MOLECULAR CLONING AND CHARACTERIZATION OF AN AUXIN-RESISTANCE GENE IN *ARABIDOPSIS.*

Leyser, H.M.O., Lincoln, C.A., Timpte, C., Lammer, D., Turner, J., and M. Estelle. Department of Biology, Indiana University, Bloomington, IN 47405

The plant hormone auxin plays a central role in many aspects of plant growth and development. To identify genes which are involved in auxin response, we have screened for mutants of *Arabidopsis* which are auxin-resistant. Recessive mutations in one of these genes (*axr1*) , confer resistance to the roots, rosettes, and inflorescences of mutant plants. In addition, the*axr1* mutants are deficient in auxin-regulated gene expression and have a number of morphological defects, all of which are consistent with a reduction in auxin sensitivity. We have isolated the *AXR1* gene using a map-based approach. The gene encodes a novel protein with significant similarity to ubiquitin-activating enzyme (E1). However, the AXR1 protein is highly diverged from other E1 enzymes and lacks a cysteine residue known to be essential for E1 activity. Thus, AXR1 may have a novel function in cellular regulation. The significance of these results with respect to auxin action will be discussed.

Stimulus-response Interactions in Roots

Kiyotaka Okada, Takuji Wada, Tokitaka Oyama, Nobuyoshi Mochizuki,
Sumie Ishiguro, and Yoshiro Shimura
(National Institute for Basic Biology, Okazaki, 444 Japan)

Roots change their growth patterns in response to physical stimuli such as gravity, light, humidity and touching to obstacles. In addition to the gravitropic, phototropic, and touching-induced waving growth, growth pattern of root hairs is shown to be modulated by physical and chemical stresses. Root hairs of young seedlings are usually about 0.4mm in length and are formed perpendicular to the root surface. However, root hairs get longer to about 0.8mm and grow obliquely to the root surface under several unusual growth conditions.
In order to dissect genetic regulatory networks controlling the stimulus-response interactions in roots of young seedlings, we have developed a series of experimental procedures using agar plates, and screened mutants with aberrant responses to the stimuli. Further characterization of the mutants and a suggested genetic networks of the stimulus-response interactions will be presented.

Steps of abscisic acid action

Erwin Grill. Thomas Ehrler, Knut Meyer, and Martin Leube,
Federal Institute of Technology Zürich, Institut für
Pflanzenwissenschaften, Switzerland

We are using *Arabidopsis* mutants to study steps mediating ABA action. ABA mediates three easily scorable responses: seed dormancy, regulation of water status, and growth inhibition. We isolated *Arabidopsis* mutants which are insensitive to ABA by using growth inhibiting ABA concentrations. The mutants fall into three subgroups: pleiotropic mutants in which all three ABA responses are impaired including the known mutants *abi-1* and *abi-2*, mutants with two impaired responses and those which are ABA-insensitive solely towards the growth response. Genetic complementation analyses indicate that several *loci* establish the mutant phenotype. Currently, we are characterizing the mutants, mapping the *loci* on the genome, and trying to clone the *loci*. Based on genetic markers we have cloned *abi-1* and final proof by DNA-transfer into plants is in progress.

CLONING AND CHARACTERIZATION OF THE *ETR1* GENE

Caren Chang, Shing F. Kwok, Anthony B. Bleecker *, Elliot M. Meyerowitz
Biology Division, California Institute of Technology, Pasadena, California 91125; * Department of Botany, University of Wisconsin-Madison, Madison, Wisconsin 53706

We are interested in understanding the molecular basis for ETR1 action in ethylene signal transduction. The *Arabidopsis ETR1* gene was defined by dominant mutations that cause insensitivity to ethylene. ETR1 acts early in ethylene signal transduction, perhaps as a receptor for ethylene, since mutants lack a variety of ethylene responses in all tissues examined and show a reduction in saturable binding of ethylene.

We have cloned *ETR1* based on the genetic map position of the gene. Starting with a genetically linked RFLP marker, we obtained ordered cosmid clones in the chromosomal region. The *ETR1* locus was confined to a single cosmid by RFLP mapping of meiotic recombination break points. This cosmid detected a single cDNA clone from a seedling library. We determined the DNA sequence of both the cDNA and the corresponding genomic DNA. We demonstrated that this clone represents the *ETR1* gene since: (1) in each of four *ETR1* mutant alleles the DNA sequence encodes a single amino acid substitution compared to the wild type, and (2) dominant ethylene insensitivity is conferred to wild type plants when the mutant *etr1-1* gene is introduced by transformation. The *ETR1* gene has similarity to a family of signal transduction genes previously uncharacterized in plants. This gene family and potential modes of *ETR1* action will be discussed.

Mutations at the *SPINDLY* locus of *Arabidopsis thaliana* alter gibberellin signal transduction.

Steven E. Jacobsen and Neil E. Olszewski. Department of Plant Biology and Plant Molecular Genetics Institute, University of Minnesota, St. Paul, MN 55108.

Three independent recessive mutations at the *SPINDLY* (*SPY*) locus of *Arabidopsis thaliana* confer resistance to the gibberellin biosynthesis inhibitor, paclobutrazol. The strongest allele, *spy1-1*, is partially epistatic to the *ga1-2* mutation. *ga1-2* plants are deficient in gibberellins and develop as dark green dwarves which do not bolt, are male sterile, and, when treated with gibberellins, produce seeds that require exogenous gibberellins for germination. In contrast, *spy1-1, ga1-2* double mutants have bolts that are approximately one half the height of wild type bolts, bear partially fertile flowers, and produce seeds that germinate in the absence of applied gibberellins. *spy* mutants have a "GA overdose" phenotype. Relative to wild type, *spy* mutants exhibit longer hypocotyls, leaves which are a lighter green color, increased bolt elongation, earlier flowering, parthenocarpy, and partial male sterility. All of these phenotypes are also observed when wild type *A. thaliana* plants are repeatedly treated with gibberellic acid (GA_3). The *ga1-2* mutation and paclobutrazol inhibit different steps in the gibberellin biosynthesis pathway. Since *spy1-1* can simultaneously suppress the effects of *ga1-2* and paclobutrazol, it is likely that *spy1-1* activates a basal level of gibberellin signal transduction, which is independent of gibberellin. Furthermore, analysis of GA_3 dose response experiments suggests that GA_3 and *spy1-1* interact in an additive manner. Two models which are consistent with these results are 1) that SPY functions to regulate a portion of a redundant GA perception and signal transduction pathway or 2) that SPY functions to regulate cross talk between a non-GA regulated pathway and the GA signal transduction pathway. A fourth allele, similar to *spy1-1*, has recently been isolated from the T-DNA insertion lines available from the ABRC at Ohio State. Recent experiments indicate that this line carries a single functional kanamycin resistance locus, and that kanamycin resistance cosegregates with the *spy* phenotype. Experiments are currently in progress to isolate the *SPY* gene.

Abstracts for Session II: **Photobiology**

Session Chair: Judy Brusslan

Friday, August 20

10:30 am - 12:30 pm

Weigel Hall Auditorium

Genetic Analysis of the Plant Photoreceptors Controlling Photomorphogenesis.
Brian M. Parks, Department of Plant Biology, Ohio State University, Columbus, Ohio.

Light is arguably one of the most important environmental factors regulating plant growth and development. Although much is known about the developmental responses controlled by light, our understanding of the photoreceptors involved in these responses and their mode of action is still incomplete. As a model genetic system, *Arabidopsis* has had a profound impact on our ability to study plant photoperception. Through the analysis of photomorphogenetic mutants of *Arabidopsis* and other higher plants, it is now apparent that there are at least three classes of photoreceptors controlling photomorphogenesis; the phytochromes, the blue-light photoreceptor(s), and the UV-A photoreceptor(s). Genetic analysis of the phytochrome photoreceptor family has been crucial in recent years toward revealing that at least two of the five separate phytochromes in *Arabidopsis* (phytochrome A and phytochrome B) have distinct roles in plant growth and development. Although a blue-light photoreceptor is still unidentified, several loci have been discovered which are important for response to blue-light signals. Mutants have been identified in *Arabidopsis* which are separately affected in etiher blue-light-induced inhibition of hypocotyl elongation (*blu*1, *blu*2, and *blu*3) or phototropism (JK218 and JK224). Very recently, the analysis of the phytochrome/blue-light double mutants has provided good evidence that plants contain a separate photosensing pathway for response to UV-A light. Data will be presented for both an overview of the genetic analysis of plant photoreceptors and the current genetic work aimed at probing questions centering on how these multiple photoreceptor systems may work in concert to effect normal plant growth and development in response to light.

GENETIC INTERACTIONS CONTROLLING ARABIDOPSIS PHOTOMORPHOGENESIS.

J. Chory, T. Elich, H-m. Li, A. Pepper, D. Poole, J. Reed, V. Vitart, T. Washburn, M. Furuya[@], and A. Nagatani[#], Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800, USA; [@]Hitachi Ltd., Hatoyama, Saitama 350-03, JAPAN, and [#]Frontier Research Program, RIKEN, Wako-shi, Saitama 351-03, JAPAN.

We are taking a combined genetic and molecular biological approach to identify components of the light-signal transduction pathways. Our studies have identified a class of Arabidopsis mutants that show many characteristics of light-grown plants even when grown in complete darkness. The mutants define 4 complementation groups, designated *det*1, *det*2, *det*3, *det*4 (*de-et*iolated). Because these mutations are both pleiotropic and recessive, we have hypothesized that *DET* genes play a negative regulatory role in photoregulated leaf and chloroplast development in Arabidopsis. To determine the molecular mechanisms of light signal transduction, we have identified small regions of yeast artificial chromosomes that contain the *DET*1 and *DET*2 genes. Our progress towards identifying these genes will be updated. In a separate set of experiments, we have identified mutations in two of the 5 Arabidopsis phytochrome apoprotein genes. Our results suggest that phytochrome B plays a general role in cell elongation in response to light and also controls flowering time and chlorophyll accumulation. In contrast, phytochrome A appears to play a highly specialized role in Arabidopsis development. The *phy*A mutants are characterized by an insensitivity to far-red light, including decreased cell growth inhibition, seed germination, and accumulation of RubisCO. We have also utilized a second genetic approach that focuses on one particular downstram light-regulated response, the transcription of the *CAB*3 promoter. Using a transgenic line, we have identified mutations that define at least 5 complementation groups in which the *CAB*3 promoter is expressed at aberrant high levels in the dark. Some of the mutations also affect the levels of *RBCS* mRNAs in dark-grown mutants, while other affect only *CAB*. The results from epistasis studies with these and the photomorphogentic mutations suggest a hierarchical regulatory network among these genes in the control of the downstream light-regulated responses.

Characterization and molecular cloning of *COP9*, a genetic locus involved in light-regulated development and gene expression in Arabidopsis

Ning Wei and Xing-Wang Deng, Department of Biology, Yale University, New Haven, CT 06511

An Arabidopsis light-regulatory locus, *COP*9, has been identified and characterized. The *cop*9 seedlings grown in the dark as if they were grown in the light conditions. The mutants exhibit light-grown characteristics including short hypocotyls and open and enlarged cotyledons with cell-type and chloroplast differentiation regardless of light signals. The *cop*9 mutation also leads to high-level expression of light-inducible genes in the absence of light. These properties imply that the mutation in the *COP*9 locus uncouples the light/dark signals from morphogenesis and gene expression. In addition, light-grown *cop*9 mutants are severely dwarfed and are unable to reach maturation and flowering, indicating that the *COP*9 locus also plays a critical role for normal development of the light-grown plant. Comparison and relationship of *cop*9 with other photomorphogenic mutants of Arabidopsis such as *cop*1, *det*1 and *det*2 will be discussed. The *COP*9 locus has recently been cloned in our lab. The molecular characterization and the expression pattern of the *COP*9 gene will also be discussed.

A nuclear encoded protein of *Arabidopsis thaliana* passes through microsomes prior to uptake in isolated chloroplasts.

Ann Van Gysel, Katia Litière, Dirk Inzé & Marc Van Montagu
Laboratorium voor Genetica, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium)

We have isolated a negatively photo-regulated gene from *Arabidopsis thaliana*. The gene product, a 20.5-Kda protein (BCB), shows extensive homology with blue copper binding enzymes such as plastocyanin and stellacyanin.

Two different polyclonal antisera raised against two independent hydrophillic regions of the protein specifically recognize a 22-Kda and a 50-Kda protein. Subcellular fractionations and electron microscopic immunolocalisations, demonstrate that these proteins are associated with the thylakoid membranes.

Unexpectedly, the amino acid sequence deduced from a full length *bcb*-cDNA clone, does not contain a canonical chloroplast transit peptide. The N-terminal sequence of the protein instead resembles an ER signal peptide. We have shown that this peptide is sufficient for import in the ER. Furthermore we have substantial *in vitro* evidence that the BCB protein translocates through microsomes, where it is glycosylated, prior to uptake in isolated chloroplasts. These data strongly support the presence of an alternative pathway for import in chloroplasts, moreover since the BCB protein seems to be glycosylated *in vivo* as well, which can be expected to take place in the ER.

OVEREXPRESSION OF MUTANT phyA SEQUENCES REVEALS NOVEL FEATURES OF PHYTOCHROME FUNCTION IN ARABIDOPSIS. Margaret Boylan and Peter H. Quail, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

We are interested in structure-function relationships of phytochrome A, the major member of the photoreceptor family found in etiolated plant tissues. Transgenic Arabidopsis seedlings that overexpress this protein are much shorter than wild-type plants, when grown in white light, because hypocotyl elongation is strongly suppressed. Phytochrome A overexpression also causes an exaggerated far-red light high irradiance response (FR-HIR) that is easily recognized in the transgenic plants. We are currently using these growth defects as an assay system to monitor the biological activity of phyA sequences that have been mutagenized in vitro. Certain mutations, targeted at selected regions of the overexpressed polypeptide, postulated from prior studies to have potential functional importance, generate phytochrome A molecules which interfere in a dominant-negative fashion with the ability of the plants to respond normally to light. Our results suggest a model for phytochrome A structure where the molecule consists of separate domains responsible for controlling plant growth under white light vs. FR-HIR conditions. In addition, we are currently screening an EMS-generated M2 population of phytochrome A overexpressors for revertant seedlings that have tall hypocotyls. From this screen, we expect to obtain phytochrome A polypeptide mutants as well as second site suppressor mutations which will facilitate characterization of the cellular reaction partner for phytochrome A signal transduction.

Abstracts for Session III: Metabolism

Session Chair: Chris Somerville

Friday, August 20

1:45 pm - 3:45 pm

Weigel Hall Auditorium

Molecular-genetics of nitrogen assimilation into amino acids in *Arabidopsis thaliana*.

Gloria Coruzzi, Carolyn Schultz, Karen Coschigano, Hon-Ming Lam, Rosana Oliveira and Sheila Peng.
New York University, Department of Biology, 1009 Main Building, New York, N.Y. 10003

In all higher plants, nitrogen is first assimilated into the amino acid glutamine, and is subsequently channeled into the amino acids glutamate, aspartate, and asparagine. These amino acids serve as nitrogen donors and/or nitrogen transport compounds in plants. The enzymes involved in the biosynthesis of these amino acids are present as multiple isoenzymes, which are located in several different subcellular compartments. By studying the genes and mutants for all these enzymes in a single species such as Arabidopsis, we hope to be able to determine whether these multiple isoenzymes perform distinct physiological roles, and whether subsets of genes for these enzymes are co-regulated. We have begun to study the Arabidopsis genes encoding the enzymes involved in the biosynthesis of the above amino acids namely, glutamate synthase (Fd-GOGAT and NADH-GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AspAT), asparagine synthetase (AS), and glutamine synthetase (GS). In addition to studying the above genes and their patterns of regulation, we have begun to isolate mutants which are lacking a particular isoenzyme activity (e.g. AspAT and GDH) as well as to characterize previously isolated mutants (e.g. Fd-GOGAT) with regard to the affected gene. Since several of the enzymes in this pathway are encoded by multigene families in Arabidopsis, we are currently identifying which structural gene is affected in a particular isoenzyme mutant. By studying the phenotype of these mutants, we should be able to assign a physiological role to each isoenzyme.

THE GENETICS AND BIOCHEMISTRY OF LIPID METABOLISM IN *ARABIDOPSIS*

John Browse

Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340

We have isolated a series of *Arabidopsis* mutants with defects in the synthesis of membrane lipids. These mutants have contributed to our understanding in three main areas. First, they have provided an alternative approach to elucidating the mechanisms regulating synthesis and desaturation of membrane glycerolipids. Second, the availability of a series of mutants with specific alterations in leaf lipid composition offers a novel method for studying the role of lipids in the structure and function of plant membranes. Finally, because of the advantages of *Arabidopsis* as a model for molecular biology, the mutations have been used as markers to facilitate the cloning of the desaturase genes by chromosome walking and gene tagging. I shall briefly summarize the overall lipid compositions of the mutants that have been characterized to date, and then use specific examples to illustrate the ways in which the genetic approach is being used to elucidate the biochemistry and physiology of mutant plants and to clone genes encoding the fatty acid desaturases.

Isolation of a mutant *Arabidopsis* plant that lacks N-acetyl glucosaminyl transferase I and is unable to synthesize Golgi-modified complex N-linked glycans

Antje von Schaewen[1], Arnd Sturm[2], Jason O'Neill, and Maarten J. Chrispeels

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The complex Asn-linked glycans on plant glycoproteins, characterized by the presence of β1->2 xylose and α1->3 fucose residues are derived from typical $Man_9GlcNac_2$ N-linked glycans through the activity of a series of glycosidases and glycosyl transferases in the Golgi apparatus. By screening leaf extracts with an antiserum against complex glycans, we isolated a mutant of *Arabidopsis thaliana* that is blocked in the conversion of high mannose to complex glycans. In callus tissues derived from the mutant plants, all glycans bind to concanavalin A. These glycans can be released with endoglycosidase H and the majority has the same size as $Man_5GlcNac_1$ glycans. In the presence of deoxymannojirimycin, an inhibitor of mannosidase I, the mutant cells synthesize $Man_9GlcNac_2$ and $Man_8GlcNac_2$ glycans, suggesting that the biochemical lesion in the mutant is not in the biosynthesis of high mannose glycans in the ER, but in their modification in the Golgi. Direct enzyme assays of cell extracts show that the mutant cells lack N-acetyl glucosaminyltransferase I, the first enzyme in the pathway of complex glycan biosynthesis. The mutant plants are able to complete their development normally under several environmental conditions, suggesting that complex glycans are not essential for normal developmental processes. By crossing the complex-glycan deficient strain of *A. thaliana* with a transgenic strain that expresses the glycoprotein phytohemagglutinin, we obtained a unique strain that synthesizes phytohemagglutinin with two high mannose glycans, instead of one high mannose and one complex glycan (von Schaewen, Sturm, O'Neill and Chrispeels - submitted).

Expression Cloning Of A Plant Amino Acid Transporter By Functional Complementation Of A Yeast Amino Acid Transport Mutant

Li-Chu Hsu[2], Tzyy-Jen Chiou[2], Lishan Chen[2] & Daniel R. Bush[1,2] Department of Plant Biology[2] & USDA/ARS[1], University of Illinois, Urbana IL 61801

Although plants are photoautotrophic organisms, they are composed of many heterotrophic tissues systems that are dependent upon sugar and amino acid import for normal growth and development. Recent biochemical studies have identified four proton-amino acid symports that mediate amino acid flux into the cell. We report here the successful cloning of a neutral amino acid carrier by functional complementation. A histidine transport deletion mutant of *Saccharomyces cerevisiae* was transformed with an *Arabidopsis* cDNA library constructed in a yeast expression vector. Forty transformants, out of 10^5, allowed growth on a histidine limiting medium. The acquired ability to grow on low histidine was shown to be strictly dependent on the protein encoded by the expression plasmid. Histidine and alanine transport activity were 20-fold greater in the transformants. The transport kinetics, inhibitor sensitivity, and substrate specificity are virtually identical to those of Neutral System II, a carrier we previously described in plasma membrane vesicles isolated from leaf tissue. We have designated this porter NAT2, for neutral amino acid transporter 2. The cDNA insert is ≈ 1.7 kb with an ORF that codes for a protein composed of 486 amino acids. Hydropathy analysis of the deduced amino acid sequence suggests this is an integral membrane protein with 11 to 13 membrane spanning alpha-helices. Southern analysis indicates this carrier may be part of a small gene family. Overall, the sequence of this amino acid carrier is not closely related to any other protein sequences in the Genbank database suggesting NAT2 belongs to a class of membrane transport proteins that have not been previously identified. Interestingly, however, there are three regions of sequence that exhibit significant levels of similarity with at least seven other integral membrane proteins. We have also identified a T-DNA tagged *Arabidopsis* mutant lacking Neutral System I, the second carrier associated with neutral amino acid transport in plants. Further analysis of the expression, regulation, and function of these carriers should provide new insight into their roles in assimilate partitioning and plant cell biology.

trp4, a suppressor of blue fluorescence, is an Arabidopsis anthranilate synthase mutation

Brian Keith[a], Krishna K. Niyogi[b], Robert L. Last[c], and Gerald R. Fink[b]
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To identify new structural and regulatory genes involved in aromatic amino acid biosynthesis in plants, we have isolated suppressors of the blue fluorescence phenotype of *Arabidopsis thaliana trp1-100* mutants. Two suppressor mutations defined a new gene, *TRP4*. The *trp4*, *trp1-100* double mutants required tryptophan for growth, whereas *trp4-1* and *trp1-100* single mutant plants grew normally. Tryptophan-requiring mutants were underrepresented in genetic crosses due to a female-specific defect in transmission of the double mutant genotype, suggesting a role for the tryptophan pathway in female gametophyte development. Extracts from *trp4-1* mutants were deficient in anthranilate synthase enzyme activity. Arabidopsis genes encoding the β subunit of anthranilate synthase (ASB) were isolated by complementation in *E. coli*. The abundantly expressed *ASB1* gene was identified as the defective gene in trp4 mutants. Despite the existence of a multigene family encoding the anthranilate synthase β subunit, the screen for loss of fluorescence successfully identified mutations in *TRP4/ASB1*, demonstrating the power of suppressor analysis in the study of plant metabolism.

Abstracts for Session IV: **Stress/Pathogenesis**

Session Chair: Brian Staskawicz

Friday, August 20

4:15 pm - 6:15 pm

Weigel Hall Auditorium

Downy mildew of *Arabidopsis thaliana*: Towards the isolation and characterization of host genes involved in resistance.

Brigitte Mauch-Mani, Hans-Jürgen Joos, Grazyna Rempulska Bujas and Alan John Slusarenko, Institut für Pflanzenbiologie, Cytologie, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, SWITZERLAND.

We have studied the genetic control of resistance of different *A.thaliana* accessions to an isolate of the downy mildew fungus *Peronospora parasitica* by following the inheritance of resistance in the progenies of crosses between susceptible and resistant plants and in backcrosses.

We have followed the co-segregation of the phenotypic resistance trait with mapped RFLP markers with the goal of localizing and, using a map-based cloning strategy, eventually isolating and characterizing the loci involved in resistance .

In another approach we are screening T-DNA tagged lines of *A.thaliana* accession Wasselijewskija (resistant to our *P.parasitica* isolate) for mutants to susceptibility. Such mutants should allow the identification of classical resistance genes as well as genes playing a role in the putative signal transduction pathway between perception of the pathogen and activation of host defense genes.

Loci for Disease Resistance and Intercellular Signalling

Jeff Dangl, Bob Dietrich, Murray Grant, Laurence Godiard, Claudia Ritter, Jürgen Lewald, and Esther Straube

Max-Delbrück Laboratory, Carl-von-Linné Weg 10, D-50829-Köln, Germany

The number and function of plant genes necessary for a disease resistance response is unknown. Genetic analyses in many systems over the last 50 years have demonstrated that recognititon functions are provided by dominant alleles of genes in the plant (Resistance, or *R*-genes) which interact, either directly or indirectly, with either the direct or indirect product of a single pathogen avirulence (*avr*) gene. Although a great deal is known about the multitude of genes activated subsequent to triggering of the defense response, virtually nothing is known regarding the number or nature of downstream transduction steps which are truly necessary for a resistance reaction.

We use *Arabidopsis* to identify genetically plant loci necessary for a resistance reaction against phytopathogenic bacteria and fungus. Our work revolves around five themes. First, identification and isolation of recognition function genes (*R* or Resistance genes). We defined and are in the process of cloning the *RPM1* locus, which conditions resistance to pathogenic*Pseudomonas syringae* strains carrying the corresponding *avrRpm1* gene. Second, we used mutation analysis to identify loci which lose *avrRpm1* recognition. These mutations should map to either *RPM1* or to loci involved in either specific or general interpretation of *RPM1*-dependent function. Mutants in each class have been isolated, and thus far each silmutaneously loses the ability to recognize bacteria carrying either *avrRpm1* or *avrB* . The dual resistance specificity encoded at *RPM1* is of particular interest (see also abstracts Godiard et al. and Innes et al.) Third, we isolated four mutants exhibiting phenotypes reminiscent of "lesion mimic" mutants which exist in many plant species, notably maize, rice, tomato, and barley. These have potentially constitutive or "hair-trigger" activation of some step in one or more pathway(s) leading to cell death and fomation of an HR-like lesion. These mutants are resistant to otherwise virulent bacterial and fungal pathogens. Fourth, we are identifying the bacterial pathogen genes which are causal to triggering of a specific plant defense response. These fall into two categories: classically defined *avr* genes, and, using Tn*phoA* as a mutagen, genes encoding membrane localized or secreted products necessary for delivery of a specific *avr* function. Our current progress in these topic areas will be described.

Systemic Acquired Resistance in *Arabidopsis*. Terrence Delaney, AnnMarie Winter, Kris Weyman, Scott Uknes, Eric Ward and John Ryals. Department of Molecular Genetics, Ciba Geigy Agricultural Biotech., 3054 Cornwallis Road, Research Triangle Park, NC

Following pathogen infection, plants can mount an acquired resistance response protecting them against subsequent attack. This phenomenon of systemic acquired resistance (SAR; Ross, 1961) is an important part of a plant's disease resistance strategy. SAR can be induced by a broad range of pathogens, and the subsequent resistance acts against a diverse range of pathogens including those not involved in the primary attack. Work in our lab and others has identified many genes that are induced during SAR, in a range of plant species. The importance of some of the SAR gene products in resistance has been elucidated by experiments demonstrating *in vitro* antimicrobial activity of members of the chitinase, glucanase and permatin protein families. Recently, we demonstrated that transgenic plants expressing high levels of the tobacco pathogenesis related (PR) protein PR-1a are resistant to oomycete fungal pathogens (Alexander et al. PNAS, in press).

Much of the work in our laboratory is directed at understanding the signal transduction pathways regulating SAR. Correlative evidence has implicated salicylic acid (SA) as an important signaling molecule in SAR, since levels of SA are elevated in plants during SAR, and SA can induce the expression of PR genes and confer protection to plants against pathogen attack. To directly assess the role of SA in SAR, we recently introduced into tobacco plants a bacterial gene that converts SA into the inactive derivative catechol. These transgenic plants fail to mount an SAR response to pathogen attack (Gaffney et al. Science, in press). These data demonstrate that SA plays an important role in induced resistance.

To identify components of the signal transduction pathways regulating SAR, we are combining genetic and molecular biological approaches, to identify genes important for induced resistance. Toward this end, we have demonstrated that *Arabidopsis* demonstrates a typical SAR, in response to pathogens or treatment with resistance-inducing chemicals. SA appears to play a role in *Arabidopsis* as well. We are conducting mutant screens aimed at identifying plants that fail to develop SAR, plants that appear constitutive for SAR, and plants that exhibit disease-like symptoms in the absence of pathogen. The current status of *Arabidopsis* as a model system for SAR, and some of the mutant screen results will be presented.

ISOLATION AND CHARACTERIZATION OF PHYTOALEXIN DEFICIENT MUTANTS OF *ARABIDOPSIS THALIANA*. Jane Glazebrook and Frederick M. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, 02114.

Phytoalexins, small molecules with anti-microbial activity that are synthesized by plants in response to pathogens, have often been implicated as important components of plant defenses against microbial pathogens. However, there is little direct evidence supporting this idea. To investigate the role of phytoalexins in the interaction between the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* and *Arabidopsis thaliana*, we have isolated *Arabidopsis* mutants that fail to synthesize the *Arabidopsis* phytoalexin, camalexin, in response to attack by strain *P.s.m.* ES4326. Phytoalexin-deficient mutants were identified by infecting plants grown from M2 EMS mutagenized seed with *P.s.m.* ES4326 to induce camalexin synthesis, preparing extracts from infected leaves, subjecting the extracts to thin layer chromatography, and assessing the presence of camalexin based on its characteristic fluorescence. Three mutants, *pad1*, *pad2*, and *pad3* were identified among 7,000 plants screened. In *pad1* and *pad2,* camalexin accumulates to 30% and 10%, respectively, of the levels reached in the Col-0 wildtype. Camalexin is undetectable in *pad3.* In *pad1* and *pad2* mutants, *P.s.m.* ES4326 grows to densities that are 10-50 fold higher than those in Col-0, but in *pad3* growth of *P.s.m.* ES4326 is similar to that in Col-0. Possible explanations for this lack of correlation between camalexin levels and increased sensitivity to *P.s.m.* ES4326 will be discussed. Like the wild-type, all of the *pad* mutants limit the growth of *P.s.m.* ES4326 carrying the cloned avirulence gene *avrRpt2*, suggesting that camalexin is not required for resistance mediated by specific resistance gene-avirulence gene interactions. Genetic analyses of the *pad* mutants have shown that the *pad* mutations are recessive alleles of single nuclear genes, and that they define three different complementation groups. The *pad1* mutation is tightly linked to, or is identical with, a mutation that causes altered leaf morphology, while the *pad2* and *pad3* mutants are morphologically indistinguishable from Col-0. A PCR-based mapping strategy recently developed in this laboratory was used to determine that *pad1* and *pad2* are located on chromosome IV, and that *pad3* is located on chromosome III.

Characterization of genes differentially expressed in Arabidopsis roots upon progressive drought stress.

Françoise Gosti, Nathalie Bertauche, Michelle Bouvier, Nicole Vartanian and Jérôme Giraudat
Institut des Sciences Végétales, CNRS, 91198 Gif sur Yvette Cedex

The experimental system used aims to mimic the physiological conditions experienced by plants in the field during natural periods of drought, i.e. a progressive reduction of water available from the soil. Arabidopsis plants were grown in a sandy soil under humid conditions for one month, and then subjected to a progressive soil water depletion in dry (50% RH) atmosphere. A progressive decrease in the soil moisture content was achieved by protecting the soil surface from evaporation, thus allowing gradual water loss through plant transpiration only. Under such conditions, Arabidopsis develops an adaptive response at the root level in particular. The new lateral roots emerging from a threshold soil humidity, remain short, do not form hairs and often take a tuberized shape as long as drought persists; but they retain the ability to rapidly resume elongation and hair formation upon rehydration.

Two cDNA libraries have been constructed, starting from the root systems of drought adapted or control well-watered plants respectively. Samples of both libraries have been submitted to a first differential screening using radiolabeled cDNA made from roots of control or adapted plants. Numerous clones have been identified, which correspond to at least four different transcripts. The abundance of two of these transcripts is decreased in response to progressive drought stress, wherease the level of the other two increases. The contribution of abscisic acid (ABA) to the regulation of these genes has been investigated by comparing the level of their transcripts in wild-type and in the ABA-insensitive abi mutants (Koornneef et al., 1984, Physiol. Plant. 61, 377-383), both in plants submitted to progressive drought and in seedlings treated with exogenous ABA.

Abstracts for Session V: **Cell Differentiation**

Session Chair: Robert Goldberg

Saturday, August 21

8:00 am - 10:00 am

Weigel Hall Auditorium

Trichome Formation as a Model for Cell Differentiation.

M. David Marks, John C. Larkin, David G. Oppenheimer, William G. Rerie, and Susan Pollock.
School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

In *Arabidopsis*, non-glandular, unicellular trichomes are found on most of the aerial surfaces of the plant, including the surfaces of the leaves, stems, and sepals. Mutations have been identified that affect trichome initiation, expansion, maturation, spacing, and location on the plant. Our goal is to learn how the genes defined by the trichome mutations interact to mediate the development and placement of a single cell type. We believe that the trichome developmental pathway will serve as a useful paradigm for the understanding of how cell fate is controlled.

The first trichome gene isolated was *GL1*. This gene, which is required for the initiation of trichome development, has been found to encode a member of the *myb* class of transcription factors. *In situ* hybridization has been used to localize *GL1* expression in developing trichomes and leaf primordia (see poster by Oppenheimer *et al.*). To assess the effect of ectopic *GL1* expression on trichome formation, we have made plants that contain *GL1* under the control of the CaMV 35S RNA promoter (*35SGL1*). These plants have a reduced number of leaf trichomes and exhibit a small number of adventitious trichomes on the cotyledons and the abaxial surface of the first leaf pair. To attempt to manipulate the expression of the adventitious trichomes, we have EMS mutagenized *35SGL1* seeds. We have identified several plants in the mutagenized population that exhibit novel patterns of adventitious trichomes. In addition, mutations that result in clustering of trichomes have also been identified (see poster by Larkin *et al.*).

We have also recently isolated the *GL2* gene. Mutations at the *GL2* locus result in an alteration of normal trichome expansion. Two types of altered trichomes are found on these plants. Some of the trichomes expand along the surface of the leaves instead of perpendicular to the leaf surface. The other type of trichomes expand in the correct orientation, but they have fewer branches than normal trichomes. In addition to the trichome phenotype, mutations at this locus result in loss of the seed coat mucilage. The gene encodes a highly conserved homeobox domain. Analysis of the gene is continuing.

An update on the progress toward the isolation of other trichome genes will be presented.

MOLECULAR GENETIC ANALYSIS OF THE MICROTUBULE CYTOSKELETON IN *ARABIDOPSIS THALIANA*

Carolyn Silflow[1,2,4], Terry Wilson[1], Nancy Haas[1], Boyang Chu[3], Mae Hee Chiang[1], John Carter[2,3], Susan Wick[2] and D. Peter Snustad[1,4]
Department of Genetics and Cell Biology[1], Department of Plant Biology[2], Department of Horticultural Science[3] and Plant Molecular Genetics Institute[4], Univ. of MN, St. Paul, MN

A variety of tubulin isotypes are expressed during plant development. In order to better understand the genetic information encoding tubulin in higher plants, our groups have examined the structure and expression of the genes encoding α-tubulin and β-tubulin in *Arabidopsis thaliana*. In the small genome of *Arabidopsis*, we detected and characterized six α-tubulin genes and nine β-tubulin genes, all of which are expressed.

The spatial and temporal patterns of gene expression have been studied using RNA hybridization with gene-specific probes. Results from these studies showed that the expression of most genes was constitutive in roots, leaves, and flowers, whereas The *TUA1* gene was expressed specifically in flowers and the *TUB1* gene was expressed preferentially in roots. We have examined the activity of the promoters of several tubulin genes in transgenic plants containing fusion genes constructed from tubulin gene promoters and the β-glucuronidase coding region. The promoter of the *TUA1* gene, which encodes the most divergent α-tubulin isoform, was active primarily in pollen which had completed the mitotic divisions. Among the genes which are expressed in all three organs, we have found three contrasting patterns of promoter activity in roots. The activity of the *TUA2* promoter was localized to the dividing and elongating cells of the root tip. No activity of the *TUB1* promoter was detected in the root tip, but activity was detected in the mature portion of the root and in root hairs. The *TUB8* promoter showed activity only in the vascular tissue of the root.

To identify components that may participate in nucleating microtubule assembly in plant cells, we have examined two *Arabidopsis* genes encoding γ-tubulin, a protein localized in microtubule organizing centers in animal and fungal cells. Genomic blot hybridization results suggest that the genome may contain as many as five γ-tubulin sequences.

GENETIC AND MOLECULAR ANALYSIS OF ROOT HAIR DEVELOPMENT IN *ARABIDOPSIS*.

John Schiefelbein, Susan Ford, Moira Galway, Jim Masucci, Mark Kinkema, and Daphne Foreman. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Root hair formation is being used in our laboratory as a model for studying plant cell differentiation. The overall process of root hair development can be divided into several distinct phases: (1) cell-type specification, in which the fate of each root epidermal cell becomes defined (as either a root-hair-forming or hairless cell), (2) root hair initiation, whereby a bulge forms at an appropriate position on the cell, (3) hair elongation, which occurs by growth at the tip of the hair cell, and (4) hair maturation, whereby tip growth ceases and the cell wall attains its final form. Root hair development (*rhd*) mutants of *Arabidopsis* have been isolated that affect one or more of these phases. These mutants have been useful in characterizing the spatial and temporal aspects of each developmental phase, and the affected *RHD* genes have been organized into a developmental genetic pathway. Several root hair mutants were isolated from T-DNA-mutagenized populations, and these mutants are being used in molecular cloning experiments to isolate the corresponding *RHD* genes. In a related project, genes encoding the actin-associated molecular motor, myosin, have been isolated from *Arabidopsis*. These cloned genes are being used to explore the role of plant myosins in cell morphogenesis and intracellular motility. Recent progress on the morphological and molecular characterization of the *rhd* mutants and the myosin genes will be presented.

AN *ARABIDOPSIS* MUTANT WITH CLUSTERED STOMATA

Ming Yang & Fred Sack, Department of Plant Biology, Ohio State University, Columbus, OH 43210 USA

Little is known at a genetic level about how the identity and differentiation of plant cells is controlled. Seedlings from 7,000 parental groups of EMS-treated seeds were screened by light microscopy to identify mutants with altered stomatal morphology. One mutant, *tmm* ("too many mouths"), was isolated which has stomata arranged in clusters. The phenotype is best described if each cluster of stomata (*tmm*) or single isolated stoma (wild-type) is termed a "unit"; thus in the WT, each unit consists of one stoma containing two guard cells. In a *tmm* cotyledon, over 50% of the units contain clusters of 2-8 adjoining stomata. The clusters have the spacing pattern characteristic of the WT, with each cluster positionally equivalent to a single stoma in the WT. In some cases stomata are only connected by a thin cellular bridge. Stomatal clusters are present on all leaves but their frequency appears to decrease in later-produced leaves. The external appearance of the plant is normal. Genetic analysis indicates *tmm* is a nuclear recessive mutation. A putative mutant ("twins") was also isolated in which many stomatal units contain two adjacent stomata. Units with 3 or 4 stomata occur but much less frequently than in *tmm*. Other abnormalities present in "twins" are (1) 3 adjacent guard cells and (2) a single kidney-shaped guard cell not adjacent to another guard cell. Genetic analysis is in progress to determine whether the "twins" phenotype is due to a single locus. *Tmm* and "twins" complement each other and apparent double mutants have a synergistic phenotype. Further analysis may determine how stomatal meristemoid number and divisions are regulated.

An *Arabidopsis* T-DNA mutant with impaired root hair formation.

Claire Grierson; Catherine Duckett; Keith Roberts and Liam Dolan, Department of Cell Biology, John Innes Institute, JI Centre , Colney Lane, Norwich NR4 7UH, UK.

Arabidopsis root hairs are produced as outgrowths at the distal ends of specialised cells of the root epidermis. The results of time-lapse video analysis show that normal hair formation occurs in at least two phases. In the first phase, hair growth is very slow. Hairs in this phase have cytoplasm indistinguishable from that in the remainder of the hair-forming cell. In the second phase, hair growth proceeds much more rapidly. Hairs in this phase have characteristic cytoplasm, which is densely packed with machinery for the synthesis and export of cell wall components.

cow, an *Arabidopsis* mutant with impaired root hair development, has been isolated from T-DNA mutagenised populations generated by Ken Feldmann. This mutant produces root hairs that are short and fat, but grow to the same final volume as hairs of the parental line WS. Time-lapse video analysis of mutant root hairs shows that they grow at a rate similar to that of the first phase of growth of wild type hairs. *cow* root hairs never achieve the high growth rate characteristic of the second phase of wild type hair formation. This result suggests that *cow* hairs may be persisting with the first phase of hair growth throughout their development. The ultrastructure of mutant root hairs is being examined to see whether it resembles that of wild type hairs in the early phase of growth. The results of this ultrastructural analysis will be presented along with preliminary data concerning the map position of the mutation and the linkage of the phenotype to T-DNA insertions carried by the line.

Abstracts for Session VI: **Embryos/Early Development**

Session Chair: David Meinke

Saturday, August 21

10:30 am - 12:30 pm

Weigel Hall Auditorium

ARABIDOPSIS FUSCA MUTANTS: PHENOTYPES AND CLONED GENES

Linda A. Castle and David W. Meinke, Department of Botany, Oklahoma State University, Stillwater, OK 74078

Fusca mutants are characterized primarily by inappropriate accumulation of anthocyanin in the cotyledons of developing embryos. Mutant lines differ in the extent of anthocyanin accumulation and seedling development, but most die shortly after germination. Double mutant analysis shows that anthocyanins are not the cause of lethality. Fusca mutations are recessive and form twelve complementation groups in *Arabidopsis*. Five fusca mutants isolated from T-DNA mutagenized families will be described to introduce this phenotypic class. We examined *fus6* (*emb78*) in most detail. Mutant embryos have a defective apical meristem and fail to develop leaves after germination. Root development is also abnormal. We cloned and sequenced the *FUS6* gene following plasmid rescue from a T-DNA tagged line. A second tagged allele was used to verify the gene identity. *FUS6* is expressed in light-grown leaf, flower, root and silique tissues. It does not appear to be expressed in the dark. *FUS6* encodes a novel protein of 50 KDa that is hydrophilic, α-helical, contains consensus phosphorylation sites, and may bind Zn, Cu, and ATP or GTP. Fusca genes appear to have important roles in the transduction of environmental and developmental signals. We found that several mutants defective in light transduction (*cop1*, *cop9*, and *det1*) are also fusca mutants that accumulate anthocyanin during embryogenesis. Two models for the biological roles of fusca genes in plant embryo and seedling development will be addressed. The first model proposes that fusca genes regulate developmental processes either through signal transduction or repression of stress response pathways. In the second model, disruption of a limited number of metabolic pathways leads directly to lethality while inducing anthocyanin production indirectly. Clearly, fusca genes are essential for seedling viability, but models of fusca gene function also need to account for their role during embryogenesis. This work was supported by NSF grant DCB-8905137.

GENETIC ANALYSIS OF PATTERN FORMATION IN THE EMBRYO

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The seedling represents the primary body organisation in higher plants. Two patterns can be distinguished in the seedling, one along the apical-basal axis of polarity and the other perpendicular to the axis. The latter (radial) pattern comprises the main tissue types, such as epidermis, ground tissue and vascular tissue, which are arranged in concentric layers. The apical-basal pattern is a linear array of elements including shoot meristem, cotyledons, hypocotyl, embryonic root (radicle) and root meristem. To investigate mechanisms that generate the body organisation in the *Arabidopsis* embryo, we are using a genetic approach. We have isolated and characterised a fairly large number of EMS-induced putative pattern mutants which affect three different aspects of the body organisation: apical-basal pattern, radial pattern and shape (Mayer et al., 1991, *Nature* **353**, 402-407).

Mutations in five genes alter the apical-basal pattern of the seedling, and each gene has its own distinct phenotype. Developing mutant embryos were analysed in order to determine the origin of the pattern defects. The results suggest that four of the genes direct the development of specific regions while one gene, *gnom*, plays a role in the very early partitioning of the axis. Additional observations support the notion that the *gnom* gene acts at a different level than the other genes. The implications for apical-basal pattern formation in the embryo will be discussed.

T-DNA-mediated promoter trapping to identify embryonic genes in *Arabidopsis*

Keith Lindsey and Jennifer F. Topping
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The complexity of gene expression during embryogenesis has been indicated by the large number of embryonic-lethal and patterning mutants generated in *Arabidopsis* by chemical mutagenesis[4]. While the study of such mutants is invaluable for the genetic dissection of embryonic processes, the subsequent isolation of the mutant genes has to be carried out by the time-consuming process of chromosome walking. To allow the more rapid isolation of genes expressed during early embryogenesis and seed development in *Arabidopsis,* we are using a T-DNA-mediated promoter trapping approach[1,3]. A promoterless *gus*A reporter gene was introduced into a population of *Arabidopsis.* Activation of this gene results following its transcriptional or translational fusion with endogenous plant promoters; GUS fusion expression in embryos and embryonic insertional mutants can be screened for. Of over 2,000 independent transgenic lines generated[2], approx. 400 have been screened for GUS activation in seeds. Two lines carrying single copy T-DNAs show activity in early embryos, and one shows activity in the endosperm. The T-DNA flanking sequences have been cloned by inverse PCR, and genomic clones corresponding to the flanking sequences have been isolated from a wild-type lambda library. Three classes of embryonic and seed mutant have been identified in the transgenic population: pigment mutants (*albino, transparent testa*); embryonic lethal; and putative embryonic pattern formation mutants. Of the latter, three are apical-basal mutants, with two lacking cotyledons and one lacking roots; one is a possible radial mutant; and one is a 'grotesque', comprising a cotyledon only. These are being characterised for linkage to T-DNA sequences and for GUS fusion activity during embryogenesis.

Refs.: 1. Topping *et al.* (1991) *Development* **112**, 1009-1019; 2. Clarke et al. (1992): *Plant Mol. Biol. Reporter* **10**, 178-186; 3. Lindsey *et al.* (1993) *Transgenic Research* **2**, 33-47; 4. Lindsey and Topping (1993) *J. Exp. Bot.***44**, 359-374

TESTA DEVELOPMENT IN ARABIDOPSIS: STUDIES WITH A SEED SHAPE MUTANT

K.M. Léon-Kloosterziel[1], C.J. Keijzer[2] and M. Koornneef[1]
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A seed shape mutant of *Arabidopsis* was isolated from an EMS treated population. Genetic analysis revealed that the heart-shape phenotype was maternally inherited, showing that this is a testa mutant. This indicates the importance of the testa for the determination of the seed shape. This *ats* (aberrant testa shape) mutation was located in the middle of chromosome 5. A comparison was made between ovules and developing and mature seeds of the wildtype and of the mutant using Scanning Electron Microscopy and Light Microscopy. It was shown that the mutant seed shape is established during the first few days after fertilization, when the embryo occupies only a very small part of the seed. Mutant *ats* ovules and seeds lack one or more layers of the inner or outer integument, whereas the the outer layer is only slightly affected. In double mutants the effect of *ats* is additive to other testa mutations such as *ttg, gl2* and *ap2*. The *ats* mutation results in a maternally inherited reduced dormancy. This effect of a testa mutation on germination is also to be seen in *ttg* seeds, in which the outer layer of the testa is disturbed, indicating the importance of the testa as a component of dormancy in *Arabidopsis*.

Mutations that affect the switch from embryonic to vegetative development in *Arabidopsis thaliana*. Laura J. Conway, Danny Gerber and Scott Poethig, Plant Sciences Institute, University of Pennsylvania.

The embryonic and vegetative phases of development are distinct in most plants. Regulation of the switch between these phases is poorly understood. We have isolated two mutations of *Arabidopsis thaliana, extra cotyledon* (*xtc*)*1* and *2*, that affect this switch. The first two true leaves of a wild-type plant are visible approximately five days after the seeds are planted. These leaves appear simultaneously and are perpendicular to the cotyledons (embryonic leaves). In plants homozygous for either *xtc1* or *xtc2*, the shoot apical meristem produces one or two organs with cotyledon characteristics in the position of the first leaves. Specifically, these organs lack trichomes, have a simple venation pattern and contain storage protein and lipid bodies. The *xtc2* mutation also affects embryogenesis. *xtc2* embryos fail to make the morphological transition from the heart to torpedo stages of development. We believe that this transition must occur before the embryonic program of development can be terminated. In the absence of the *xtc2* gene product, the first leaf primordia are formed when the embryonic program is still in effect. These organs consequently express embryonic traits. These data demonstrate that the vegetative phase can be initiated prior to termination of the embryonic phase. We conclude that these two phases are independently regulated.

Abstracts for Session VII: **Reproductive Development**

Session Chair: Renee Sung

Saturday, August 21

1:45 pm - 3:45 pm

Weigel Hall Auditorium

MOLECULAR GENETICS OF CELL INTERACTIONS IN *ARABIDOPSIS* Robert E. Pruitt*, Martin Hülskamp, Steven D. Kopczak, Nora K. Murphy and Kay Schneitz, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, USA.

Many events in plant development are regulated by the interactions of neighboring cells. We are interested in determining what sorts of molecules act as signals and/or receptors in these interactions and how these mechanisms relate to those used in animals and fungi. We are presently working on two different types of systems to try to address this question. In one case we are starting at the molecular level and characterizing a family of receptor protein kinase genes which seem natural candidates for mediating cellular interactions. By analyzing the expression patterns of these genes as well as the phenotypes of transgenic plants bearing altered genes we hope to determine what roles these proteins play in plant development. In the second case we are starting from the organismic level and using genetics to identify genes essential to a whole range of cellular interactions which are required for proper male gametophyte development during reproduction. These interactions involve both recognition of the pollen grain to verify that it is from the correct species and also a transfer of positional information from the female to the male which first allows the pollen tube to determine the polarity of the stigmatic cell on which it has germinated and later provides 'guidance' for the elongating tube to find the ovule.

Cell cycle regulation during *Nicotiana tabacum* and *Arabidopsis thaliana* pollen devolopment.

Erwin Heberle-Bors, Dang Thi Cam Ha, Viktor Zarsky, Laci Bögre, Cathal Wilson, Oscar Vicente, Heribert Hirt. Vienna Biocenter, Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.

We have recently isolated a number of cell cycle regulatory genes and are studying their expression during the cell cycle and *in planta*. Gamete formation in plants, and pollen development in particular, is composed of a number of tightly regulated cell divisions and thus an interesting system to study cell cycle regulation. In addition, immature tobacco pollen can be induced in vitro to form haploid embryos. We found that the vegetative cell in tobacco pollen is arrested in the G1-phase of the cell cycle while classical studies had indicated a G2-arrest. During induction of pollen embryogenesis by a starvation treatment the cell cycle arrest was found to be released. A tobacco *cdc2* gene that encodes the protein kinase activity of the eucaryotic mitosis promoting factor has been isolated. It is highly homologous to a *cdc2* gene that we had previously isolated from alfalfa. *cdc2* transcripts of different size were detected in leaf, pollen and embryogenic pollen. A second tobacco *cdc2* cDNA clone has been isolated that contains a few amino acid exchanges but has a completely different 3' non-coding region. Control of mRNA stability by 3' end regulation has been observed in the proto-oncogenes c-fos and c-myc, and is a characteristic feature of animal gametogenesis. Further data on *cdc2* gene expression during pollen development will be presented. A MAP-kinase gene has been isolated from tobacco that is constitutively expressed on the RNA level. In other eucaryotes, MAP-kinase genes are involved in the establishment and release of a cell cycle arrest. Another protein kinase gene has been isolated from tobacco that is homologous to rat glycogen synthase kinase and *Drosophila "shaggy"*. Expression of this gene is suppressed during induction of pollen embryogenesis. A genomic cyclin clone has been isolated from *Arabidopsis thaliana* that is homologous to a previously isolated mitotic cyclin gene of alfalfa. Its promoter region was fused to the GUS reporter gene and was transferred into *A. thaliana* and tobacco plants. A histochemical analysis of GUS expression during pollen development will be presented.

Molecular-genetic analysis of flowering-time in *Arabidopsis*: Isolation of the *CO* locus and identification of early-flowering mutants

Joanna Putterill, Frances Robson, Karen Lee, Sudhansu Dash and George Coupland.

Cambridge Laboratory, The John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

Arabidopsis flowers much earlier under long than short days. In order to study this process we are investigating the molecular-genetic basis of mutations that result in an altered response to daylength.

One class of mutants are those which flower late under long days. The *co* mutant falls in this class and is nearly day-neutral. We have analysed the behaviour of double mutants carrying *co* and other mutations which disrupt hormone and phytochrome systems. This demonstrated a strong interaction between *co* and the *gibberillic acid insensitive* (*gai*) mutation: the double mutant is later flowering than *co* plants, and exhibits a more extreme *ga* phenotype than *gai* mutants. We have used chromosome walking with YACs to isolate *CO*. A 1700 kb contig was made in the vicinity of *CO* on chromosome 5, and the gene located on a 40 kb cosmid contig contained within this region. Introduction of these cosmids into *co* mutants showed that two overlapping cosmids were capable of complementing the mutation. This located the gene to the 6 kb overlap, which is now being sequenced. In addition two c-DNAs which appear to span the overlap region were isolated and are being sequenced.

A second class of mutants are those which flower early under short days. We have isolated 5 such mutants. Our progress in performing allelism tests between and mapping of these mutations will be presented, as will an analysis of double mutants.

THE ROLE OF *CLV1* IN FLORAL AND APICAL MERISTEM DEVELOPMENT

Steven E. Clark, Mark P. Running and Elliot M. Meyerowitz
California Institute of Technology, Division of Biology 156-29, Pasadena, CA 91125

We have investigated the effect of mutations in the *CLV1* locus on *Arabidopsis* development. *clv1* plants have enlarged apical meristems compared to wild type as early as 8 days after germination. Later, *clv1* inflorescence meristems can remain enlarged in weak alleles, become fasciated in intermediate alleles, and become massively proliferative in severe alleles. Growth under short day conditions indicate that *clv1* plants can fasciate prior to the transition to flowering. An analysis of early flowers by confocal laser scanning microscopy revealed that *clv1* floral meristems are slightly wider and much taller than wild-type floral meristems at the earliest stages of organ initiation (stage 3). As *clv1* flowers progress, we observe a fifth whorl developing interior to the normally central fourth whorl carpels. This fifth whorl later develops into a separate gynoecium interior to the gynoecium formed by the fourth whorl carpels. In addition, *clv1* flowers develop extra floral organs in every whorl. Double mutant combinations with *ag*, *ap2*, *ap3* and *pi* indicate *clv1* affects the underlying floral meristem structure upon which these homeotic genes act. Examinations of RNA expression by *in situ* hybridizations demonstrate that *clv1* flowers display altered patterns of *AG* and *AP1* expression. Double mutant combinations with *ap1* and *lfy* suggest that *CLV1* plays a previously unsuspected role in floral meristem identity. In *clv1; ap1* flowers, the center of the flower occasionally develops into a new inflorescence meristem with developing flowers. In this case, only the outer portion of the floral meristem adopts a floral fate. *clv1; lfy* plants generate mainly bracts and filamentous structures. The few flowers that do form exhibit significant transformations into shoot structures: organ are intiated in a spiral pattern, outer organs are leaf-like sepals with stipules and stellate trichomes, inner organs are leaf-like and tipped with stigmatic tissue, and the flowers are indeterminate.

THE *TOUSLED* GENE ENCODES A NOVEL PROTEIN KINASE IN *ARABIDOPSIS*

Judith L. Roe, *Carol Rivin, Allen Sessions, ‡Kenneth Feldmann and Patricia C. Zambryski, Dept. of Plant Biology, University of California, Berkeley, CA 94720, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, ‡Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721

The *tousled* mutation of *Arabidopsis thaliana* exhibits a complex phenotype during development. More rosette leaves are formed in *tsl* than in wild-type plants, and the leaves of the mutant are abnormal, displaying deeper serrations on their margins than wild-type leaves. Flowers of homozygous *tsl* plants each contain a random set of floral organs (sepals, petals, stamens and a bicarpellate gynoecium); their positions are correct, but their number is reduced. Scanning electron microscopy of *tsl* floral meristems indicates that the *TSL* gene acts early during flower development and affects the number of primordia that are initiated and the positioning of the primordia in the floral meristem. Later organ development is also affected. A *tsl* mutant line has been generated which contains a T-DNA insertion element that cosegregates with the mutant phenotype. The T-DNA has been used to identify and characterize the *TSL* locus. A fragment of wild-type DNA from the region has been transformed into *tsl* plants and rescues the mutant phenotype. The *TSL* gene encodes a 78 kDa protein with three structural domains. The C-terminal half of the protein shares the conserved residues found in the catalytic domain of known protein kinases, and is most homologous to the serine/threonine class. It does not fall into any known subgrouping of protein kinases, however, and may be the first representative of a new family. The N-terminal half of the protein consists of two sub-domains. A glutamine-rich domain is followed by an α-helical domain which contains two segments predicted to participate in coiled-coil structures. The *TSL* gene is most abundantly expressed in developing floral meristems, but the transcript can also be detected at lower levels in both roots and leaves. The *TSL* gene product may participate in a signalling pathway acting during several stages of normal plant development.

Abstracts for Session VIII: **Vegetative Development**

Session Chair: George Rédei

Saturday, August 21

4:15 pm - 6:15 pm

Weigel Hall Auditorium

Patterns and signals in the vegetative shoot apex. June Medford, Joseph Callos, Bruce Link, Fred Behringer, Bibo Xu and Daniel Stewart. Department of Biology, The Pennsylvania State University, University Park, PA 16802 USA.

In vegetative development the shoot apical meristem produces a series of tissues and organs that form the basal rosette. The plant proceeds through two distinct stages in vegetative development: a juvenile stage where the meristem is rectangular and leaves are initiated opposite from one another and an adult stage where the meristem is rounded and the leaves are initiated at positions 137° apart, producing a spiral pattern. In the Forever young (Fey) mutant leaf primordia form with an abnormally large number of cells and the meristem fails to maintain itself as a formative (stem cell) region. The first detectable alteration in the mutant is necrosis in a single cell in the apical meristem at three days. Leaf primordia are formed with an abnormally large number of cells and in positions which, at times, causes a reversal in the spiral pattern. The disruption in organ position does not continue in floral development suggesting that there is distinct information directing patterns in vegetative and floral development. A second mutant, Schizoid (Shz) suggests that there are signals produced outside the meristem which are perceived in the meristem. In Shz1-2 mutants young leaf primordia degenerate and the apical meristem soon follows. With further growth, the Shz1-2 mutant will at times produce a loose callus or shoots from vascular tissue. Analysis of the Fey and Shz gene products, isolated by their disruptive T-DNA inserts, will be described and related to the functions of the meristem.

Liam Dolan, Ian Cooper, Kate Duckett, Claire Grierson, Paul Linstead, Katharina Schneider, Mimi Tasimoto and Keith Roberts.
Department of Cell Biology, John Innes Institute, Norwich NR4 7UH U.K.

Epidermal Differentiation in the Arabidopsis root

The small size of the Arabidopsis root meristem makes it an ideal system for the study of many aspects of pattern formation and differentiation in higher plants. As a first part of such a study a detailed description of the organization of the root meristem and its derivatives was made. These studies revealed that cell file number is relatively constant in primary roots and they appear to be derived from a small number of initial cells surrounding the four celled quiescent centre. The cellular organization of the root meristem and the preliminary results of a clonal analysis will be described.

The regular pattern of cell division and the patterning of the cell types in the epidermis is the focus of the research in this laboratory. We are characterizing the development of the two cell types in this lineage with a variety of techniques including clonal analysis. The preliminary results of genetic screens for mutations that alter pattern formation and differentiation will also be presented.

The Genetic Control of Chloroplast Development in *Arabidopsis*. K.A. Pyke, S.M. Rutherford and R.M. Leech, Department of Biology, University of York, Heslington, York, UK. Y01 5DD.

The division and development of chloroplasts is central to the competent functioning of the leaf mesophyll cell. We have isolated mutants from both EMS-mutagenised and T-DNA tagged populations of *Arabidopsis* in which the chloroplast division process has been perturbed such that the number of chloroplasts per mesophyll cell is significantly different to that found in wild type cells. These mutants have been termed *arc* (*a*ccumulation and *r*eplication of *c*hloroplasts) mutants and so far 10 different *arc* loci have been identified. Analysis of these mutants has revealed a 100 fold variation in mean chloroplast number per mesophyll cell, ranging between 1 and 120 chloroplasts per cell. This variation in chloroplast number is compensated for by a 100 fold variation in chloroplast size ranging between $25 \mu m^2$ and $2500 \mu m^2$ mean chloroplast plan area, compared to wild type ($50 \mu m^2$) The compensation between chloroplast number and size in this mutant collection is closely controlled so that the total size of chloroplast compartment for a given cell size is similar for all of the *arc* mutants. This suggests that chloroplast division is not an essential prerequisite to mesophyll cell development in higher plants if compensated for by changes in chloroplast size. In several *arc* mutants, chloroplast division is severely restricted since there is no significant increase in chloroplast number during mesophyll cell expansion. Cells of one such mutant, *arc*5, contain 13 chloroplasts which are six-fold larger than wild-type yet 50% show a degree of central constriction similar to the division profile of normal dividing chloroplasts. This suggests that the *arc*5 mutation specifically interrupts the chloroplast division process. *arc*5 is not a null mutation for chloroplast division, since a double mutant combination with *arc*1, a mutant in which the rate of chloroplast development is reduced, results in 45 chloroplasts per cell. Cells of the *arc*6 mutant contain only 1-3 very large chloroplasts which do not increase in number during cell expansion..These *arc* mutants represent the first large stably inherited changes in chloroplast number in higher plants. The isolation of T-DNA tagged *arc* alleles will facilitate the isolation and characterisation of *arc* genes.

A novel 154 kd protein encoded by *sabre* affects cell elongation in *Arabidopsis thaliana*

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Plant morphology is determined by four principal factors: Cell division activity, the plane of cell division, cell expansion, and cell differentiation. The *sabre* mutant, which was identified among T-DNA transformed *Arabidopsis* lines (K.Feldman), shows aberrant cell expansion. Mutant roots are much shorter than wild type roots, but radially expanded. Root tips appear normal and the aberrant expansion becomes only apparent at the position where root cells would normally elongate. Fresh sections through the mutant root showed that cortex cells and, to a lesser degree, also other root cells, were expanded radially. In etiolated *sabre* seedlings, the hypocotyl is short, but wider than wild type. However, the mutant tissue has the same volume and cell number as wild type tissue. Interestingly, the cotyledons and hook formation appear normal and the aberrant cell expansion is apparent only after the curvature of the hook, where in the wildtype, cells elongate longitudinally. True leaves and cauline leaves are shorter than wild type leaves. In the flower, sepals and petals are also shorter and rounder and therefore fail to encapsulate the stamens and carpel. *Sabre* siliques do not elongate and do not contain any seeds. *Sabre* appears to be both male and female sterile, although pollen, in lesser amounts than wild type, is present in the mutant.

The *Sabre* genomic region was isolated using the T-DNA as a tag. Four overlapping cDNAs mapping to this region were isolated. Using a RACE-PCR protocol, the exact RNA start site was determined. *Sabre* produces a 4.4 kb long mRNA which encodes a novel 154 kd protein, for which no homology to known proteins has been found. About 2 kb of the *Sabre* promoter region have been sequenced.

RNA-PCR experiments showed that no functional *Sabre* mRNA can be made presumably due to the T-DNA insertion, although aberrant RNA is expressed from both the 5' and 3' ends of the *Sabre* coding region. Two additional alleles of sabre have been identified. Their phenotypes are very similar to that of *sab-1*.

Sabre mRNA cannot be detected in northern experiments and appears to be expressed at a very low level and/or highly localized. It is possible that the Sabre protein may only be needed in the few cells of the root and shoot elongation zone, as well as other longitudinally elongating cells. RNA in situ experiments are in progress in order to determine the expression pattern.

amp1 - a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis, and precocious flowering

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amp1, a mutant of *Arabidopsis thaliana* has a phenotype altered in three different aspects of plant development; spatial pattern, photomorphogenetic growth, and initiation of flowering. While fewer than 0.1% of the seedlings of wild-type plants are non-dicot as many as 20% of the seedlings of the *amp1* mutant are tricot or tetracot. The rate of leaf initiation is faster and vegetative phyllotaxy is altered in *amp1*. When grown in the dark *amp1* seedlings show morphogenetic properties similar to light grown wild-type plants; they do not form an apical hook, have hypocotyls shorter than wild-type plants and form etiolated true leaves. *amp1* mutant flowers significantly earlier than congenic *Amp1* plants and has no photoperiodic response. The mutant has six times more cytokinin than wild type indicating that endogenous cytokinin levels might play an important role in mediating these different developmental processes. *AMP1* might code for a negative regulator of cytokinin biosynthesis, or may be required for the degradation of cytokinin.

Abstracts for Session IX: **Gene Regulation**

Session Chair: Elizabeth Dennis

Sunday, August 22

8:00 am - 10:00 am

Weigel Hall Auditorium

Mobile Elements and Inducible Genes in the Nitrate Assimilation Pathway of Arabidopsis

Nigel Crawford[1], Yi-Fang Tsay[1], Mary Frank[1], Julian Schroeder[1], Caroline Dean[2] and Kenneth Feldmann[3]
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Structural genes of the nitrate assimilation pathway have been cloned and characterized from *Arabidopsis*. The expression of these genes respond to environmental signals including nitrate, light and CO_2 as well as to internal signals from plastids, circadian rhythms and carbon metabolism. Evidence for both transcriptional and post-transcriptional regulation has been obtained for nitrate reductase. Even though the NR gene is tightly regulated, one can delete the *NIA2* NR gene encoding for 90% of shoot NR activity and still retain viability even when nitrate is the sole source of nitrogen. A point mutation the MoCo domain of a second NR gene, *NIA1*, has been identified in a *NIA2* deletion background and found to reduce NR activity levels to 0.5% the wt level. *nia1nia2* double mutants can no longer grow on nitrate. Mutations in the *CHL1* gene have no effect on NR activity but reduce nitrate and chlorate uptake. The *CHL1* gene was cloned using a T-DNA tagged mutant. The *CHL1* gene encodes a protein with twelve putative membrane spanning segments characteristic of cotransporters. Electrophysiological studies demonstrated that *CHL1* mRNA encodes an electrogenic, nitrate transporter in *Xenopus* oocytes. *CHL1* mRNA is found predominantly in roots and increases rapidly in response to nitrate and to acidic pH. Two additional alleles of *chl1* were found. One is a deletion of *chl1* generated by γ-rays. The second was found in a line *Arabidopsis* containing an active maize *Ac* element. This mutant had an insertion of 3.3 kb, which was not homologous to *Ac*, in the fourth intron of *chl1*. Analysis of the insert showed that it is a transposon with short inverted repeats that duplicated genomic target sequences upon integration. The element, called *Tag1*, excises from the *chl1* gene producing revertants (25-30% of the total progeny) with a small "footprint" left in the gene. *Tag1* is present in the Landsberg ecotype but not Columbia or WS.

Mechanisms of mRNA instability in plants. Pamela J. Green, Crispin B. Taylor, Pauline A. Bariola, Pedro Gil, Michael L. Abler, Michael L. Sullivan, Christie J. Howard, Linda Danhof, Thomas C. Newman, and Masaru Ohme-Takagi. MSU-DOE Plant Research Laboratory and Department of Biochemistry, Michigan State University, E. Lansing, Michigan 48824-1312.

A long-term goal of our research is to elucidate the mechanisms that target highly unstable transcripts for degradation because these mechanisms provide plants with a means to make rapid changes in gene expression in response to various stimuli. To this end we have identified two types of sequences that can markedly accelerate the decay of reporter transcripts in tobacco. These are repeats of the AUUUA motif, known to function as an mRNA instability determinant in mammalian cells, and DST sequences which may be unique to a set of auxin-inducible plant transcripts. We have identified and characterized a Small Auxin Up RNA gene of *Arabidopsis* called SAUR-AC1 that contains a DST sequence in the 3' UTR. The properties of this gene indicate that it will serve as a useful molecular genetic tool to study the auxin response and mRNA instability. A selection strategy designed to isolate mutants that lack the ability to recognize AUUUA instability sequences will also be described.

Another objective of our research is to differentiate the ribonucleases (RNases) that function in mRNA decay from those that play other roles in RNA metabolism in plants. As a first step, we have begun the biochemical characterization of *Arabidopsis* RNases and have isolated three RNase genes, designated *RNS1*, *RNS2*, and *RNS3*. *RNS2*, the gene that we have studied in the most detail, may be involved in phosphate remobilization in *Arabidopsis* because it is induced during senescence and during phosphate starvation. Using an RNase activity gel assay, we have screened extracts from 2500 M2 *Arabidopsis* plants for those exhibiting alterations in the typical RNase profile. This approach has led to the isolation of several mutants that have bred true in the next generation. The potential use of these mutants to address the functions of *Arabidopsis* RNases will be discussed.

Molecular Characterization of the *TCH4* Gene of *Arabidopsis*: Genomic Organization, Sequence Similarity to *meri-5* and *gusA*-Fusion Expression Patterns

Wei Xu, Diana Polisensky, Stefan Wawersik and Janet Braam

Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251

The *TCH4* was identified as a gene whose expression is highly regulated by stimuli such as touch, rain, wind and darkness (Braam and Davis, Cell, 60, 357-364, 1990). It is also highly regulated in cultured cells by temperature shifts (Braam, PNAS, 89, 3213-3216, 1992; Polisensky and Braam, Unpublished). Unlike *TCH1*, 2 and 3 that are calmodulin or calmodulin-related genes, *TCH4* gene has no sequence similarity with calmodulin.

A nearly full-length cDNA of *TCH4* has been sequenced; it contains one open reading frame and the deduced *TCH4* protein has 284 amino acids. The amino terminus of TCH4 shows striking similarity to the protein of *Meri-5* (Medford, *et al.* Plant Cell, 3, 359-370, 1991). *Meri-5* is an *Arabidopsis* gene that is expressed in the shoot apical meristematic dome and branching points in the shoot and root, and encodes a protein related to a cell wall expansion enzyme (de Silva, et al., Plant Journal, in press).

To determine the structure of the *TCH4* transcription unit, we isolated and sequenced the *TCH4* genomic locus. The *TCH4* transcription unit is about 1.1kb in length and contains one intron. There are two 5' transcription start sites as determined by primer extension. To begin to dissect the mechanism(s) of *TCH4* regulation and the *cis*-regulatory elements, DNA fragments derived from 5'-flanking region with and without a translated portion of *TCH4* were fused to the bacterial β-*glucuronidase* gene (*gusA*). Transgenic *Arabidopsis* plants have been generated by *Agrobacterium*-mediated transformation. The *TCH4-gusA* fusion expression patterns suggest that TCH4 is regulated during development at points of mechanical stress (e.g., branch points) in addition to the regulation by external mechanical stimuli. The expression patterns and inducibility of the *TCH4-gusA* fusions will be presented.

This work was supported by NIH grant R29GM46346 and NASA grant NAGW-3139.

Circadian regulation of *cab2* gene expression in *Arabidopsis thaliana*. I. A. Carré, S. L. Anderson, S. Martino and S.A. Kay. Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22903.

Higher plant cab genes encode the chlorophyll a/b-binding polypeptides that form part of the light-harvesting complexes of photosystems PSI and PSII. The transcription of the *Arabidopsis cab2* gene occurs rhythmically, with a period that matches the natural 24 h day-night cycle. This rhythm has been shown to be under the control of an endogenous circadian pacemaker, since it persists with a period of approximately 24 h in the absence of environmental time cues. In order to investigate the pathway through which the circadian clock regulates *cab2* transcription, the *Arabidopsis cab2* promoter was fused to the firefly luciferase gene, and a series of 5'- deletions were analysed in transgenic tobacco. This study demonstrated that a strong positive element (CUF1, *cab* upstream factor 1) corresponds to a G-box-type sequence located between -142 and -133, and that a weaker positive element is located between -321 and -198. The promoter region corresponding to -111/-33 was sufficient for the circadian regulation of the luciferase reporter gene.

Gel shift analysis using both Tobacco nuclear extracts and Arabidopsis whole cell extracts indicated that at least two complexes bound to the CUF1 site. A smaller complex (SCF, small *cab* factor) bound within the -111/-33 region, and thus may be responsible for the circadian regulation of *cab2* expression. An additional complex (CGF, *cab* GATA factor) was identified in Tobacco extracts, that bound to a triple GATA sequence located between -79 and -44. We wish to determine how the binding of these different transcription factors is modulated as a function of circadian time.

Pleitropic effects of a T-DNA tagged gene encoding a regulatory protein with ß-transducin repeats

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A transgenic *Arabidopsis* carrying the T-DNA of pPCV6NFHyg*lux* gene fusion vector segregates at 3:1 ratio a recessive mutation causing pleitropic phenotypes. During germination the mutant shows a fusca habit, but develops to a dwarf plant displaying reduced root elongation, increased trichome density, reduced flower size, light-red seed colour etc. These phenotypes are severely influenced by enviromental factors such as salinity or draught in soil. In sterile culture the mutant exhibits an increased sensitivity to exogeneously provided sucrose, glucose, potassium salts and auxins. Segregation analysis of F2 progeny from crosses between wild type and mutant indicated a tight linkage between mutation and the T-DNA encoded Hyg^R selectable marker. Molecular characterization of the mutant revealed an insertion of a trimeric tandem T-DNA repeat in a gene that proved to be a member of a gene family represented by 20 to 25 homologous copies in the *Arabidopsis* genome. A DNA sequence motif conserved in this gene family was identified as a repeat showing homology to genes encoding the ß subunit of trimeric G-proteins, bovine ß-transducin, yeast transcription factors TUP1, Ste4, CDC4 and the enhancer split factor of *Drosophila*. Insertion of the T-DNA occured close to ß-transducin repeats within the C-terminal coding region of *Arabidopsis* gene by resulting in the synthesis of an altered protein. Changes in the expression of a large set of genes in the mutant suggest that factors with ß-transducin repeats play a central role in transcriptional regulation in response to metabolic, enviromental, hormonal and developmental stimuli.

Abstracts for Session X: **New Technologies**

Session Chair: Ken Feldmann

Sunday, August 22

10:30 am - 12:30 pm

Weigel Hall Auditorium

MAPPING THE ARABIDOPSIS THALIANA GENOME WITH SIMPLE SEQUENCE LENGTH POLYMORPHISMS

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Our objective is to construct a physical map that is tightly aligned to the genetic linkage map. This will require a large number of mapped molecular markers and to this end we are adding simple sequence length polymorphisms (SSLPs) to the existing collection of RFLPs. Several dinucleotide and mononucleotide repeats were initially found in a database search of *Arabidopsis* sequences. These were subsequently found to vary in length between the two commonly used ecotypes, Landsberg and Columbia, and were placed on the meiotic map by scoring alleles amplified from genomic DNA of a set of selfed recombinant inbred lines originating from a Landsberg-Columbia cross. After showing the existence and length variation of microsatellites we began to systematically screen for $(CA)_n$ and $(GA)_n$ clones in marker-selected plasmid libraries [1] and a small-insert lambda ZapII genomic library. After sequencing these clones, PCR primers were chosen using the PRIMER program (S. Lincoln, M. Daly and E. Lander, Whitehead Institute) and PCR was carried out to determine polymorphisms between the Landsberg and Columbia parental lines. Once length variation between the parental lines was established, the repeats were typed on 48 recombinant inbreds and placed on the linkage map in relation to existing RFLP markers using RI Plant Manager [2] and MAPMAKER 3.0 [3] software. Surprisingly, of twenty $(CA)_n$ clones ($n > 14$) only one was polymorphic. In contrast, over 80% of $(GA)_n$ repeats are polymorphic and these are being systematically placed on the linkage map. As estimated by plaque hybridization, $(GA)_n$ and $(CA)_n$ repeats are relatively abundant in *Arabidopsis*, although much less so than in mammalian genomes, with, on average, one repeat every 240 and 430 kilobase pairs respectively. The isolation of further $(GA)_n$ clones is continuing and we intend to create a dense map of SSLPs which will serve as a resource for the rapid mapping of new mutations, and provide a set of STS markers for anchoring yeast artificial chromosomes.

1. E. A. Ostrander et al (1992). Proc. Natl. Acad. Sci. USA 89, 3419
2. K.F. Manly and. R.W. Elliot (1991). Mammalian Genome 1, 123.
3. S. Lincoln et al (1992). Whitehead Inst. Tech. Report, 3rd ed.

Progress in Transposon Tagging and Physical Mapping.

Caroline Dean, Ian Bancroft, Francois Belzile, Anuj Bhatt, Paul Jarvis, Clare Lister, Karina Love, Tania Page, Renate Schmidt and Jo West.
AFRC, IPSR, Cambridge Laboratory, John Innes Centre, Norwich, U.K.

An effective transposon tagging system using derivatives of the maize transposons *Ac* and *Ds* has been developed in Arabidopsis. The transposition frequency of these elements in Arabidopsis has been increased both by modifying the elements and isolating Arabidopsis mutants. Over 1500 families (of the Landsberg *erecta* ecotype) carrying transposed elements have been screened for segregating mutations. Over 30 mutations have been identified. Currently, we know that two are tagged with either *Ac* or *Ds* - *drl1* (deformed roots and leaves, no inflorescence produced) and *dif1* (determinate inflorescence, reduced fertility) and two others -- *wlc1* (waxy leaves, cotyledons furled back) and *pcm1* (leaves initially wild-type, but turn pale green after *ca.* 3 weeks growth on culture medium) -- are at least closely linked to a *tDs* and therefore are likely to be tagged. These and other mutations will be described and the current status of the frequency of tagged mutations presented.

We are also involved with the physical mapping of the Arabidopsis genome. Our efforts have focused on chromosomes 4 and 5. YAC contigs have been identified around RFLP markers mapping to these chromosomes and a combination of approaches is being used to join these contigs. One involves the identification and use of new RFLP markers mapped using recombinant inbred lines generated from a cross between Landsberg *erecta* and Columbia. The second involves chromosome walking experiments. Currently, there is >80% YAC coverage of chromosome 4 and the top half of chromosome 5. These YAC contigs are being integrated with the Goodman cosmid contigs. In preparation for large scale genomic sequencing, cosmid clones covering a 1500kb of chromosome 4 are being identified (in collaboration with the groups of H. Goodman, MGH Boston and C. Cobbett, University of Melbourne) and restriction maps constructed.

Isolation of a *MALE STERILITY* gene using a two element *En-I* transposon tagging system

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Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Postbus 16, 6700 AA, Wageningen, The Netherlands.

We are using the non-autonomous counterpart of the maize *Enhancer* or *Suppressor-mutator (En)* element, namely the *Inhibitor* or *defective Spm (I)* element, for transposon tagging in Arabidopsis. Transformation of Arabidopsis with a T-DNA construct containing both a CaMV 35S promoter controlled immobile *En*-transposase source and a transposable *I*-element without selection marker has yielded a transgenic plant containing several copies of the T-DNA with a high frequency of transposition of *I*-elements. These continue to transpose over at least four subsequent generations without detectable loss.

In the T_3 generation of the transgenic plant a recessive male sterile mutant producing no pollen was detected. One of the few obtained selfed progeny plants (terminal flowers produce some pollen) displayed variegation for male sterility, suggesting transposon tagging of a gene we called *MALE STERILITY 2*. In outcross F_2 progeny the mutant phenotype segregated closely with one *I*-element. Transposon flanking DNA was obtained by Inverted PCR and used as a probe to isolate the corresponding cDNA from an Arabidopsis flower cDNA library. The transposon turned out to be inserted in the 3' part of the translated region of the cDNA. By sequencing empty donor sites of various revertant and male sterile progeny without transposase genes we could demonstrate a strict correlation between male sterility and excision footprints leading to reading frame aberrations. Fertility was always correlated with either wildtype DNA sequence or a minor change in amino acid composition. This analysis proved that insertion of the *I*-element in the cloned gene had indeed caused the male sterile phenotype. Characterization of the gene is still ongoing in our laboratory, as is the study on the behaviour of the transposon tagging system in Arabidopsis.

Large scale sequencing of *Arabidopsis thaliana* var. Columbia cDNAs and generation of expressed sequence tags. Thomas C. Newman and Christopher Somerville. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing MI. 48824-1312.

With the progress in automated fluorescent sequencing and the access to fast homology algorithms it is possible to identify the functions of cDNAs by sequencing a portion of the clone. Our initial goal is to generate Expressed Sequence Tags (ESTs) (Adams *et al.* 1991. Science 252,1651-1656) from the 5' ends of directionally cloned Arabidopsis cDNAs and distribute the data to dbEST and make the clones readily available to all researchers through the Arabidopsis Resource Center (ARC). The collaboration of 10 laboratories at MSU and Ernie Retzel's biocomputing group at the U. of Minnesota has identified >400 cDNA homologs (BLASTX score >80) to genes from a wide spectrum of organisms. The choice of material to make the cDNA was based on the idea to maximize the number of represented mRNAs in our library. The cDNA was made with oligo dT primed mRNA derived from aerial and rosette material from staged plants (grown under 2 different light regimens) as well as etiolated seedling and tissue culture grown roots (both harvested at 5 and 7 days). The library (λPRL-2) is constructed as Sal-Not directional inserts in λZipLox. This vector has a Cre-Lox mediated autoexcision of the cDNA containing plasmid for easy and efficient isolation of DNA for sequencing. The cDNA insert sizes range from 400 to > 2000 bases with a number of full length clones identified. The λPRL-2 and associated bacterial strains are available from the ARC. About 40% of the clones sequenced have signifcant homology to known gene products. Redundant sequences have not yet become a problem but with an estimate of 20,000 unique cDNAs to sequence, the following observations suggest that the question of redundancy must be addressed. Redundant ESTs include: 27 CAB ESTs, (10 different genes); 10 Rubisco ESTs (3 different genes); 8 Cytochrome P-450 ESTs (4 different genes). We are in the process of generating a "normalized" library (Sankhavaram *et al.* 1991. PNAS 88,1943-1947) where the most abundant product is only twice as abundant as the least. 25% of the ESTs had homologies to non-plant entries, and another 50% had similarity to plants other than *Arabidopsis thaliana* in the NCBI non-redundant database. Interesting ESTs include homologs to: hemoglobinase, cyc-7, laminin receptor, acyl-CoA binding protein, thaumatin, proteosomes, placental protein 15, prohibitin, zeta-crystallin, and "T-cell specific protein".

SPECTRUM OF INSERTIONAL MUTAGENESIS IN *ARABIDOPSIS* USING *AGROBACTERIUM*-MEDIATED ZYGOTIC EMBRYO TRANSFORMATION METHODOLOGY. R.S. SANGWAN, I. VELU, P. COBANOV, B. VILCOT, Y. BOURGEOIS and B.S. SANGWAN-NORREEL. Université de Picardie Jules Verne, Androgenèse et Biotechnologie (AEB), 33, rue Saint-Leu, 80039 AMIENS Cédex, FRANCE

The T-DNA of *Agrobacterium*, transferred and normaly integrated stably at low copy numbers into the genome of *Arabidopsis*, provides a molecular tool to "tag" genes. Several T-DNA mutants have recently been obtained using both *in situ* seed infection and *in vitro* leaf/root transformation techniques. Employing the zygotic embryo transformation protocol in *Arabidopsis* and using "binary" and "gene fusion vectors" we have generated more than 5000 independent transformants. Seeds of 1400 R_1 and R_2 families were germinated in large petri-dishes to identify early (at seedling stage) and late (mainly aerial and floral stages) developmental mutants. Our current genetic and molecular analysis of over 1000 transformants have shown : 1- low frequency (<5%) of tissue culture induced somaclonal variation, particularly tetraploidy, 2- generally a low copy number (1 to 5) of T-DNA inserts, 3- about 50% of these mutants fall into the previously described categories of mutants i.e. from embryo lethal to flowering abnormalities, as described by Feldman 1991, Koncz et al 1992. 4- However, many of the developmental mutants (about 20%) did not fit into any of the classes listed before. In this meeting we will present the analysis of only three of these mutants e.g. a) all lanceolated leaves with abnormal flowers, b) short hypocotyl, and c) fasciated-stem with abnormal inflorescences.

Poster Abstracts

(Holiday Inn Ballroom)

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Exhibitors

Arabidopsis Biological Resource Centers
Conviron
Environmental Growth Chambers
IN/US Systems, Inc.
Lehle Seeds
Life Technologies GIBCO/BRL
Percival Manufacturing Company

001

Analysis of a nitrilase gene family in Arabidopsis

Bonnie Bartel and Gerald R. Fink
Whitehead Institute for Biomedical Research, Cambridge, MA 02142

Auxins are phytohormones that affect many plant processes, including gravitropism, cell elongation, and apical dominance. Although the pathway of auxin biosynthesis has not been definitively determined in any plant, it is thought that indole-3-acetonitrile (IAN) may be the immediate precursor of the most abundant auxin, indole-3-acetic acid (IAA), at least in the Brassicaceae. We are using transgenic plants to examine this hypothesis. A cDNA that encodes an activity that hydrolyzes IAN to IAA was recently cloned from Arabidopsis (Bartling *et al.*, 1992, *Eur. J. Biochem. 205*, 417–424). Using this cDNA as a probe, we cloned four nitrilase (*NIT*) genes from Arabidopsis genomic and cDNA libraries. *NIT1*, *NIT2*, and *NIT3* are arranged in a tandem array on chromosome III, and encode proteins that are ~85% identical to each other. *NIT4* is ~65% identical to the other members of the family. Northern analyses using gene-specific probes indicate that the nitrilase genes are differentially regulated. Transgenic plants containing promoter-reporter gene fusions are being constructed to further examine these differences. We are also making transgenic plants that over-produce the various nitrilase cDNAs in the sense and antisense orientations. Quantitation of IAA and IAN in the resultant plants will provide evidence as to whether IAN serves as a precursor to IAA. In addition, morphological phenotypes of the transgenic plants may provide insight into the regulation of this important hormone in Arabidopsis.

002

Arabidopsis Root Tropism's: A Model for Plant Cell Signalling and Growth.

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Root tropisms provide an attractive model to study how a variety of environmental signals, such as gravity, are perceived and communicated by the plant to modify its growth and development. Plant roots perceive the gravity vector using specialised columella cells, that sense the sedimentation of intracellular starch filled plastids, termed statoliths, and signal this information to the cells of the elongation zone. Numerous physiological experiments have suggested gravisignalling roles for various growth regulators, notably auxin, during a tropic response (Evans (1991) Plant Physiol., **95**, 1-5). A genetic approach in *Arabidopsis* has generated a number of agravitropic mutations, including ***aux1***. The nature of the ***aux1*** mutation suggests that the ***Aux1*** gene product functions specifically in the hormonal regulation of root gravitropism (Klee and Estelle (1991) Ann. Rev. Plant Physiol. Plant Mol. Biol., **42**, 529-551). To obtain a greater understanding of the tropic signalling pathway(s), we have screened T-DNA mutagenised *Arabidopsis* populations for mutants defective in their root tropic behaviour. One mutant is allelic with *Aux1*. A number of other mutants feature an impaired gravitropic response, affecting both the rate of gravitropic curvature and root growth. The poster will discuss the morphological, physiological and molecular characterisation of the various T-DNA induced agravitropic mutants.

003

PROTEIN KINASES WITH CALMODULIN-LIKE DOMAINS FROM *ARABIDOPSIS THALIANA*.

Brad M. Binder, Estelle M. Hrabak, John S. Satterlee, Jeffrey F. Harper[1] and Michael R. Sussman. Department of Horticulture, Univ. of Wisconsin, 1575 Linden Dr., Madison WI 53706-1590 and [1]Scripps Research Institute, Dept. of Cell Biol., 10666 N. Torrey Pines Rd., LaJolla, CA 92032.

A. thaliana contains a family of genes encoding calcium-dependent protein kinases (CDPK). These kinases contain a catalytic domain linked via a junction domain to a carboxy-terminal calmodulin-like regulatory domain. Several cDNA and/or genomic clones that represent isoforms of this protein kinase family have been identified and are designated AK-1 (*Arabidopsis* Kinase-1), AK-2, AK-3, AK-4 and AK-5. The predicted amino acid sequences of AK-1 and AK-2 are >90% identical except in the first ~130 amino acid residues preceding the catalytic domain, which are highly diverged. The predicted amino acid sequences of AK-3, AK-4 and AK-5 are <90% identical to AK-1.

We are further characterizing the biochemical properties of a full-length AK-1 protein which has been expressed and purified as a fusion protein in *E. coli*. Using syntide-2 as substrate, AK-1 was stimulated by calcium up to 50-fold and further stimulated by specific lipids another 2-5 fold. The maximal specific activity was ~400 nmole/(min·mg) at 20°C. Stimulatory lipids included the phosphoinositides (PI, PIP, PIP_2), lysophosphatidylcholine and lysophosphatidylinositol, but not other lipids such as phosphatidylcholine, phosphatidylserine or phosphatidyl-ethanolamine. Interestingly, AK-1 was potently inhibited by poly-L-lysine with an IC_{50} of ~2nM. Lipid reversed this inhibition and increased the IC_{50} to ~340 nM. Thus, one effect of lipid on AK-1 may be to alleviate the inhibition produced by highly basic protein substrates.

004

CHARACTERISATION OF SUPER ROOT, AN AUXIN-OVERPRODUCING *ARABIDOPSIS* MUTANT

Wout Boerjan[1], Walter Dewitte[2], Harry Van Onckelen[2], Marc Van Montagu[1] and Dirk Inzé[1]
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Unravelling the mechanisms by which auxin influences a diverse array of cellular and developmental processes greatly depends on the isolation and characterisation of mutants. Modulation of the level of free auxin seems one important mechanism to control different auxin-dependent processes. We have isolated an *Arabidopsis* mutant that displays several characteristics reminiscent to auxin effects. This mutant, called Sur (SUPER ROOT), develops excess lateral roots. Up to four days after germination, the Sur mutant develops as a WT seedling. Then, the hypocotyl elongates up to twice the length of a WT hypocotyl. Furthermore, the coherence of the cortical and epidermal cells at the base of the hypocotyl disintegrates, a process which occurs concomitantly with the development of numerous adventitious roots by the hypocotyl. The aerial parts of the mutant are underdeveloped and no inflorescence is produced. The mutant phenotype can be mimicked by incubating four-day-old WT seedlings on auxin-containing medium. Root explants of Sur mutants can be propagated independently of exogenously applied phytohormones and can be induced to produce flowering shoots upon incubation on cytokinin containing medium. In contrast, WT roots require a cultivation period on auxin- prior to cytokinin-containing medium in order to develop shoots. Analyses of endogenous phytohormone levels indicate elevated auxin levels.

005

Isolation and Characterization of *Arabidopsis* Mutants Resistant to IAA-phenylalanine.

James J. Campanella and Christopher D. Town, Biology Department,
Case Western Reserve University, Cleveland, OH 44106.

Almost all the indole-3-acetic acid (IAA) in plants is found not as the free acid, but conjugated either to carbohydrates through ester linkages or to amino acids through amide linkages. In *Arabidopsis* seedlings the amounts of free, ester-conjugated and amide-conjugated IAA are approximately 0.029, 0.18 and 17.1 ug per gram fresh weight respectively. The levels of free and conjugated IAA in different circumstances are presumably controlled by the relative activities of the enzymes which form and hydrolyze IAA conjugates. In order to identify genes involved in auxin conjugate hydrolysis, we have selected mutants resistant to growth inhibition by IAA-phenylalanine. Mutagenized seed were plated on agar containing *Arabidopsis* nutrient salts supplemented with 150-250 uM IAA-phenylalanine. After 3-4 weeks' incubation, healthy individuals from among the dying population were transferred to non-selective conditions and allowed to set seed. The resulting seed families were re-tested for resistance to IAA-phenylalanine using the root inhibition assay. Resistant mutants were tested for their response to free IAA and those resistant to IAA-phenylalanine but sensitive to free IAA were retained for genetic and phenotypic analysis. Mutants were obtained from EMS- neutron- and T-DNA-treated seed. Segregation data shows that the primary resistant mutant isolated from the T-DNA population is an embryo lethal, but preliminary evidence suggests that the conjugate resistance phenotype is segregating from the kanamycin resistance and embryo lethality. Experiments are in progress to determine whether the conjugate resistance mutation is tagged with a cryptic DNA fragment. Mapping of the mutant loci and characterization of the other strains is in progress.

006

An Arabidopsis Mutant that Initiates Large Numbers of Lateral Roots in the Absence of Exogenous Auxin

John L. Celenza, Paula Grisafi and Gerald R. Fink, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.

The auxin indole-3-acetic acid (IAA) can regulate plant growth by initiating cell division. In Arabidopsis roots, exogenous IAA specifically initiates cell division in a tissue layer called the pericycle. These localized areas of cell division are lateral root primordia, which upon removal of IAA will develop into lateral roots. If the IAA concentration is maintained these lateral root primordia accumulate along the root as tumorous growths.

In order to study this hormonal regulation of cell division we have taken a genetic approach in which we have identified mutants that have increased numbers of lateral roots in the absence of exogenous IAA. One mutant in this class, *alf3* (for *a*berrant *l*ateral root *f*ormation), initiates large numbers of lateral root primordia that usually do not continue to develop into mature lateral roots. *alf3* plants maintained in an optimum growth environment fail to develop extensive root systems and instead have several short roots that are covered with undeveloped lateral roots. Presumably because of this underdeveloped root system the mutant plants have small rosettes, flower early, and are anthocyanic. We are further characterizing this mutant by monitoring the expression of genes that control the cell cycle such as *CDC2A* and *CDC2B*, as well as an auxin-induced gene. Preliminary results suggest that *CDC2B* transcription is several-fold higher in *alf3* roots as compared to wild-type roots. Long term goals include determining if the defect in *alf3* is in the production or response to endogenous IAA, or if the mutant has an IAA-independent defect in cell division.

007

EFFECTS OF SUCROSE ON *ARABIDOPSIS* SEEDLING GROWTH

Mee-Rye Cha & Roger P. Hangarter, Department of Plant Biology, The Ohio State University, Columbus, Ohio 43210

Sucrose has frequently been included in agar growth media used for *Arabidopsis* research. However, it's effect on seedling development has not been well characterized. Therefore, we compared growth of *Arabidopsis* seedlings on MS media with or without 2% (w/v) sucrose. Germination was delayed by 6 to 8 hrs in the presence of sucrose in light or darkness. In darkness, sucrose resulted in inhibition of hypocotyl elongation and hook opening was delayed. However, sucrose promoted radial growth of the hypocotyl, cotyledon expansion, and root growth. In blue light, sucrose did not inhibit hypocotyl elongation in wild type but it did reduce elongation in the *blu1* mutant, which lacks blue light responses. In red light, hypocotyl elongation was inhibited by sucrose in both genotypes. Light-grown, wild-type seedlings produced as much as 8 times more anthocyanin in the presence of sucrose than in the absence of sucrose. Glucose caused similar effects to sucrose but mannitol only caused a slight reduction in hypocotyl elongation. Therefore, most of the effects of sucrose were not a result of osmotic stress.

008

ANALYSIS OF ETHYLENE MEDIATED GENE EXPRESSION IN *ARABIDOPSIS THALIANA*. Qianhong G. Chen and Tony Bleecker. University of Wisconsin, Department of Botany, Madison, Wisconsin 53706.

Many ethylene responses in plants are mediated in part at the level of gene expression. One class of genes that are known to be induced by ethylene treatment are members of the pathogenesis-related (PR) gene families. We have examined one such system in Arabidopsis by comparing the expression patterns of the endogenous basic chitinase (chit-B) gene of Arabidopsis (Samac et al., 1990) with a heterologous reporter gene consisting of the promoter of the basic chitinase gene from bean (Broglie et al., 1989) fused to the coding sequence of the bacterial glucuronidase gene (chit-GUS). In transgenic Arabidopsis carrying the heterologous reporter gene, transcripts for both the endogenous chit-B and the chit-GUS reporter showed constitutive expression in root and ethylene-inducible expression in inflorescence stems. In contrast, expression of chit-B was not induced by ethylene in rosette leaves whereas the chit-GUS was. Dose response analysis of GUS expression in stem indicated wounding of stem tissue caused a shift in sensitivity of stem tissues to ethylene of up to two orders of magnitude. We suggest that the responsiveness of the chit-B gene to ethylene in stem but not in leaf tissue may be indicative of a generally stronger defense response in stems relative to leaves of Arabidopsis. If true, this may have implications for the use of Arabidopsis as a model system for plant pathogen interactions.

1. Broglie, K.E., Biddle, P., Cressman, R., and Broglie, R. (1989) The Plant Cell 1, 599-607.
2. Samac, D.A., Hironaka, C.M., Yallaly, P.E., and Shah, D.M. (1990) Plant Physiol. 93, 907-914.

009

The casein kinase II beta subunit homologue from *Arabidopsis thaliana*

Margaret A. Collinge and John C. Walker
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Casein kinase II is a serine threonine protein kinase that occurs in all eukaryotes. In animals, its substrates include proteins involved in gene expression and protein synthesis and components of signal transduction pathways. In plants, casein kinase II has been shown to phosphorylate the Arabidopsis G-box binding factor, GBF1, stimulating its DNA binding activity, and nuclear lamin proteins. The enzyme from most sources is composed of two alpha and two beta subunits. The alpha subunit is catalytic; the beta subunit is phosphorylated by the alpha subunit and considered to have a regulatory function. The enzyme has been purified from a number of plant sources and alpha subunits have been cloned from *Zea mays* and *Arabidopsis thaliana*. To date there is no evidence for a beta subunit in plants.

In the yeast *Saccharomyces cerevisiae*, two catalytic subunits alpha and alpha' are encoded by *CKA1* and *CKA2*. Strains carrying disruptions of both genes are not viable. We expressed an *A. thaliana* cDNA library in a *S. cerevisiae cka1Δ cka2Δ* haploid strain which carries a temperature sensitive *cka2* allele on a plasmid. This strain (kindly provided by Dr. C. V. C. Glover, University of Georgia) grows normally at 30°C but does not grow at 37°C. A cDNA clone was isolated that allowed the strain to grow at the restrictive temperature. The C-terminal portion of the protein encoded by this cDNA has a high degree of homology to casein kinase II beta subunits.

Our current work is concentrated on isolating and sequencing the full length cDNA of this putative beta subunit and determining whether it suppresses the *cka2* temperature sensitive allele independently or in cooperation with the mutant protein.

010

Cytokinin regulation of anthocyanin accumulation in *Arabidopsis thaliana.*

Jill Deikman, Philip E. Hammer and Michelle L. Kneissl, Department of Biology and Biotechnology Institute, Pennsylvania State University, University Park, PA 16802

Cytokinins influence many aspects of plant growth and development from cell division and organogenesis to chloroplast development, apical dominance and senescence. Cytokinins and light have similar effects on a number of plant developmental processes. Despite the importance of cytokinins to plant development very little is known about how they act in plant cells or about their role in light-regulated development. We are studying cytokinin induction of anthocyanin accumulation in *Arabidopsis thaliana* in order to address these questions. Seedlings grown in the presence of 0.1 mg/l benzyladenine for 12 days accumulate about 37 times more anthocyanins than do controls grown without exogenous hormone. Chalcone synthase is a key enzyme for anthocyanin biosynthesis. We have found that treatment of Arabidopsis plants with exogenous cytokinins causes increased chalcone synthase mRNA accumulation, and we are studying the mechanism of this regulation. Chalcone synthase gene expression is also regulated by light and comparison of cytokinin and light regulation of chalcone synthase gene expression will permit us to focus on how cytokinins and light interact in regulating expression of this gene. Alterations in anthocyanin accumulation are relatively easy to detect and can be used for mutant screens. We have identified a mutant that produces more anthocyanins than wild-type plants in response to exogenous cytokinins but which produces normal amounts of anthocyanins in the absence of exogenous cytokinins. We are in the process of characterizing this mutant and of screening for additional mutants with altered ability to produce anthocyanins in response to cytokinins. These mutants will be valuable for studying steps in the cytokinin response mechanism.

011

VIDEO DIGITIZER ANALYSIS OF SHORT-TERM RESPONSES OF ARABIDOPSIS ROOTS TO AUXIN: COMPARISON OF WILD TYPE AND AUXIN RESISTANT MUTANTS.
Michael L. Evans,* Hideo Ishikawa,* and Mark A. Estelle**, Department of Plant Biology, The Ohio State University, Columbus, Ohio 43210* and Department of Biology, Indiana University, Bloomington, Indiana 47405**

We modified a video digitizer system to allow short-term high resolution measurements of root elongation in intact seedlings of *Arabidopsis thaliana*. We used the system to measure the kinetics of promotion and inhibition of root elongation by applied auxin and to determine the dose response relationship for auxin action on elongation in roots of wild type and auxin resistant mutant seedlings. Low concentrations (ca. 10^{-11} M) of auxin induced substantial promotion of root elongation in the wild type and in the mutants axr2 and aux1-7 but not in axr1-3. Roots of all three mutants maintained more rapid rates of elongation in the presence of high concentrations of auxin than did roots of the wild type. Because the auxin dose response curves for axr2 and aux1-7 showed peak promotion of elongation over the same concentration range as in the wild type, it appears that the mutations do not alter the affinity of the auxin receptor.

012

MOLECULAR GENETIC ANALYSIS OF ABSCISIC ACID SIGNAL TRANSDUCTION IN ARABIDOPSIS,
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Abscisic acid (ABA) regulates many aspects of plant growth and development, including embryogenesis, water relations and tolerance of a variety of environmental stresses. Although many ABA effects are well-documented, the chain of events between recognition and cellular response remains obscure. We have used a genetic approach to identify elements of the signal transduction pathway(s), working with previously isolated ABA insensitive (*abi*) mutants (alleles of *ABI1*, *ABI2* and *ABI3*) and isolating mutants at previously unidentified loci. Our long term goal is to understand the biochemical function of these gene products. Therefore, our mutant screening has focused on populations mutagenized by techniques that should cause physical rearrangements of the genome, thereby simplifying cloning of the affected genes. From an M2 population produced by gamma-irradiation we have isolated mutants in at least three new loci. In addition, we have screened pools comprising over 12,000 T-DNA insertion lines (produced by Ken Feldmann, available through the Arabidopsis Biological Resource Center at Ohio State and by agreement with Du Pont) and identified eight mutants affecting at least two additional loci. We are in the process of cosegregation analysis to determine if any of these *abi* mutants contain T-DNA insertions. Further genetic and physiological characterization will be described.

013

ISOLATION AND CHARACTERIZATION OF NPA RESISTANT AND AGRAVITROPIC MUTANTS OF *ARABIDOPSIS THALIANA*.

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We have used a variety of screening techniques to isolate *Arabidopsis* mutants exhibiting defects in auxin sensitivity and auxin-mediated responses. We have isolated four mutants resistant to the phytotropin naphthylphthalamic acid (NPA) by screening T-DNA-mutagenized stocks for growth in the presence of 1.7×10^{-5}M NPA. Roots of these mutants show abnormal elongation and/or thigmotropism in the presence of NPA. We have also screened T-DNA-mutagenized populations for abnormal root gravitropic responses. We have isolated seven mutants with reduced root gravitropism, and have characterized the behavior of two of these mutants in gravitropism, hormone resistance and wavy growth assays (for assay see K. Okada and Y. Shimura, Science 250: 274 - 250). One mutation exhibits a pleiotropic phenotype including abnormal waving growth, reduced root length and plant stature, cotyledon and leaf bleaching, delayed senescence and reduced seed set. Some of these defects can be partially corrected by growth conditions. This mutation is semi-dominant and is likely to be tagged. A second mutant is defective in gravitropism of root and hypocotyl and in waving. A third mutant (*rgr-1*) is described separately (see poster by C.R. Simmons *et. al.*). Three additional mutants are deficient in starch synthesis.

014

Gibberellin is essential for development of floral organs in the *ga1* mutant of *Arabidopsis thaliana*.

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A gibberellin-deficient dwarf mutant, *ga1-1* (NG5) of *A. thaliana*, produces only undeveloped floral organs (petals, stamens and stigma; sepals are normal). Growth of the petals is arrested, being scaly and translucent. Stamens are abortive since filaments do not elongate, nor is pollen formed. The stigma is defective, and its papillae remain underdeveloped. However, when pollinated by wild-type pollen, a small number of seed do set, and ovules grow normally.

The above defects of the floral organs could be completely eliminated by gibberellin (GA) treatment, allowing good seed set by self-pollination. The most effective GAs were, in order of efficacy:2,2,-dimethyl GA4 > GA4 ≥ GA7 ≥ GA3 > GA5. If GA4 is used, normal development was obtained for filaments and pollen (0.1 to 1 ng), petals (0.1 ng), Stigmata (0.01 ng). However, for the flower to develop normally, the floral buds do require a continuous (weekly) supply of the GA.

We thank Dr. M. Koornneef for providing us with the *ga1-1* mutant, and Prof. L. N. Mander for provision of the GAs.

015

Genetic and molecular characterisation of the gibberellin-insensitive (*gai*) locus of *Arabidopsis*.

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The *gai* mutant of *Arabidopsis* displays a phenotype similar to that of gibberellin (GA) deficient plants, being dwarf, dark green, having reduced apical dominance, and exhibiting a delayed flowering time in short days. Unlike the GA deficient mutants, *gai* is insensitive to the effect of externally added GAs and contains high levels of endogenous active GAs. These properties suggest that *gai* identifies a locus that modulates the response of plant cells to GA. We have isolated γ-irradiation induced apparent derivative alleles of *gai*. Since these alleles confer a wild-type phenotype we suggest that *gai* is a gain-of-function mutation affecting the responses of the plant to GA, and that the wild-type allele (GAI) is dispensible. *gai* maps close to *ga4* (a GA deficiency mutation) which lies between RFLP markers m219 and g2395 on the top arm of chromosome 1. We are using these RFLP markers to isolate yeast artificial chromosomes (YACs) covering the *gai-ga4* region. Since the *gai*-derivatives alleles confer a wild-type phenotype it is likely that a transposon insertion inactivated allele will also confer a wild type phenotype. We have attempted to generate such alleles by transposition of a Ds element located approximately 10cM distal of *gai* on chromosome 1. A Ds insertion mutant allele will enable us to clone the *gai* locus. Recent progress in this area will be described.

016

Isolation and characterization of a T-DNA tagged auxin-resistant mutant

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An auxin-resistant mutant of *Arabidopsis* with defects in gravitropism was isolated from the collection of T-DNA transformed lines at DuPont. A second gamma-ray-induced allele with an identical phenotype was recently identified. Genetic analysis shows that these mutants define a new locus involved in auxin response, called *axr4*. Both alleles are recessive. The mutations confer resistance specifically to auxin as the mutant plants show essentially wild-type levels of sensitivity to other plant hormones. The AXR4 gene maps very close to the *ch1* marker near the centromere of chromosome 1. Using the T-DNA insertion as a molecular tag, the AXR4 gene is being cloned. Characterization of this gene at the molecular level should give new insights into the molecular mechanism of auxin action.

017

Molecular and Genetic Analysis of the Constitutive Ethylene Response Mutants

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The simple gas ethylene influences a diverse array of plant growth and developmental processes, including germination, senescence, epinasty, abscission and fruit ripening. The molecular mechanisms regulating biosynthesis and perception of this or any plant hormone are poorly understood. We have used a response of dark-grown seedlings to ethylene, the "triple response", as a model system to isolate mutants affected in ethylene biosynthesis and perception. This response is extremely amenable to genetic analysis due to its high reproducibility and ease of screening very large numbers of seedlings. We and others have used this response to identify mutants that are insensitive to ethylene (Bleecker *et al.*, Science **241:** 1086-1089, 1988; Guzman and Ecker, Plant Cell **2:** 513-523, 1990). A second class of mutants that constitutively display this response have also been isolated (Kieber *et al.*, Cell **72:** 427-441, 1993). These mutants either overproduce ethylene (Eto) or affect ethylene signal transduction (Ctr). All of the latter mutants are recessive and fall into a single complementation group called *ctr1*. The *ctr1* mutation has profound effects on the growth and development of both seedlings and adult plants. The gene corresponding to the *ctr1* mutation was cloned and found to encode a protein that resembles the Raf family of serine/threonine protein kinases. The putative CTR1 kinase is postulated to act as a negative regulator in the ethylene signal transduction chain. We have raised antibodies against *E. coli*-expressed CTR1 and are also using the baculoviral system to express this protein. We have generated transgenic *Arabidopsis* expressing the whole CTR1 protein and also the kinase domain alone under the control of the 35S promotor, as well as fusions of the CTR1 promotor to the GUS reporter gene. Work in progress using the antibodies, purified protein and transgenic plants will be discussed.

018

A non-dormant *Arabidopsis* mutant which is sensitive to ABA

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One approach to understanding the molecular and biochemical basis of ABA action in higher plants has been the isolation and characterization of mutants of *Arabidopsis* that germinate in the presence of exogenously supplied ABA. Mutations in 3 loci (designated *abi*) all reduce dormancy suggesting ABA is a key player in the establishment of this physiological condition. This screen necessarily identifies ABA insensitive mutants that turn out to be non-dormant, however, it will not identify non-dormant mutants which are ABA sensitive.

Wild-type *Arabidopsis* seeds are 100% dormant for 25 days after pollination. We screened a population of immature M2 seeds (generated from 6000 M1) for mutants that would germinate within 2 days of imbibition. Of the 20 mutants generated in this screen one, designated 10-2, has been further characterized. It appears to have normal levels of ABA and does not germinate in the presence of 3 μM ABA. The 10-2 mutant seed also has reduced seed storage protein accumulation, is desiccation intolerant and has reddish purple cotyledons. A double mutant of 10-2 and abi3-3, a mutation with similar phenotypes which is insensitive to exogenous ABA, suggests 10-2 operates in a different developmental pathway than abi3-3. The double mutant embryo is black, highly non-dormant and insensitive to ABA. Double mutants of 10-2 and other ABA mutations are currently underway to determine genetic relationships. To our knowledge, this is the first report of a highly non-dormant mutant of *Arabidopsis* that still synthesizes and retains it sensitivity to ABA.

019

Genetic and Molecular Analysis of the Hookless Mutants in *Arabidopsis thaliana*

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The "triple response" in *Arabidopsis thaliana* occurs in response to the plant hormone ethylene and is characterized by three distinct changes in the morphology of etiolated seedlings. These include, exaggeration of the apical hook, radial swelling of the hypocotyl, and inhibition of root and hypocotyl elongation. One class of mutants in this response is the hookless mutants. Mutations at two different loci have been identified. These mutants lack an apical hook in air and exhibit different hook responses in the presence of ethylene. Characterization and phenocopy of these mutants has shown auxin to be important for hook formation. The *hookless1* (*hls1*) mutation is a single recessive mutation (1). The alleles of *hls1* fall into two distinct classes. The more severe class has no bend in the hook in the presence of ethylene; the less severe class exhibits a partial bend in the hook in the presence of ethylene. The *hls3* mutant has no apical hook in the presence of air or ethylene and produces an excess of lateral roots which eventually transform the young plant into a mass of roots. A weak allele of *hls1* (*hls1-2)* was isolated from a T-DNA tagged population and cloned by left-border rescue from the T-DNA which inserted upstream of the coding region. Cloning of the *hls1* gene has been confirmed by examination of two X-ray alleles which each have a deletion in this gene. The steady state level of *hls1* mRNA is increased by ethylene. Sequence analysis of the *HLS1* cDNA has revealed a novel gene. Further characterization of this gene and the HLS1 protein should prove helpful in understanding development of the hook and may provide information about the interaction between ethylene and auxin in differential cell growth processes.

1. Guzman and Ecker. (1990). Exploiting the triple response of *Arabidopsis* to identify the ethylene-related mutants. The Plant Cell 2: 513-523.

020

ABSCISIC ACID SIGNALLING AND CHROMOSOME WALKING TOWARDS THE *ABI*1 LOCUS IN *ARABIDOPSIS THALIANA*

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Abscisic acid, or ABA, plays an essential role in normal seed development and germination, and adaptive response to a diversity of environmental stress during subsequent vegetative growth. To date, the repertoire of components in mediating ABA perception, as well as those in transducing the appropriate physiological signals are only beginning to be defined at the molecular level.

What are some of the components involved in ABA perception and signal transduction? We approach this problem by targetting our efforts towards characterizing and cloning some of the mutations in *A. thaliana* known to confer resistance to toxic levels of ABA at the germination stage (ABA-insensitive mutants: *abi*-1,-2 and -3; Koornneef *et al.* 1984). All of these mutants contain normal content of ABA; also, as their phenotypic defects are pleiotropic and cannot be restored by exogenous supply of ABA, these mutations probably impair some early steps in ABA signal perception or transduction. One of the loci involved in regulating seed development, known as *ABI*3, has recently been cloned in our laboratory (Giraudat *et al.*, 1992). To gain a more comprehensive analysis of the ABA signalling by way of comparisons, we have also initiated the cloning of an other locus, called *ABI*1. The mutant transpires excessively upon drought stress suggesting a defect in water relations. Indeed, recent experiments by others have demonstrated that the expression of several "stress-related" genes are clearly reduced in the *abi*1 mutant.

We are currently extending the analysis of the mutant at the level of both genetics and physiology in order to define the putative functions of *ABI*1 during normal course of plant development and during stress. In parallel, we have also undertaken a chromosome walk towards the locus. Thus far, 13 overlapping YAC clones encompassing at least 500 kb have been isolated. Using ends of the YAC clones as probes and mapping them against restriction polymorphisms (RFLP), we have obtained evidence that the target locus is likely to be contained within the walk. We are in the process of narrowing down the relevant region by further RFLP mapping, assembling this relevant region in the forms of cosmids and phage clones from both wild-type and mutant DNA, which might be suitable towards complementation analysis by plant transformation.

021

GENETIC AND PHYSIOLOGICAL ANALYSIS OF *sar1*, A SUPPRESSOR OF THE *axr1* MUTANTS OF ARABIDOPSIS THALIANA

Cindy Lincoln, Doug Lammer, Alex Cernac, Mark Estelle, Indiana University, Bloomington, Indiana, 47405.

The AXR1 locus encodes a 540 amino acid protein with regions of significant sequence similarity to the ubiquitin activating enzyme, E1. Mutations in this gene result in plants with a series of morphological and physiological abnormalities, all of which are consistent with a decrease in auxin sensitivity or response. We are using genetic approaches to increase our knowledge of the auxin response pathway in which AXR1 acts. *axr1-3* seedlings were mutagenized with EMS and M2 seedlings were screened for restoration of auxin sensitivity using a root elongation assay. Eleven lines, representing a minimum of 3 unique loci, were identified. One of these loci, *sar1* (*s*uppressor of *a*uxin *r*esistance 1) has been characterized genetically and physiologically. *sar1* is a recessive suppressor of the auxin resistant phenotype. It is gene-specific, but not allele-specific in its ability to suppress auxin resistance. *sar1* homozygotes are not auxin supersensitive, as measured by inhbition of root elongation, and show a unique phenotype including small stature, and early flowering. We are currently mapping this mutation as a first step towards walking to the SAR1 gene.

022

Regulation of gene expression by the Arabidopsis ABI3 (abscisic acid insensitive) gene: analysis of mutant and transgenic plants.

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The molecular cascades that mediate the various physiological roles of abscisic acid (ABA) remain largely unknown. Arabidopsis is so far the only species where multiple classes of ABA response mutants have been identified (Koornneef et al., 1984, Physiol. Plant. 61, 377-383). Characterization of these Arabidopsis abi (for ABA-insensitive) mutants and genes thus allows to investigate both the nature and diversity of ABA signalling cascades. Mutations at any of the abi loci (abi1, abi2, abi3) confer an increased resistance to toxic levels of ABA at the germination stage and a decreased seed dormancy. However, the additional phenotypes which have been evidenced in various laboratories, are confined to vegetative tissues in the case of the abi1 and abi2 mutants; in contrast, to siliques and young seedlings for abi3 mutants.

In order to refine the description of the processes controlled by ABI3 in seeds, we studied the effects of mutations at this locus on the expression of putative target genes.The accumulation kinetics of various cloned Arabidopsis mRNAs representative of distinct stages of seed development have been monitored by Northern analysis in siliques of both the wild-type and the more severe abi3-4 mutant allele recently isolated by K.Léon & M.Koornneef. Abundance of the ABI3 transcript has been followed in parrallel using the ABI3 clone that we have isolated (Giraudat et al., 1992, Plant Cell 4, 1251-1261). Transgenic Arabidopsis carrying ABI3 promoter - GUS fusions have been produced to further analyze the temporal and spatial expression pattern of ABI3 throughout the plant life cycle, in particular in tissues where no mutant phenotype has yet been observed. Transgenic Arabidopsis carrying the ABI3 cDNA placed under the control of a constitutive promoter have also been obtained, to study the effect of an ectopic production of ABI3 on the expression of genes normally restricted to seeds.

023

The Role of Ethylene in Floral Organ Abscission in ***Arabidopsis thaliana***

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During development, specific tissues in plants may become detached from the main body of the plant by a developmentally controlled process known as abscission. A well characterized mutation in *Arabidopsis thaliana* which confers ethylene insensitivity (*etr1-1*) delays the normal floral organ abscission process. Abscission of sepals, petals, and stamens occurs within two to three days of anthesis in wild-type plants. In mutant *etr1-1* plants abscission is delayed by an additional two to three days. Treatment of plants with low levels ethylene ($\leq$ 50ppm) hastens abscission in wild-type plants, but has no significant effect on the mutant *etr1-1* plants. In order to more fully characterize this process, we have examined GUS expression patterns in transgenic plants carrying the bacterial glucuronidase reporter gene (GUS) fused to an ethylene responsive promoter (chitinase). Histochemical staining indicates GUS expression is localized to the abscission zone cells of the receptacle tissue. Expression patterns and levels of expression are developmentally regulated and correlate with anthesis and abscission in wild-type plants. Ethylene treatment results in increased levels of GUS expression as well as earlier petal abscission and earlier tissue specific expression of GUS. An anatomical analysis of the floral organ abscission process in response to ethylene and the expression pattern of the GUS reporter gene will be presented.

024

A MOLECULAR APPROACH TO ETHYLENE PHYSIOLOGY IN HIGHER PLANTS

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To decipher some important aspects of plant development, it is necessary to clarify the molecular basis of plant hormone biosynthesis and action. Concerning the plant hormone ethylene two basic questions can be raised: (a) In which way are the molecular mechanisms of ethylene formation controlled in plants?; (b) How is this gaseous hormone perceived and its signal transduced resulting in the well characterized responses? The first question concerning the molecular analysis of ethylene biosynthesis is under study after the cloning of genes encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase), the key regulatory enzyme in ethylene formation, and ACC oxidase. Our group has characterized a genomic clone of one member of the *Arabidopsis thaliana* (L.) Heynh. ACC synthase gene family (*ACS1*). Genomic DNA gel blotting suggested the existence of an ACC synthase multigene family in Arabidopsis. The other members are distantly related to *ACS1*. The existence of at least another gene was confirmed by the isolation of a cDNA (*ACS2*) from a flower specific cDNA library. Expression of the *ACS1* gene was studied by reverse transcription-PCR on total RNA. The mRNA accumulated strongly in young leaves and flowers. Wounding of young leaves did not induce the *ACS1* gene. An ACC synthase promoter-GUS fusion has been made, and provided us some new insights about the temporal and spatial regulation of this gene in Arabidopsis. The expression of the Arabidopsis *ACS1* gene is under developmental control both in shoot and root, confirming results obtained by RT-PCR analysis. Since both ACC and ACC oxidase are present in young tissues a role for *ACS1* in the developmental control of ethylene synthesis is suggested.

025

A Genetic Analysis Of Ethylene Insensitive Mutants In *Arabidopsis thaliana* ; Gregg Roman, and Joseph R. Ecker, Department of Biology, Plant Science Institute, University of Pennsylvania, Philadelphia, PA 19104

We are investigating the role of ethylene signals in the *Arabidopsis thaliana* triple response through mutant analysis. In the presence of ethylene, etiolated *Arabidopsis* seedlings undergo drastic morphological changes. This seedling phenotype consists of an exaggerated apical hook, radial swelling of the hypocotyl, and an inhibition of root and hypocotyl elongation. We have screened large numbers of x-ray and diepoxybutane (DEB) mutagenized M_2 seedlings in search of mutants which are deficient in aspects of the triple response. The mutants found fall into one of two broad classes. The first class of mutants, ethylene insensitive (*ein*), fail to form any aspects of the triple response in the presence of ethylene. Five ethylene insensitive (*ein*) loci have been identified: *ein1*, *ein2*, *ein 3*, *ein4*, and *ein5*. The second class consists of mutants with tissue specific defects in the triple response.

As part of our analysis of ethylene signal transduction, we are attempting to clone the *ein2* locus by map-based strategies. In our screens we have identified five X-ray and five DEB alleles of *ein2*, which should facilitate the positional cloning of this gene. Additionally we have identified two novel *ein* loci: *ein4* and *ein5*. These loci are currently being mapped and genetically characterized. We have also identified a tissue specific mutation which has an elongated root in the presence of ethylene. This mutant, designated *eir* (ethylene insensitive root), is recessive, represented by two alleles, and maps to the bottom of chromosome 5.

026

Characterization of Mutants of *Arabidopsis thaliana* with Altered Responses to Auxin Transport Inhibitors. Max O. Ruegger, Lawrence J. Hobbie, and Mark Estelle. Department of Biology, Indiana University, Bloomington IN 47405.

Auxin transport is believed to be central to the regulation of many developmental processes. However, very little is known about auxin transport at the molecular level. We are using a genetic approach to study auxin transport in *Arabidopsis*. When wild-type *Arabidopsis* seedlings are grown on auxin transport inhibitors (ATIs), both root growth and root gravitropism are inhibited. We have isolated three classes of mutants with altered root responses to ATIs. Mutants of the first class are specifically resistant to the ATI chlorflurenol methyl (CFM). Roots of *cmr1* seedlings elongate and are gravitropic on concentrations of CFM which are inhibitory to wild-type roots. However, *cmr1* seedlings have a wild-type response to the hydrolyzed form of CFM (thought to be the active species) and to other ATIs. This result suggests that the *cmr1* mutation prevents the plant from hydrolyzing CFM to the active species. Mutants of the second class are hypersensitive to inhibition of root growth by several ATIs. At least one locus, *tis1*, has been identified. Mutants of the third class are resistant to ATI inhibition root-growth. These mutants fall into at least three complementation groups, the *tir* loci. Recently, an allele of *tir1* was recovered from a T-DNA-mutagenized population. Preliminary results indicate that a T-DNA insert is linked to the *tir1* locus. The genetic and physiological characterization of the *tir* and *tis* mutants and the molecular characterization of the *tir1* locus should provide insight into the mechanism of auxin transport.

027

MOLECULAR AND PHYSIOLOGICAL ANALYSIS OF AUXIN RESISTANT AND GRAVITROPISM MUTANTS IN *ARABIDOPSIS THALIANA*.

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We have isolated seven mutants with reduced root gravitropism from the DuPont and Ohio State *Arabidopsis* T-DNA mutagenized collections. One mutant, which we call *rgr-1*, for reduced gravitropism, is deficient in both gravitropism and the wavy phenotype. The *rgr-1* roots coil in a clockwise rotation instead of waving as do wildtype roots. By the root growth inhibition assay, *rgr-1* is resistant to exogenous auxins; it has 8-fold resistance to IAA, 4-fold resistance to 2,4-D, and 2-fold resistance to NAA. Hypocotyl elongation is also resistant to exogenous auxin but to a lesser degree. The *rgr-1* mutant has otherwise normal morphology and development. Genetic cosegregation analysis with kanamycin resistance indicates that *rgr-1* is recessive and probably tagged. Results of allelism tests with other auxin resistant and agravitropic mutants will be presented, as will genetic chromosomal mapping of the *rgr-1* locus. Progress on the molecular analysis of the mutant and wild-type genes will also be reported. Three of the other mutants isolated are deficient in starch synthesis, one of which has a non-tagged mutation in ADP-glucose pyrophosphorylase. There are three other agravitropic mutants of unknown physiological defects. (See also abstract Garbers *et al.*).

028

Approaches to determining the function of the auxin resistance gene, AXR1

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The AXR1 gene is defined by several recessive alleles which confer auxin resistance to the roots, rosettes and inflorescences of mutant plants. Additionally, *axr1* mutant plants have several morphological defects which suggest a decrease in auxin sensitivity. The AXR1 gene has recently been characterized and found to have significant similarity to the ubiquitin activating enzyme, E1. However, AXR1 has diverged substantially from E1, since it is approximately half the size of E1 and does not encode certain residues which are critical for ubiquitin activation. We are currently attempting to elucidate the biochemical activity of the AXR1 protein. Initially, the cellular localization of AXR1 will be determined using an antibody directed against a bacterially-produced AXR1 fusion protein. Fractionated cells and various plant tissues are being examined. The AXR1 gene appears to be a member of a gene family. Another member of the AXR1 family has been isolated and the deduced amino acid sequence has diverged from both AXR1 and the *Arabidopsis* E1. The transcription of this gene throughout plant development will be analysed. The AXR1 gene family may define a novel class of proteins which are important in plant growth regulation. Analysis of the AXR1 protein and other AXR1 family members will provide insight into the mechanism of this regulation.

029

Generation of transgenic *Arabidopsis thaliana* plants harbouring *Agrobacterium* tumor-inducing genes.

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The *Arabidopsis* leaf transformation method described by van Lijsebettens et al.(TAG 81, 1991:277-284) was improved on several steps in the protocol. Optimizations were made concerning the growth conditions of the plant source, callus induction prior to infection and seed set, resulting in eventually 2-3 shoots per leaf 4 weeks after infection.
Transgenic *Arabidopsis* plants were generated harbouring the *Agrobacterium tumefaciens* genes *iaaM* and *iaaH*, gene *ipt*, gene 6b and the *A.rhizogenes* gene *rolC* under the control of their natural promoters to study the effect of altered phytohormone content on the morphology of *Arabidopsis*. Also the effect of exogenously applied phytohormones on root development in wild type and transgenic seedlings was tested.

030

TOWARDS THE IDENTIFICATION OF THE MOLECULAR MECHANISM OF AUXIN SIGNAL TRANSDUCTION.

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A reporter construct containing the promoter of the auxin inducible tobacco *NT103* gene fused to the *gusA* (ß-glucuronidase) gene, was introduced into *Arabidopsis thaliana* via cocultivation of roots with *Agrobacterium tumefaciens*. Histochemical analysis showed that the GUS activity was limited to the lateral root cap and the transition region. The GUS activity was enhanced after addition of different auxins or high concentrations of ABA, kinetin, $CuSO_4$, benzoic acid or TIBA. The transgenic plants were used to study the signal transduction pathway leading to the activation of this auxin inducible promoter. To this end genes involved in the regulation of this reporter construct were identified after crossing with different well known *Arabidopsis* (hormone) mutants and after EMS mutagenesis.
Genes homologous to the tobacco *NT103* gene were isolated from *Arabidopsis*. One of these genes was found to encode an enzyme with glutathion S-transferase activity like the tobacco gene. A difference in RNA expression was found between tobacco and *Arabidopsis*. The tobacco *NT103* gene was only expressed in roots while the *Arabidopsis* genes were constitutively expressed in leaves and one gene could be induced by auxins and some other compounds in roots. The genes were sequenced and the expression of the genes in cell-suspensions and in (hormone) mutants of *Arabidopsis* was studied in more detail.

031

STRUCTURE AND REGULATION OF GENES ENCODING PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (POR) IN *ARABIDOPSIS THALIANA*

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The enzyme NADPH-protochlorophyllide oxidoreductase is a key control point for the light-regulated synthesis of chlorophyll in higher plants and the differentiation of etioplasts in dark-grown seedlings to chloroplasts. POR is nuclear-encoded but is localized in plastids, and accumulates massively in etiolated seedlings. Although POR requires light for the conversion of protochlorophyllide to chlorophyllide, enzymatic activity and protein levels decrease rapidly and dramatically upon illumination.

We have identified and characterized cDNAs and genes encoding POR in the angiosperm *Arabidopsis thaliana* to address the question of how higher plants maintain chlorophyll synthesis in the light. A previous study from this laboratory described an *A. thaliana* cDNA encoding POR. Using PCR-based approaches we have now identified and characterized genomic and cDNA sequences corresponding not only to the previously described cDNA (*porB*), but also to a closely related sequence, *porA*. Genomic Southern analysis supports the conclusion that *A. thaliana* contains only these 2 genes encoding POR-related proteins. *porB* and *porA* contain 3 and 4 introns, respectively, in conserved positions. The genes are 83 % identical at the nucleotide level and are predicted to encode polypeptides that are 88 % identical. Both genes are expressed in etiolated seedlings. *porA* mRNA disappears after exposure of the seedlings to white light, however. The regulation of the *porA* and *porB* genes in response to environmental and developmental cues, and the potential physiological roles of the PorA and PorB proteins will be discussed.

032

Translation Initiation in *Chlamydomonas* Chloroplasts

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To better understand translation initiation in chloroplasts, we chose to use *Chlamydononas reinhardtii*, a unicellular eukaryotic green alga, as a model system. With the development of both nuclear and chloroplast transformation, *Chlamydomonas* has become an attractive molecular genetic system to study photosynthesis and the control of chloroplast gene expression. Our strategy for studying translation initiation in *Chlamydomonas* chloroplasts is to introduce mutations that affect translation initiation into a chloroplast gene essential for photosynthesis, and then isolate suppressors, which may help identify components involved in chloroplast translation initiation. We made AUG --> AUC and AUG --> AUU changes in the initiation codon of the *petD* gene, which codes for the subunit IV of the cytochrome b6/f complex. *Chlamydomonas* strains carrying these chloroplast mutations accumulated 10-20% of wild-type levels of subunit IV, which is sufficient for photosynthetic growth at 24°C. At 35°C, the mutants only accumulated trace amounts of subunit IV and were unable to grow phototrophically. Using this photosynthetic temperature sensitivity, we isolated more than 100 suppressor strains from the AUU mutant strain. These suppressor strains showed increased levels of subunit IV synthesis and accumulation compared to the AUU mutant. At least two suppressors have been found to be nuclear genes. More genetic analyses are being carried out to determine the genomic location of the other suppressor mutations and to place nuclear suppressors into complementation groups.

033

Light modulation of *Arabidopsis* PRP gene expression during seedling growth.

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PRPs have been characterized as a family of proline-rich structural cell wall proteins that are differentially expressed during plant growth and development. The cell type-specific expression of individual PRPs and their insolubilization within the plant extracellular matrix is thought to play a critical role in determining cell form and function. Analysis of PRP expression in seedlings has shown that individual gene family members are highly expressed in the actively growing regions of the root and hypocotyl. We are currently using *Arabidopsis* seedlings as a model system for investigating the relationship between PRP expression and factors that influence cell elongation. We have cloned the four PRP genes encoded by *Arabidopsis* and have analyzed the expression of two of these genes during seedling growth, using gene-specific probes. *Arabidopsis* seedlings were grown in darkness for 36 h after which half of the seedlings were transferred to white light. Light- and dark-grown seedlings were then collected every 24 h for 4 d and analyzed for PRP expression, using northern hybridization. AtPRP3 mRNA was detected in light-grown seedlings throughout the time course, while the steady-state level of this transcript decreased over time in dark-grown tissue. In contrast, the steady state levels of AtPRP2 mRNA were not affected by white light. These data indicate that PRP expression is differentially regulated during seedling growth in *Arabidopsis* and that AtPRP3 expression in seedlings is controlled, in part, by light. We are currently investigating the expression patterns of the other members of the *Arabidopsis* PRP gene family in light- and dark-grown seedlings and whether the light responses of different AtPRPs are controlled by phytochrome- or blue light-dependent signal transduction pathways.

034

A MUTATION IN *Arabidopsis thaliana* THAT AFFECTS XANTHOPHYLL COMPOSITION CONFERS SENSITIVITY TO HIGH LIGHT

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Carotenoids, mainly xanthophylls, protect leaves from damaging oxygen radicals that are produced in high light. To study the role of xanthophylls in this process we have isolated light sensitive (*ls*) mutants in *Arabidopsis thaliana*. These mutants demonstrate normal pigmentation when grown under low light intensity (photon-flux density [PFD] of 10 $\mu E.m^{-2}.sec^{-1}$), but become chlorotic when grown at 100 $\mu E.m^{-2}.sec^{-1}$. Leaves of wild-type plants, grown at PFD of 100 $\mu E.m^{-2}.sec^{-1}$, have normal appearance and contain 25% more chlorophyll a and b than leaves of plants that grow at 10 $\mu E.m^{-2}.sec^{-1}$. However, when exposed to light intensity of 500 $\mu E.m^{-2}.sec^{-1}$, a decrease of about 50% in chlorophyll content is observed. In contrast, leaves of the mutant *ls*-15, grown at 10 $\mu E.m^{-2}.sec^{-1}$, contain 50% the amount of chlorophyll as compared to the wild-type at the same condition, but their chlorophyll content decreases when grown under 100 $\mu E.m^{-2}.sec^{-1}$ and they suffer a severe chlorosis at 500 $\mu E.m^{-2}.sec^{-1}$. This chlorophyll bleaching is caused by oxygen radicals that are produced by light. The bleaching is related to mainly chlorophyll b, as can be estimated from the chlorophyll a/b ratio, which increases about two fold in leaves grown at high light. The β-carotene content demonstrated similar pattern of changes in response to illumination conditions. The amount in wild-type plants of the xanthophyll cycle components: violaxanthin, antheraxanthin and zeaxanthin, is higher when grown at high light as compared to low light. Following exposure of leaves of wild-type plants to excessive PFD of 1500 $\mu E.m^{-2}.sec^{-1}$ for 4-6 hours, an increase in antheraxanthin and zeaxanthin was observed as a result of the light-dependent conversion of violaxanthin to zeaxanthin, *via* antheraxanthin. In contrast, leaves of mutant *ls*-15, grown for several days at 500 $\mu E.m^{-2}.sec^{-1}$ contain less violaxanthin and antheraxanthin than the wild-type, but completely lack zeaxanthin. These results suggest that conversion of violaxanthin to zeaxanthin is impaired in mutant *ls*-15 and demonstrate the role of zeaxanthin in excess light-energy dissipation. The segregation in F2 of crosses of wt with *ls*-15 indicated that the mutant phenotype is determined by a single recessive gene.

035

Genetical, physiological and molecular analysis of phytochrome mutants of Arabidopsis.

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Phytochrome is a family of photoreceptors that regulates plant photomorphogenesis. At present there are five defined family members (phytochromes A,B,C,D and E). Recently, Arabidopsis mutants displaying elongated hypocotyls in defined light conditions have been shown to be specifically deficient in one or other of two of these family members, phytochrome A and phytochrome B. We have identified novel mutations at three Arabidopsis loci (*fhy1*, *fhy2* and *fhy3*) which confer an elongated hypocotyl in far-red but not in white light. *fhy2* mutants (obtained following γ-irradiation mutagenesis) are phytochrome A deficient, have reduced or indetectable levels of *PHYA* transcripts and contain structural alterations within the *PHYA* gene. These *fhy2* alleles confer partially dominant phenotypes in far-red light, suggesting that the levels of phytochrome A in wild-type hypocotyls is approaching saturation of the far-red mediated hypocotyl growth inhibition response. Mutants at the other two loci (*fhy1* and *fhy3*) have normal levels of functional phytochrome A, suggesting that the *FHY1* and *FHY3* gene products may be involved with the transduction of the far-red signal from phytochrome A to downstream processes involved in hypocotyl growth regulation. The properties of the *fhy1*, *fhy2* and *fhy3* mutants will be compared with those of other photomorphogenetic mutants of Arabidopsis, including the phytochrome B deficient *hy3* mutants.

036

GENETIC EVIDENCE THAT THE PR FORM OF PHYTOCHROME B MODULATES GRAVITROPISM IN *ARABIDOPSIS*

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Hypocotyls of dark-grown *Arabidopsis* seedlings exhibit strong negative gravitropism, growing away from the gravitational vector in a unidirectional manner. When illuminated with continuous or pulsed red light, growth direction is randomized with respect to the gravity vector. The onset of randomized growth upon exposure to red light is prevented if red light pulses are immediately followed by far-red light pulses, indicating that this red light-dependent response is mediated by phytochrome. Analysis of three phytochrome-deficient mutant strains of *Arabidopsis* (*hy2*, chromophore-deficient; *hy3*, phytochrome B-deficient; *hy8*, phytochrome A-deficient) indicate that the red light-absorbing form (Pr) of phytochrome B regulates normal hypocotyl gravitropism in darkness, and that depletion of the Pr form by photoconversion to the far-red light-absorbing form (Pfr) upon absorption of red light, attenuates hypocotyl gravitropism. These studies provide genetic evidence that the Pr form of phytochrome has an active function in plant development.

037

MEASUREMENTS OF GROWTH DURING PHOTOTROPISM OF *Arabidopsis thaliana* SEEDLINGS

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Changes in elongation rates on two opposite sides of hypocotyls of seedlings of *Arabidopsis thaliana* during phototropism were investigated. Curvature toward the light source was a consequence of an increase in the elongation rate of the shaded side and a decrease in the elongation rate of the lighted side in first positive phototropism, second positive phototropism and in red light-enhanced first positive phototropism. The phase of straightening that followed maximum curvature in each of these responses resulted from a decrease in the elongation rate of the shaded side and an increase of the elongation rate of the lighted side. Based on these data we conclude that for all three types of blue light induced phototropism that we studied here and straightening, distribution of growth along the hypocotyl is consistent with the predictions of Cholodny-Went theory.
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038

UV-B-INDUCIBLE AND CONSTITUTIVE GENES THAT MEDIATE REPAIR AND TOLERATION OF UV-DAMAGED DNA IN *ARABIDOPSIS*. Qishen Pang and John Hays, Department of Agricultural Chemistry, Oregon State University, Corvallis OR 97331.

Cells can repair UV-light damage to their DNA by directly reversing, by (light-dependent) enzymatic photoreactivation, the principal UV photoproduct in DNA, cyclobutane pyrimidine dimers (CPDs), and/or remove CPDs and other photoproducts by excision repair. They may tolerate unrepaired photoproducts by recombinational "filling" of replication-block-induced daughter-strand gaps (sister-chromatid exchange). Plant responses to UV-induced DNA damage are of particular interest, because projected depletion of stratospheric ozone will increase terrestrial UV-B irradiance. We previously showed that whole *Arabidopsis* plants remove CPDs from their genomes primarily by photoreactivation; excision repair is about 5% as efficient. *Arabidopsis* photolyase is developmentally regulated (more activity in older than in younger plants), and inducible by UV treatment of plants.

In order to gain information about the range of DNA-damage-repair/toleration (DRT) activities in *Arabidopsis*, and to obtain *DRT* gene probes for regulatory studies, we recently selected for *Arabidopsis* cDNAs that partially corrected the phenotypes of *E. coli* mutants lacking repair/toleration activities. Of the six cDNAs isolated, three - *DRT100*, *DRT111* and *DRT112* - appear to mediate either strand-exchange or resolution steps of homologous recombination. *DRT101* and *DRT102* may encode UV-specific excision repair activities. Four of the DNA sequences - *DRT100, 101, 111* and *112* - predict chloroplast-targeted proteins.

We now find levels of *DRT100* and *DRT101* mRNA to be increased 3- to 4-fold by UV irradiation of whole plants. *DRT100* and *DRT101* induction patterns differ from one another and from the pattern for the UV-inducible *CHS* gene: (1) All three genes are induced by UV light, only *DRT100* and *DRT101* by the DNA crosslinking agent mitomycin C, and only *DRT100* by the alkylating agent methylmethane sulfonate. (2) *CHS* and *DRT101* mRNA levels are maximal immediately after a 2-h UV-B pulse, whereas *DRT100* mRNA peaks 3 h later. (3) A UV-B pulse induces *CHS* mRNA maximally in 7-day-old plants, and much less in older plants, whereas *DRT100* and *DRT101* show higher inducibility at 14 days and 21 days. This last result, and the developmental pattern of photolyase expression, suggest that older *Arabidopsis* plants may rely more on DRT activities, and perhaps less on shielding by flavonoids, than younger ones. Supported by USDA Competitive Grants 90-37280-5597 and 92-37100-7725.

039

Phenotypic analyses of phytochrome-deficient mutants.

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In *Arabidopsis*, five known genes encode phytochromes, the photoreceptor proteins that mediate developmental responses to red and far-red light. Evidence to date suggests that the multiple phytochromes encoded by these genes have different functions, but in general it is not known which phytochrome(s) mediate which red light responses. We are approaching this issue by examining the phenotypes of mutants deficient in particular phytochrome molecules. We have previously described the isolation of lines carrying mutations in the *PHYB* gene, and of mutants that are specifically deficient in PhyA spectral activity and protein accumulation. The *phyB* mutants have numerous elongated tissues and increased apical dominance, and they flower early. The PhyA-deficient mutants, by contrast, have few obvious morphological phenotypes under standard growth conditions. However, they appear to have deficiencies in germination and de-etiolation, suggesting that PHYA mediates light responses early in development. In addition, these mutants are affected in the night-break response to floral induction, suggesting that PhyA is involved in coupling sensation of day length to the circadian rhythm. Analysis of phenotypes of double mutants deficient in both PhyA and PhyB will be presented.

040

CHARACTERIZATION OF *ARABIDOPSIS THALIANA* MUTANTS AFFECTED IN THE GRAVITY RESPONSE

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Four agravitropic mutants were identified in the Du Pont collection of *Arabidopsis thaliana* T-DNA insertional mutants (screening done in collaboration with Tim Casper, DuPont Co. and Carl Simmons, Yale Univ.). These mutants were named 5549, 68-11, 6728, and 1113. Phenotypic characterization revealed the following: 5549 is partially agravitropic in the root only, shows normal sensitivity to exogenous 2,4-D, and is allelic to Agr 2. 68-11, which is allelic to 6728 and possibly a sib, is partially agravitropic in both the root and hypocotyl. Both 68-11 and 6728 show normal sensitivity to exogenous 2,4-D. 1113 exhibits slow bending in the root upon 90° reorientation to the gravity vector and has increased resistance to exogenous 2,4-D. Tagging analysis of 5549 and 68-11 is in progress.

041

In vivo phosphorylation of the COP1 protein during light-regulated seedling development

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We are interested in elucidating the role of protein phosphorylation as a regulatory device during photomorphogenesis. Previous analysis established that the COP1 protein represses photomorphogenic seedling development in the absence of light and that the light signal acts to overcome this inhibitory activity. Molecular analysis of the *COP1* gene revealed that the encoded protein contains both a domain homologous to the β-subunit of G-proteins and a novel zinc-binding motif [Cell 71, 791-801 (1992)]. Northern- and western-blot analyses clearly indicated that both mRNA and protein levels of COP1 are not affected by the presence or the absence of light. Therefore, the mechanism of light modulation of COP1 activity must entail post-translational events, possibly modification of the COP1 protein. To investigate the phosphorylation status of COP1 a method based on immunoprecipitation and gel electrophoresis was developed to analyse protein phosphorylation in germinating seedlings. It was used to analyse the phosphorylation status of COP1 in dark-grown and light-treated seedlings of wild-type, COP1-deficient mutant, and COP1-overexpressing genotypes. Our results clearly indicate that COP1 is phosphorylated in nuclear fractions of an overexpressing genotype. We are currently investigating whether the degree of phosphorylation is modulated by light signals.

042

IDENTIFICATION OF FUNCTIONALLY IMPORTANT STRUCTURAL DOMAINS OF PHYTOCHROME B

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The phytochrome photoreceptor family mediates many responses of higher plants to light, such as seed germination, hypocotyl elongation, and flowering. Recently it has been possible to assign specific photomorphogenic responses in green plants to one member of the phytochrome family, phytochrome B (1,2,3,4). *Arabidopsis* plants overexpressing *Arabidopsis* phytochrome B show increased inhibition of hypocotyl elongation in white and red light (1,3) , whereas the *hy3* mutant of *Arabidopsis*, which is deficient in phytochrome B (2,4) shows decreased inhibition of hypocotyl elongation in white and red light (3). Thus this response is not only controlled by phytochrome B, but highly dependent on the level of phytochrome B.

We are now trying to understand the properties of this photoreceptor. Using the phytochrome B overexpression system we have characterized the behavior of the phytochrome B holoprotein in the light and analyzed its spectral properties (1). Currently we are employing the phytochrome B overexpression system to define structural domains of phytochrome B which are important for its function as a photoreceptor. Results of this analysis will be presented.

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4 Reed, J.W.; Nagpal, P.; Poole, D.S.; Furuya, M.; Chory, J. (1993) Mutations in the gene for red/far-red receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. The Plant Cell 5, 147-157.

043

Properties of cytokinin resistant mutant, *ckr1*, indicate interaction of cytokinin and light on photomorphogenesis

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Cytokinin is known to mimic light effects on many aspects of photomorphogenesis, including inhibition of hypocotyl elongation, opening of cotyledonary leaves, seed germination, leaf enlargement, and greening process. These properties were studied in the cytokinin insensitive mutant, the *ckr1*, to understand the interaction of light and cytokinin. To our surprise, almost all of the properties tested were altered in the *ckr1* mutant. First of all, hypocotyl growth of the *ckr1* mutant was resistant to cytokinin compared with wild type seedling grown in the dark. Secondly, seeds of *ckr1-109* mutant germinated very poorly in total darkness, yet germination rate of the mutant can be rescued by light but not by cytokinin. Thirdly, unlike wild type cotyledon growth, some alleles of the *ckr1* mutants opened their cotyledonary leaves constitutively and without hook formation in the dark. Fourthly, the pattern of leaf enlargement and greening were also altered in a way that the difference of leaf size and chlorophyll content between mutant and wild type was more evident in dimmer light condition or in the dark. Finally expression of some of the light-regulated genes, such as *rbcS* and *cab* gene, was also altered. Our results are consistent to the hypothesis that light and cytokinin interact in many aspects of photomorphogenesis. A model to explain the results will be presented.

044

GENETIC VARIATION FOR UV-B SENSITIVITY IN A CENTRAL ILLINOIS POPULATION OF *ARABIDOPSIS THALIANA*.

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Arabidopsis thaliana seeds were collected from a population in central Illinois. Nine maternal lines were grown in the greenhouse under supplemental UV-B irradiation simulating current midsummer ambient conditions and a 20% reduction in stratospheric ozone over central Illinois (6 and 12 kJ m^{-2} $UV\text{-}B_{BE}$). Based on morphological, phenological, and reproductive characteristics, these lines were clustered into three distinct biotypes. The high UV-B treatment caused significant reductions in fruit number and mass, plant height, rosette mass, and inflorescence mass. In addition, there was a significant UV-B x biotype interaction for fruit number, indicating genetic variation for UV-B sensitivity in *A. thaliana*. The high UV-B treatment reduced fruit number (relative to the 6kJ treatment) in the sensitive biotype by 20% while this variable was essentially unchanged in the tolerant biotypes (reductions of 2%% and 5%). There was also a trend toward a significant UV-B x biotype interaction for total height, rosette mass, inflorescence mass, and fruit mass. The sensitive biotype exhibited the largest reduction for all variables. The sensitive and resistant biotypes did not differ with respect to kaempferol concentration, a putative UV-B protective filter.

045

TRANSGENIC COMPLEMENTATION OF A *phyB* MUTATION AND PHYSIOLOGICAL RESPONSE TO *PHYB* GENE COPY NUMBER.
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The apoprotein portions of the phytochrome red light photoreceptors in *Arabidopsis* are encoded by a family of five genes. This diversity of receptor structure suggests that plant red/far-red light regulated physiological responses may be mediated differentially by individual phytochrome forms. We have used an *Arabidopsis* mutant, *hy3*, that is a null allele at the *PHYB* locus as a host for a mini-*PHYB* transgene in order to demonstrate that the diverse altered light-controlled phenotypes of this mutant are indeed the result of deficiency for light-stable *PHYB* phytochrome and to investigate the effects of *PHYB* gene copy number on plant photoresponses.

A *PHYB* minigene (m*PHYB*) consisting of the complete *PHYB* cDNA fused to the *PHYB* promoter was constructed and introduced into wild-type and *hy3* mutant plants. The transformed *hy3*(m*PHYB*) progeny show gene dosage-dependent complementation of several red light-controlled mutant phenotypes including hypocotyl length and cell size, flowering time, and response to end of day far red light. The gene dosage-dependent nature of *PHYB* function is further observed in wild-type strains containing one or two extra copies of the *PHYB* gene which show quantitatively exaggerated photoresponses. Determination of transgene copy number and blot analysis of *PHYB* protein levels confirmed that, in the case of *PHYB*-mediated responses, sensitivity of plants to light cues is strongly influenced by photoreceptor levels.

046

LIGHT-REGULATED TRANSCRIPTION FACTOR GENES FROM *ARABIDOPSIS THALIANA*

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Many processes in the plant life cycle are controlled by light. The effect of light as a signal is especially dramatic in case of the etiolated seedling where growth is arrested and further development is completely dependent on light. Our hypothesis is that this process of light-dependent seedling development involves the regulated expression of transcription factor genes. To isolate such transcription factor genes an *Arabidopsis* genomic library enriched in transcription factor genes has been constructed. A screening procedure was developed to isolate from this library light regulated clones and we currently have isolated five genes which encode proteins with DNA-binding domains homologous to the probes used (myb, homeodomain, MADS, bZIP). Two genes are induced within one hour after transfer of etiolated seedlings to the light. One gene is transiently expressed and detectable only one hour after transfer. We are currently testing whether these genes are under the control of known photomorphogenetic systems by testing *Arabidopsis* mutants. In addition, we are investigating the expression pattern and biological function of these genes by deregulated expression in transgenic plants.

047

EXPRESSION OF LIGHT-INDUCED GENES IN BLUE LIGHT RESPONSE MUTANTS OF ARABIDOPSIS, Jeff C. Young and Roger P. Hangarter, Dept. of Plant Biology, Ohio State University, Columbus, OH 43210

To determine the role of the blue light photosensory systems in gene regulation, the spectral and time dependent expression of numerous light regulated genes was measured in several blue light response mutants (blu1, blu2, blu3, hy4), a phytochrome deficient mutant (hy2), and a blue light photosystem::phytochrome double mutant (blu1::hy6). Northern analysis showed that although blue light induced many genes, blue light-induced gene expression in seedlings from the blu mutant and hy2 backgrounds was similar to wild-type in most cases. However, expression in the double mutant was altered for some of these genes. These results suggest that many light regulated genes are coordinately regulated in blue light by the blue light photosystem and phytochrome. The regulation of one gene, Chalcone Synthase, was specifically altered in the blu mutants and in hy4 in blue light. However, it appears that the induction of Chalcone Synthase by UV-A light is similar in the mutants and wild-type, suggesting that a separate photosystem operates in the UV-A spectral region.

048

Isolation of *Arabidopsis* light response mutants by screening for altered transgene expression

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We are using a combined molecular and genetic approach to investigate the UV/blue light-regulation of gene expression. One objective in this research is to isolate *Arabidopsis* mutants altered in the regulation of chalcone synthase (CHS) gene expression. To this end we produced transgenic *Arabidopsis* plants expressing the β-glucuronidase (GUS) reporter gene driven by a CHS promoter. We demonstrated that expression of the transgene is induced substantially by blue and high fluence rate white light but not significantly by red light. A single diploid individual homozygous for the transgene was selfed to produce an isogenic line and seed from this line was used for mutagenesis. A method was developed to screen for mutants using the fluorescent GUS assay in microtitre dishes. Putative mutants have been selected which show altered light-induction of CHS-GUS transgene expression. Putative mutants with very low GUS activity have normal expression of the endogenous CHS gene and hence are not regulatory mutants. Experiments with 5-azacytidine suggest that methylation of the transgene contributes to the low GUS activity. A mutant selected for high GUS activity was found to have increased light-induction of both anthocyanin synthesis and endogenous CHS transcript levels. This mutant retains light-regulation of the CHS gene and CHS-GUS transgene but the response is increased at low-medium fluence rates of light. This mutant is diploid and is not altered in the organisation of the transgene in genomic DNA. This mutant has slower germination, is altered in leaf shape and has fewer trichomes and these traits segregate with high CHS-GUS expression in crosses in a manner consistent with a single recessive Mendelian character. In further crosses we have shown that the transgene does not segregate with the mutant phenotype. Although we cannot exclude the possibility that the different characteristics of the mutant phenotype are caused by mutations in tightly linked genes, we believe it is likely that the mutant is altered in a gene product which is involved in both the light regulation of CHS promoter activity and other developmental processes.

049

A family of proteins containing a serine/threonine kinase and/or an EGF repeat has members in the plasma membrane and chloroplast. Bruce D. Kohorn and Masaaki Fujiki. Duke University, Botany Department, Durham, NC 27708. To identify proteins that either modify or bind LHCP we developed a general selection scheme for the isolation of cDNAs that encode proteases and binding peptides for known target peptide sequences. (Smith and Kohorn 1991, Proc. Natl. Acad. Sci USA 88:5159). We have used this scheme to isolate an *Arabidopsis* cDNA encoding a protein with specificity for the amino terminal 50 amino acids of pLHCP, a region that should interact with transit peptidases, receptors, chaperones, or kinases with different specificities (Kohorn, Lane and Smith 1992, Proc. Natl. Acad. Sci. USA 89: 10989). The *Arabidopsis* cDNA inhibits a transcriptional activator GAL4-LHCP fusion protein, but not native GAL4 protein. The cDNA encodes a 595 amino acid protein (*ca*. 70 kDa) with at least two functional domains, one with similarity to the family of serine/threonine kinases and another that contains an Epidermal Growth Factor (EGF) repeat. In *Arabidopsis*, a labile 70 kDa protein is detected in the plasma membrane/cell wall fraction on immunoblots probed with antiserum to protein expressed in *E. coli*. Immuno-electron microscopy shows staining on both the plasma membrane and within the chloroplast. In pea leaves, which are easier to fractionate, the antiserum detects two proteins on immunoblots; one of 70 kDa in the plasma membrane, and one of 73 kDa in the chloroplast stroma. In *Chlamydomonas*, only the 73 kDa chloroplast stromal protein is detected on immunoblots. We have been unable to detect high levels of the 73 kDa stromal protein in *Arabidopsis* tissues, but we believe that this protein is extremely labile and likely to be developmentally regulated. Immuno-electron microscopy does, however, detect protein in both the plasma membrane and chloroplasts of young leaves. Our current hypothesis is that the isolated cDNA clone encodes the plasma membrane 70 kDa protein, as the size and protein structure predicted by the clone suggests a receptor-like function with an extracellular domain. The 73 kDa stromal protein must share at least one region of similarity to that found in the plasma membrane as it reacts as strongly with the antiserum. We predict that this shared domain is responsible for the genetic interaction with LHCP in yeast, and possibly in the chloroplast stroma. It is possible that this domain encodes the kinase, but we are in the process of cloning a gene for the 73 kDa species.

050

Molecular Genetic Characterization of 5-Fluoroindole Resistant Mutants of *Arabidopsis*

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Tryptophan synthase ß (TSB) catalyzes the conversion of indole and serine to tryptophan. Because the tryptophan pathway has a role in protein synthesis and in providing precursors for indolic secondary compounds, it is important to understand how this enzyme is regulated. It was previously reported that *trp 2-1* is a TSB deficient tryptophan auxotroph, which has a mutation at the *TSB1* locus of *A. thaliana*[1]. We utilized the indole analog 5-fluoroindole (5FI) to obtain a collection of TSB structural gene mutants and to find mutations that effect the expression of TSB.

The basis for the selection is that 5-fluoroindole is converted to 5-fluorotryptophan, which is cytotoxic. Therefore, wild type seedlings die on medium containing 5FI, whereas seedlings with lower levels of TSB activity are able to survive. Genetic and molecular biological approaches are being used to characterize twenty 5-fluoroindole resistant (FiR) mutants. Approximately one-half of the FiR mutants are recessive and prototrophic, suggesting that they are leaky *trp 2* alleles. Genomic Southern blot analysis show that one *trp 2* allele causes an EcoRI polymorphism. *TSBI* mRNA levels were found to vary substantially among the 20 lines. A genetic analysis of the mutants is in progress, and preliminary F_2 data suggest that at least 10 FiRs are allelic to *trp 2-1*. Interestingly, two FiRs are recessive and not allelic to *trp2-1*, and four FiRs might be dominant. Further genetic and DNA sequence analysis will reveal whether the mutations are *TSB1* structural mutations or regulatory mutations.

[1] Last *et al* (1991). The Plant Cell 3:345-358.

051

Arabidopsis thaliana Mutants Deregulated for Tryptophan Biosynthesis

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In plants aromatic amino acids serve as biosynthetic precursors for a host of compounds including hormones, phytoalexins, and alkaloids. We have isolated mutants of *Arabidopsis thaliana* with deregulated biosynthesis of the aromatic amino acid tryptophan. These mutants will elucidate how plants regulate the tryptophan pathway and how the tryptophan pathway feeds into synthesis of secondary metabolites.

We have used two screens to identify mutants with altered tryptophan regulation (ATR). First, we have screened for mutants that are resistant to the false tryptophan feedback inhibitor, 5-methyl-tryptophan. Second, we have screened for mutants that fail to sense feedback inhibiting levels of tryptophan using an *Arabidopsis* strain (*trp1-100*) that under normal growth conditions accumulates a fluorescent intermediate in the tryptophan pathway, but in the presence of high levels of tryptophan is feedback inhibited so that very little fluorescent intermediate is made. We have isolated mutants of this strain that are fluorescent even in the presence of high tryptophan levels. Mutants obtained from both screens should include feedback inhibition resistant plants and plants that have an increased flow of metabolites through the tryptophan pathway.

A mutant isolated from the fluorescence screen, *ATR1*, has a number of phenotypes consistent with a plant that has increased tryptophan biosynthesis. The *ATR1* mutation is dominant, and in addition to conferring resistance to 5-methyl-tryptophan, specifically confers resistance to other toxic analogues of metabolites in the tryptophan pathway. However, the mutation is not linked to genes encoding anthranilate synthases (*ASA1* and *ASA2*), the known targets of tryptophan feedback inhibition. We have mapped this mutation to the bottom of chromosome V between the markers *cer3* and *lfy*. We are now walking towards the *ATR1* gene and characterizing the biochemical nature of the *ATR1* mutant using Northern blot analysis and tryptophan biosynthetic enzyme assays.

052

Genetic Dissection of Carotenoid and Chlorophyll Synthesis in *Arabidopsis thaliana*

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My laboratory has undertaken a long-term program of study directed at increasing our understanding of pigment biosynthesis in higher plants at the molecular level. A necessary first step is the isolation and characterization of biosynthetic and regulatory genes affecting carotenoid and chlorophyll synthesis. Our approach to this task utilizes HPLC-based screening of the T-DNA mutagenized *Arabidopsis thaliana* population to circumvent many of the problems that have historically plagued such studies in higher plants. Individual mutant lines with altered pigment profiles are identified by HPLC and studied at the genetic, molecular and biochemical levels. Based on these results, a limited number of tagged loci affecting pigment synthesis are selected and their genes physically isolated by virtue of the associated T-DNA element. Our long-term goals are to characterize the structure, expression, function and developmental regulation of these genes and use this knowledge to rationally manipulate pigment biosynthesis in plants.

During the past year we have been systematically screening the T-DNA tagged *Arabidopsis* mutant population by HPLC for lines exhibiting pigment profiles consistent with mutations affecting carotenoid and/or chlorophyll biosynthesis. To date, several interesting lines have been identified and partially characterized including two carotenoid biosynthetic loci, *pds1* and *pds2 (pds=phytoene desaturation)*, a potential regulatory locus, *snow white1*, and a chlorophyll b deficient mutant, *chlb-*. *pds1* and *pds2* accumulate phytoene and genetically define distinct loci involved in phytoene desaturation in *Arabidopsis*. *snow white1* completely lacks chlorophylls and β-carotene and has characteristics consistent with a regulatory mutation affecting overall pigment biosynthesis or a very early step in chloroplast biogenesis. Preliminary data suggests that the *chlb-* locus is not allelic with several known chlorophyll b deficient loci (*ch1*, *ch42* and *ch5*). Genetic, molecular and biochemical studies of these four mutant lines will be presented.

053

GENETIC ANALYSIS OF STARCH SYNTHESIS AND ACCUMULATION

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In order to characterize starch biosynthesis and the genes involved in this process, we are interested in identifying mutants which are altered in their ability to accumulate starch. In order to efficiently screen for these mutants, we have developed a highly simplified screening procedure for starch mutants which takes advantage of the large amount of starch accumulated in root caps. *Arabidopsis* seedlings are grown in vertically oriented Petri plates, such that the roots grow along the surface of the agar, and after 4 days the plates are flooded with iodine stain. The starch-filled root caps of the wild type are intensely stained by the iodine. Mutants which have altered starch contents can be readily identified and recovered since the iodine does not kill the seedlings. Using this technique and an automated protocol for sterilizing large numbers of independent seed lots, about 4000 lines from the DuPont T-DNA insertional mutant collection have been individually screened for starch mutants. Ten mutants have been recovered including 3 with no detectable starch, 6 with reduced starch accumulation and 1 which accumulates starch in specific areas of the root where the WT does not normally accumulate any. The mutants in the starchless class are similar to previously characterized Arabidopsis starchless mutants, but the latter two classes have novel phenotypes and identify new genes affecting starch accumulation. Cosegregation results indicate that at least one of these 10 lines contains a T-DNA which is very tightly linked to the starch mutation.

054

Molecular-genetic analysis of two Arabidopsis Fd-GOGAT genes and their roles in photorespiration.

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Ferredoxin-dependent glutamate synthase (a.k.a. ferredoxin-dependent glutamine-oxoglutarate amino transferase or Fd-GOGAT) is a necessary component of the photorespiratory cycle. Fd-GOGAT, working coordinately with glutamine synthetase, functions to reassimilate the large amounts of nitrogen released in the form of ammonia during photorespiration. In the absence of Fd-GOGAT activity, the levels of amino donors required for photosynthetic CO_2 fixation rapidly decline, ultimately resulting in death of the plant.

We have isolated two clones, GGT1 and GGT2, from an Arabidopsis cDNA library which, by sequence and structure comparison, encode two Fd-GOGAT isoforms. Northern analysis reveals that GGT1 is highly expressed in light but not dark conditions, suggesting a role in photorespiration. GGT2 is expressed at lower levels and does not appear to be regulated by light. The existence of two Fd-GOGAT genes is surprising since many Arabidopsis mutants have been isolated which are lacking Fd-GOGAT activity.

Approximately thirty photorespiratory mutants deficient in Fd-GOGAT enzyme activity, isolated over a decade ago, have been obtained from Dr. Chris Somerville. Since little is known about GOGAT activity in Arabidopsis, the enzymatic activities of both Fd-GOGAT and NADH-GOGAT in wild-type and mutant plants grown under various physiological conditions (i.e. light versus dark, low versus high CO_2) will be determined by HPLC. The mutants will also be more thoroughly characterized by Northern analysis with gene-specific probes for GGT1, GGT2, NADH-GOGAT and the glutamine synthetase genes to determine how the expression of these related genes are affected in the photorespiratory mutants. Finally, RFLP mapping of the mutants will be used to classify them into linkage groups and to determine which GGT gene is affected in the mutants and hence involved in photorespiration.

055

The Search for Chloride Channel Genes in *Arabidopsis thaliana*

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In order to clone an *Arabidopsis thaliana* chloride channel that has sequence homology to a large superfamily of highly conserved proteins, the ATP-binding cassette (ABC) transporters, two molecular approaches are being attempted: (i) PCR mediated cloning, and (ii) yeast functional complementation.

Two primers from highly conserved regions were used to amplify an *Arabidopsis* cDNA library using polymerase chain reaction (PCR). Sequencing of an PCR product did not reveal any homology to the available Genebank sequences. The PCR product is presently being used to isolate a cDNA clone from an *Arabidopsis* library.

The yeast member of the ABC transporter family, STE6, mediates export of a-mating factor. A mutation in this gene can be functionally complemented by another member of the ABC transporter family, such as the mammalian multidrug resistance (mdr) gene (Raymond M et al., 1992, Science 256:232-234). We have undertaken complementing a yeast ste6 mutant by an *Arabidopsis* expression library. Preliminary results have identified a candidate clone. PCR analysis of this clone using the above mentioned primers gave a PCR product, thus implying possible sequence homology with the ABC transporter superfamily. Southern blot analysis suggests that this clone is different from the one obtained by PCR mediated cloning. In addition to the sequencing, the experiments are being carried out to verify that the candidate *Arabidopsis* clone does indeed functionally complement the yeast STE6 gene.

056

A late flowering starch excess mutant in Arabidopsis thaliana ecotype RLD

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A mutant (KE21) has been identified by screening the M2 of EMS mutagenized plants for leaf starch contents. In comparison to the wild-type the mutant KE21 shows a high amount of leaf starch after a prolonged dark phase judged by iodine staining. This phenotype becomes distinguishable from the wild-type only after approximately 3-4 weeks of growth. Furthermore, the KE21 mutant is also delayed in bolting (lagging behind the wild-type for more than 10 days). Back crosses revealed the recessive character of both phenotypes. Eight late flowering mutants received from the Ohio stock center have been tested for leaf starch contents. Two of them showed an increased staining of leaves with iodine after a prolonged dark phase. However, these late flowering mutants were stained more intensely after 1-2 weeks of growth. The KE21 mutant has been crossed with different marker lines for chromosome mapping and with late flowering mutants and starch excess mutants for examination of complementations.

057

Isolation and Characterization of *Arabidopsis* TrpG cDNAs by Complementation of a Mutant of *S. cerevisiae*.

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Anthranilate synthase converts chorismic acid to anthranilic acid, and is the first committed step in the biosynthesis of tryptophan in both microorganisms and plants. The enzymatic activity consists of two components. The large sub-unit (component I) is capable of converting chorismic acid to anthranilic acid using ammonia as amino donor, but only under non-physiological conditions. The second sub-unit (component II, glutamine amidotransferase) permits the utilization of glutamine as amino donor under physiological conditions. Two genes for the large sub-unit (ASA1 and ASA2) have previously been isolated and described by Niyogi and Fink. We have also been interested in the regulation of anthranilate synthase activity in both mutant and wild-type plants and callus. In order to have both DNA sequences and ultimately antibodies against both components of AS, we have isolated cDNA clones capable of complementing a TrpG mutant of *Saccharomyces cerevisiae* from an *Arabidopsis* cDNA library. From 500,000 transformants, we have isolated 55 TRP$^+$ lines. One of the first three lines characterized contained an approximately 900 bp insert which hybridized to both Southern and northern blots of *Arabidopsis* nucleic acid. Further characterization and sequencing of this and other isolates is in progress.

058

REGULATION OF ASPARTATE KINASE IN *ARABIDOPSIS THALIANA* (L.) Heynh..

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Though amino acid biosynthesis is a fundamental process for plant growth and development, its physiological and biochemical regulation, as well as its molecular basis and genetic control are until now poorly understood compared to microorganisms. Feedback inhibition properties of key enzymes (aspartate kinase (AK), dihydrodipicolinate synthase, homoserine dehydrogenase) of the aspartate family pathway have made it attractive for selecting mutants with altered enzyme regulation. In *Arabidopsis* we have obtained a set of mutants resistant to toxic amino acid analogues (S-2-aminoethylcysteine) or amino acid combinations (lysine plus threonine) and the feedback sensitivity of AK has been studied in these mutants. Among them the mutant 5 FALT 40/6/1, resistant to toxic concentrations of lysine plus threonine, was characterized by a threonine overproduction. Therefore, AK, the first key enzyme of the aspartate pathway, has been studied in detail in the wild type and in this mutant. In *Arabidopsis* this enzyme is present under the form of three isoenzymes. The first isoenzyme is threonine-sensitive and represents about 54 % of the total enzyme activity. Compared to most other plant species, *Arabidopsis* is thus characterized by a relative high amount of threonine-sensitive enzyme. The second isoenzyme is lysine-sensitive and is responsible for a third of the enzyme activity, and the last isoenzyme is insensitive to both lysine and threonine and stands for 12 % of the total enzyme activity. In 5 FALT 40/6/1 the lysine-sensitive form is less sensitive to lysine than in the wild type and the insensitive isoenzyme represents up to 19 % of the total enzyme activity. So, the resistance and overproduction of this mutant might be associated with a mutation at the AK-level.
A linkage tester line (W 100) was used for the determination of the map position of the involved gene. As a linkage was found with specific markers (er, py) on chromosome 2, a more detailed analysis based on other markers (hy-3, cer-8, as, cp-2) on that chromosome is presently carried out.

059

Christopher S. Johnson and Brenda W. Shirley, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406. Analysis of protein-protein interactions between the first two enzymes of flavonoid biosynthesis.

Biochemical and immunocytochemical studies have indicated that the flavonoid biosynthetic pathway functions as a multicatalytic complex loosely associated with the endoplasmic reticulum. However, interactions between the individual components have been difficult to analyze with available techniques, presumably due to the relatively tenuous associations between these proteins. We have used a yeast two-hybrid system, in which fusion proteins are tested for the ability to activate transcription of reporter genes, to identify a specific interaction between the Arabidopsis chalcone synthase and chalcone isomerase enzymes. Deletion mutagenesis is being used to define specific domains involved in this interaction. These studies will allow us to investigate the role of protein-protein interactions in assembling and compartmentalizing the flavonoid biosynthetic machinery and in regulating flavonoid biosynthesis during development and in response to environmental cues.

060

Temporal and Spatial Regulation of HMG CoA Reductase Gene Expression in *Arabidopsis thaliana*

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Long-term regulation of HMG CoA reductase in plants appears to involve controlling the levels of the enzyme by transcriptional and post-transcriptional mechanisms that are not currently understood. Using both biochemical assays and microtechniques (such as *in situ* hybridization and histochemical staining), we have compiled a systematic description for the pattern of HMG CoA reductase gene expression during the *Arabidopsis* life cycle. Even though HMG CoA reductase is considered a "housekeeping gene", we have found that the levels of HMG1 gene expression are dramatically different in different tissues and exhibit specific patterns of expression at different developmental stages. Thus, the patterns of HMG1 gene expression appear to be regulated temporally and spatially during development.

061

Regulation of HMG CoA Reductase Gene Expression by Environmental and Developmental Cues

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Current studies indicate that in plants, just as in animals, mechanisms exist for both long- and short-term regulation of HMG CoA reductase. Long-term regulation of HMG CoA reductase in plants appears to involve controlling the levels of the enzyme by transcriptional and post-transcriptional mechanisms that are not currently understood. As a first step towards elucidating this regulatory circuit, we have used a variety of assays to compile a systematic description for the pattern of HMG CoA reductase gene expression during the *Arabidopsis* life cycle. Surprisingly, even though HMG CoA reductase is considered a "housekeeping enzyme", we have found that the levels of HMG1 gene expression are dramatically different in different tissues and exhibit specific patterns of expression at different developmental stages.

Moreover, our studies have shown that HMG CoA reductase gene expression is also subject to control by environmental signals, and in particular, is responsive to changing light conditions. In the absence of light, HMG1 mRNA accumulates over a period of 12-18 hours to steady state levels approximately 5-10-fold higher than the levels present in light-grown plants. Conversely, when etiolated or dark-adapted plants are transferred into the light, the amount of HMG1 mRNA decreases to the basal level. Furthermore, in transgenic plants carrying chimeric gene in which the *Arabidopsis* HMG1 promoter controls expression of the *gusA* gene from *E.coli* , β-glucuronidase activity is also responsive to light conditions, suggesting that the regulation of HMG1 gene expression by light is mediated, at least in part, at the transcriptional level. Based on the relatively slow kinetics of this response to light, we speculate that modulation of HMG1 promoter activity by light may be one mechanism by which the plant maintains MVA homeostasis during the diurnal cycle.

In light of these findings, we are continuing to investigate the molecular and genetic basis of the control systems responsible for regulating HMG CoA reductase expression in Arabidopsis.

062

Characterization of Triplicate Genes Encoding Phosphoribosylanthranilate Isomerase of the Arabidopsis Tryptophan Biosynthetic Pathway

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The tryptophan biosynthetic pathway in higher plants provides the essential amino acid tryptophan, as well as precusors for the plant hormone indole 3-acetic acid (IAA) and other secondary metabolites including phytoalexins and glucosinolates. One of the intermediate steps in this pathway (the conversion of 5-phosphoribosyl anthranilate to 1-(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate) is catalyzed by phosphoribosylanthranilate isomerase (PAI). *Arabidopsis thaliana* PAI cDNAs have been cloned from a cDNA expression library by suppression of an *E. coli* trpC$^-$ mutation. Unlike in most microorganisms, where the PAI is fused with indole-3-glycerolphosphate synthase (InGPS), which catalyzes the next reaction in the pathway, there is no evidence that the Arabidopsis PAI is fused with InGPS. Genomic Southern analysis reveals that there are three non-allelic copies of PAI in the Arabidopsis genome. DNA sequence analysis of cDNA and genomic DNA indicates that *PAI1* and *PAI2* are virtually identical with only a single conservative change in the total 275 deduced amino acids. However, *PAI3* shows about 90% identity with *PAI1* and *PAI2*. All three PAI polypeptides possess a putative plastid target sequence at the amino-termini, suggesting that these PAI isoenzymes function in plastids.

063

Arabidopsis Dissimilates L-Arginine and L-Citrulline for Use as N-Source

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Contrary to widespread belief that higher plants exclusively grow on inorganic N, *Arabidopsis* ecotype Columbia plants grew perfectly well on several amino acids as sole N source. When aseptically grown on defined medium with either L-arginine, L-citrulline, or nitrate as sole N source, *Arabidopsis* plants grew and developed normally. Three catabolic activities, L-arginine iminohydrolase, L-ornithine carbamoyltransferase, and carbamate kinase, were found in stromal fractions of purified *Arabidopsis* chloroplasts. These activities dissimilate L-arginine and/or L-citrulline into L-ornithine, ammonium, bicarbonate, and ATP. In physiological tests with purified, intact *Arabidopsis* chloroplasts, L-[guanido-^{14}C]-arginine was rapidly taken up and some 10% was decomposed, releasing $^{14}CO_2$. Therefore, chloroplasts can take up and dissimilate L-arginine. In principle, chloroplast arginine dissimilation allows *Arabidopsis* to use L-arginine and/or L-citrulline as general N-sources for growth. However, plants rarely encounter exogenous L-arginine and/or L-citrulline in amounts exceeding their biosynthetic needs. Rather, L-arginine and L-citrulline might serve as endogenous N-sources. Specifically, we have hypothesized that L-citrulline and/or L-arginine might participate in endogenous CO_2 recycling by mitochondria and chloroplasts, and that exogenous L-citrulline and L-arginine only incidentally input such endogenous CO_2 recycling.

064

Suppressors of a tryptophan auxotroph

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Indole-3-acetic acid (IAA)is part of the signal response mechanism in many cell processes in *Arabidopsis,* including cell division and geotropism. The hormone IAA is a secondary metabolite of the tryptophan pathway. Elucidating the regulation of tryptophan biosynthesis is essential for understanding the regulation of IAA biosynthesis. Three tryptophan auxotrophs of *Arabidopsis* (*trp1-1*, *trp2-1*, and *trp3-1*) were isolated in our lab. These plants have phenotypes consistent with a perturbation in auxin levels. Biochemical results from this lab confirm that the auxotrophs have altered levels of IAA. My interest is in the *trp2-1* auxotroph which is defective in one of the two genes encoding tryptophan synthase β (*TSB1*), the last step in the tryptophan biosynthetic pathway. The *trp2-1* plants are small, pale, slow growing and accumulate indole and IAA (J. Normanly and G. Fink, unpublished results). Since *trp2-1* plants accumulate indole and IAA, it is thought that indole is the major precursor to IAA in *Arabidopsis.* I have isolated eight independent prototrophic revertants of the *trp2-1* mutation. Both dominant and recessive suppressors were isolated. One recessive suppressor is agravitropic, which suggests a perturbation of auxin metabolism. Another mutant is dominant and has drastically altered levels of *TSB* mRNA, which suggests that it is a regulatory mutant. These suppressors of *trp2-1* (*sot*) mutants have defined new genes which will enable me to learn more about the regulation of transcription and biosynthesis in the tryptophan and IAA biosynthetic pathways. Presently, I am characterizing the *sot* mutants by completing their genetic and biochemical analysis.

065

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC): A POWERFUL TOOL TO SCREEN FOR MUTANTS ALTERED IN PHENYLPROPANOID BIOSYNTHESIS

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Phenylpropanoid metabolism and its branch pathways produce a large number of diverse compounds that play important roles during normal plant growth, development and during plant responses to biotic and abiotic stresses. We are using High Performance Liquid Chromatography (HPLC) to screen EMS mutagenized and T-DNA tagged populations of *Arabidopsis thaliana* to isolate mutants altered in phenylpropanoid metabolism. HPLC is a powerful technique for detecting changes in the production of soluble phenylpropanoid derivatives because of its ability to resolve small quantities (as little as 100 fmoles) of a large number of compounds (100 different aromatic compounds) in a relatively short time (20 minutes). Since, each HPLC profile provides a simultaneous analysis of many different individual compounds, a single HPLC chromatogram allows one to screen multiple phenotypes concurrently. This screen also allows direct observation of the product affected by a mutation and thus give some insight into the nature of the mutation. We are using this screen to try to isolate three classes of mutants: 1) mutants with significantly reduced or enhanced levels of a specific phenylpropanoid derivative; 2) mutants which have completely lost or have acquired a new specific phenylpropanoid product; and 3) mutants which show a more general alteration in several phenylpropanoid products. The long term goal of the study is to clone genes that will allow us to control and modify the production of phenylpropanoid derivatives in plants.

066

INTRAGENIC RECOMBINATION IN THE *CSR1* LOCUS OF *ARABIDOPSIS*

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Four classes of herbicides are known to inhibit acetolacetate synthase (ALS), the first enzyme in the biosynthetic pathway of the branched-chain-amino acids. In *Arabidopsis*, ALS is encoded by a single gene, *CSR1*. The dominant *csr1-1* allele encodes an ALS resistant to chlorsulfuron and triazolopyrimidine sulfonamide while the dominant *csr1-2* allele encodes an ALS resistant to imazapyr and pyrimidyl-oxy-benzoate. The molecular distance between the point mutations in *csr1-1* and *csr1-2* is 1369 base-pairs. Here we used multiherbicide-resistance as a stringent selection to measure the intragenic recombination frequency between these two point mutations. The frequency of the double-mutant recombinants was 4×10^{-5}. From that we estimated a genetic distance of 0.01 cM and 502 cM for the *CSR1* locus and the whole genome, respectively. The recombinant multiherbicide-resistant allele, *csr1-4*, provides an ideal marker for plant genetic transformation.

067

ISOLEUCINE-INENSITIVE MUTANTS OF *ARABIDOPSIS*

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Threonine dehydratase/deaminase (TD), the first enzyme in the isoleucine-biosynthetic pathway is feedback-inhibited by isoleucine. From screening M2 populations of EMS-treated *Arabidopsis thaliana* Columbia wild type seeds, we isolated five independent mutants that are resistant to L-O-methlythreonine, an isoleucine structural analogue. The resistance was due to a single nuclear dominant gene, *omr1*, which we mapped to linkage group 3. Growth was 50- to 600-fold more resistant to L-O-methylthreonine in *omr1/omr1* than in the wild type, *OMR1/OMR1*. TD activity in extracts from young leaves was 20- to 50-fold less sensitive to inhibition by isoleucine in *omr1/omr1* than in *OMR1/OMR1*. We compared the biochemical characteristics of TD extracted from *omr1/omr1* and *OMR1/OMR1*. L-O-methylthreonine-resistance is probably due to a mutant form of TD enzyme molecule encoded by *omr1*. Amino acid analysis from both *omr1/omr1* and *OMR1/OMR1* is underway. The mutant allele, *omr1* will provide a new selectable marker for genetic transformation and possibly for engineering plants to overproduce isoleucine.

070

Complementation of a Mutant Deficient in Polyunsaturated Fatty Acid Synthesis by Transformation with Cloned FAD2.

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Desaturation of fatty acids from 18:1 (oleic acid) to 18:2 (linoleic acid) is the critical step in the formation of polyunsaturated fatty acids. This reaction can be catalyzed by the ω-6 desaturase, *FAD2*. While linoleic acid is an essential nutrient for animal nutrition, among higher eukaryotes, ω-6 desaturation occurs only in higher plants. After gas chromatographic screening of lipids extracted from T-DNA tagged plants, we identified a mutant (*fad2*) deficient in production of linoleic acid. A fragment of *Arabidopsis* DNA was rescued from this fad2 mutant and used to isolate wild-type *FAD2* cDNA clones. *FAD2* is predicted to encode an approximately 350 amino acid polypeptide which shows low but signigicant homology to other desaturases, including desA from *Synecocystis* and the plant ω-3 desaturases *FAD3* and *FADD*. Hydropathy calculations reveal several possible membrane spanning regions. An *Agrobacterium* vector containing *FAD2* under control of the CaMV 35S promoter was used to transform a *fad2 Arabidopsis* mutant. From *fad2* explants, transgenic plant tissue was recovered containing wild-type levels of linoleic acid, demonstrating that the cloned gene has an essential role in the formation of polyunsaturated fatty acids. We predict that *FAD2* encodes the microsomal, membrane bound, ω-6 desaturase.

071

Isolation and Characterization of Tryptophan Synthase α from *Arabidopsis thaliana*

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The complexities of microbial tryptophan synthase have been explored for decades. The prokaryotic enzyme is an $\alpha_2 \beta_2$ heterotetramer, catalyzing the overall reaction of

INDOLE-3-GLYCEROL PHOSPHATE + SERINE --> TRYPTOPHAN.

The α protein catalyzes **INDOLE-3-GLYCEROL PHOSPHATE --> INDOLE.**
The βprotein catalyzes **INDOLE + SERINE --> TRYPTOPHAN.**
An intimate communication between the α and β proteins is required for full activity of the α protein, whereas the β protein is active as a β_2 dimer (1). Given the very low activity of isolated prokaryotic tryptophan synthase α (TSA) in the absence of tryptophan synthase β (TSB) and the lack of conserved sequences in the micobial *TSA*s, we suspected that cloning the first plant *TSA* would require an innovative approach. We isolated Arabidopsis *TSA* by complementing an *E. coli ΔtrpBA* mutant that expressed active Arabidopsis TSB with an Arabidopsis cDNA expression library in λYES (2). We sequenced a 1.2kb cDNA and found that all of the amino acids known to be essential in microbial TSA were conserved. We are now sequencing 8kb of genomic sequence isolated in a screen of an Arabidopsis genomic library with the cDNA.

The *Arabidopsis thaliana* tryptophan-requiring mutant *trp3-1* (3) appears to be defective in TSA activity. Two approaches are being pursued to test the hypothesis that the *trp3-1* mutation is indeed a *TSA* structural gene mutation. We are introducing genomic clones of *TSA* into *trp3-1* via Agrobacterium-mediated transformation and are conducting genetic mapping studies to test whether the cloned *TSA* gene is linked to *trp3-1*.

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068

FOLATE MUTANTS OF *ARABIDOPSIS*

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Dihydropteroate synthase (DHPS), an early enzyme in the folate pathway, -catalyzes the condensation of p-aminobenzoic acid (PABA) and 2-amino-4-hydroxy-6-pyrophosphoryl-methyldihydropteridine (AHPM) to give 7,8 dihydropteroic acid, the dihydrofolate precursor. The antibacterial compounds, sulfonamides, have structural resemblance to PABA and are known to be specific competitive inhibitors of DHPS. We have isolated a wide array of independent sulfadiazine-resistant mutants by screening M2 populations of EMS-treated seeds of *Arabidopsis thaliana* Columbia wild type. Growth resistance to sulfadiazine was due to a single dominant nuclear gene which was denoted *sdr1*. Homozygous *sdr1* mutants were crossed to the multimarker line, W100 and are at the final stages of being mapped to one of the five linkage groups. The phenotypes of the mutants, *sdr1/sdr1*, ranged from partial to complete male/female sterility, minor to gross flower-organ deformities, and medium to very small, bushy and completely male sterile plants. DHPS activity and the folate pools in mutants *sdr1/sdr1* and wild type *SDR1/SDR1* are currently being measured.

069

A TRYPTOPHAN AUXOTROPH REVEALS TWO AUXIN BIOSYNTHETIC PATHWAYS IN *ARABIDOPSIS THALIANA*.

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We used a tryptophan auxotroph to test the hypothesis that tryptophan is the precursor to the auxin indole-3-acetic acid (IAA). Quantitative GC-MS reveals that the trp2-1 mutant of *Arabidopsis thaliana*, defective in the conversion of indole to tryptophan, accumlates amide-linked (IAA) and ester-linked IAA at levels 25-fold and 10-fold, respectively, above those of wild type. Additionally, the trp2-1 mutant shows a 30-fold accumulation of indole. To determine if tryptophan has the capacity to serve as a precursor to IAA, the trp2-1 mutant was grown in the presence of ^{15}N anthranilate and $^{2}H_5$ typtophan. IAA, tryptophan and indole were isolated, and the relative ^{15}N and $^{2}H_5$ enrichments of these molecules determined via GC-MS. $^{2}H_5$ IAA comprised 15% of the amide linked IAA pool and 8% of the ester-linked IAA pool. From this we conclude that tryptophan can act as a precursor to IAA. However, the ^{15}N enrichment of tryptophan, 10%, was less than the ^{15}N enrichment of both the amide-linked and ester-linked IAA pools (20% each), therefore, tryptophan is not the only precursor to IAA. Another biosynthetic pathway to IAA with indole as the precursor is consistent with our data. That two IAA biosynthetic pathways exist explains why, to date, an auxin auxotroph has been unattainable. Brassica, the family to which *Arabidopsis thaliana* belongs, is reputed to contain high levels of indole-3-acetonitrile (IAN). Since IAN is one of the intermediates postulated in the conversion of tryptophan to IAA, we quantitated IAN in the trp2-1 mutant. Suprisingly, the IAN level in the trp2-1 mutant was six-fold above that in wild-type. Double-labelling experiments with ^{15}N anthranilate and $^{2}H_5$ tryptophan reveal that IAN could act as an intermediate in either of the two IAA biosynthetic pathways.

072

Ectopic Expression of HMG CoA Reductase Gene in Transgenic *Arabidopsis* Plants

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We have examined the effects of ectopic HMG CoA reductase (HMG1) expression on the physiology of *Arabidopsis* by using the CaMV 35S promoter to drive transcription from the HMG1 gene in transgenic plants. Several transgenic Arabidopsis lines were isolated that exhibit elevated levels of HMG CoA reductase activity (approximately 3-fold greater than wild-type strains), and consequently, show resistance to mevinolin, a potent and specific inhibitor of reductase. Interestingly, despite the higher levels of HMG CoA reductase in the transformed plants, chlorophyll and carotenoid levels remain the same as wild-type plants. We are currently collating data regarding changes in the steady state levels of sterols in these transgenic lines. The results of these experiments will allow us to confirm whether HMG CoA reductase is the rate limiting step in sterol biosynthesis, and to address the possibility that there are multiple pools of MVA in the cell that are sequestered into different branches of the pathway.

Ectopic expression of HMG1 has also revealed two unexpected features of this complex regulatory circuit. First, blot hybridization analysis has revealed that Arabidopsis plants carrying the CaMV35S::HMG1 transgene express 10-20 times the amount of HMG1 mRNA as wild-type plants, even though enzyme levels are elevated only three-fold. This observation sugggests that control of HMG CoA reductase may be implemented by post-transcriptional, as well as, transcriptional mechanisms. Second, we have shown using high resolution S1 nuclease analysis that high levels of expression from both the transgene and the HMG1 locus contribute to HMG1 mRNA accumulation in these transgenic plants. Consequently, we speculate that the expression of the CaMV35S::HMG1 transgene results in co-activation of the HMG1 promoter, and the highly elevated levels of HMG1 mRNA.

073

Cloning and Analysis of cDNAs coding for GAR synthase, AIR synthase and GAR transformylase: Three enzymes involved in the de novo purine biosynthesis pathway.

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Purine biosynthesis is essential for most organisms. Beyond the obvious function of supplying precursors for both DNA and RNA synthesis, purine bases, ribosides and nucleotides are extremely important in a number of plant processes. GAR synthase (EC 6.3.4.13), AIR synthase (EC 6.3.3.1), and GAR transformylase (EC 2.1.2.2) are the second, third and fifth enzymes in the 10 step de novo purine pathway to IMP; the central intermediate in AMP and GMP biosynthesis. In all eukaryotes so far studied, GAR synthase is present in two forms; as a monofuntional enzyme and as either a trifunctional enzyme with AIR synthase and GAR transformylase or as a bifunctional enzyme consisting of GAR synthase and AIR synthase. Prokaryotes have separate, monofunctional enzymes.

In order to begin to understand the organization, regulation, and gene enzyme relationships of purine biosynthetic genes in plants, the three above genes were cloned from *Arabidopsis thaliana*, by functional complementation of *E. coli* mutants. The Arabidopsis GAR synthase gene has been completely sequenced. Sequence comparisons of the *Arabidopsis* GAR synthase with corresponding enzymes from prokaryotic and eukaryotic sources indicate a conserved homology of about 45% at the amino acid level. Assays from extracts of *E. coli* expressing the complementing clone verified the specific enzymatic activity of *Arabidopsis* GAR synthase. Arabidopsis has a single and monofuntional GAR synthase similar to prokaryotes. The AIR synthase and GAR transformylase clones also appear to code for single and monofunctional enzymes. Sequence analysis, as well as Northern and Southern data suggest that the gene organization of these three purine biosynthesis genes is different from all other eukaryotes studied. Cloning of the corresponding genomic clones for each of the cDNAs is in progress.

074

CELL WALL MUTANTS OF *ARABIDOPSIS.* Wolf-Dieter Reiter, Clint Chapple, and Chris Somerville, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824-1312

The primary cell wall of higher plants plays numerous roles during plant growth and development such as the determination of cell shapes and sizes; however, very little is known about biochemical and regulatory aspects of its synthesis. We have used a genetic approach to address these questions by isolating mutants of *Arabidopsis thaliana* with alterations in the composition of cell wall polysaccharides. More than 5000 EMS-mutagenized plants were screened for changes in the relative amounts of the cell wall-derived monosaccharides rhamnose, fucose, arabinose, xylose, mannose, and galactose, leading to the isolation of 38 mutant lines representing more than ten complementation groups. The observed alterations in monosaccharide composition encompass the complete absence of a monosaccharide, reductions or increases in the relative amounts of specific monosaccharides, or more complex changes in sugar composition values. Some, but not all, of the observed cell wall mutations correlate with morphological or physiological abnormalities. We obtained several alleles of a locus designated *mur1* which cause the virtual absence of L-fucose in the shoot apparently due to a lesion in the *de novo* synthesis of L-fucose. The fucose content in the roots of *mur1* plants is only slightly reduced, suggesting the presence of a second fucose-biosynthetic gene with root-specificity. The *mur1* mutation causes structural alterations in pectic and hemicellulosic polysaccharides, leads to a more than twofold decrease in the mechanical strength of elongating regions of inflorescence stems, and correlates with a slightly dwarfed growth habit. Overall, this genetic approach should help elucidate the roles of individual polysaccharide components for cell wall structure and function, and permit the isolation of cell wall-related genes.

075

"The Gene Defective in Blue Fluorescent Arabidopsis Tryptophan Mutants"

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To understand the tryptophan biosynthetic pathway and its regulation we have isolated Arabidopsis mutants defective in tryptophan biosynthesis, and are characterizing the genes defective in these mutants. The *trp1* mutants are blue fluorescent, due to an accumulation of anthranilate, a fluorescent intermediate in the tryptophan pathway. We cloned and characterized the Arabidopsis gene (*PAT1*) encoding phosphoribosyl anthranilate transferase, the enzyme affected by the *trp1* mutations. *PAT1* is a single-copy gene that complements all visible phenotypes caused by two different *trp1* mutations (Rose *et al.*, 1992, Plant Physiology **100**: 582).

Experiments to determine the regulation of the *PAT1* gene are in progress. The wild-type *PAT1* promoter, and several promoter deletions, have been fused to the β-glucuronidase gene and transformed into Arabidopsis. Several *PAT1* gene constructs containing the same promoter deletions are also being introduced into *trp1* mutant plants. These constructs should reveal the normal tissue-specific expression of the *PAT1* gene and may identify promoter elements that control this pattern.

A number of *trp1* mutants have been isolated that are fluorescent, but differ in other respects. Some are small, bushy, and virtually sterile auxotrophs, while others are prototrophs that grow like wild-type plants. Each member of this allelic series is being analyzed for PAT enzyme activity and level of accumulated anthranilate compounds. The *PAT1* gene is being cloned from the *trp1* mutants, with the aim of understanding the molecular basis of the different mutant phenotypes.

076

Genetics of iron uptake in *Arabidopsis thaliana*

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Iron features in a number of important processes in higher plants, such as photosynthesis and respiration. However, at biological pH, iron is present in the soil predominantly in the form of stable ferric salts which are unavailable for uptake by the plant. Higher plants overcome the problem of iron availability by increasing the activity of ferric reductase at the root surface. This process reduces ferric iron to the ferrous state and allows ferrous uptake mechanisms to move the iron across the plasma membrane. Plants also increase the rate of iron uptake by the extrusion of protons from the roots into the rhizosphere. This results in a change in soil pH that favours ferrous iron formation.

We are studying the genetics and molecular biology of iron uptake in *Arabidopsis thaliana*. Root ferric reductase activity has been assayed under a variety of growth conditions. Mutant screens have been performed for plants that have a defective or altered (higher or lower) ferric reductase activity than wild type. The ferric reductase activity of a variety of *Arabidopsis* ecotypes has been determined and hairless root mutants have been analysed for ferric reductase activity.

077

ISOLATION OF PURINE BIOSYNTHETIC GENES OF *ARABIDOPSIS THALIANA* BY COMPLEMENTATION OF *ESCHERICHIA COLI* AUXOTROPHS AND ANALYSIS OF EXPRESSION PATTERNS IN TRANSGENIC *ARABIDOPSIS* PLANTS,

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In bacteria, a series of 12 enzymatic steps are involved in the *de novo* synthesis of purine nucleotides. These nucleotides are essential precursors to DNA synthesis as well as cofactors for a variety of other enzymatic processes. In tropical legumes, purine biosynthesis plays an additional, essential role in the nitrogen fixation pathway, providing precursors for the biosynthesis of ureides. We have undertaken an analysis of *de novo* purine biosynthesis in *Arabidopsis thaliana* using cDNA expression library to isolate clones which complement particular *E. coli* mutants. We have previously presented data on the cloning and characterization of two genes, those encoding AIR synthetase (*purM* or *PUR5*) and SACAIR synthetase (*purC* or *PUR7*). Transgenic plants have been generated which fuse promoter fragments up to the initiator ATG with the β-glucuronidase gene from *E. coli*. Initial experiments with a *PUR5* construct which contains approximately 1kb of upstream sequence show a highly specific pattern of expression. Low levels of expression are apparent in lateral root initials in young seedlings, cotyledon leaves and irregular patches of GUS expression are apparent on true leaves. In mature plants, the only staining observed is in pollen. Pollen staining is evident in later development of flowers. We are currently generating additional constructs which contain larger promoter regions as well as generating plants containing upstream regions of the *PUR7* gene. The promoter regions will be sequenced and compared for similarities between the various genes.

A PCR probe for the *purF* gene has been generated from an *Arabidopsis* cDNA library and is being used to screen both cDNA and genomic libraries for the *Arabidopsis purF* gene. This enzymatic step is known to be subject to regulation in bacteria, fungi and animals and will likely be subject to control in *Arabidopsis* as well. Once isolated, the promoter region will also be fused to GUS and transgenic plants will be analyzed for expression patterns. Ultimately, attempts will be made to induce expression of these promoters by using various growth conditions of purine starvation or excess. Hopefully, these studies will help elucidate the mechanisms regulating purine gene expression in plants.

078

Genetic Approaches to Flavonoid Research in *Arabidopsis thaliana*.

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Flavonols are the most abundant class of flavonoids. They protect plants from UV damage[1], but their absorption favors UV-A, and ozone depletion is preferentially increasing UV-B radiation at temperate latitudes. Flavones and flavanones absorb UV-B radiation more efficiently, but efforts to enhance their production have been hampered by ineffective methods of visualization. In response to this situation, a selective staining procedure was developed. It identified plants blocked in several steps of flavonoid biosynthesis[2]. A T-DNA insertional mutant library was also screened. Two flavonoid 3'hydroxylase (F3'OH) mutants were isolated by the characteristic green fluorescence of their stained flavonoids, and attempts to clone the gene are in progress. We are focusing on these mutants because F3'OH is a key enzyme in flavonoid biosynthesis. It is hypothesized that by switching its position within membrane aggregates, F3'OH shuttles flavonoids into several flavonoid classes, including the flavones and flavanones. The staining procedure also facilitates genetic manipulation of flavonoid biosynthesis, and the thousands of structures that are possible will make flavonoid biosynthesis a model system for secondary metabolism in higher plants.

1. Li, J., Ou-Lee, T.-M., Raba, R., Amundson, R.G., and Last, R.L. (1993). *Arabidopsis thaliana* flavonoid mutants are hypersensitive to UV-B irradiation. The Plant Cell. 5(2), 171-179.

2. Sheahan, J.J., and Rechnitz, G.A. (1993). Differential Visualization of *Transparent testa* mutants in *Arabidopsis thaliana*. Anal. Chem. 65(7), 961-963.

079

ANALYSIS OF THE STRUCTURE AND EXPRESSION OF GENES ENCODING E2s FROM *ARABIDOPSIS THALIANA*

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In eukaryotes, conjugation of ubiquitin to a protein acts as a commited step for intracellular protein degradation. Ubiquitin-conjugating enzymes (E2s) are involved in the covalent attachment of ubiquitin to protein substrates. In *Arabidopsis*, there are at least 15 E2 genes, which fall into five subclasses. These subclasses differ with respect to size, amino acid sequence, requirement for ubiquitin-protein ligase (E3), and potential function. Genomic clones corresponding to six E2s have been isolated and sequenced. These genes fall into two of the five subclasses; UBC 1-3 contain only a core region, whereas UBC 4-6 contain an acidic C-terminal extension in addition to the core region. Southern analysis indicates that UBC 1-3 and UBC 4-6 constitute their entire respective gene families. Northern analysis was used to examine the expression of these genes in a variety of tissue types. The temporal and spatial regulation of UBC 1-6 was examined by analysis of β-glucuronidase (GUS) activity in transgenic *Arabidopsis* containing UBC promoter-GUS fusions.

080

5-Fluoro-2'-deoxyuridine resistant mutants segregate as single recessive nuclear mutations

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The major cytotoxic effect of 5-fluoro-2-'deoxyuridine (FUdR) is associated with its anabolite 5-fluorodeoxyuridine monophosphate (FdUMP), which inhibits thymidylate synthase. Thymidine kinase (TK) is responsible for the conversion of FUdR to FdUMP intracellularly. Our initial interest is to isolate Arabidopsis mutants which are defective in TK activity. Two independent selected FUdR-resistant mutants of Arabidopsis (FUD-1 and FUD-2) were isolated from EMS mutagenized populations. Genetic analysis indicates that these two mutants are due to a single recessive nuclear mutation. All the F1 progeny of the crossing between FUD-1 and FUD-2 are resistant to FUdR. This indicates that these mutations are in the same gene locus, which was designated fur1. Biochemical studies demonstrate that the FUdR resistance in these two mutants is not due to a defect of TK activity but because of decreased drug uptake. Currently, genetic mapping is in progress.

081

Cloning the *cer2* locus of *Arabidopsis* by chromosome walking.

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Prior to initiating a chromosome walk to *cer2*, a detailed genetic map was constructed of the region of chromosome 4 that contains *cer2*. Approximately 100 genetic recombinants involving *ag, im, cer2,* and *ap2* were analyzed with several RFLP markers from this region. RFLP marker 600 is approximately 2.5 cM from *cer2*. Because on average there are 170 kb/cM in *Arabidopsis*, we estimated this marker to be approximately 425 kb from *cer2*. RFLP marker 600 was used to screen *Arabidopsis* YAC libraries; four YACs were identified that contained sequences homologous to RFLP marker 600. Both ends of each YAC were rescued by either plasmid rescue or lambda subcloning. The YACs were oriented relative to the genetic map by using the rescued YAC ends as RFLP markers to analyze the collection of genetic recombinants. The YAC end closest to *cer2* was used to isolate a collection of YACs closer to *cer2*. This cycle was repeated five times. We have analyzed genetic recombinants on both sides of *cer2* with RFLP probes isolated from our YAC contig. This analysis has established that one of our YACs includes the *cer2* locus. This YAC is currently being subcloned into the cosmid vector pLZO3 which will enable us to generate a library in *Agrobacterium* strain AGL1. Isolates from this *Agrobacterium* library will be tested for their ability to complement *cer2* following transformation of *Arabidopsis*. In parallel, we have used this YAC to screen a silique-specific cDNA library.

082

The Study of the Arabidopsis Tryptophan Biosynthetic Pathway by Immunological Techniques

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The tryptophan biosynthetic pathway provides plants with precursors for the growth hormone auxin and many secondary products in addition to tryptophan. In order to understand how a plant regulates the tryptophan biosynthetic pathway to fufill these diverse functions, it is important to monitor the enzymes of the pathway at the protein level. We will present our progress towards making and characterizing polyclonal antibodies against the following enzymes of the tryptophan biosynthetic pathway: phosphoribosylanthranilate transferase (PAT), phosphoribosylanthranilate isomerase (PAI), tryptophan synthase α (TSA) and tryptophan synthase β (TSB). The cDNAs encoding these proteins were inserted into the *E. coli* overexpression vector pGEX1 or pGEX2T to make fusion proteins with glutathione S-transferase. The fusion proteins were used to make polyclonal antibodies in rabbits.

Previous results from transgenic plant analysis indicates that TSB is subject to cell-type specific regulation (1). The TSB promoter directs the expression of β-glucuronidase in vascular tissues, meristems and other specialized tissues in transgenic Arabidopsis plants. The TSB antibody will be used in immunohistochemistry experiments to confirm these results. In addition, immunolocalization studies with immunogold antibodies will enable us to examine the subcellular distribution of these tryptophan biosynthetic enzymes.

1. Pruitt, K. D. and Last, R. L. Expression patterns of duplicate tryptophan synthase β genes in *Arabidopsis thaliana*. Plant Physiol. In Press

083

ARABIDOPSIS THALIANA-ERYSIPHE CRUCIFERARUM AS A MODEL SYSTEM FOR MAP-BASED CLONING OF PLANT DISEASE RESISTANCE GENES.

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Race-specific disease resistance in plants is triggered following specific recognition of pathogenic agents. This type of plant-microbes interaction has been described by the gene-for-gene hypothesis, based on genetic analysis in numerous systems. Molecular analysis of plant genes that confer resistance to specific pathogenic agents would thus enhance our understanding of this interaction. *Erysiphe cruciferarum* is an obligate fungal pathogen that causes the powdery mildew disease on *Arabidopsis thaliana*. Most ecotypes of *A. thaliana* are susceptible to infection, except for nine which present various degrees of resistance. These lines have been grouped into three categories based upon their disease reaction (DR) scores. Fully susceptible ecotypes, including Columbia-gl, have been given a DR score of 4, that is characterized by extensive fungal growth and conidiation, with no apparent host reaction (chlorosis or necrosis). At the other end, a DR score of 0 was given to fully resistant lines that support no visible growth of *Erysiphe*. A DR score of 1-3 has been associated with increasing *Erysiphe* growth and conidiation, and corresponding reaction from the host (chlorosis, necrosis). Genetic analysis is currently being performed to determine the number of distinct disease resistance loci represented among these ecotypes as well as to allow future map-based cloning of the genes involved. All resistant ecotypes have been crosses to the susceptible strain Columbia-gl, and the first generation (F1) allowed to self-fertilized to generate a F2 population. The recessive marker gl (trichrome development) is being used as a marker in crosses. An F2 population (n-250) has been scored for DR. Testcrosses of the F1 plants with Col-gl provide an independent confirmation of the mode of inheritance of the resistance genes.

084

TRANSGENIC *ARABIDOPSIS* PLANTS THAT CONSTITUTIVELY EXPRESS THE COLD-REGULATED GENE, COR15

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Arabidopsis is capable of withstanding freezing temperatures after exposure to a low, nonfreezing temperature. During cold acclimation, certain cold-regulated (COR) proteins are induced. We have generated transformant *Arabidopsis* that express the chloroplast-targeted protein, COR15, at warm temperatures. We are currently performing a variety of tests on these plants aimed at determining the function of COR15. Results from some of these experiments, including chlorophyll fluorescence measurements after a freeze-thaw, will be presented.

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085

Characterization of lipoxygenase gene expression in *Arabidopsis*. Erin Bell and John E. Mullet, Department of Biochemistry and Biophysics, Texas A & M University, College Station, TX 77843.

Lipoxygenases (LOX) are non-heme iron-containing enzymes which catalyze the incorporation of molecular oxygen into unsaturated fatty acids, and can also cause carotenoid co-oxidation. Based on these activities, roles for LOX in the biosynthesis of jasmonic acid, traumatic acid, and abscisic acid have been proposed. In addition, increases in LOX activity in response to wound and pathogen stresses have been reported. In order to examine specific roles for LOX in normal growth and in response to stress conditions, we have isolated an *Arabidopsis* cDNA (*AtLox2*) which encodes a 102 kD protein with strong similarity to known LOX sequences. The deduced amino acid sequence also contains features that suggest that the protein may be chloroplast localized. *AtLox2* mRNA levels are high in leaves and inflorescences but very low in seeds, roots, and stems. Further accumulation of *AtLox2* mRNA in leaves occurs within 3 hours in response to wounding, and within 2 hours in response to methyl jasmonate treatment. We are continuing to determine the effect of different stress treatments on *AtLox2* expression in order to identify conditions where a specific role for the protein may exist. In addition we are beginning to characterize transgenic *Arabidopsis* containing *AtLox2* constructs to examine the effects of altered *AtLox2* expression on developmental and stress responses.

086

MOLECULAR GENETIC ANALYSIS OF DISEASE RESISTANCE IN *ARABIDOPSIS - PSEUDOMONAS* INTERACTIONS: THE *RPS2* LOCUS.

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A highly effective disease resistance response is induced when plants, through the action of a "resistance gene," recognize the presence of a pathogen expressing a particular avirulence gene. We have identified *RPS2*, an *Arabidopsis* locus that controls resistance against *Pseudomonas syringae* bacteria expressing the *avrRpt2* avirulence gene. Both mutants and natural ecotypes have been identified that lack resistance against bacteria expressing *avrRpt2;* these plants retain resistance against bacteria expressing avirulence genes *avrB* or *avrRpm1*. The *RPS2* locus has been mapped to chromosome 4, and YAC and cosmid contigs that span the *RPS2* locus have been identified. Our progress toward molecular isolation of the *RPS2* locus will be presented. Work on additional plant loci controlling resistance and on physiological parameters associated with defense will also be discussed.

087

ISOLATION AND CHARACTERIZATION OF ARABIDOPSIS MUTANTS SUSCEPTIBLE TO *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

Karen Century, Andrew Bent, and Brian Staskawicz. Department of Plant Pathology, University of California, Berkeley, CA 94720.

The interaction between Arabidopsis and the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) has been developed as a model system to study plant pathogen interactions. Arabidopsis ecotype Col-0 is resistant to Pst strain DC3000 harboring the avirulence gene *avrB*. To identify plant genes involved in disease resistance, M2 Col-0 plants derived from fast neutron and ethyl methanesulfonate mutagenized seeds were screened for susceptibility to DC3000(*avrB*). Three putative mutants have been isolated and are currently being characterized further. M3 progeny of two of the putative mutants, FNB21 and CEB3, are fully susceptible to DC3000(*avrB*), as demonstrated by analysis of bacterial growth *in planta*. These putative mutants are also susceptible to DC3000(*avrRpm1*), while they both retain resistance to DC3000 expressing *avrRpt2*. These results support data which suggest that resistance to bacteria expressing *avrB* or *avrRpm1* maps to a single locus or tightly linked loci in Col-0 (Innes, et. al., submitted for publication). Another putative mutant, FNB22, has lost resistance to DC3000 expressing any one of the three avirulence genes, indicating that this mutation may be blocking a more general step in the resistance process. Crosses of these putative mutants with resistant and susceptible ecotypes are underway to determine the number and location of genes controlling the above resistance phenotypes.

088

Cadmium-sensitive mutants of *Arabidopsis thaliana*

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A major component of the response of plants to heavy metals such as Cd is the induction of Cd-binding peptides. These peptides have been called phytochelatins and consist of a repeating γ-glutamylcysteine unit with a terminal glycine residue ($[\gamma EC]_n G$). Unlike the metallothioneins of animal cells which are encoded directly by genes, the ($[\gamma EC]_n G$) peptides are synthesised enzymatically from glutathione (γECG).

To identify genes involved in responses to Cd a screening procedure to identify Cd-sensitive mutants of *Arabidopsis* has been devised. Two loci, *CAD1* and *CAD2*, mediating these responses have been identified. The phenotypic characterisation of *cad1* and *cad2* mutants will be described. Cd uptake experiments indicate the mutants are less able to sequester Cd than the wildtype. Gel-filtration chromatography of Cd-binding peptides shows the *cad1* mutant lacks both the high and low MW forms of Cd-binding complex observed in the wildtype while the *cad2* mutant lacks only the HMW form. These mutants appear to be deficient in some aspect of biosynthesis or sequestration of Cd-binding peptides.

The *CAD1* locus has been mapped to the *TT3* region of chromosome 5 and a chromosome walk in the region to isolate the gene has been initiated. Mapping of the *CAD2* locus will be described.

089

Mutations In a Single *Arabidopsis* Locus Abolish Resistance to *Pseudomonas* Strains Expressing Two Different Avirulence Genes: A Gene-for-*Genes* Interaction

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We have isolated 3 independent mutations at the *RPS3* locus of *Arabidopsis*. This locus confers resistance to *Pseudomonas syringae* strains that carry the avirulence gene *avrB*. To identify mutations at this locus, we inoculated mutagenized populations of *Arabidopsis* ecotype Col-0 with *P. syringae* pv. tomato strain DC3000(pAvrB) and scored for conversion to susceptibility. Progeny of selfed mutant plants were inoculated with *P. syringae* strains carrying either *avrB, avrRpt2,* or *avrRpm1*. All mutants that were susceptible to strain DC3000(pAvrB) were also susceptible to strain DC3000(pAvrRpm1). These mutants were still resistant to DC3000(pAvrRpt2), however, indicating the mutation was not in a generalized resistance response. Genetic complementation analysis of the mutants with the naturally occurring susceptible ecotype Bla-2 revealed that all mutations were allelic and that they mapped to the *RPS3* disease resistance locus. Furthermore, we determined that *RPS3* co-segregates with *RPM1,* which confers resistance to *P. syringae* strains carrying *avrRpm1*. Our data, therefore, indicate that *RPS3* and *RPM1* are the same gene. This implies that a single plant resistance gene can interact with two different pathogen avirulence genes.

090

Genetic analysis of *Arabidopsis* mutants involved in systemic acquired resistance

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In order to initiate a genetic analysis of the mechanism(s) of systemic acquired resistance (SAR), we have used two novel approaches to identify related *Arabidopsis* mutants. First, we have devised an approach to efficiently infect a large number of mutagenized *Arabidopsis* plants with a *Pseudomonas* bacterial pathogen, *P. syringae* pv. *maculicola*, and identify mutants that have enhanced resistance or tolerance to the pathogen. This approach involves vacuum infiltrating a bacterial suspension into young *Arabidopsis* seedlings grown on a petri dish and screening for reduced disease symptoms. Using this approach, we have isolated a number of *Arabidopsis* mutants that show reduced symptoms and decreased *in planta* bacterial growth upon *Pseudomonas* infection. Northern analysis of a number of mutants showed constitutive expression of the PR-1 gene, whose expression has been correlated with SAR. The second approach focuses on mutants of a specific signal transduction pathway activated during SAR. *Arabidopsis* ß-1,3-glucanase (*BGL2*) gene has been shown to be induced by SA, INA and pathogens and probably involved in SAR. A transgenic *Arabidopsis* line carrying a *BGL2-GUS* chimeric gene has been constructed and shown to respond dramatically to SAR induction. Mutants are being isolated which have either constitutive *BGL2-GUS* expression or noninducible *BGL2-GUS* expression in response to SAR-inducing signal (SA or INA). Using a quick assay of the *GUS* reporter gene, we have screened through 10,039 M2 *Arabidopsis* mutants and identified 119 putative mutants constitutively expressing *BG2-GUS*. Progeny of 10 putative mutants obtained from the primary screen have been retested. One of them seems to be a homozygous mutant that constitutively expresses the *BGL2-GUS* fusion gene in every progeny produced by selfing. Molecular genetic analysis of mutants isolated using these two approaches are underway to elucidate the signal transduction mechanism of SAR.

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CHARACTERIZATION OF A HOST LOCUS ASSOCIATED WITH A GENOTYPE SPECIFIC INTERACTION BETWEEN *Arabidopsis thaliana* AND *Albugo candida* (WHITE BLISTER).

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Resistance of *Arabidopsis thaliana* (*At*) to *Albugo candida* (*Ac*) appears to be less common than resistance to *Peronospora parasitica* (*Pp*). Consequently, we have concentrated our efforts on the analysis of resistance of a single *At* accession (Kes37) to a single *Ac* isolate (*Acem1*). The phenotype associated with this interaction is characterised by necrotic flecks and a chlorotic halo. Using a cross between Wei0 (susceptible) and Kes37 (resistant), a single resistance locus (*RAC1)* has been identified and mapped to chromosome 1. *RAC1* lies between the loci defined by RFLP markers M335 and M253. This cross has also been useful in mapping genes that code for resistance to *Pp*. Kes37 carries an allele at locus *RPP2* (see abstract by Holub *et.al.*). Wei0 carries an allele at *RPP9* associated with delayed, sparse sporulation. This locus appears to cosegregate with *RAC1*.

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TOWARDS MAP-BASED CLONING OF THE *RXC1* GENE THAT CONFERS TOLERANCE IN *ARABIDOPSIS THALIANA* TO *XANTHOMONAS CAMPESTRIS* pv *CAMPESTRIS*. C. Robin Buell and Shauna C. Somerville, DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 USA.

The interaction between *Arabidopsis thaliana* and *Xanthomonas campestris* pv *campestris* provides a model system to isolate and characterize a plant resistance gene that confers tolerance to a bacterial pathogen. Resistance in *Arabidopsis thaliana* ecotype Columbia to *X. c. campestris* isolate 2D520 is conditioned by a single dominant allele termed *rxc1-1* at the *RXC1* locus. The recessive allele *rxc1-2* is found in the susceptible ecotype Pr0, resulting in spreading chlorosis and necrosis following inoculation with *X. c. campestris* 2D520. Genetic mapping using morphologically marked lines of *Arabidopsis* placed *RXC1* on chromosome II. RFLP markers that map to chromosome II have been used to map *RXC1* relative to known molecular markers as a first step in map-based cloning of *RXC1*. Fine mapping and/or chromosome walking techniques utilizing yeast artificial chromosomes (YACs) will be employed to further map *RXC1* within 0.5 cM of flanking molecular markers. To determine if the interaction between the *RXC1* gene in ecotype Columbia and *X. c. campestris* 2D520 involves a gene-for-gene interaction, the pathogen *X. c. campestris* 2D520 is being examined for a putative avirulence gene that is complementary to the resistance gene *RXC1*. A cosmid library made from *X. c. campestris* 2D520, has been constructed in the broad host range vector pIJ3200 and will be mobilized into the recipient strain *X. c. campestris* 8004 which is able to cause chlorosis on ecotype Columbia. Transconjugants will be screened for the putative *avrRXC1* gene by inoculation into leaves of ecotype Columbia. *X. c. campestris* 8004 transconjugants that contain the putative *avrRXC1* gene are predicted to prevent chlorotic symptom development on ecotype Columbia, an ecotype that is susceptible to *X. c. campestris* 8004 but resistant to *X. c. campestris* 2D520.

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The oxidative stress response in Arabidopsis.

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We have initiated a study on the response of Arabidopsis to oxidative stress. Agents of oxidative stress produce toxic oxygen free radicals that cause cellular damage. The Halliwell/Asada pathway (a.k.a. glutathione/ascorbate cycle) acts to rid the cell of the superoxide radical (O_2^-) with the ultimate expenditure of NADPH. Isoforms for the enzymes in this pathway exist in at least two subcellular compartments - the chloroplast and the cytoplasm. We wish to determine if the chloroplast and cytoplasm respond differently to various oxidative stresses.

The redox-active herbicide paraquat causes oxidative stress predominantly in the chloroplast. We isolated paraquat resistant Arabidopsis mutants and are currently characterizing these mutants. This characterization is focused on the genes that encode the different Halliwell/Asada pathway enzymes. An investigation to determine if the genes encoding these isoenzymes are differentially induced by various oxidative stresses (paraquat and ozone) is also underway. We currently have evidence that the RNA levels for two of the isoenzymes (cytosolic Cu/Zn-superoxide dismutase, chloroplastic ascorbate peroxidase) increases with a low level ozone fumigation.

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Genetic Approaches to Specific Resistance of *Arabidopsis thaliana* Against Phytopathogenic Pseudomonads

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The number and function of plant genes necessary for a disease resistance response is unknown. Genetic analyses in many systems over the last 50 years have demonstrated that recognititon functions are provided by dominant alleles of genes in the plant (Resistance, or *R*-genes) which interact, either directly or indirectly, with either the direct or indirect product of a single pathogen avirulence (*avr*) gene. Although a great deal is known about the multitude of genes activated subsequent to triggering of the defense response, virtually nothing is known regarding the number or nature of downstream transduction steps which are truly necessary for a resistance reaction.

We use *Arabidopsis* to identify genetically plant loci necessary for a resistance reaction against phytopathogenic bacteria and fungus. Our work revolves around five themes. First, identification and isolation of recognition function genes (*R* or Resistance genes). We defined and are in the process of cloning the *RPM1* locus, which conditions resistance to pathogenic*Pseudomonas syringae* strains carrying either the corresponding *avrRpm1* gene, or the sequence unrelated *avrB* gene. Second, we used mutation analysis to identify loci which lose *avrRpm1* recognition. These mutations should map to either *RPM1* or to loci involved in either specific or general interpretation of *RPM1*-dependent function. Mutants in each class have been isolated, and thus far each silmutaneously loses the ability to recognize bacteria carrying either *avrRpm1* or *avrB* . The dual resistance specificity encoded at *RPM1* is of particular interest (see also abstracts Godiard et al. and Innes et al.) Third, we isolated four potentially T-DNA tagged mutants exhibiting phenotypes reminiscent of "lesion mimic" mutants which exist in many plant species, notably maize, rice, tomato, and barley. These have potentially constitutive or "hair-trigger" activation of some step in one or more pathway(s) leading to cell death and fomation of an HR-like lesion. At least two of the four constitutively express resistance against otherwise virulent bacterial and fungal pathogens. Fourth, we use a generalizable genetic approach to assess whether a particular activated defense response is strictly tied to *R*-gene mediated recognititon. Fifth, we are identifying the bacterial pathogen genes which are causal to triggering of a specific plant defense response. These fall into two categories: classically defined *avr* genes, and, using Tn*phoA* as a mutagen, genes encoding membrane localized or secreted products necessary for delivery of a specific *avr* function. Our current progress in these topic areas will be described.

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STRESS-INDUCED CHANGES IN MEIOTIC RECOMBINATION FREQUENCY IN *ARABIDOPSIS THALIANA*.

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Meiotic recombination occurs through a coordinated series of steps that are under enzymatic control. Recombination frequencies are relatively stable, allowing genetic maps to be made, but map distances can be altered by genetic and environmental factors. These variations are biologically significant since the gene combinations transmitted to gametes are also modified. We are using *Arabidopsis* as a model system to identify factors that can change meiotic recombination frequencies. We have developed an efficient recombination assay that uses two marker genes on chromosome 3 (*Csr* and TT_5). In a first experiment, salicylic acid treatment, but not nutrient deficiency, produced large increases in recombination frequencies. Salicylic acid is likely a natural transduction signal for pathogenesis-related proteins. These results are consistent with current evolutionary theory which predicts that functional relationships will be found between pathogen-induced stress responses and recombination mechanisms.

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ISOLATION OF *ARABIDOPSIS THALIANA* MUTANTS HYPERSENSITIVE TO GAMMA RADIATION

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We have identified twelve mutations in *Arabidopsis thaliana* that confer hypersensitivity to gamma radiation. The mutations represent at least four independently segregating loci, designated *rad1*, *rad2*, *rad3*, and *rad4*. The *rad* mutations produce symptoms of severe radiation damage in seedlings treated with sublethal doses of gamma radiation but do not induce hypersensitivity to UV-C. In the absence of radiation, the mutant plants resemble the wild-type except they have lower growth rates in stressful environments. Our results indicate that mutants hypersensitive to high-energy radiation occur frequently in EMS-treated populations of *Arabidopsis*. By analogy with radiation-sensitive mutants in bacteria, fungi, insects, and mammals, we expect at least some of these genes to function in DNA repair, recombination, transposition, transformation, or cell cycle control. We have chosen the *rad3* mutation for further characterization. Experiments are underway to map the *rad3* gene and to test its effect on meiotic and mitotic recombination, double-strand break repair, stress responses and development.

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ANALYSIS OF GENE ACTIVITY IN NEMATODE-INDUCED FEEDING STRUCTURES, USING PROMOTER-GUS FUSIONS AND PROMOTER TAGGING.

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The formation of nematode feeding structures in plant roots after infection with cyst or root-knot nematodes is accompanied by severe changes in gene regulation. To monitor these changes at the molecular level, a variety of promoter-GUS fusions were introduced into Arabidopsis and analysed for GUS activity after infection with either *Heterodera schachtii* or *Meloidogyne incognita*. Furthermore, a large number of transgenic *Arabidopsis* plants were generated using *Agrobacterium* harbouring a binary vector with a promoterless GUS gene located at the right border sequence. Using this approach we were able to tag regulatory sequences that give rise to high GUS activity inside the nematode feeding structures. After isolation with inverted PCR, these tagged sequences are now subject to a more detailed analysis. The latest results from both approaches will be summarized on the poster.

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Molecular and Genetic Analysis of the Arabidopsis RPM1 Disease Resistance Locus

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We and others have shown that *Arabidopsis* is a host for phytopathogenic isolates of *Pseudomonas syringae* pathovar *maculicola* (Psm), normally pathogenic on Brassicas and tomato. Based on a series of "differential responses", we began to ask whether or not single loci in both plant and pathogen controlled the generation of a hypersensitive resistance response (HR) as predicted by the gene-for-gene hypothesis. We mapped one resistance locus, which we named *RPM1*, to an interval of around 5 map units near the top of chromosome three. Concurrent with this plant genetic analysis, we cloned the avirulence gene from the appropriate Psm isolate which triggers the HR in combination with the *RPM1* product (*avrRpm1*). We further demonstrated that the cloned *avrRpm1* gene was, in fact, responsible for *RPM1*-mediated recognition. We subsequently showed that the *avrRpm1* and the highly related *avrPpiA1* from *P. s.* pv. *pisi* are both recognized by specific cultivars of pea, bean, and soybean. This finding clearly suggests that *Arabidopsis* has functional, and possibly molecular, homologs to resistance genes in those species.

We are enlarging our YAC contig around *RPM1*. Progress in map-based cloning of this locus will be detailed. We have also isolated and are characterizing mutants of the resistant ecotype Col-0 unable to recognize the *avrRpm1* signal. These mutants should either be in the *RPM1* gene, and their analysis will further understanding of the critical recognition event mediated by its product, or they will be in loci necessary to transduce the *RPM1* signal. Mutants of each type have been isolated, and genetic analyses have begun. A particularly interesting point is that all of our mutans also lose the ability to recognize the sequence unrelated *avrB* gene from *P. syringae* pv. *glycinea*. This finding, and data from the labs of Roger Innes at Indiana University and Brian Staskawicz at UC Berkeley, show that *RPM1* , or a very closely liinked gene, also determines resistance to bacteria containing *avrB* . Whether or not the same, or a very closely linked, gene product determines resistance to both *avr* gene products awaits cloning of the *RPM1* locus.

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ARABIDOPSIS METALLOTHIONEIN GENES ARE FUNCTIONAL HOMOLOGUES OF ANIMAL AND FUNGAL METALLOTHIONEIN GENES

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Metallothioneins (MTs) are cysteine-rich proteins required for heavy metal tolerance in animals and fungi. In plants, phytochelatins, cysteine-rich non-protein peptides which bind cadmium and other metals, have been demonstrated to be important in heavy metal tolerance. Two cDNAs, MT1 and MT2, encoding proteins with significant homology to animal and fungal metallothioneins, have been isolated from *Arabidopsis*. The proteins predicted from these two cDNAs are 45 and 81 amino acids long, and are homologous in their N-terminal and C-terminal cysteine-rich domains. MT2 has a third central domain which lacks cysteine but contains aromatic amino acids, and is more homologous to MT genes in other plant species than to MT1. In contrast, MT1 does not have this central domain. When expressed in a MT-deficient yeast mutant, both MT1 and MT2 complemented the mutation and provided a high level of resistance to $CuSO_4$ and moderate resistance to $CdSO_4$. The MT-deficient yeast cells were not viable in the presence of 300 µM $CuSO_4$ or 5 µM $CdSO_4$. Cells expressing MT1 were able to grow in medium containing 3 mM $CuSO_4$ and 10 µM $CdSO_4$, and those with MT2 grew in the presence of 3 mM $CuSO_4$ and 100 µM $CdSO_4$. In plants, MT1 and MT2 mRNAs were induced by $CuSO_4$ but not $CdSO_4$ suggesting that *Arabidopsis* MTs are responsible for copper tolerance. The expression of MT genes in *Arabidopsis* is also developmentally regulated. MT1 mRNA was more abundant in roots and etiolated seedlings than in leaves. In contrast, MT2 mRNA was more abundant in leaves than in roots and dark grown seedlings. We conclude that both MT1 and MT2 are functional homologues of animal and yeast MTs. These results argue against the hypothesis that phytochelatins are the sole molecules involved in heavy metal tolerance in plants.

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Approaches to the analysis of the host-parasite interaction between *A. thaliana* and gall- and cyst-forming plant-parasitic nematodes

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A. thaliana has been shown to be an optimal model plant to study different aspects of the interaction with gall- and cyst-forming sedentary plant parasitic nematodes. The specific properties of the plant have led to the application of some useful methodological approaches to analyse the structural and molecular basis of this system.
The simple structure of the thin and translucent root is an optimal basis to describe and analyse the tremendous histological and cytological changes caused by the nematode with the formation of a feeding structure within the central cylinder. Due to the particular properties of the root, in vivo observations of the parasitic nematode behaviour and the plant response can be performed with the aid of video light microscopy. Thus the complete live cycle of the cyst nematode *Heterodera schachtii* and early events of the infection by the root-knot nematode Meloidogyne incognita could be analysed in detail. The in vivo observations allow the exact timing of sampling material for embedding procedures. Classical light and electron micrographs give insights to the histological and cytological events. By direct microinjection into the feeding structure the nutrient uptake by the nematodes and the response of plant and nematode to the injected substances are now studied. Feeding structures can easily be identified and sampled in agar cultures. Thus enough material for protein assays can be collected in order to determine biochemical changes at the protein level. The agar culture is also the basis for an extensive screening of nematode infected *A. thaliana* mutants in order to identify plant genes which are essential for a functional host-parasite interaction.

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HARPIN$_{PSS}$, THE *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE HRPZ* PRODUCT THAT ELICITS THE HYPERSENSITIVE RESPONSE IN HIGHER PLANTS. Sheng Yang He[1,2], Hsiou-Chen Huang[3], Gail M. Preston[2], Robert L. Last[4], and Alan Collmer[2]. [1]Department of Plant Pathology, University of Kentucky; [2]Department of Plant Pathology, Cornell University; [3]Agricultural Biotechnology Laboratories, National Chung Hsing University, Taiwan; [4]Boyce Thompson Institute.

The ability of *Pseudomonas syringae* to elicit the hypersensitive response (HR) in nonhost plants or pathogenesis in host plants is controlled by a 25-kb cluster of *hrp* genes. The cosmid pHIR11 carries the entire *hrp* gene cluster of *P. s.* pv *syringae* 61 and enables saprophytic bacteria, such as *Pseudomonas fluorescens* and *Escherichia coli*, to elicit the HR in higher plants including *Arabidopsis thaliana*. We report here the identification of the *hrp* gene (*hrpZ*) that encodes an elicitor (harpin$_{Pss}$) of the plant HR. *hrpZ* was identified by screening a partial *Sau*3A library (in pBluescript) of pHIR11 for HR-eliciting activity in tobacco leaves. Harpin$_{Pss}$ is a 34.7 kDa extracellular protein, heat stable, glycine rich, and is dissimilar to any known proteins. Harpin$_{Pss}$ is only produced in planta or in an HR-inducing medium; its secretion is dependent on *hrpH*, which shows significant sequence similarity with genes involved in protein secretion in other Gram-negative bacteria. The carboxyl-terminal 148 amino acid portion of harpin$_{Pss}$ is sufficient and necessary for elicitor activity and contains two directly repeated sequences of GGGLGTP and QTGT. *hrpZ* homologues have been cloned from *P. s.* pv. *tomato* DC3000 and *P. s.* pv. *glycinea* race 4. The HR elicited by harpin$_{Pss}$ can be inhibited by plant metabolic inhibitors and therefore is an active response of the plant. To understand the pathway(s) of harpin$_{Pss}$-mediated plant responses, we have developed a vacuum-infiltration-based screening procedure, which allowed us to obtain several putative *A. thaliana* Co-1 mutants that are impaired in hypersensitivity to *P. fluorescens*(pHIR11), which produces and secretes harpin$_{Pss}$. Further characterization of harpin$_{Pss}$, its interactions with Avr proteins, and the *A. thaliana* mutants will help reveal the role and function of harpin$_{Pss}$ in plant-bacterial interactions.

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AvrRps4 CONFERS AVIRULENCE TO *PSEUDOMONAS SYRINGAE* IN AN ECOTYPE-SPECIFIC MANNER

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Race-specific disease resistance in plants has been studied using *Pseudomonas syringae*-Arabidopsis interactions as a model system. Arabidopsis ecotype Po-1 is susceptible to strains of the bacterial plant pathogen *Pseudomonas syringae* which carry the avirulence genes *avrRpt2, avrRpm1*, or *avrB*. In order to identify new avirulence gene specificities, 54 *P. syringae* strains were inoculated into Po-1. One strain, *P.s.* pv *pisi* strain 151, was chosen for further study as it induced a hypersensitive response in Po-1. A cosmid from a library constructed in pLAFR3 with DNA from this strain converted the normally virulent *P.s.* pv *tomato* strain DC3000 to avirulence in Po-1. This locus has been designated *avrRps4*. Initial evidence suggests that *avrRps4* is carried on an endogenous plasmid in *P.s.* pv *pisi* strain 151. DNA from representatives of all seven known *P.s.* pv *pisi* races hybridize to a 3kb active subclone. *In planta* growth curves confirm that after four days, DC3000 (*avrRps4*) grows approximately 50 fold lower in ecotypes Po-1 and Col-0 than does DC3000(pLAFR3). DC3000 (*avrRps4*) is virulent in tomato and Arabidopsis ecotype RLD as confirmed by measurement of *in planta* growth. Col-0xRLD F2 plants are currently being screened for segregation of resistance in order to identify and map a resistance locus that corresponds to *avrRps4*.

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CHARACTERIZATION OF HOST LOCI ASSOCIATED WITH GENOTYPE SPECIFIC INTERACTIONS BETWEEN *Arabidopsis thaliana* AND *Peronospora parasitica* (DOWNY MILDEW).

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Genotype specific interactions in a wild pathosystem involving *Arabidopsis thaliana (At)* and the biotrophic oomycete *Peronospora parasitica (Pp)* offer an exciting opportunity to investigate the molecular basis of disease resistance. Several different interaction phenotypes have been characterized using a cotyledon assay, each being genotype specific. Phenotypes include early, profuse sporulation; necrotic flecks without sporulation or with delayed, sparse sporulation; necrotic flecks developing into discrete necrotic cavities; and more severe necrotic pits that expand until the entire cotyledon has died. In each case, seedlings are capable of reproduction, except infection resulting in profuse sporulation can dramatically reduce plant vigour. At least nine different matching pairs of host and parasite loci are predicted thus far following analysis of a half diallel cross between eight accessions of *At* inoculated with three isolates of *Pp* . Four host loci are being mapped using molecular markers and progeny from a single *At* cross (Col-*gl1* x Nd0). A locus from Nd0, *RPP1*, on chromosome 3 (between *gl-1* and M249) is identified by an allele associated with necrotic pits. Two loci from Col-*gl1*, *RPP2* and *RPP4* , are closely linked on chromosome 4 (near M557). These loci are associated with necrotic flecks. However, the allele at *RPP4* permits delayed, sparse sporulation whereas the one at *RPP2* does not. A third locus from Col-*gl1*, *RPP7*, is associated with a phenotype similar to *RPP2* but its location has yet to be determined. Fine scale mapping of alleles at *RPP1* and *RPP2* is underway as a prelude to cloning and further molecular characterization.

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Characterization of an *Arabidopsis* Mutant Hypersensitive to Ultraviolet Light

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Our laboratory is interested in the mechanisms of DNA repair and genetic recombination in higher plants. Toward this end, we have isolated several UV light hypersensitive (*uvh*) *Arabidopsis* mutants from an EMS mutagenized seed stock. These mutants turn brown and shrivel at doses that do not affect wildtype plants. The ultimate goal of this analysis is to identify targets of UV light damage in plants and learn how these targets are protected and repaired. In *E. coli* and yeast, it has been observed that DNA is the primary target of UV damage and that UV sensitive mutants are often defective in DNA repair. Although it is likely that DNA repair mutations will be represented in our mutants, it is also likely that mutations in mechanisms unique to plants will be obtained. UV hypersensitive mutations in plants could involve UV protective pigments, chloroplast DNA repair, and UV sensitive targets unique to plants such as photosystem II. These unique mechanisms make it unlikely information from other systems will be sufficient to explain UV sensitivity in plants. We have chosen to focus our study on the *uvh6* mutation because it has several interesting properties in addition to UV hypersensitivity. First of all, *uvh6* plants are sensitive to gamma rays, which cause strand breaks in DNA. Sensitivity to both gamma rays and UV light suggests that DNA repair is defective in *uvh6.* This mutant also appears to have altered accumulation of pigments, including chlorophyll. These changes cause the mutant to appear yellow green compared to wildtype. *uvh6* is also conditionally lethal, dying when incubated at 37°C for 4 days. Whether this complex mutant phenotype is due to one or more mutations is being investigated by genetic analysis. We are currently mapping the mutation, selecting for suppressor mutations and comparing the rate of meiotic recombination to that of wildtype plants. We are also examining sensitivity to free radical producing herbicides, UV protection, and pigment levels in this mutant. We believe the *uvh6* mutation will help elucidate the unique physiological responses of plants to UV light.

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UV-B Stress Induction of Antioxidant Enzymes in Arabidopsis

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UV-B radiation may exert its deleterious effects on cells at two levels: 1) primary damage caused by the direct absorption of UV-B photons by cellular chromophores such as proteins and nucleic acids, and 2) secondary damage caused by free radicals produced when excited chromophores pass electrons to molecular oxygen. Arabidopsis mutants with altered sensitivity to UV-B radiation are being used to investigate the biochemical mechanisms that protect plants against UV-B. Aromatic compounds such as flavonoids and sinapate esters act as a barrier in the leaf epidermal cells by absorbing UV-B photons. Mutations that reduce levels of flavonols and sinapate esters cause increased UV-B sensitivity (Li et al., 1993, Plant Cell 5: 171-179). We are investigating the induction of enzymes such as superoxide dismutase and the enzymes of the ascorbate/glutathione cycle, which may protect against the damaging effects of free radicals, in Arabidopsis foliage exposed to growth-limiting levels of UV-B radiation. Antioxidant enzyme activities and levels of mRNA transcripts are being compared in ecotype Landsberg erecta and *tt5*, a chalcone isomerase mutant of Landsberg erecta.

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USE OF *ARABIDOPSIS THALIANA* RECOMBINANT-INBRED LINES TO LOCALIZE RESISTANCE GENES TO BACTERIAL PLANT PATHOGENS. Gerard R. Lazo and Richard A. Dixon. Plant Biology Division, The S. R. Noble Foundation, Inc., P. O. Box 2180, Ardmore, OK 73402.

Bacterial pathogens of crucifers have been screened on a number of ecotypes of *Arabidopsis thaliana*, and many ecotypes have been found susceptible to these pathogens. Race-specific resistance has also been identified to these pathogens. Having identified resistant/susceptible interactions for the *A. thaliana* ecotypes to these pathogens, recombinant-inbred lines of *A. thaliana* (Reiter *et al*, 1992) were used to localize resistance genes. Six replicates of the 155 available recombinant-inbred lines (ABRC) were screened against *Xanthomonas campestris* pv. *armoraciae*, *X. campestris* pv. *campestris*, and *Pseudomonas syringae* pv. *maculicola*. The recombinant-inbred lines were from parents of *A. thaliana* ecotypes Wassilewskija and Landsberg *erecta* backgrounds containing nine phenotypic genetic markers. These lines were scored for disease phenotypes and the resulting information was analyzed for linkage against the genetic markers. Additionally, more than 60 RFLP markers have been scored against interactions with the tested pathogens. Using data derived solely from the screening of the recombinant-inbred lines, all of the races of *X. campestris* pv. *armoraciae* interacted similarly and were placed into a single group. Races of *X. campestris* pv. *campestris* and *P. syringae* pv. *maculicola* appeared independently grouped. Interactions to the *X. campestris* pv. *armoraciae* strains mapped to linkage group I and appeared to be closely linked to each other. Resistance to the strain of *X. campestris* pv. *campestris* was mapped to linkage group II. Resistance to *P. syringae* pv. *maculicola* was mapped to linkage group I, although it was independently grouped from interactions of *X. campestris* pv. *armoraciae*. While previous reports of resistance to a race of *P. syringae* pv. *maculicola* have been mapped to linkage group III (Debener *et al*, 1991), perhaps resistance to the strain tested here is due to another race-specific resistance gene. Likewise, it has not been resolved if the linkage of resistance to *X. campestris* pv. *armoraciae* is due to a single resistance gene, or several clustered race-specific resistance genes. Other plant crosses with appropriate markers are being evaluated to help resolve these observations.

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Infection of *Arabidopsis thaliana* by the geminivirus, beet curly top virus

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The infection of susceptible plant hosts by single-stranded DNA viruses in the geminivirus group depends on the interaction of host and viral factors for the replication of viral DNA, the expression of viral genes, and viral movement between plant cells. We have shown that specific isolates of the geminivirus, beet curly top virus (BCTV), are capable of infecting some land races of *Arabidopsis thaliana* but not others. Symptoms appear on susceptible plants approximately 2-3 weeks after inoculation with BCTV-Logan and 10-15 days with BCTV-CFH and are characterized by leaf curling and stunted, deformed inflorescence structures. BCTV-CFH causes more severe symptoms than BCTV Logan. Analysis of viral DNA accumulation indicate that symptom development and severity is correlated with the accumulation of viral DNA in the plants. In Columbia, viral DNA begins to accumulate between 10 and 15 days after inoculation, and continued to accumulate over the 4 week period after inoculation. Levels of BCTV-CFH DNA were approximately 5 times higher than that observed for BCTV Logan 28 days after inoculation. Viral DNA is undetectable in phenotypically resistant land races, indicating that BCTV replication and/or movement is blocked in these plants. The identification of these resistant and susceptible land races provides an excellent model system that will allow the use of genetic approaches to determine the molecular basis of disease resistance to this important group of viral plant pathogens.

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Molecular Cloning of Hsc70 in *Arabidopsis*

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We have isolated cDNA clones of the 70 kD heat shock protein "cognate" (hsc70) from a cDNA library made with four week old soil-grown seedlings of *Arabidopsis thaliana* ecotype Columbia. Sequence comparison shows that the clones are over 90% homologous to other hsc70's isolated from plant species at the nucleotide level, and also contain 70 to 80% homology at the amino acid level to those reported for animal systems, such as human and *Drosophila*. These cDNAs were expressed at similar level in root, stem, leaf and flower tissues, but not in siliques of different stages. Antisense expression of the hsc70s and the expression of their mutants are analyzed in transgenic *Arabidopsis* to demonstrate the function of these genes in normal cells.

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New fungal pathogens of *Arabidopsis thaliana*.

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Until recently, investigations concerning mechanisms of disease resistance in *A.thaliana*, the model plant for plant molecular biology, were hindered by the lack of suitable pathogens. The first well described fungal pathogen of *A.thaliana* was *Peronospora parasitica* (causing downy mildew). We will describe additional fungal pathogens that were isolated from naturally infected plants grown in the greenhouse or growth chamber.

We will report on infections caused by Ascomycetes/Deuteromycetes, e.g. two *Fusarium* species, one *Peziza* species, one *Hypoxylon* species, and one *Cladosporium* species and by Oomycetes (two different *Pythium* species). The causal agent of a further disease has tentatively been identified as *Pyrenopeziza brassicae*.

The availability of easily cultured and possibly transformable pathogens should facilitate the work with *Arabidopsis* / pathogen systems and allow genetic investigations of the pathogen as well as of the host.

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CELL CYCLE CONTROL IN INITIAL PHASES OF NEMATODE INDUCED NURSE CELL FORMATION.

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In order to successfully accomplish their parasitic life cycle, sedentary plant-endoparasitic nematodes need to induce highly specialised cell types within the roots of their hosts. These multinucleated "nurse-cells" from which the parasite feeds, are, for most of these nematodes functionally equivalent yet their ontogeny varies significantly. Root-knot nematodes for example, induce giant cells by repeated mitosis without cytokinesis, while cyst-nematodes induce a syncytium by cell wall breakdown, protoplast fusion and DNA endoduplication.
To further characterise these key steps in the establishment of a successful compatible plant-nematode interaction and especially the unusual induced plant cell cycle in nurse cells, we decided to study the expression of two essential cell cycle genes, cdc2 and cyclin, after both root-knot and cyst nematode infection. For this purpose Arabidopsis plants containing chimaeric gus constructs under the control of the promoters of the Arabidopsis cdc2a and mitotic cyclin cyc1a, were infected with the root-knot nematode Meloidogyne incognita and the cyst nematode Heterodera shachtii. Spatial and temporal induction patterns will be discussed.

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OVEREXPRESSION OF A CHLOROPLAST SMALL HEAT SHOCK PROTEIN IN TRANSGENIC ARABIDOPSIS. Katherine W. Osteryoung, Nadja Wehmeyer and Elizabeth Vierling, Department of Biochemistry, University of Arizona, Tucson, AZ 85721.

In plants, the most abundant among the proteins induced in response to high temperature stress are the small heat shock proteins (sHSPs). Although the function of the sHSPs is unknown, the presence of distinct members of this protein family in the cytoplasm, endoplasmic reticulum and chloroplast suggests that the sHSPs are crucial to the ability of plants to withstand exposure to elevated temperatures. We are taking a number of approaches to assess the role of the chloroplast sHSP, HSP21, in the acquisition of thermotolerance. HSP21 is encoded in the nucleus as a large precursor and is processed to its mature size of 21 kDa upon import to the chloroplast. In its native form, HSP21 is a complex of 200-300 kDa. The protein is not detectable in the absence of heat stress, but accumulates in the presence of heat stress in proportion to the maximum temperature. We have generated several independent lines of transgenic Arabidopsis plants that constitutively overexpress HSP21. In all these lines, HSP21 accumulation in the absence of heat stress is at least five-fold higher than in wild-type plants heat-stressed at 40°C. The protein is processed to its mature size implying cleavage of the chloroplast transit peptide and correct localization within the chloroplast. The native size of the protein is identical to that in wild-type plants, indicating that proper assembly of the native complex can occur in the absence of heat stress. We are currently analyzing these plants for their growth properties, chloroplast ultrastructure, photosynthetic characteristics and responses to heat stress. Attempts to down-regulate HSP21 expression during heat stress using antisense techniques have met with only limited success, possibly due to the very long half-life of HSP21 *in vivo*.

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Progress towards map-based cloning of *RPP5*, an *Arabidopsis* gene that confers resistance to *Peronospora parasitica* race NoCO-2.

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In the course of studies of *Xanthomonas/ Arabidopsis* interactions, land race Columbia (Col-0) plants developed a downy mildew caused by *Peronospora parasitica*. Spores from infected leaves led to further disease on reinoculation of Col-0, but not of Landsberg (La-er). F2 progeny from a La-er X Col-0 F1 segregated 3:1 for resistance and sensitivity, indicating a single resistance gene. Close inspection of the La-er X Col-0 F1 plants showed they were less resistant than La-er, indicating a semi dominant resistance gene, designated *RPP5*.

To clone *RPP5* based on its map position, 297 recombinant inbred lines from the Col-0 X La-er cross were analyzed for segregation of resistance and sensitivity, and linkage to known RFLP markers. *RPP5* lies in a ~1 cM interval between the markers m226 and g3845 on chromosome 4. A RAPD marker $OPC18_{650}$ lies unbreakably linked to the resistance gene in this population. We are using the linked visible markers *cs* and *ara*, as well as primers that amplify distinguishable alleles of the *AGAMOUS* gene, to identify more recombinants in this region, so that binary vector cosmid clones that might carry *RPP5* can be identified. The easily transformable land race No-0 is sensitive to NoCO2, and will be used for these transformation experiments.

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Molecular cloning and characterization of low temperature induced genes in etiolated seedlings of Arabidopsis.

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Arabidopsis is able to survive freezing temperatures after being exposed to low nonfreezing temperatures, in a process termed cold acclimation. By screening a cDNA library prepared from poly$(A)^+$ RNA from cold-acclimated etiolated seedlings of Arabidopsis with a subtracted cDNA probe, we have isolated a set of genes which expression is induced by low nonfreezing temperatures. Sequence data indicate that these genes are different to those already described as low temperature inducible in Arabidopsis. The results obtained when both the levels and the temporal patterns of expression were studied, show that these are different between the genes; moreover, the responsiveness of these genes to stimuli other than low temperature, i.e. light, anaerobiosis, ABA, drought, etc, is also markedly different. The analysis of their expression in ABA deficient and insensitive mutants reveals a different involvement of ABA in the inducibility of these genes by low temperature. Although the participation of these genes in freezing tolerance remains to be demonstrated, their differential expression patterns might indicate that they have somewhat different roles in freezing tolerance and/or, in general, in other environmental stress responses.

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TMP-A and *TMP-B*, TWO TURGOR-RESPONSIVE cDNA CLONES OF THE *TMP* GENE-FAMILY ENCODE TRANSMEMBRANE PROTEINS WITH AN MIP-MOTIF

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We have isolated two cDNA clones of putative turgor-responsive genes in *Arabidopsis thaliana*. The nucleotide sequences of these clones termed *TMP-A* and *TMP-B* were determined, and amino acid sequences were deduced. The amino-acid sequences of the 30 kDa proteins encoded by these clones show 93% identity and 96.5% similarity to each other. However, nucleotide sequences of the coding region show only 74% identity where most changes are in the third position of the codons. Both proteins contain a MIP (major intrinsic protein) motif (SGGHINPAVT) found in many transmembrane proteins of animal and plant origin. Hydropathy plots revealed six stretches of hydrophobic amino acids, which could correspond to membrane-spanning domains common in ion channel proteins. Most of the potential membrane-spanning helices are bordered by charged residues or by helix breaking amino acids. We suggest that the proteins encoded by members of the *TMP* gene family might be ion transporters, possibly involved in turgor regulation.

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Ozone Induced Responses in *Arabidopsis thaliana*

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Ozone, a major air pollutant, is known to drastically affect plant growth and metabolism. However, not much is known about the molecular basis of these effects. We are currently characterizing ozone-induced responses in *Arabidopsis thaliana* ecotype Columbia. Plants grown in growth chambers for 3-4 weeks, under a 12 hour photoperiod were transferred into ozone fumigation chambers with similar growth conditions. Plants were treated with ozone (150 or 300 ppb) for 6 hours every day (9 am- 3 pm) over a two week period. Control plants were treated with ambient air. The major symptoms observed in ozone-treated plants were stunting and leaf curling. Plants treated with ozone for 2 weeks showed approximately 20-40% reduction in both fresh and dry weights of the shoots. To begin to understand the molecular basis of these responses, we are currently analysing the expression of various environmental stress-inducible genes in ozone-treated plants, including antioxidant defense genes such as peroxidase and superoxide dismutase. Experiments to identify other ozone inducible cDNA clones by differential mRNA display method are also in progress.

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ANALYSIS OF CIS-ACTING ELEMENTS INVOLVED IN THE DEHYDRATION-RESPONSIVE GENE EXPRESSION IN *ARABIDOPSIS THALIANA*.

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Under water-deficit condition various genes are induced, whose products are thought to function in protecting cells from dehydration. To understand the molecular process of signal transduction from water stress to gene expression in *Arabidopsis thaliana*. we cloned nine independent cDNAs (named RD), that are induced by dehydration stress[1]. We found that six RD cDNAs have sequence similarity with reported proteins in the database.

In the present study we report structure and expression of genes corresponding to two RDs, RD22 and RD29, which are induced by exogenous ABA. Protein synthesis is required for the induction of RD22 but not for RD29[3]. Two step induction of RD29 by water deficiency was observed[2]. We cloned and analyzed two genes for RD29, *rd29A* and *rd29B*[2], and one gene for RD22, *rd22*. The GUS reporter gene driven by these *rd* promoters was induced at significant level by dehydration in both transgenic *Arabidopsis* and tobacco. A *cis*-acting element involved in dehydration-responsive expression of *rd29A* was identified at the nucleotide sequence level. Nuclear protein(s) that bind to the cis-acting element was detected with gel retardation assay. Different cis-acting elements seem to function in the dehydration responsive expression in *rd29A*, *rd29B* and *rd22* genes.

1. Yamaguchi-Shinozaki et al.: Plant Cell Physiol. 33, 217-224 (1992)
2. Yamaguchi-Shinozaki, K. and Shinozaki, K.: Mol. Gen. Genet. 236, 331-340 (1993)
3. Yamaguchi-Shinozaki, K. and Shinozaki, K.: Mol. Gen. Genet. 238, 17-25 (1993)

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Bypass of the plant-pathogen recognition event(s) that triggers a plant cell death response (the hypersensitive response) in *Arabidopsis acd* mutants. Jean Toby Greenberg and Frederick M. Ausubel, Massachusetts General Hospital, Boston MA. Wild-type plants can undergo a rapid suicide cell death response called the hypersensitive response (HR) specifically when exposed to avirulent bacterial pathogens and not virulent pathogens. We have isolated two complementation groups of *acd* (accelerated cell death) mutants which appear to show a visible hypersensitive-like response when exposed to virulent pathogens or when they are allowed to age. The cell death in the *acd* mutants shares events characteristic of a hypersensitive response of wild-type plants. For example, both the abnormal lesions on aging *acd* leaves and wild-type leaves undergoing an HR accumulate autofluorescent material peripheral to the interior of cells, probably in the cell walls. Another marker for plants that have undergone a hypersensitive response is the induction of a select group of mRNAs (so-called pathogenesis related or PR mRNAs) in the uninfected leaves. Indeed, uninfected *acd* mutants which have the abnormally aging leaves also accumulate PR mRNAs in the apparently healthy tissue. Since the presence of the elevated levels of PR mRNAs correlates with the onset of spontaneous symptoms in the *acd* mutants, we take this as evidence that *acd* mutants can bypass the recognition of a pathogen to induce hypersensitive cell death.

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MECHANISMS FOR PLANT GROWTH AT LOW TEMPERATURE

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Plants of temperate regions have mechanisms to survive low temperatures (0-20°) that are lacking in tropical plants. We are employing a novel approach to identify the unknown processes plants require to survive chilling stress. The Feldmann collection of *Arabidopsis* mutants, generated by T-DNA insertion into the genome, was screened after growth at low temperature. Against a general background of chilling-tolerant plants, some individuals had acquired various phenotypes induced by low temperature. Phenotypes included chlorosis, no or slow growth, aborted flowering and split bolts.

This molecular genetic approach has several advantages. The chilling-tolerant and -sensitive plants are near-isogenic, differing in a single mutagenic event. Insertion mutagenesis should generate loss of function rather than change of function mutations and allow simple isolation of the mutated gene. Genes are identified whose products are required at low but not normal or freezing temperature. Genomic sequence associated with the T-DNA insert of one mutant has been isolated and is being characterized to determine genetic identity and relation to the phenotype.

The long term goals are three-fold: 1) determine the processes of low temperature adaptation in temperate species, 2) identify the molecular basis for chilling injury of various subtropic crop species, and 3) show the feasibility of conditional lethal mutants to elucidate metabolic and developmental processes.

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PROLINE ACCUMULATION IN *ARABIDOPSIS THALIANA* AFTER SALT STRESS

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In *Arabidopsis thaliana* (L.) proline can account for up to 20% of the free amino acid pool after salt stress. The precise role of proline during stress is not understood yet and several adaptative roles have been proposed. The main source of proline accumulation is its *de novo* synthesis from glutamic acid. Our main goal is to study the proline biosynthesis in *Arabidopsis thaliana* and the mechanisms of its enhancement during salt stress. *De novo* transcription and *de novo* translation are necessary for proline accumulation to occur. We cloned the *proc-1* gene (and corresponding cDNA) which encodes the last enzyme of the proline biosynthetic pathway, namely pyrroline-5-carboxylate reductase (P5CR) (L-proline:NAD(P)-5-oxidoreductase; EC 1.5.1.1). The P5CR encoded by *proc-1* is most probably cytosolic. *proc-1* mRNA level is higher in roots and ripening seeds than in green tissue, and is enhanced by a salt treatment. Southern blot analysis has suggested that at least one gene homologous to *proc-1* exists within the *Arabidopsis* genome. Its cloning and expression studies are in progress.

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ARABIDOPSIS HAS BOTH CYTOPLASMIC AND CHLOROPLAST HOMOLOGS OF THE HSP100 OR CLPA/B FAMILY OF PROTEINS

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Plants and other eukaryotes synthesize a 100 kDa protein in response to heat stress. The expression and structure of HSP100 is best characterized in *Saccharomyces cerevisiae*, where it has been shown to be required for development of thermotolerance. HSP100 is homologous to the *E. coli* clpB protein which is a member of the clpA/B family of proteins. ClpA modulates activity of an *E. coli* protease, but the function of clpB is not clear. An *Arabidopsis thaliana* gene with homology to the clpA/B family was isolated previously and designated clpC (Squires & Squires, J. Bact., 1992). The clpC protein is chloroplast-localized as evidenced by the presence of a chloroplast transit peptide. We have isolated a related gene from an *Arabidopsis* heat shock cDNA library which is more homologous to yeast HSP100 than to *Arabidopsis* clpC. We have designated this gene as *Arabidopsis* HSP100. The cDNA is 3.0 kb and contains a full-length coding region for a protein that does not have an amino-terminal transit peptide. Northern analysis reveals that *Arabidopsis* clpC mRNA is present at normal temperatures and is reduced by heat shock treatment. In contrast, the *Arabidopsis* HSP100 transcript is strongly heat-induced and undetectable prior to stress. Thus, *Arabidopsis* has at least two genes in the clp family of proteins, one which is not heat-regulated and encodes a chloroplast-localized protein, and a second which is strongly heat-regulated and most likely encodes a cytoplasmic protein. It will be of interest to determine if HSP100 is essential for plant thermotolerance as it is in yeast.

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A visual screen for freezing-sensitive mutants of *Arabidopsis*: preliminary results.

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We have screened a pedigreed collection of mutagenized *Arabidopsis* germ plasm for freezing sensitivity. The collection consists of 1931 families of M3 seed resulting from EMS mutagenesis of an M1 seed population (James & Dooner, 1990). Seedlings were cold-acclimated and then screened by freezing to -6°C, thawing, and visual inspection 48 hours later. 32 putative mutants have been identified by the gross shriveling and/or necrosis of their leaves. 14 of the 32 could be resuscitated and taken to seed; for the remainder, siblings were planted from the corresponding families and their progeny are being tested to prove heritability of the phenotype and recover the mutation responsible.

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ARABIDOPSIS THALIANA AS A MODEL ORGANISM TO STUDY PLANT RESPONSE TO SALINITY, Joanna E. Werner and Ruth R. Finkelstein, Dept. of Biological Sciences, University of California, Santa Barbara, CA 93106.

Salinity is one of the most important environmental factors limiting crop productivity. In spite of great research attention, the mechanisms which impart salt tolerance to some plants are still not well understood. The most studied crops include barley, rice and tomato. We have chosen *Arabidopsis* as a model plant to select mutant lines that can tolerate the presence of salt in a medium. Three populations of M2 seeds produced by gamma-irradiation of *A.t.* ecotype Columbia were screened for their ability to germinate on minimal medium supplemented with 1.5% NaCl . Six individuals were selected out of 130,000 seeds and rescreened as salt tolerant in the next generation. These are subject to genetic, biochemical and physiological analyses. The germination rate on 1.5% NaCl medium after 7 days varies among lines from 22 to 75%; in contrast, wild type does not germinate. There is significant variation among lines in the germination response to salt dosage on NaCl medium containing from 1 to 2% of salt, and their bleaching rate over time. Although some of the ABA insensitive (*abi*) mutants germinate more readily than wild type on salt, none of the new mutant lines appears to be ABA insensitive. The allelic relationship among lines and their further physiological characteristics will be presented.

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Towards understanding trichome differentiation in Arabidopsis: the cloning of ttg (transparent testa, glabra).

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Trichomes or leaf hairs are single cell structures that protude from leaves and stems in Arabidopsis. They develop very early in the leaf primordia from the L1 cell layer which also gives rise to the epidermal cells and the stomatal complex. A number of loci influence trichome differentiation and development in Arabidopsis. We have taken a molecular genetic approach in an attempt to understand the relationship between the products of these genes and their role in trichome formation.

The ttg locus was originally described by Koornneef, 1981 (Arab Inf Serv 18, 45-51) as a "complex syndrome" because the gene product affects several apparently unrelated pathways. Mutants at the ttg locus lack trichomes, anthocyanin in the seed coat and the plant body and seed mucilage. The function of the ttg gene was unknown though recent evidence from Lloyd et al, 1992 (Science 258, 1773-5) suggests that it is functionally similar to the R gene from maize which regulates anthocyanin biosynthesis. Because the chromosomal location of ttg was well characterised in relation to other genetic loci, we decided on a chromosome walking approach together with RFLP analysis of recombinants between ttg and nearby marker genes, ms-1 and ga-3 to isolate ttg. Using over 60 recombinants on either side of ttg and spanning 10 cM of chromosome 5 we have mapped ttg within a 60 kb region. At present we are using inserts from overlapping lambda clones to complement the ttg mutation by Agrobacterium-mediated transformation.

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Differences in morphology of the pedicel-primary stem juncture associated with mutations of the bp locus in Arabidopsis thaliana.

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Mutations of the bp locus display altered morphological patterns which are expressed primarily in the pedicel. Plants harboring these mutations of the bp locus display a shortened pedicel and altered trophic orientation. The altered trophic orientation is exhibited in the growing flower and maturing silique and result in the downward orientation of both the flower and fruit. A total of eight different mutant alleles of bp have been isolated in two different parental ecotypes using both chemical and ionizing radiation techniques. Examinations of both plants and tissues from these mutant alleles using light microscopy of embedded tissue sections show that cell enlargement of cells along the adaxial surface of the pedicel is responsible for the altered tropism. Further examinations employing scanning electron microscopy show that mutations of the bp locus affects not only the pedicel but also the junction regions with the primary stem. These results suggest that mutations of this locus are responsible for affecting the epidermis and may be causal in directing the morphology of other specific tissues of the plant.

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Cell-Cell Interactions In *Arabidopsis* Root Development - A Microinjection Study.

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Local interactions between cells are an important feature of plant development and are probably involved in the mechanisms by which neighbouring cells aquire different fates. In *Arabidopsis* roots, epidermal cells differentiate into cells with or without root hairs. These two cell types lie in discrete files. The root hair cell file has a characteristic set of developmental traits that reflects a divergent pathway acting early in epidermal cell differentiation.

The root hair cell files are located in the cleft formed between two cortical cell files while the other epidermal cell files abut only the outer periclinal wall of one cortical cell file. Previous studies indicate that the precise positioning of the different epidermal cells may be important in local cell interactions (Bünning, 1951). During development of the root, cells need to register their local position with respect to other cells as part of the process of specifying final cell fate. Such communications could involve matrix-matrix, matrix-cell or cell-cell interactions. Cell-cell communications might involve signalling molecules moving through the apoplast or directly through the plasmodesmata. These possibilities are being investigated using microinjection techniques and results will be presented.

Bünning, E. (1951) Über die Differenzierungsvorgänge in der Cruciferenwurzel. Planta, 39 126-153.

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ROOT HAIR STRUCTURE AND DEVELOPMENT IN NORMAL AND MUTANT SEEDLING ROOTS OF *ARABIDOPSIS THALIANA*

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Root hairs are tip-growing tubular outgrowths formed by specialized root epidermal cells (trichoblasts) which greatly increase the area of contact between plant and soil. The accessibility and continuous initiation of hairs on growing roots makes them suitable subjects for investigating cellular differentiation and the process of tip growth. In *Arabidopsis*, trichoblasts differ from other epidermal cells in shape, position and delayed vacuolation. Nuclei migrate into root hairs growing from the surfaces at the distal ends of trichoblasts. Electron microscopy of selected mutant and wild type root hairs prepared by rapid freezing and freeze substitution has revealed a highly polarized organization of cytoplasm like other tip-growing cells. Secretory vesicles, ribosomes, microfilaments and peripheral axially oriented microtubules occupy the extreme tips of growing hairs. Between the extreme tip and the major vacuolated portion of hairs the plasma membrane is frequently clathrin-coated, and the cytoplasm in this area contains endoplasmic reticulum and Golgi bodies (which typically have 4-5 cisternae, plus prominent trans-Golgi networks), as well as multivesicular bodies and partially coated reticula. These organelles also extend into the vacuolated region, in which mitochondria, plastids and nuclei are also found. Large dark vesicles are often aligned along bundles of axially oriented microfilaments in this area. Evidence for vacuolar biogenesis suggests that the formation of new vacuoles contributes to the extension of the vacuolar system in growing root hairs.

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Immunophilins/peptidyl-prolyl *cis-trans* isomerases of Arabidopsis. Charles S. Gasser, Irene Chou, Veronica Lippuner, Victoria A. Vucich, Steven M. Theg and Sydney V. Scott
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Immunophilins (cyclophilin, and the FK506-binding protein) are a class of ubiquitous proteins first identified in mammals as the specific targets of T-cell active immunosuppressive drugs. The mode of action of these drugs indicates that the immunophilins can interact with and modulate intermediates in Ca^{++}-dependent signal transduction pathways. Immunophilins have additionally been shown to have peptidyl-prolyl *cis-trans* isomerase (rotamase) activity, which is necessary for the efficient folding of a number of proteins. We are studying the biochemistry and molecular biology of the immunophilins of Arabidopsis. Arabidopsis contains a small family of four to six cyclophilin genes. We have isolated cDNA and genomic clones of four of these genes. Two genes, designated *ROC1* and *ROC4*, have been further characterized. *ROC1* is expressed in all parts of the plant and appears to encode the primary cytosolic cyclophilin in *Arabidopsis*.. Recombinant ROC1 protein, produced in *E. coli*, is an active rotamase with properties similar to human cytosolic cyclophilin. The protein product of *ROC4* includes an amino-terminal extension with properties of a chloroplast transit peptide. *In vitro* uptake experiments have shown that the primary product of this gene is taken up and processed by isolated chloroplasts. *ROC4* is expressed at high levels in photosynthetic organs, but expression was not detected in roots. We conclude that the *ROC4* gene encodes a novel chloroplast-localized cyclophilin isoform.

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

Two of the most fundamental problems in developmental biology are: 1. How do cells acquire different developmental fates? and 2. How is the spatial arrangement of different cell types achieved? Trichome differentiation in the *Arabidopsis* leaf provides an opportunity to examine both of these questions at the level of the differentiation of a single cell type. Approximately one in 600 epidermal cells on the adaxial surface of the *Arabidopsis* leaf is a trichome. Recessive mutations in two genes, *GL1* and *TTG*, block the initiation of trichomes. The *GL1* gene has been cloned by T-DNA tagging, and exhibits sequence similarity to the Myb class of transcriptional regulators (Oppenheimer et al., 1991). Transgeneic plants containing the *GL1* gene expressed under the control of the CAMV 35S promoter (35SGL1) were crossed with *ttg* mutant plants to test hypotheses about order of function of *GL1* and *TTG*. Plants of the genotype *ttg/ttg*; 35S*GL1*/35S*GL1* failed to produce trichomes, indicating that *TTG* does not act upstream of *GL1* in the trichome initiation pathway. Unexpectedly, in plants of the genotype *TTG/ttg*; 35S*GL1*/+ 30 % of the leaf trichomes occurred as clusters of 2-5 trichomes. In wild-type plants, only 0.6% of the trichomes occur in clusters. Genetic analysis has demonstrated that the *ttg* mutation is directly responsible for this clustering phenotype. These results suggest that the *TTG* gene may play a role in selecting which protodermal cells differentiate as trichomes, in addition to its role in trichome initiation. The unexpected clustered trichome phenotype observed in *ttg* heterozygotes in the presence of ectopic *GL1* expression suggested that other novel trichome phenotypes might be detectable in the presence of the 35S*GL1* transgene. We have recently isolated several mutants in a 35S*GL1* background that produce trichome clusters. At least one of these mutants is not allelic to *ttg*. Several of the mutants also produce adventitious trichomes on the cotyledons, hypocotyl, or siliques.

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TSO-1 MUTATION AFFECTS MERISTEM ORGANIZATION

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A mutation named *TSO-1* was isolated in a genetic screen for abnormal flower mutants. In addition to causing a fasciated inflorescence meristem, *tso-1* appears to have a major effect in flowers. Flowers in *tso-1* mutants send out 5-6 sepal-like organs arranged in a non-whorled fashion, interior to which is a callus-like cell mass that rarely differentiate into floral organs. We performed confocal laser scanning microscopy (CLSM) to examine *tso-1* mutant flowers. Propidium iodide was used to stain nuclei. We discovered that *tso-1* flowers possess abnormal cellular morphology both in the shape and size of nuclei and cells. In some instances, the floral meristem lacks typical cell layers. This finding has lead to the hypothesis that *TSO* gene products may be required to direct proper cell division, cell morphogenesis, and meristem organization. With *in situ* hybridization, we have shown that *AG* and *AP3* RNA accumulation is reduced in *tso-1* mutant flowers. However, this reduction in *AG* and *AP3* expression may be an indirect result of a defect in the earlier steps of flower development.

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Characterization and cloning of *RHD6*, a T-DNA tagged mutant causing defects in root hair formation. James D Masucci, Yonca Ilkbahar, and John Schiefelbein. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Our laboratory is interested in determining the mechanisms of root hair development: root hair specification, initiation, elongation, and completion. A number of mutant plants have been recovered which exhibit root hair defects. The *rhd6* mutant was recovered from the seed pools of T-DNA mutagenized plants created at DuPont. This mutant genetically complements all other root hair mutants in our lab. When grown on agar-containing media, *rhd6* mutants completely lack root hairs, but when grown on agarose-containing media, mutant roots form a variable number of apparently normal hairs. The regions of roots lacking hairs show no signs of root hair initiation. Other unusual aspects of the *rhd6* mutant phenotype include the formation of root hairs in adjacent epidermal cells and the initiation of hairs at inappropriate positions within the cells. Taken together, these results implicate the *RHD6* gene product in the early steps of root hair formation. The further phenotypic characterization of *rhd6* will be discussed as well as our attempts at cloning the *RHD6* locus.

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Characterization of an *Arabidopsis* T-DNA mutant which is defective in root hair formation

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A fundamental problem in developmental biology concerns the generation of cell diversity. We have chosen the *Arabidopsis* root as a model system to address this question because of its simplicity. There are only two cell types in the epidermis, those with hairs and those without. We intend to characterize different mutants generated by T-DNA insertional and EMS-mutagenesis that affect the number of root hairs, their location and their shape. By genetic analysis the pathway leading to root hair formation will be dissected, and it is our aim to clone essential gene products from T-DNA-tagged lines which have been isolated from Ken Feldmann's T-DNA mutagenized populations.
A putatively T-DNA tagged mutant line which has no root hairs is described here. Mutant root morphology has been characterized by electronmicroscopic and immunocytochemical methods. Differences in the cellular distribution of arabinogalactan proteins between mutant and wildtype suggest that the expression of these molecules reflects key steps in the determination of cell differentiation.

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The *immutans* variegation mutant is plastid autonomous and impaired in carotenoid biosynthesis

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The *immutans* (*im*) variegation mutant of *Arabidopsis thaliana* contains green- and white-sectored leaves due to the action of a nuclear recessive gene. Whereas the cells in the green sectors contain normal chloroplasts, most cells in the white sectors are homoplastidic for non-pigmented plastids that lack organized lamellar structures. However, some white cells are heteroplastidic and contain, in addition to abnormal plastids, small pigmented plastids and/or rare normal chloroplasts. This indicates that the expression of *immutans* is plastid autonomous and that the Im gene product accumulates differentially in plastids; this is the first plastid autonomous mutant that has been described in *A. thaliana*. We have also found that the white tissues of *immutans* accumulate phytoene, a non-colored C_{40} carotenoid intermediate. This suggests that the primary lesion in *im* resides in the structural gene (*PDS*) for phytoene desaturase, the enzyme that converts phytoene to zeta-carotene in higher plants. However, we have isolated an *A. thaliana PDS* gene, determined that it is present in a single copy in the *A. thaliana* genome, and found that it does not map to the *im* locus. This indicates that *im* is not the structural gene for phytoene desaturase. Therefore, *immutans* may encode a product that regulates, either directly or indirectly, this step of the carotenoid biosynthetic pathway.

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Strategies for identifying partner(s) of the *Arabidopsis* protein encoded by the *AKR* gene

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We previously showed that *AKR*, the gene encoding the *Arabidopsis* ankyrin repeat-containing protein, was involved in chloroplast differentiation (H. Zhang, D.C. Scheirer, W.H. Fowle, and H.M. Goodman [1992] Plant Cell 4:1575-1588). However, the molecular mechanism of its involvement is not known. In order to study its function and regulation at the molecular level, we are currently employing several approaches to identify its partner(s) which might give us clues on its mode of action. The first approach we have taken is the lexA-based interaction trap. In this technique, a library that conditionally expresses cDNA encoded proteins fused to an acidic transcription activation domain is introduced into a special yeast strain. This strain contains a transcriptionally inert LexA-*AKR* fusion protein (the "bait") and two different reporter genes whose transcription is stimulated if the library encoded protein complexes with the bait. One reporter, *LexAop-LEU2* gene, allows growth in the absence of leucine; the other, *LexAop-lacZ* gene, directs the synthesis of β-galactosidase. We have now identified several cDNA clones using this system, and we are in a process of analyzing these clones. The second approach is to directly label the bacterially expressed *AKR* protein *in vitro*, then screen the λgt11 expression library of *Arabidopsis*. We have successfully expressed the partial AKR protein in a bacterial host, and purified the expressed protein to almost homogeneity. We plan to label the purified protein with kinase and screen the *Arabidopsis* expression library.

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Functions of the *monopteros* gene in *Arabidopsis* development

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The formation of basal body structures is initiated as early as the octant-stage of embryogenesis recognizable as oriented cell expansions of formerly isodiametric cells. These cells go on to form an almost constant number of parallel files that are elongated by horizontal divisions. Finally, the characteristic architecture of the root meristem becomes visible. This developmental process is embryo-specific. Post-embryonic root formation can be distinguished morphologically and genetically.

The *monopteros(mp)* gene is required only for embryonic but not for post-embryonic root formation. In the absence of *mp* gene activity the formation of basal structures is not even initiated and consequently the mutant seedling consists of only the shoot meristem and the cotyledons. Post-embryonically, *mp* appears to be involved in several other developmental processes, such as the differentiation of vascular elements and flower formation.

We describe the analysis of *mp* mutants at various stages of development as well as progress made towards the cloning of the *mp* gene.

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Isolation of mutations affecting late seed development in *Arabidopsis*

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Several aspects of seed development are affected by abscisic acid (ABA), including seed storage protein (ssp) synthesis, dormancy and desiccation tolerance. Several genes have been identified which when mutated confer the ability to germinate on ABA (ABA insensitive or abi). The seeds homozygous for severe mutations in the Abi3 gene (*abi3-3*) germinate on 50 μM ABA, have reduced ssp, are desiccation intolerant and fail to mature fully. Weak Abi3 alleles (*abi3-1*) germinate on 3 μM ABA and otherwise appear normal. We exploited this difference in ABA sensitivity to screen for new mutations which interact with ABI3. We mutagenized *abi3-1* and screened immature M2 seed for mutations which enhance *abi3-1* , permitting germination on 50 μM ABA. We have isolated 7 independent mutations which resemble the strong allele, *abi3-3*. All of these fail to complement *abi3-3* and map to the same chromosomal region as Abi3, suggesting they are intragenic enhancers of *abi3-1*.

Our experience with *abi3-3* has shown that complete insensitivity causes a desiccation lethal phenotype, suggesting that severe alleles of other loci involved in seed development may also result in lethality. To circumvent the difficulties posed by lethality, we have screened for mutations which confer hypersensitivity to ABA in comparison to wildtype. This screen will identify a different spectrum of mutants than insensitivity screens and permits genetic identification of loci involved in responses to ABA without loss of the ability to respond to ABA. One mutation which appears to be recessive has been tentatively designated **aes1** (abscisic acid enhanced sensitivity). This mutant displays no obvious visible phenotypes aside from enhanced sensitivity to ABA. The construction of double mutant lines between aes1 and the abi lines will permit us to determine the relationships between these loci in response to ABA.

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Analysis of genes expressed during embryogenesis of *Arabidopsis* using promoter trapping

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A population of 750 transformed lines of *Arabidopsis* has been generated. The construct transferred via *Agrobacterium* contains a promoterless GUS gene and a kanamycin resistance gene located between the left border and the right border of the T-DNA. The ATG of the GUS gene is adjacent to the left border of the T-DNA in such a way that its expression could be driven by a native plant gene promoter. The transformants (kanamycin resistant) are screened for GUS activity which is detected in some lines. Up to now 300 lines have been screened for GUS expression and 30% showed an expression in the seeds and siliques at different stages of development. Among these lines 6 were obtained in which the expression is limited to the embryo or to the embryo and a few other organs : Line 91-7: GUS gene expression in all tissues of the immature seed including the embryo from heart to cotyledon stage.Line 115 b-4 : GUS gene expression in the whole embryo at heart and cotyledon stages. Line 233: GUS gene expression in vascular tissue of the embryo, this activity is consequently detected in the vascular tissue throughout the life cycle of the plant. Line 276-1: GUS gene expression is detected in the suspensor in globular stage and heart stage embryos. Line 276-7. The GUS gene is expressed in the root tip of the embryo as early as the torpedo stage, it remains active in the root cap throughout the whole life cycle of the plant. Line 295: GUS gene expression is first detected in the cotyledons of heart stage embryos and is present throughout seed development until the dry seed stage.

The genomic DNA adjacent to the T-DNA insertion of some lines has been isolated by IPCR. These DNA fragments will be used as probes to isolate corresponding wild type DNA.

This approach is expected to yield interesting information on the structure of promoters of genes expressed during embryogenesis

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ISOLATION OF cDNAs AS MARKERS OF EARLY EMBRYO DEVELOPMENT

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Formation of the shoot apical meristem and determination of organ primordia in the developing angiosperm embryo are poorly understood at the molecular level. Numerous mutations arresting or altering the normal progression of embryo development are known in *Arabidopsis*, yet very few molecular markers have been isolated that would assist in analysis of these mutants. Our lab has initiated work to identify such markers and to establish the relationship between gene expression and the developmental fate of cells that participate in meristem formation and maintenance. We have chosen the related crucifer, *Brassica napus*, as a source to isolate cDNAs from the early embryo. The relatively large size of the *B. napus* embryos has allowed us to isolate mRNA and construct a cDNA library from the "transition stage" embryo. At this stage of development, the embryo switches from radial to bilateral symmetry, the basic tissues are established, and the shoot apical meristem is defined. Clones are being selected by a combination of the differential display technique (Liang and Pardee, Science 257:967), Northern analysis, and *in situ* hybridization. We have compared embryos at several developmental stages with germinated seedling apices and mature vegetative organs and are currently developing an inventory of cDNA expression patterns. The information and cDNA clones obtained from this study should be extremely useful in analyzing the nature of defects in *Arabidopsis* embryo mutants.

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Genetic analysis of the fertilization process in *Arabidopsis thaliana*

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We use the small weed *Arabidopsis thaliana* as a model system for a genetic and developmental analysis of the fertilization process in plants. The first step in the fertilization is the transfer of pollen from the anthers onto the surface of the stigma. Once a pollen grain has landed on the stigma, the papillar cell releases water and nutrients. The pollen grain hydrates and germinates on the stigma surface. The pollen tube then grows down the papillar cell, enters the transmitting tract, travels up the funiculus and eventually locates the ovule, where it mediates the fertilization of the egg cell.
We are interested in identifying genes which are involved in this process in order to functionally define regulatory steps and interactions.
The best characterized step so far is the very first step: The initial interaction between the pollen grain and the papillar cell. The transfer of water from the stigma is essential for the germination of the pollen grains and represents the first opportunity for the control of the ferilization process by cell-cell interactions. We can distinguish three different complementation groups of male sterile mutants which fail to elicit water release from the stigma. However, this phenotype can be rescued efficiently by copollination with wild-type pollen. This suggests that these mutants fail to provide a signal needed for the proper recognition of the pollen grain by the papillar cell. A genetic analysis of these mutants suggests that these mutants are allelic to *cer1*, *cer2* and *cer3* though they are not genetically linked to the same map positions.

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Characterization of Arabidopsis mutants impaired in embryo development. Jim Mattsson*, Jin-Chen Cheng, Regina McClinton, and Z. Renee Sung. Department of Plant Biology, University of California, Berkeley, CA 94720. *Department of Physiological Botany, University of Uppsala, Uppsala, Sweden.

To study the genetic basis of embryo development in *Arabidopsis*, investigators have isolated "embryo-lethal" and "seedling-lethal" mutants (1,2,3,4) Embryo-lethal mutants are selected as M1 plants that segregate 1/4 brown and shrunken seeds. Embryo-lethal mutants which can germinate but cannot develop further can be categorized as a subclass of seedling-lethal mutants. Many seedling-lethal mutants do not die at the seedling stage, rather they cannot survive in soil or produce progeny. Hence they are called abnormal seedling mutants. We have isolated a large number of abnormal seedling mutants. In addition to mutants impaired in only shoot or only root growth (4), many abnormal seedling mutants were affected in both shoot and root development and often grew from abnormal embryos. Mutants aberrant in embryo development are likely the result of mutations in genes required for synthesizing essential metabolites, maintaining proper cell shape or tissue differentiation. To identify genes required only during embryogenesis, we tested the mutant callus for the ability to undergo *in vitro* organogenesis. Among the mutants tested, i.e., *monopteros*, *gnom*, *knoll*, *micky*, *fass*, *keule* (3), only *monopteros* was embryo-specific. *Monopteros* mutants could not produce embryonic roots, but monopteros callus could regenerate roots. Moreover adventitious roots could be induced on monopteros hypocotyl such that normal soil-grown plants were obtained.

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.FURTHER ANALYSIS OF THE HOMEOTIC LEAFY COTYLEDON (lec) MUTANT OF ARABIDOPSIS

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We have previously described the isolation and initial characterization of a homeotic leafy cotyledon (lec) mutant of Arabidopsis thaliana that appears to be defective in a critical regulatory gene required for the activation of a wide range of embryo-specific functions in higher plants [Meinke DW (1992) Science 258: 1647-1650]. Homozygous mutant embryos are altered in morphology, lack protein and lipid bodies, often accumulate anthocyanin and remain green late in development, and are desiccation intolerant at maturity. When rescued in culture prior to desiccation, mutant embryos produce viable plants that are phenotypically normal except for the presence of cotyledons with trichomes and other features characteristic of foliage leaves. This mutation therefore appears to interfere with very early stages of embryonic maturation, causing mutant cotyledons to revert to a more primitive and leaf-like developmental state. We describe in this poster the results of more recent studies on the response of mutant embryos to abscisic acid, the internal structure of mutant embryos, and the position of this gene relative to flanking RFLP markers. The sensitivity of mutant embryos to exogenous ABA demonstrates that lec is not simply defective in ABA response, and that abscisic acid is necessary but not sufficient for embryonic maturation in Arabidopsis. This is consistent with our model that LEC plays a more global role than ABI3 or VP1 in regulating embryonic maturation in higher plants. This research has been supported by grants from the NSF Developmental Biology Program.

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Regulation of the *LOX1* Gene of *Arabidopsis* During Seed Germination

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The *LOX1* gene of *Arabidopsis* is induced in response to ABA and methyl jasmonate as well as in response to bacterial pathogen attack. We report here that expression of the *LOX1* gene also increases during seed germination. *LOX1* mRNA was not detected in ungerminated seeds but reached a maximal level in both light- and dark-grown seedlings after 24 hours of growth. *LOX1* mRNA levels in light-grown seedlings were approximately 4-fold greater than those observed in dark-grown plants. At 24 hours the radicle had just emerged in both light and dark-grown seedlings. Similar trends were observed for lipoxygenase (LOX) enzymatic activity levels. LOX activity, which was not detected in ungerminated seeds, peaked at 48 hours in both light- and dark-grown plants and then declined. The activity in dark-grown seedlings was approximately 50% of that observed in light-grown plants. At 48 hours the cotyledons of light-grown plants were green and rapidly expanding while hypocotyl growth was extensive in dark-grown seedlings. LOX polypeptide, which was not detected in ungerminated seeds, reached a maximal level at 48 hours in both light- and dark-grown plants. These results indicate that the increase in LOX activity early in germination is due, at least in part, to increased transcription of the *LOX1* gene. *In situ* hybridization studies are in progress to determine if expression of the *LOX1* gene is localized to a particular cell-type or tissue in young seedlings.

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Identification of Stage-Specific mRNAs in Developing Seeds of *Arabidopsis thaliana*. Michael Nuccio, Tzung Fu Hsieh, Robin Lidiak, and Terry Thomas. Department of Biology, Texas A&M University, College Station, Texas, 77843.

Although most of the major discernible morphogenetic events in plants occur after germination, the overall architectural pattern of the mature plant is established during early events of embryogenesis. Following fertilization, an asymmetric division of the zygote of many dicots yields a basal cell that will produce the suspensor and an apical cell. Ensuing cleavages of the apical cell yields a radially symmetric, globular embryo with differentiated protoderm. Further cell divisions and subsequent morphogenesis breaks radial symmetry yielding the bilaterally symmetric, heart stage embryo with root and shoot apices, incipient cotyledons and provascular tissue. So far, few genes have been identified that are expressed specifically during early embryogenesis, primarily because of technical difficulties associated with the mass ratios of the embryo and the surrounding maternal tissue and the lack of molecular and cellular markers to direct screening efforts. We are using a modification of a powerful new method, called differential display of eucaryotic mRNA (Liang and Pardee, 1992), to identify mRNAs expressed in early embryos, particularly during the globular/heart transition. This method is rapid, and since it is PCR-based, it requires small amounts of starting material. In addition, amplifers representing early embryo mRNAs are easily cloned. Probes representing these mRNAs will be used for *in situ* hybridization experiments to localize expression within the embryo. Those which demonstrate tightly-regulated, well-defined expression patterns will be targeted for further analysis, including an assesment of their possible role in early development. Details of the differential display method and results to date will be presented.

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ACQUISITION OF DESICCATION TOLERANCE AND LONGIVETY IN SEEDS OF *ARABIDOPSIS THALIANA*; A COMPARATIVE STUDY USING ABA-INSENSITIVE *abi3* MUTANTS.

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Two new ABA-insensitive mutants of *Arabidopsis thaliana* affected in the *abi3* locus are described. These new mutants are severely insensitive to ABA. With the earlier described *abi3-1* and the ABA-deficient and -insensitive double mutant, *aba,abi3-1*, these new mutants vary in the extent of ABA-correlated physiological responses. Mutant seeds fail to degrade chlorophyll during maturation, show no dormancy, and desiccation tolerance and longevity are poorly developed. Carbohydrate accumulation as well as synthesis of LEA or RAB proteins are often suggested to be essential for acquisition of desiccation tolerance. Here we show that: i) Accumulation of carbohydrates as such does not correlate with the acquisition of desiccation tolerance or longevity. However, a low ratio of mono-/oligosaccharides correlates with seed longevity ii) Synthesis of a few assorted ABA responsive proteins, in the later phases of seed maturation, does not correlate with desiccation tolerance or longevity.

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ABNORMAL SUSPENSOR MUTANTS OF *ARABIDOPSIS THALIANA*

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The zygote in flowering plants divides to form an embryo composed of two parts: the embryo proper and the suspensor. In *Arabidopsis*, the suspensor normally consists of a single column of six to eight cells that persist through the heart stage of development and then degenerate. Abnormal development of the suspensor is a characteristic feature of many embryo-defective (*emb*) mutants of *Arabidopsis*. We have identified at least 15 *emb* mutants with abnormally large suspensors. We are currently focusing on three mutants (*emb76*, *emb158*, and *emb177*) that arrest around the globular stage of development. These mutant embryos consistently produce suspensors composed of many cells arranged in multiple columns and rows. We are using Nomarski optics and light microscopy of sectioned material to characterize abnormal development and to determine the extent of cellular differentiation in mutant embryos. We are also testing the developmental potential of mutant embryos in culture. Our ultimate goal is to identify and characterize genes that are essential for normal development of the embryo proper and suspensor. We have cloned genomic DNA sequences that flank T-DNA insertions in *emb76* and *emb177*. We are currently sequencing these genomic fragments and using them to probe RNA blots and silique cDNA libraries. We will also use the genomic fragments in transformation experiments to complement the *emb76* and *emb177* mutants. Supported by grants from NSF and NSERC (Canada).

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Cloning genes involved in directional cell division in *Arabidopsis thaliana* using the yeast *Saccharomyces cerevisiae* as a model system.

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Orientation of cell divisions and establishment of cell polarity are essential to the development of many organisms. The genetic basis of oriented cell division is not clear. Therefore we have decided to use the yeast *Saccharomyces cerevisiae* as a model system to understand this process in plants. Yeast cells multiply during their mitotic cell cycle in a polar cell division. Many of the genes that regulate the polar cell division in yeast have been identified. Thus it seems likely that some of the mechanism responsible for oriented cell division in yeast will be relevant to cell division in other systems. In fact several proteins that are involved in directional cell division in yeast (*BUD1*, *CDC42)* have homologs in mammalian cells.

In order to study the genes that are involved in directional cell division in plants we are using three different approaches. First, using yeast mutants involved in directional cell division we are isolating plant genes by complementation. The second approach uses PCR to isolate *BUD1* homologs from *Arabidopsis.* From preliminary results we have identified the *Arabidopsis thaliana Rap1a/BUD1* homologs. The third approach is introduction of dominant mutations in *BUD1* and *Rap1a* into *Arabidopsis* using Agrobacterium mediated transformation. Using these different approaches we hope to learn about the genetic system that regulates directional cell division in plants.

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Characterization of *Arabidopsis* mutants impaired in embryo development.

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To study the genetic basis of embryo development in *Arabidopsis*, investigators have isolated "embryo-lethal" and "seedling-lethal" mutants (1,2,3,4). Embryo-lethal mutants are selected as M1 plants that segregate 1/4 brown and shrunken seeds. Embryo-lethal mutants which can germinate would be the same as seedling-lethal mutants, which form abnormal seeds. We have isolated a large number of abnormal seedling mutants. In addition to mutants impaired in shoot but not root and root but not shoot development (4), many abnormal seedling mutants were affected in both shoot and root development and often grew from abnormal embryos. The aberrant embryo development usually resulted from mutations in genes required for synthesizing essential metabolites, maintaining proper cell shape or tissue differentiation. Few were impaired in genes specifically required for embryo development. To identify genes required only during embryogenesis, we tested the mutant callus for the ability to undergo *in-vitro* organogenesis. Among the mutants tested, i.e. monopteros, gnom, knolle, mickey, fass, keule (1,2), only monopteros was embryo-specific. Monopteros mutants could not produce embryonic root, but monopteros callus could regenerate roots. Moreover adventitious roots could be induced on monopteros hypocotyl such that normal, soil growing plants were obtained.

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TWIN: AN *ARABIDOPSIS* MUTANT THAT DISPLAYS FREQUENT POLYEMBRYONY

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Polyembryony during plant development is a widespread but infrequent phenomenon. Studies on a number of taxa have determined that supernumerary embryos can originate from a variety of sources, including haploid or fertilized synergids, maternal integument tissue, and embryonic meristems. We have identified an *Arabidopsis* mutant, *twin*, that yields viable twin and occasional triplet seedlings. *Twin* (*twn*) is a highly variable embryo-defective mutant. Embryos often appear normal, but they can also display a host of abnormalities such as irregular pigmentation, reduced, distorted, or multiple cotyledons, fat hypocotyls, and prematurely arrested development. Homozygous mutant seeds of *twn* germinate efficiently and although many seedlings are undersized, distorted, and slow to green, most develop into fully fertile plants which produce 100% mutant progeny following self-fertilization. Microscopy of developing *twn* seeds has revealed that secondary embryos consistently arise from the suspensor, an embryonic structure established following the initial zygotic cell division. Transformation of the suspensor involves suspensor enlargement and the development of characteristic embryonic features (hypophysis and protoderm), resulting in the formation of a true secondary embryo. Although a large number of *Arabidopsis* mutants with abnormal suspensors have been identified, viable mutants displaying suspensor polyembryony have not previously been described. The *twn* phenotype reveals that suspensor cells have the potential to duplicate the full spectrum of developmental programs normally restricted to the embryo proper. The *TWN* gene product may play a crucial role in maintaining cell identity and repressing embryonic potential in the suspensor.

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Genes controlling carpel identity in *Arabidopsis thaliana*

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During normal flower development the gynoecium of two fused carpels arises from the center of the flower meristem. We have isolated mutants of two genes which specifically affect development of the gynoecium. In *crabs claw* mutants the two carpels are unfused in their upper regions. They curve inward so that the top of the gynoecium resembles claw-like pincers. In *spatula* mutants the septum does not develop in the upper region. The stigma and the transmitting tissue of the style are also reduced. When *crabs claw spatula* double mutants are made, the gynoecium is much more severely affected. Instead of two carpels, unfused bract-like organs appear.

We propose that a function of the wild type *CRABS CLAW* and *SPATULA* genes is to determine that the fourth whorl of floral organs develop as carpels. The genes seem to act redundantly in that the product of one is sufficient to establish carpel identity when the other is in mutant form. The two genes also influence the development of carpels which arise in ectopic positions such as occurs in *apetala2* mutant plants.

The homeotic gene *AGAMOUS* is expressed in regions of the wild type flower primordium which will develop into carpels. We have tested if transcription of *AGAMOUS* is positively regulated by *CRABS CLAW* and *SPATULA*. This does not seem to be the case as *AGAMOUS* transcripts occur at normal levels in *crabs claw* and *spatula* mutants. Genetic experiments to test if the converse is true, i.e. if *AGAMOUS* positively regulates the activity of *CRABS CLAW* and *SPATULA*, are under way.

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MAPPING *MS-1* IN *ARABIDOPSIS THALIANA*, A GENE CRITICAL FOR MALE GAMETOPHYTE DEVELOPMENT, BY CHROMOSOME WALKING

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The genetic and ultrastructural characterization of the *ms1* mutant, which has been induced by EMS treatment (Veen & Wirtz, 1968), indicates that the *MS1* gene is critical for the development of normal microspores. *ms1* has been mapped to chromosome five and is flanked by the phenotypic markers *ttg* (yellow seed, glabrous) and *hy5* (long hypocotyls) (Koornneef *et al.*, 1983). Because the gene has been located in a region smaller than 10 cM, the region was a suitable target for chromosome walking.

RFLP markers that are genetically linked to *ms1* were used to screen three *Arabidopsis thaliana* YAC libraries. An ordered contig was then established by reprobing these libraries with end probes from the newly isolated YACs generated either by inverse PCR or rescued in *E. coli*. To delineate the *ms1* gene with respect to the linked RFLPs the male-sterile, glabra (hairless) (*ms1/ttg*; La-O ecotype) was crossed with the male-fertile, hairy (*MS1/TTG*; NO-O ecotype) strain. F2 and F3 progenies have been screened for cross-overs, using flanking RFLP markers and YAC endprobes closely linked to *ms1*. As well, *hy5/MS1* recombinants have been identified and are being mapped with respect to RFLP markers. These data lead to the identification of two overlapping YACs containing the *ms1* gene Subsequent, a cosmid library of wildtype *Arabidopsis thaliana* DNA was screened with these YACs. 30 overlapping cosmid clones were identified and used to transform *ms1* mutant plants. Two cosmid clones complemented *ms1*. Transformation of *ms1* mutants with subcloned fragments of one of the complementing cosmids is in the process.

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COMBINATORIAL GENE ACTION AND THE SPECIFICATION OF FLORAL MERISTEM IDENTITY IN *ARABIDOPSIS*. John L. Bowman, John Alvarez, Detlef Weigel*, Elliot Meyerowitz*, and David R. Smyth. Dept. of Genetics and Develop. Biology, Monash University, Clayton, Victoria 3168, Australia and *Div. of Biol. Caltech, Pasadena, CA. 91125, USA.

Mutations in the *APETALA1* gene have two effects, the partial conversion of flowers into inflorescences and the disruption of sepal and petal development. These effects have been analyzed in nine different *apetala1* mutants which can be arranged in an allelic series based on the relative severity of disruptions. Interactions between *APETALA1* and several other genes involved in floral meristem specification were studied by examining the phenotypes of plants carrying combinations of *apetala1* mutations and mutant forms of the other genes. The results indicate that the products of both *APETALA1* and *LEAFY* are required above a threshold level to ensure that primordia arising on the flanks of the inflorescence apex develop as flowers. The two genes have some distinct and some overlapping functions in specifying floral meristem identity and they reinforce each other's action synergistically. In addition, these genes appear to be activated, at least in part, by those factors that mediate floral induction in *Arabidopsis*. Another gene, *CAULIFLOWER*, also has a role in the specification of floral meristem identity by positively regulating the expression of both *APETALA1* and *LEAFY*, but this function is redundant with that of *APETALA1*. The *APETALA2* gene has an early function in reinforcing the action of *APETALA1* and *LEAFY*, especially if the activity of either is reduced or absent. Once the identity of a flower primordium has been specified, *APETALA1* has a later action in combination with *APETALA2* in controlling the development of the outer two whorls of the *Arabidopsis* flower.

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Isolation and Characterization of Vernalization Mutants of Arabidopsis

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Plant growth and development are strongly influenced by environmental conditions. For example, timing of the transition from vegetative to reproductive growth is known to be influenced by temperature and photoperiod. In Arabidopsis, mutations in at least 11 loci delay flowering, and a subset of these mutants (*fca, fpa, fve, fy, ld* and *fmc*) can be reverted to nearly wild-type flowering time by vernalization (cold treatment). In order to determine how Arabidopsis plants sense cold and use it as a signal for flowering, we have mutagenized seed from *fca* plants and screened for mutants that flower late despite vernalization treatment. A number of different vernalization mutations have been identified and they represent at least two distinct loci, designated VRN1 and VRN2. The flowering time and leaf number of *vrn1* and *vrn2* mutants have been characterized and compared to *fca* and wild-type. The *vrn* mutants have been crossed to wild-type to determine whether they exhibit a visible phenotype when not in the *fca* background, and to enable the *vrn* mutations to be crossed into other late-flowering backgrounds. We are also looking at the meristems of wild-type, *fca*, *vrn1* and *vrn2* plants, using light microscopy and EM techniques. It is believed that the vernalization signal is perceived in the apical meristem, and it may be possible to detect meristem differences between the four genotypes. The *vrn* mutations are being mapped using RFLP and visible markers to facilitate their cloning by either chromosome walking or by targeted transposon tagging.

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brevipedicellus (bp), locus responsible for shape and size of epidermal cells in the pedicel of Arabidopsis thaliana

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We have investigated the cellular nature of a mutation which affects the orientation of flower and fruit in Arabidopsis. The genetic locus under investigation is the brevipedicellus (bp) locus (4-9.6 (Koornneef, 1983)). Mutations of the bp locus are manifest in a shortened pedicel and with the localized expansion of the epidermis cells on the adaxial surface of the pedicel are responsible for the downward orientation of flower and developing silique. In this report we have isolated and describe several new mutant lines in the Landsberg erecta and Columbia ecotypes using various mutagenic treatments. In our investigations of the cellular nature of these mutant lines, we show that the localized cell expansion of one or two cell layers of the adaxial epidermal surface, rather than gravity or altered cell number, is the causal effect in the downward orientation of flower and developing fruits.

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Genetic and phenotypic characterization of new male sterile mutants of *Arabidopsis*

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Recessive nuclear mutations causing arrested development of pollen and anthers have been generated in *Arabidopsis* (Ler) by treatment of seeds with EMS or ionizing radiation. M2 generations were screened for plants that were male sterile (ms) and these were rescued by pollination with fertile Ler. Six of these mutants have been analysed in detail by light microscopy and by transmission and scanning EM. These ms lines carry mutations in sporophytically controlled *ms* genes, which prevent the correct development of the stamen. cause pollen abortion different stages or which prevent anther dehiscence. For example, the *msH* mutant produces viable pollen which is not released owing to a block in the dehiscence process, possibly mediated by structural abnormalities in the endothecial layer of the anther wall. In the *msZ* mutant. however, pollen development probably fails as a result of abnormalities in the stamen filament. SEM analysis suggests sculpturing of the pollen exine is unaffected by either of these mutations. Other *ms* mutations reflect aberrations probably occuring around meiosis. Inheritance of all the mutations is consistent with them being monogenic recessive traits. The phenotypes of these and other ms lines will be described. The map positions of some of the *ms* genes will also be presented.

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Developmental regulation of gene expression in bicellular and tricellular pollen

Abstract
The activation and developmental regulation of the promoter of the tomato *lat*52 gene was analysed in *Nicotiana tabacum* and *Arabidopsis thaliana* to investigate the conservation of transcriptional regulatory mechanisms in species with bicellular and tricellular pollen. Promoter activity in transgenic plants containing the *lat*52 promoter fused to the ß-glucuronidase (*gus*) gene was studied throughout pollen development by fluorimentric and histochemical analysis and by RNA analyses. These analyses showed that in transgenic *A. thaliana* the *lat*52 promoter was activated in uninucleate microspores immediately prior to microspore mitosis, whereas in transgenic *N. tabacum* *lat*52 promoter activity was first detectable immediately following microspore mitosis in binucleate pollen grains. Thus, *lat*52 gene activation in *A. thaliana* does not depend upon passage of the microspore nucleus through microspore mitosis. Despite this temporal difference, the pattern of *lat*52 promoter activity during vegetative cell maturation showed a very similar cumulative pattern of activity in both species, which was correlated with a similar accumulation of *gus* transcript and spore protein content. Using a novel nuclear targeted GUS fusion protein we have further shown that the *lat*52 promoter is expressed specifically in the vegetative cell of the pollen grain and not in the developing generative or sperm cells.

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AGL1 AND *AGL2*, TWO *AGAMOUS*-RELATED MADS-BOX GENES, ARE DIFFERENTIALLY EXPRESSED DURING FLOWER DEVELOPMENT

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Several floral homeotic genes, such as *AGAMOUS* and *AP3* from *Arabidopsis thaliana* and *DefA* from *Antirrhinum majus*, have been shown to be members of the MADS-box gene family, which includes the known transcription factors MCM1, a yeast protein involved in mating-type control, and SRF, the mammalian serum response factor. The MADS-box is a conserved region in MCM1 and SRF shown to be within the DNA binding and dimerization domain. Thus, the floral homeotic MADS-box genes are proposed to be transcription factors involved in regulating flower development. To determine if other MADS-box genes might also be involved, several MADS-box genes were cloned from *Arabidopsis thaliana* by their homology to *AGAMOUS* and named *AGL* genes, for *Agamous*-Like. We are characterizing two of these genes, *AGL1* and *AGL2*, which are known to be preferentially expressed in flowers. To determine what role these genes might have in flower development, we have analyzed, using RNA *in situ* hybridization with gene-specific ribo-probes, the specific expression patterns of *AGL1* and *AGL2* within the flower. Our results indicate that *AGL1* and *AGL2* are differentially expressed, both spatially and temporally, during *Arabidopsis thaliana* flower development. *AGL1* expression arises late in flower development (stage 9-10) and is restricted to the carpels. In contrast, *AGL2* expression is ubiquitous and uniformly high early in flower development (stages 2 and 7). During later stages of flower development, *AGL2* expression becomes organ-specific. Detailed analysis of the *AGL1* and *AGL2* expression patterns will be presented.

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

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curly leaf: a transposon-induced mutation affecting flower and leaf morphology of *Arabidopsis*

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To isolate and study genes controlling flower development in *Arabidopsis*, we performed a transposon mutagenesis experiment using lines carrying the maize *Ds* transposon and *Ac* transposase. In a screen of 500 families carrying independent transposon insertions, we obtained several mutations affecting flower development and/or flowering time.

Genetic and molecular analysis of one such mutation, *curly leaf*, indicate that it is caused by a *Ds* insertion and have allowed the isolation of the wild-type gene. Plants carrying the recessive *curly leaf* mutation flower earlier than wild-type and have smaller leaves which are rolled inwards along the margins. In addition, the floral organs of *curly leaf* mutants display homeotic transformations; the sepals have features of carpels and the petals are variously narrow, absent or stamen-like. Many aspects of this phenotype are also observed in transgenic plants in which the homeotic gene *AGAMOUS* is constitutively expressed. Northern analysis indicates that the *AGAMOUS* gene is expressed ectopically in leaves of the mutant, suggesting that the wild-type *CURLY LEAF* gene may repress the action of *AGAMOUS*. The *curly leaf* mutation is within 1 cM of the *Ds* element present in the mutant line and shows reversion in the presence of transposase. The DNA adjacent to the *Ds* was isolated on a lambda clone, and the final identification of the gene is underway. To analyze further the interaction of *CURLY LEAF* with other floral homeotic genes, we are also making a series of double mutants containing *curly leaf* and previously reported mutations which affect flower development.

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Ectopic Expression of PISTILLATA Gene in Transgenic *Arabidopsis*.

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The flower homeotic gene PISTILLATA (PI) of *Arabidopsis* is required for petal and stamen development in the second and third whorls. In the *pi* mutant, sepals develop in the second whorl, and carpels or filamentous organs occupy the third whorl region.

Molecular cloning of the PI gene shows that, like other homeotic genes, PI is a member of the MADS box gene family. In situ hybridization experiments revealed that PI transcripts exist in the second and third whorl after stage three in floral buds. Genetic and molecular evidence suggests that PI works with APETALA3 (AP3) to regulate gene expression.

To reveal the function of the PI gene product, we have introduced a heat-shock gene promoter(HSP 18.2)-driven PI cDNA into wild-type *Arabidopsis* (No-0 ecotype) via T-DNA mediated transformation. Transgenic *Arabidopsis* which has 35S::AP3-cDNA shows a phenotype that suggests the expansion of second and third whorl organ identities (Jack and Meyerowitz, unpublished). This means AP3 itself can specify second and third whorl organ identities, that is, petals and stamens, though to do this it needs wild type PI activity.

Our next question is whether PI itself has enough function to make morphological changes or whether it needs AP3 as a counterpart. We are now analyzingtransgenic Arabidopsis plants both morphologically and at the molecular level.

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DEFICIENS, AN *ANTIRRHINUM MAJUS* ORGAN IDENTITY GENE IS FUNCTIONAL IN *ARABIDOPSIS THALIANA*.
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There is increasing evidence that the regulatory mechanisms controlling floral morphology have been conserved throughout evolution of dicotyledonous species. *Arabidopsis thaliana* is distantly related to *Antirrhinum majus* yet both organisms specify floral organ identity in a similar manner. In both species, only three classes of genes (A, B, C) with overlapping expression patterns combine to establish unique identities for each of the organ whorls. Recessive mutant alleles of the Class B genes, *apetala3* (*ap3*) of *A. thaliana* and *deficiensA* (*defA*) of *A. majus*, result in an analogous phenotype (conversion of sepals to petals and stamens to carpels) and *AP3* and *DEFA* DNA sequences have a high degree of homology. We have tested whether the Class B genes *AP3* and *DEFA* are functionally equivalent by introducing genomic sequences of *DEFA* into wild-type and *ap3 A. thaliana* lines. In wild-type presence of *DEFA* caused a distinct floral phenotype similar to that observed in plants mutant for *flo10* a negative regulator of Class B genes. This is consistent with ectopic expression of Class B genes in the fourth whorl and suggesting that *FLO10* does not regulate *DEFA* efficiently. *In situ* hybridization results using an *DEFA* probe support this hypothesis and indicate that *DEFA* is otherwise regulated properly. The *DEFA* gene is able to partially complement the *ap3-1* allele suggesting that *AP3* and *DEFA* are functionally homologous.

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A Post-fertilization Event Influences the Mode of Inflorescence Meristem Shutdown in Arabidopsis.
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The longevity of the Arabidopsis life cycle is governed in part by the cessation of inflorescence meristem proliferative activity at a particular stage in development. The transition from vegetative to reproductive development results in the production of the highly branched inflorescence. After approximately five weeks of growth, flower production ceases on all meristems within a 24-48 hour period. SEM analyses showed that the cessation of flower production results in a cluster of buds at the apex with spiral phyllotaxy. The timing of this "global shutdown" phenomenon is remarkably reproducible. To elucidate the mechanism of inflorescence meristem arrest, we studied the differences in terminal structures and the timing of arrest between wild-type and mutant Landsberg *erecta* lines.

Initial analysis of the male-sterile line, *ms1-1*, showed that in the absence of fertilization, an "asynchronous termination" versus global shutdown of plant inflorescence meristems occurs. Secondly, when floral production ceased from the male-sterile primary inflorescence, irregularly patterned structures formed and a cluster of buds was never found at the terminal meristem. A third characteristic distinguished male-sterile from wild-type inflorescence growth--under constant growing conditions, the primary inflorescence of the male-sterile plant produces approximately twice the number of flowers as wild-type, even though flower production rates are equivalent. Thus, in the absence of fertilization, the "signal" to halt meristematic cell division is <u>not present</u> or <u>not recognized</u>. When a male-sterile plant is hand-fertilized total flower production decreases and wild-type shutdown structures are produced at the terminal meristem.

We have isolated a mutant line, *fec* (fecundity), that has asynchronous termination characteristics yet produces wild-type levels of seed/silique, thus uncoupling fertilization from "global shutdown." The primary inflorescence of the *fec* mutant produces greater than two-fold the number of wild-type flowers and forms irregular floral structures at the terminal meristem. Further analysis of the *fec* mutant line, as well as other mutant lines, may lead to determining what factors play a role in controlling meristematic proliferation.

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TWO PATHWAYS FOR FLOWERING IN ARABIDOPSIS Eva Huala and Ian M. Sussex, Department of Plant Biology, University of California, Berkeley, CA 94720.

Arabidopsis (ecotype Landsberg *erecta*) is a facultative long day plant that flowers after 15 days under continuous illumination, whereas under 8 hr photoperiods flowering is delayed until plants are about 8 weeks old. This flowering behavior could be due to the presence of two separate pathways for the initiation of flowering in Arabidopsis; a photoperiod-inducible pathway and a constitutive pathway. The phenotype of the late flowering mutant *fb*, which flowers after about the same number of vegetative nodes under long or short photoperiods, suggests that it may be deficient in photoperiod-inducible flowering[1]. A gibberellin responsive dwarf carrying a mutation at the *ga1* locus[2] fails to flower when maintained in short photoperiods, suggesting that this mutant may be blocked in a constitutive flowering pathway[3].

To test whether the *ga1* and *fb* mutations define two separate pathways for the initiation of flowering, we have constructed an *fb ga1* double mutant. Some double mutant individuals grown in long days flowered much later than either *fb* or *ga1*, while other individuals never flowered, confirming that the GA1 and FB gene products act in two separate pathways for the initiation of flowering. To further define these two pathways we have analyzed other double mutant combinations including several late flowering and early flowering mutants. These results have been incorporated into a model for the induction of flowering in Arabidopsis. In addition, we are characterizing several new mutants that flower earlier than wild type when grown in 8 hr photoperiods. These mutants may be deficient in an inhibitor of flowering that operates in short day conditions but not long day conditions.

[1] M. Koornneef et al. (1991) Mol. Gen. Genet. 229:57-66.
[2] M. Koornneef and J. H. van der Veen (1980) Theor. Appl. Genet. 58:257-263.
[3] R. Wilson et al (1992) Plant Physiol. 100:403-408.

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Characterization of AG target sequences

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The deduced product of the floral homeotic gene *AG* shares substantial similarity with the DNA-binding domains of known transcription factors SRF and MCM1, suggesting that AG is also a transcription factor. To test whether AG is a sequence-specific DNA-binding protein, and to determine the sequence(s) of AG target sites, we have performed in vitro DNA binding studies. We first tested the ability of AG to bind sequence, CCATTAATGG, using a gel mobility shift assay, and we found AG indeed binds this sequence. As a control, we performed a parallel binding experiment with the sequence GGATGCATCC, and the result was negative. It has been shown that, although SRF and MCM1 can bind to some of each other's natural target sites, they have different spectra of binding sites. The consensus for MCM1 is CC(T/C)(A/T)$_3$NNGG, determined after screening pools of random oligonucleotides. To characterize the spectrum of AG target sites, we screened for AG binding sequences among a pool of random oligonucleotides, essentially as described by Pollock and Treisman. A pool of random single-stranded oligonucleotides each having constant ends of 25 bases and a variable center of 26 bases was converted to double-stranded sequences. The pool of sequences was subjected to binding with AG, and the DNA-protein complexes were separated from unbound DNA using PAGE. The shifted band was cut out and the bound oligonucleotides were eluted for PCR amplification. The PCR products were then used for a subsequent round of the AG binding reaction. After five such rounds, the PCR products were cloned and analyzed by DNA sequencing. We find that the AG binding site sequences are more similar to the MCM1 than SRF target sequences.

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MOSAIC ANALYSIS OF THE HOMEOTIC PISTILLATA GENE IN ARABIDOPSIS.

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Mosaic analysis is a powerful tool for defining the biological function of a gene product. We have are examining the function of the *pistillata* (*pi*) gene of Arabidopsis by constructing plants that are genetically mosaic for the *pi* mutation: Plants mutant for *pi* display a homeotic conversion of petals into sepal-like organs, while stamëns are converted into carpelloid structures. Seed heterozygous for *pi* and a closely linked albino mutation are irradiated, and plants are recovered that contain hemizygous albino sectors. Numerous sectors have been recovered that include part of an inflorescence, part of a flower, or a single floral organ. L3 sectors in the flower have not been recovered, suggesting that primarily L1 and L2 derived cells contribute to the flower. The sectored tissues are also genetically mutant for *pi*, and such mosaics can be used to assess whether the *pi* gene functions in a cell-autonomous or non-autonomous manner. Examination of sectored flower buds indicates that the *pi* gene behaves in a non-autonomous fashion. This result suggests that the *pi* gene is necessary for cell-cell interactions required to establish the normal pattern of floral organ development. We will also report on our progress in examining the cellular autonomy of other homeotic genes in Arabidopsis.

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Ectopic Expression of Floral Homeotic Genes

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The three homeotic genes *APETALA3*, *PISTILLATA*, and *AGAMOUS* are expressed exclusively in a subset of the floral organs in the wild-type flower. To investigate the phenotypic consequences of ectopic expression of these genes, we fused the coding regions of *APETALA3* and *AGAMOUS* to heterologous promoters. When *APETALA3* is placed under the control of the constitutively expressed 35S promoter of cauliflower mosaic virus, the resulting transgenic plants exhibit dramatic defects in the fourth whorl of the flower where the carpels are converted into an additional whorl of stamens. In the 35S-*APETALA3* flowers, transcripts for *PISTILLATA* are also misexpressed in the fourth whorl, but not in the first. In addition, expression of *PISTILLATA* in the fourth whorl is necessary for the phenotypic transformations observed in the fourth whorl. In a second transformant line the *AGAMOUS* cDNA was fused to the *APETALA3* promoter; ectopic expression of *AGAMOUS* specifically in the second whorl results in flowers that most frequently lack second whorl organs, although occasionally the second whorl develops into stamens rather than petals.

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UFO, a gene involved in inflorescence and floral meristem development

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Arabidopsis flower development can be divided into four stages: floral induction, initiation and formation of floral meristems, formation of floral organ primordia, and determination of organ identity and differentiation into the corresponding organ type. The Arabidopsis *UFO* (unusual floral organs) gene is likely to be involved in the last three steps. *ufo* mutations cause defects in the formation of both inflorescence and floral meristem structures. *ufo* mutants exhibit the following inflorescence defects. First, the apex of the inflorescence meristem often ends in a carpelloid structure due to variable premature termination of the formation of floral meristem primordia. Second, a bract subtends the first flower on about two-thirds of inflorescences. Third, a filamentous structure may form in place of a flower. Fourth, filamentous structures develop variably on inflorescences and pedicels of flowers. The first three inflorescence defects have also been observed previously in *leafy (lfy)* mutants. *ufo* mutants exhibit variable defects in all four whorls of the flower with the second and third whorls showing the most frequent and dramatic alterations. Mutant flowers often contain mosaic organs and/or fusions of two adjacent organs. The number and shape of the first whorl sepals can be affected. The second whorl organs are most often sepals or petalloid sepals. The third whorl organs are most often carpels, stamens, filaments, or mosaic combinations of these three structures. The fourth whorl occasionally has one or three carpels, instead of two seen in wild-type flowers.

To further analyze the role of this gene, we constructed doubly mutant strains with other mutations affecting inflorescence and floral meristem development. An *agamous; ufo* double mutant forms fasciated flowers indicating that the *UFO* gene has a role in controlling growth of the floral meristem. A *superman; ufo* double mutant has a novel phenotype indicating some synergistic interaction between these two genes. In a *lfy; ufo* double mutant, the *lfy* mutation is epistatic to the *ufo* mutation, indicating that loss of *UFO* function in the absence of *LFY* function has no effect on the phenotype of the plant and that perhaps *UFO* functions downstream of *LFY.* We will also present results of *in situ* hybridization and antibody experiments to show the effects of a *ufo* mutation on the pattern of expression of other genes involved in inflorescence and floral meristem development.

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GENETIC IDENTIFICATION AND CHARACTERIZATION OF *LEUNIG*, A CLASS A GENE IN *ARABIDOPSIS* FLOWER DEVELOPMENT

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Three classes of homeotic genes (A, B, C) specify organ identity during flower development in *Arabidopsis*. Class A genes are required for sepal and petal development in the first and second whorls of a flower. We have been screening for enhancer or suppressor mutations of a weak A class mutant *ap2-1*. We have isolated two recessive mutations that define an extragenic enhancer locus of *ap2-1*.

The mutant enhancer locus causes the phenotype of *ap2-1* to resemble the more severe alleles of *ap2*. When separated from *ap2-1*, the mutant enhancer locus causes a weak homeotic conversion of sepals into carpel-like organs in the first whorl. In addition, all floral organs and cauline leaves exhibit an elongated and pointed morphology and most flowers have unfused carpels. The mutant phenotype and map location resembles that of *leunig* (Dr. David Smyth, pers. comm.). Complementation tests confirmed that the enhnacer locus is allelic to *leunig*.

With *in situ* hybridization, we tested *AG* and *AP1* expression in *leunig*. In wild type, *AG* expression is confined to the third and fourth whorls of a flower; in *leunig* mutants *AG* transcripts are expressed in all four whorls of a flower. Thus, together with *AP2*, *LEUNIG* normally functions in the first two whorls to repress *AG* expression. Further, *leunig* enhances the phenotype of another class A mutant, *ap1*. *ap1-1*; *leunig* double mutants exhibit more severe homeotic transformation than either mutant alone, and are similar to *ap2-1 leunig* double mutants. While wild type flowers express *AP1* in the first two whorls, *AP1* mRNAs were absent in the first two whorls of *leunig* flowers, suggesting that *LEUNIG* is required for *AP1* expression in the first two whorls. Finally, *ag-1;leunig* double mutants exhibit a phenotype similar to *ag-1* single mutants, suggesting that the effect of *leunig* mutations is mediated by ectopic *AG* expression. All of the above results suggest that *leunig* is required in the first two whorls for proper floral organ specification and thus *leunig* is likely another class A gene.

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ANALYSIS OF TRANSGENIC *ARABIDOPSIS THALIANA* CARRYING *GLOBOSA*, A FLORAL CLASS B GENES OF *ANTIRRHINUM MAJUS*. S.E. Kohalmi[1], Z. Schwarz-Sommer[2], H. Sommer[2], G.H. Haughn[3], [1]Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0; [2]Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, Germany; [3]Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 2B1.

There is increasing evidence that the regulatory mechanisms controlling floral morphology have been conserved throughout evolution of dicotyledonous species. *Arabidopsis thaliana* is distantly related to *Antirrhinum majus* yet both organisms specify floral organ identity in a similar manner. In both species, three classes of genes (A, B, C) with overlapping expression patterns combine to establish unique identities for each of the four organ whorls. The individual genes have been identified by mutation and an increasing number has been sequenced. For both, *A. thaliana* and *A. majus*, mutations in genes of the same Class cause similar floral phenotypes and the responsible genes show a high degree of homology. For example, recessive mutant alleles of the Class B genes, *pistillata* (*pi*) of *A. thaliana* and *globosa* (*glo*) of *A. majus*, result in a similar phenotype (conversion of sepals to petals and stamens to carpels). We are attempting to determine whether the *A. majus* Class B gene *GLO* will be regulated and function appropriately in *A. thaliana*. To test this possibility, genomic sequences of *GLO* were introduced into wild-type and *pi A. thaliana* lines. Results from the characterization of these transgenic lines will be presented.

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Functional Analysis of the Floral Homeotic Gene *AGAMOUS*.

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The homeotic gene *AGAMOUS* (*AG*) is necessary for normal development of reproductive floral organs (stamens and carpels). The AG protein shares striking similarity with the DNA-binding domains (designated MADS-box) of transcription factors from humans and yeast, suggesting that the AG protein is also a transcription factor. To study the function of *AG* in flower development, we have examined the effects of ectopically expressing *AG* in transgenic plants. We observed that the transgenic plants produce flowers similar to those from *ap2* mutants, supporting the proposed inhibition of *AP2* function by AG (Mizukami and Ma, 1992, Cell, 71:119-131). Similar results were obtained in transgenic tobacco by Yanofsky and colleagues (Mandel et al., 1992, Cell, 71:133-143). AG is required for normal development of stamens and the ovary, as well as for determinant growth in the flower. We have generated transgenic plants carrying antisense *AG* RNA; these plants have a range of floral phenotypes resembling those of *ag* mutant flowers to various degrees. All of the abnormal transgenic flowers have reduced determinacy, while some of these have normal carpels and even more have normal stamens. These results suggest determinacy requires more AG activity than organ morphogenesis. To study the function of various regions of the AG protein, we have created mutations which lead to truncated AG proteins lacking part or all of the N-terminal region, as well as those missing progressively more regions from the C terminus. Truncated AG proteins missing the N-terminal region, when expressed from the 35S promoter, also resulted *ap2*-like flowers in transgenic plants, suggesting that this region is not essential for inhibition of *AP2* function. We have also observed abnormal floral phenotypes in transgenic plants which express AG protein truncated from the C-terminus. The results from these plants and their implications will be presented.

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Molecular Characterization of *Luminidependens*; A Floral Induction Gene in *Arabidopsis thaliana*

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ABSTRACT

Plants have evolved the ability to regulate flowering in response to environmental signals such as low temperature and photoperiod. The physiology and genetics of floral induction have been extensively studied, but the molecular mechanisms of this process remains unknown. To study this process at a molecular level, we isolated a gene, *luminidependens*, which is involved in the floral induction pathway. *ld* is one of the late-flowering mutations in *Arabidopsis thaliana* which was first described by Redei. The physiological analysis of the *ld* mutant indicated that *LD* is involved in sensing and responding to inductive photoperiods. The *LD* gene was isolated by genomic and cDNA library screening followed by plasmid rescue from T-DNA tagged *ld-3* mutant. The 5'-end of *LD* cDNA was obtained by anchored PCR. The isolation of the *LD* gene was confirmed by molecular complementation with 9.4 kb genomic DNA fragment which covers *LD* gene using *Agrobacterium tumefaciens*-mediated transformation. The nucleotide sequence of *LD* cDNA contains 3185 bp which is in close agreement to the size of the mRNA as determined by RNA gel blot analysis (3.2 kb). The predicted *LD* protein displays a peculiar QPVNG repeat sequence and glutamine rich region. Northern blot analyses at different developmental stages and in different photoperiodic conditions indicated that mRNA of *LD* is expressed constitutively.

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Morphological and Genetic Analyses of Three Male-Sterile *Arabidopsis thaliana* Mutants Generated by T-DNA Insertional Mutagenesis. Christopher A. Makaroff, Brenda N. Cleaver, Heather A. Owen, Craig H. Dodrill, and Dawn M. Paxson. Department of Chemistry, Miami University, Oxford, OH, 45056 USA

Three nuclear male-sterile *Arabidopsis thaliana* lines which were generated by T-DNA insertional mutagenesis are currently being studied in our laboratory. The three mutant lines (7219, 7593, and 8048) have been analyzed using both classical genetics and molecular techniques. Based on segregation ratios and Southern analyses, each mutant appears to contain a single T-DNA insert consisting of multiple T-DNA's. A morphological study of the mutants has been initiated using semi-thin sections (0.5µm) of mutant and wild-type anthers that have been fixed with glutaraldehyde and osmium and embedded in Spurr's resin. The first sign of developmental aberration appears at different stages in the three mutants. In mutant 7219 the microsporocyte wall, which is no longer visible following tetrad formation in wild-type anthers, persists throughout development, preventing release of microspores from tetrads. In some cases, the unreleased microspores fuse together inside the persistent microsporocyte wall on which sporopollenin is deposited. These multi-nucleate cells collapse prior to anthesis. Mutant 7593 also produces abnormal microsporocytes, which divide asymmetrically to produce variable sizes of microspores. These develop abnormally and are covered by a darkly-staining wall that is not evident in wild-type development. In mutant 8048 microsporogenesis appears normal through release of microspores from tetrads. In contrast to the deposition of sporopollenin as a continuous layer on released microspores in wild-type development, sporopollenin is deposited as discontinuous aggregates on the surface of the mutant microspores. These deposits increase in size until collapse of the cells prior to anthesis. Lambda libraries for all three mutants have been constructed and clones containing T-DNA border sequences have been isolated and analyzed for the presence of flanking plant DNA. Experiments to isolate the wild-type copies of the mutant genes are currently in progress.

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THE *ARABIDOPSIS* FLORAL HOMEOTIC GENE *APETALA1* IS NEGATIVELY REGULATED BY *AGAMOUS*

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Genetic studies indicate that the *Arabidopsis* floral homeotic gene *APETALA1* is one of several genes involved in the generation of floral meristems, the first step in flower development. Molecular analyses indicate that *APETALA1* RNA is uniformly expressed in young flower primordia and in sepals and petals, and that *APETALA1* encodes a putative transcription factor with a MADS-domain. These molecular studies, together with the *apetala1* mutant phenotype, suggest that *APETALA1* acts locally to specify floral meristem identity, and to determine sepal and petal development. Further studies demonstrate that the floral organ identity gene *AGAMOUS* negatively regulates *APETALA1* RNA accumulation in the two inner whorls of wild-type flowers.

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Genetic control of the floral transition in Arabidopsis: The role of the *FVE* locus.

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Floral induction is a complex developmental process that depends on the interaction between genetic and environmental factors. In Arabidopsis, the transition from vegetative to reproductive growth is followed by the elongation of the stem and is induced by both long photoperiods and low temperature treatment (vernalization). Natural populations of Arabidopsis show an important amount of natural variation in their response to those environmental factors. Mutant analysis has allowed the identification of more than twenty loci whose mutations produce an altered flowering phenotype. Mutations at the *FVE* locus produce a delay in flowering time that can be rescued by a vernalization treatment. We have performed a physiological and morphological characterization of two independently isolated mutants at this locus. Moreover, we have also characterized the effect of the mutations at the *FVE* locus in the flower morphology of *ap1* and *ap2* mutants. The results obtained indicate that this locus plays a role all along the development of the plant from the initial phases of rosette development till the transition from the inflorescence to the flower meristem. In order to clone the *FVE* locus we are currently following a chromosome walking approach and the progress on these experiments will also be reported.

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Identification of a New Late-Flowering Locus: *FLC*

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A new late flowering locus, *FLC*, has recently been identified in *Arabidopsis*. The presence of *FLC* is required for the late flowering phenotype of *FLA* and *ld*. *FLC* has been observed to act in a manner consistent with it being an inhibitor of flowering. Preliminary studies have mapped *FLC* to the top of chromosome five, and also suggest that *FLC* may exhibit gene dosage effects. Experiments are currently in progress to study the physiological effect of *FLC* on flowering behavior.

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BEL1 Gene Regulates Ovule Development in Arabidopsis thaliana. Zora Modrusan[1], Leonore Reiser[2], Kenneth. A. Feldmann[3], Robert Fischer[2], and George W. Haughn[1]; [1]Botany Department, University of British Columbia, Vancouver, B.C., V6T 1Z4, Canada; [2]Department of Plant Biology, University of California, Berkeley, CA 94720, USA; [3]Department of Plant Sciences, The University of Arizona, Tuscon, AZ 86721, USA.

In order to determine the mechanisms controlling ovule development we have characterized Bel1-2 and Bel1-3 mutants defective in this developmental event. Both mutants were independently isolated from a collection of Arabidopsis thaliana plants transformed with Agrobacterium. Genetic analysis demonstrated that both Bel1-2 and Bel1-3 are the result of recessive alleles of the same nuclear gene BEL1 genetically linked to a T-DNA insert.

The morphological and histological changes of Bel1 ovules from initiation to degeneration were examined by SEM and light microscopy. Ovule development deviates from wild type at the stage of integument initiation. The inner integument fails to form and outer integument develops abnormally. Within the nucellus the formation of embryo sac is arrested during megagametogenesis. Many of these deformed Bel1 ovules degenerate as the flower senesces. However the outer integument of some Bel1 ovules develop into distinctive carpel-like structure (CLS) having cell types characteristic of ovary, style, and stigma.

Characterization of double mutant combinations with floral-organ identity genes AP2, AP3, and PI revealed that development of morphologically normal ovules is dependent on BEL1 expression regardless of the whorl in which the ovule develops. Moreover, in situ hybridization analysis with AG and AP3 probes demonstrated that the spatial pattern of their transcription in Bel1 ovules is not completely disrupted.

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Ultrastructure of Microsporogenesis in *Arabidopsis thaliana* (L. Heynh.) ecotype Wassilewskija. (Brassicaceae). Heather A. Owen and Christopher A. Makaroff, Chemistry Dept., Miami University, Oxford, OH 45056. USA.

One of the primary research interests in our laboratory is the study of nuclear sporogenous male-sterile mutants of *Arabidopsis thaliana* that have been generated by T-DNA insertional mutagenesis. In order to provide a basis for comparison, an ultrastructural study of microsporogenesis in wild-type *A. thaliana* has been conducted.

To obtain a complete developmental sequence all buds were removed from single inflorescences and kept in order throughout processing. Whole buds were measured, fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in Spurr's resin. Ultrathin sections (silver to pale gold) were cut with a diamond knife on a Reichert Jung Ultracut ultramicrotome, stained with uranyl acetate and lead citrate and viewed with a Zeiss EM10C transmission electron microscope operating at 60 kV. A complete developmental profile of microsporogenesis, including cellular changes in the tapetum, will be presented.

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A GENETIC, MOLECULAR AND PHYSIOLOGICAL ANALYSIS OF FLOWERING TIME IN ARABIDOPSIS.

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At least 11 loci have been identified of which the mutants delay flowering in long days in the Landsberg erecta (Ler) "wild type". The epistatic relations between these genes, as well as their response to vernalization and daylength have been determined. In addition, it was found that lateness in other ecotypes such as Columbia and Estland may depend on the co-action of two genes, which differ from the 11 loci identified in Ler. Flowering time of the phytochrome deficient *hy2* and *hy3* mutants as well as the *hy2/hy3* double mutant was studied in various environments and in the background of some late flowering mutants and indicated that phytochrome deficiency resulted in earliness suggesting that phytochrome suppresses flowering. Earliness of the *hy* mutants is expressed both in the background of late flowering mutants and in "extreme" short day conditions. Progress in cloning the late flowering *FWA* locus by means of "chromosome walking" will be reported together with information about its physiological mode of action.

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Cell-cell Communication in Arabidopsis: Genetic Analysis of Fertilization

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Fertilization in flowering plants requires proper communication between pollen and the pistil, yet few of the molecules that mediate this process have been identified. To address this question we have isolated several sterile Arabidopsis mutants, each of which is defective in pollen-pistil interactions. Initially, we have focused on a defect that disrupts pollen communication with the stigma by eliminating the extracellular pollen coat. Stigma cells that contact this mutant pollen produce callose, a carbohydrate normally synthesized in response to foreign pollen grains. The mutant pollen fails to germinate because it is not hydrated by the stigma, yet germinates in vitro, indicating it is viable. The mutation is also conditional; high humidity results in pollen hydration and successful fertilization.

Complementation tests showed the defect is due to a mutation in the *CER6* gene, a locus previously shown to be required for long-chain lipid biosynthesis. Analysis of Arabidopsis pollen confirmed that the wild-type pollen coat contains long-chain lipids, while they are virtually absent from the mutant pollen grains. In addition, while immature mutant pollen grains have some of the protein that is normally present in the coat, this protein is degraded during pollen development. These results demonstrate that the pollen coat is critical for pollen-stigma interactions and suggest that long-chain lipids are required for fertilization, either by directly signaling the stigma or by stabilizing other components on the pollen surface. Extraction of the pollen surface has yielded a small number of polypeptides. Each of these can be obtained in large quantities, and protein sequence analysis is currently in progress.

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ADENINE PHOSPHORIBOSYLTRANSFERASE IS REQUIRED FOR NORMAL POLLEN DEVELOPMENT IN *ARABIDOPSIS THALIANA*

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In order to understand the role of the purine salvage pathway in plants, a mutant has been isolated that is deficient in adenine phosphoribosyltransferase (APRT), an enzyme responsible for the conversion of adenine to AMP. In *Arabidopsis*, the mutant is male sterile due to aborted pollen development. Cytochemical staining has shown that the course of pollen development in the mutant begins to diverge from that in the wild type soon after meiosis. Although the majority of the morphological changes appear in the developing pollen, the region directly affected by the APRT deficiency could be any of the tissues of the anther. In order to gain insight into the mechanism by which the APRT deficiency causes male sterility, an *Agrobacterium*-mediated transformation experiment using the cDNA for APRT has been performed. Promoters that are spacially and temporally regulated in the anther have been fused to the antisense orientation of the cDNA, and transformed into wild-type plants. Several transgenic plants have been generated and are now being analysed for male sterility.

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GENETIC ANALYSIS OF OVULE DEVELOPMENT. Kay Robinson-Beers, Daphne Preuss*, Robert E. Pruitt†, and Charles S. Gasser, Section of Biochemistry and Biophysics, University of California, Davis, CA 95616; *Department of Biochemistry, Stanford University, Stanford CA 94305-5307; †Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

We are combining genetic methods with anatomical and morphological analyses to dissect ovule development in Arabidopsis. Chemically mutagenized populations have been screened for female-sterile mutants through 1) the failure of siliques to elongate after self pollination and 2) reciprocal crosses used to distinguish gynoecial vs. androecial defects. To date, ten female-sterile mutants (*bel1-1, bel1-4, bel1-5, hai, ino, oni, sin1, ste17, ste112, 184-20*) that segregate as single gene recessives and exhibit altered ovule development have been independently isolated. In addition, five other independently isolated ovule mutants have recently been identified. These mutants exhibit a range of distinct morphological abnormalities affecting either individual or both integuments. For example, the *ino* (*inner no outer*) mutant produces an apparently normal inner integument, but the outer integument of *ino* mutants is initiated in a position 180° opposite normal and fails to develop. Conversely, the *oni* (*outer no inner*) mutant appears to lack an inner integument and produce only an outer integument. One of our most well studied classes, the *bel* mutants (*bel1, bel1-4, bel1-5*), appear to cause a homeotic conversion of the outer integument into a carpeloid structure, and ovules of the *sin1* (*short integuments*) mutant initiate two integuments which appear to undergo cell division without the accompanying pattern of cell elongation necessary for normal integument formation. Neither of the latter two mutants forms an embryo sac indicating that normal morphological development of the integuments and proper embryo sac formation may be interdependent processes or in part governed by common pathways. Genetic analyses indicate that *bel1* and *sin1* define two loci which are essential for normal ovule development[1]. We are continuing to screen for additional mutants, and complementation analyses and mapping studies on the mutants in hand are in progress. Our long range goal is to understand the molecular genetic mechanisms that regulate ovule development.

1. Robinson-Beers et al., (1992) The Plant Cell. 4:1237-1249.

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GENETIC AND MOLECULAR STUDIES OF *PERIANTHIA*, A NOVEL GENE THAT SPECIFICALLY AFFECTS FLORAL ORGAN NUMBER

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While much is known about the genes responsible for floral organ identity, very little is known about an earlier process of flower pattern formation: establishment of the correct number and position of organs in individual whorls. While floral organ identity is relatively well conserved among flower species, floral organ number is much less conserved, suggesting that the genes involved in organ number determination may be important in the evolution of flower form. We describe here mutations in the *PERIANTHIA* (*PAN*) gene, a novel gene that specifically affects floral organ number. Flowers of plants mutant in *pan* exhibit a consistent increase in perianth organ number: 5 sepals and 5 petals are most commonly observed, instead of the 4 sepals and 4 petals seen in wild-type. In addition, stamen number is decreased from 6 to 5 in most flowers. Double mutant studies, as well as the single mutant phenotype, indicate that *PAN* may interact with the homeotic genes to regulate their domains of function, specifically in regulating the position of the boundary between the A function genes (which specify sepal and petal organ identity) and the C function genes (which specify stamens and carpels). Alternatively, *PAN* may affect the floral meristem structure independent of the homeotic genes. Studies of *pan* flower development using confocal laser scanning microscopy and scanning electron microscopy will be discussed, as well as RNA expression patterns of the homeotic genes in *pan* single and double mutants. We are in the process of cloning the gene via chromosome walking.

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HANABA TARANU, a gene controlling the floral meristem development with *LEAFY*.

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Several mutants strains showing abnormal floral development were isolated through T-DNA mutagenesis. Some exhibit phenotypes which cannot be characterized as results of the abnormal gene expression of the homeotic or meristem identity genes. One of them has a reduced number of floral organs without showing homeotic transformation (hence the mutant name *hanaba taranu*: too few floral leaves). All four floral whorls, especially the second and third, are affected by the spatial reduction.

In order to elucidate a possible interaction with other genes, a series of genetic analyses was performed involving construction of the double mutants of *hanaba taranu* with the previously characterized floral mutations. An additive phenotype was observed with the homeotic mutations, but the *hanaba taranu; leafy* double mutants showed a striking phenotype: In combination with a strong *leafy* allele the inflorescence shoots of the double mutant could not develop any floral organs or bracts, except occasionally filaments were formed. An enhanced defect in floral organ development was also seen in the double mutant of *hanaba taranu* with a weak *leafy* allele. As the gene *LEAFY* has been shown to be expressed in the very beginning of floral meristem development, controlling its identity, it is likely that *HANABA TARANU* acts with *LEAFY* at the same early stage, by controlling the size and growth of the floral meristem.

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Genetic control of inflorescence initiation and development.

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Photoperiodic regulation plays a fundamental role in the initiation of flowering of many angiosperms. In *Arabidopsis*, floral initiation is promoted by exposure to long-day photoperiods such that plants grown in long days flower earlier and after the production of fewer leaves than plants grown in short days. We are investigating the photoperiodic sensitive pathway of inflorescence initiation and development by studying mutants defective in this process. Early-flowering (*elf*) mutants initiate inflorescence development earlier than wild-type plants and produce normal inflorescence structure. The *elf3* mutant is insensitive to photoperiod with regard to floral initiation. Surprisingly, *elf3* mutants are largely defective in blue light-mediated inhibition of hypocotyl elongation, and do not exhibit the phenotypes characteristics of the known phytochrome mutants of *Arabidopsis*. The pleitropic phenotype of *elf3* mutants suggests that one of the signal transduction pathways controlling photoperiodism is regulated, at least in part, by blue light. *terminal flower* 1 (*tfl1*) mutants also initiate flowering earlier than wild-type, but prematurely terminate inflorescence development with the formation of a terminal flower. Thus, the *TERMINAL FLOWER 1 (TFL1)* gene is required for normal inflorescence meristem function. The *LEAFY (LFY), APETALA 1 (AP1),* and *APETALA 2 (AP2)* genes have been shown to be required for normal floral meristem function. We have found that the acquisition of meristem identity during inflorescence development is likely to be mediated by antagonistic interactions between *TFL1* and *LFY*, and between *TFL1* and *AP1/AP2*. This proposal will be discussed with regard to other genes that are likely to be part of the genetic hierarchy regulating the establishment and maintenance of inflorescence and floral meristems.

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Use of Cre/Lox and Flp/Frt to generate genetic chimeras in *Arabidopsis*

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We are using the site specific recombinase systems Cre/Lox (of phage P1) and Flp/Frt (of yeast) to generate genetic chimeras. These systems each contain two components: the first component is a site specific recombinase (Cre and Flp) and the second component is the target site for the recombinase (Lox and Frt). Constructs that place the recombinase genes, Cre and Flp, under the control of the Arabidopsis heat shock promoter HSP18.2, have been made and introduced into plants. We have also made constructs that place 35S-GUS between direct repeats of the target sequences, Lox and Frt. These have also been introduced into plants. Currently, plants carrying these constructs are being analyzed for copy number and are being crossed to generate F1 plants that contain both components of a system (i.e. Heat shock-Cre with Lox constructs and Heat shock-Flp with Frt constructs). Exposure of these F1 plants to heat-shock conditions should induce expression of the recombinase, which will then act on the two target sites to delete the intervening sequences. By controlling the level of induction, we should be able to find conditions that result in excision in only a subset of cells, thus resulting in plants that are chimeric for 35S-GUS. Excision will be assessed by staining for GUS activity and monitoring white (non-staining) sectors. Analysis of these plants should allow us to understand the clonal relationship between cells of the inflorescence and flower meristem and the cells that are incorporated into the mature flowers. Through these studies, we hope to determine whether the *Arabidopsis* inflorescence meristem is organized into L1, L2 and L3 layers as has been described for other plants. We have also made a series of constructs that contain both 35S-GUS and the *AGAMOUS (AG)* gene between target site direct repeats. Plants containing these constructs, and plants containing the heat shock-recombinase constructs, are being crossed into an agamous mutant background. Eventually, we will obtain an F1 plant in which the only functional *AG* allele is located between the recombinase target sites. Heat-induction of the recombinase in these plants should result in excision of both the 35S-GUS and the functional *AG* gene. Through analysis of flower development in these plants, we hope to assess cell autonomy of *AG* and identify the subset of cells in which AG expression is essential for normal differentiation.

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Detailed mapping and chromosome walking to the *ms1* locus.

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The recessive mutation *ms1* [Van der Veen and Wirtz (1968) Euphytica 17: 371-377] results in male sterile plants due to a breakdown in the later stages of pollen development, which occurs soon after microspore release; *ms1* homozygotes are characterized by abnormalities in the tapetal layer of the anther and the microspore wall. The *ms1* gene has been mapped to the top arm of chromosome 5 [Koorneef et al.(1983) Heredity 74:265-272]. This region has been characterized using in excess of 100 recombinants between the phenotypic markers *Ch7*, *lu*, *ms1* and *ttg*. The order and map distances between RFLP markers linked to *ms1* have been determined and compared to physical distances as established by PFGE. RFLP probes flanking *ms1* have been used to identify YAC clones for a chromosome walk in this region. Three YAC libraries have been used: EG [Grill and Somerville (1991) MGG 226: 484-490], EW [Ward and Jen (1990) PMB 14: 561-568] and yUP (Joe Ecker, University of Pennsylvania). Positive YAC clones have been characterized for overlap and sized. End-probes have been generated from the YACs by plasmid rescue and vectorette PCR and used to walk out from the starting clones. The progress of this work will be described.

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Molecular Analysis of a T-DNA Insertion Mutant of *Arabidopsis thaliana* Displaying the *erecta* Phenotype

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Our major interest is to define the molecular process of inflorescence development. The initial and key step to elucidate this process is the isolation of genes affecting the inflorescence and characterization of their expression in wild type and other mutant backgrounds displaying abnormal development of inflorescence.

We have transformed *Arabidopsis* WS-ecotype *in planta*, using T-DNA harboring hygromycin resistant gene as an insertional mutagen, and obtained a mutant displaying *erecta* phenotype. The phenotypic traits of this line included compact inflorescences, short petiole and reduced height, and this phenotype cosegregated with hygromycin resistance. Complementation tests by genetic crossing with Landsberg *erecta* indicate that the mutational defects are allelic. We have therefore designated the allele in this insertion mutagenized line *erecta-104*.

Genomic Southern blots, probed with either T-DNA or just the *hph* gene, indicate a complex insertion with three T-DNA copies with different lengths integrated tandemly at a single site. At least one of them is estimated to be a full length T-DNA. Utilizing plasmid rescue, we have obtained plasmids containing all T-DNAs together with flanking sequence. Subcloning and restriction analysis of this flanking region is now in progress.

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ACTIVATION OF FLORAL HOMEOTIC GENES DEPENDS ON THE MERISTEM IDENTITY GENES *LEAFY* AND *APETALA1*

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The identity of floral organs in the weed *Arabidopsis thaliana* is determined by floral homeotic genes, which are expressed in specific regions of the developing flower. Several studies have shown that the region-specific expression is brought about mainly by negative interactions; for example, expression of the homeotic gene *AGAMOUS (AG)* is repressed in the outer whorls by the activity of the homeotic gene *APETALA2*, and expression of the homeotic genes *APETALA3 (AP3)* and *PISTILLATA (PI)* is repressed in the inner whorl by the cadastral gene *SUPERMAN*. These studies, however, did not reveal anything about the initial activation of homeotic genes by positive regulators. Candidates for such activators are two genes, *LEAFY (LFY)* and *APETALA1 (AP1)*, which promote floral over shoot identity, and which are expressed before the homeotic genes. We have studied how mutations in the *LFY* and *AP1* genes affect the spatial and temporal pattern of homeotic gene expression.

We found that activation of the homeotic genes *AP3*, *PI*, and *AG* depends on the activity of the meristem identity genes *LFY* and *AP1*. Furthermore, our results show that homeotic genes differ in their requirement for *LFY* and *AP1* activity. None of the homeotic genes studied is strongly affected by loss of *AP1* activity alone. In the case of *AG*, *AP1* and *LFY* act largely redundantly, and elimination of both *AP1* or *LFY* activity is required to abolish the normal pattern of *AG* expression. By contrast, *LFY* is a major activator of *AP3* and *PI*, as indicated by the severe reduction in their RNA levels in strong *lfy-6* single mutants, with *PI* being possibly more sensitive to loss of *LFY* activity than *AP3*. *AP1* is required to much a lesser extent than *LFY* for the activation of *AP3* and *PI*, and a strong effect of *AP1* on *AP3* and *PI* expression is observed only when *LFY* activity is reduced or eliminated. That—albeit very rarely—some remnants of the normal pattern of homeotic gene expression are observed even in strong *lfy ap1* double mutants suggests that yet other factors act in concert with *LFY* and *AP1* to activate homeotic gene expression.

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PHENOTYPIC AND GENETIC ANALYSIS OF TWO NOVEL FLOWER MUTATIONS IN ARABIDOPSIS

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In *Arabidopsis*, a limited number of floral homeotic genes have been identified that control organ identity. However, questions of how the known floral homeotic genes are activated and how they are interpreted by the early flower are not as well understood. In an attempt to find genes that affect other aspects of flower development, several mutations have been identified that affect floral organ number. This work reports the phenotypic analysis of two such mutations. The first, called *snowball* (*sno*), is characterized by the presence of extra sepals and a variable number of petals and stamens. Other defects include misshapened sepals that fail to completely close during stage 6, and very small siliques. The second mutation, called *wendell* (*wen*), is characterized by a reduced number of organs in the second and third whorls. Double mutants with these genes and some of the known homeotic and meristem identity genes are presented. The possible roles of these two genes in determining floral organ number are discussed.

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Genetic characterization of embryonic flower (*emf*) mutants in *Arabidopsis*

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EMF is a gene required for vegetative development in *Arabidopsis thaliana* (Sung *et al.*, 1992, *Science* 258:1645-1647). A total of six independent *emf* mutants, all segregated as monogenic recessive traits, were isolated from ethyl methane sulfonate (EMS), diepoxybutane (DEB) or gamma ray-mutagenized *Arabidopsis* seeds. *emf* mutants produced floral organs upon germination, bypassing the rosette, suggesting that the reproductive development is the basal state of the shoot apex. Genetic complementation tests among these six mutants demonstrated the involvement of two loci, *EMF1* and *EMF2*, in regulating the vegetative/reproductive switch. All mutants lost the ability to produce rosette, yet they exhibited variable effects on the extent of reproductive organogenesis. A strong allele such as *emf1-2* bypassed all inflorescence and floral organ development and resulted in the formation of carple-like structures directly from the embryo. Weaker alleles, such as *emf1-1* and alleles of *emf2* gave rise to reduced inflorescence and variable floral organ development. *emf1-2* is a gamma ray-induced mutant, thus likely a null mutation. Based on the phenotype of *emf1-2* homozygous mutant, we propose that rosette development depends on the vegetative program and carpel formation is controlled by the reproductive program alone. However, inflorescence and floral organogenesis result from an overlapping expression of both the vegetative and reproductive program. In *emf1-2* homozygous mutant, the total loss of vegetative state eliminated not only the rosette, but also the inflorescence and floral organogenesis, resulting in carpel formation only. In the mutants homozygous of weaker alleles, residual expression of the vegetative state enabled the development of some inflorescence and floral organs. The characterization of double mutants within two different *EMF* loci and the possibility of interactions between *EMF1* and *EMF2* will be discussed in detail. The progresses on genetic mapping and cloning of *EMF* genes also will be presented.

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Expression of the cauliflower mosaic virus derived gene VI in its host *Arabidopsis* results in morphological and developmental changes

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Expression of ORF VI of cauliflower mosaic virus (CaMV) in transgenic *Arabidopsis thaliana* resulted in a new plant phenotype. Besides stunting, vein clearing, chlorosis abnormal leaf shape and production of secondary stems (tillering), developmental changes were observed in the Arabidopsis plants expressing gene VI: the plants had a reduced fertility and flowered late.

In correlation with expression from the ORF VI transgene, the flowering time (FT) and the number of rosette leaves (LN) increased, giving a typical late flowering phenotype which has been observed for certain mutants.[1]

FT and LN were studied with three different ORF VI transgenics under four treatments: long and short day with and without vernalization. Under long day conditions, the gene VI plants showed a FT and a LN up to 3 times higher than those observed for WT plants. Vernalization of the seeds did not revert this effect. Under short day conditions the effect was not observed and WT plants flowered as late as gene VI transgenics.

[1] Koornneef, M., Hanhart, C.J. and van der Veen, J.H. Mol Gen Genet (1991) 229: 57-66

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ANALYSIS OF *dwf4*, A DWARF MUTANT WITH A POSSIBLE LIGHT-REGULATORY PHENOTYPE.
Ricardo Azpiroz, Yewen Wu, Burkhard Schulz and Kenneth A. Feldmann. Department of Plant Sciences, University of Arizona.

Primary dwarfs are defined as plants with a short, robust stem and short, dark green leaves. A number of primary dwarf mutants of Arabidopsis generated by T-DNA insertion mutagenesis are currently under investigation in our laboratory. *dwf4*, the focus of this study, normally attains a height of approximately 3 cm. at five weeks, as compared to wild-type Wassilewskija, which normally grows to >25 cm. Regardless of the growth conditions, all organs in this mutant are shorter than those of wild-type, and this reduction in length is not reversed by application of gibberellic acid. *dwf4* flowers are essentially infertile, since the filaments are shortened and shed their pollen onto the ovary wall. This mutant displays a prolonged vegetative phase with a concomitant delay in senescence. Furthermore, apical dominance is reduced in *dwf4*, since mutant plants develop three times as many inflorescences as wild-type. Comparison of fresh weight and dry weight between *dwf4* and wild type plants indicates that mutant plants have a higher water content. Cell size determinations from seedling hypocotyls and inflorescence stems show that the dwarf phenotype is at least in part due to a reduction in cell size. Whole plant chlorophyll content and chlorophyll a/b ratios do not differ between *dwf4* and wild-type, however, suggesting that the dark green color of the mutant is solely due to wild-type chlorophyll amounts present in smaller cells. An additional phenotype of *dwf4* is the opening of cotyledons in dark-grown seedlings. The various phenotypic features of*dwf4* overlap those of *det2*, *cop2* and *cop3* mutants, which are affected in the regulation of light-induced responses. *dwf4* may therefore represent a novel class of light-regulatory mutant in Arabidopsis.

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EMF gene activation and shoot determination during embryogenesis.
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We have isolated a set of *Arabidopsis* mutants, called *embryonic flower*, *emf*, that cannot produce vegetative organs, only reproductive organs (Sung et al. 1992 Science 258: 1645-1647). The phenotype of these mutants indicates that the wild type*EMF* alleles are required for the development of the rosette. The loss of *EMF* function did not stop growth or cause callus growth, but resulted in inflorescence development. Hence the *EMF* genes function to initiate vegetative development by suppressing reproductive development. Under this scenario, reproductive state would be the basal or default state of the shoot apex, the vegetative state is activated by the expression of the *EMF* gene. Wild-type *Arabidopsis* plants would flower when *EMF* genes are suppressed by developmental and photoperiod-mediated signals. To study the time of *EMF* gene activation during embryogenesis, we used the divergent pattern of shoot apex development as an assay to distinguish the mutant from the wt embryos. We found that an *EMF* gene acted as early as the late heart-stage embryogenesis. While the tunica-corpus was well developed in the wt embryos, mutant embryos exhibited irregular cellular arrangement at the shoot apex as early as the heart-stage. This finding suggests that the shoot apex was determined as early as, if not before heart-stage embryogenesis. Although the tunica-corpus cellular arrangement was disturbed in early development, the mutants formed proper inflorescence and floral meristems before flowering. The altered shoot apices of the mutant embryos are believed to result from precocious development of the inflorescence, because similar alterations were found in the axillary buds of inflorescence shoot that develop directly into conflorescence, bypassing the vegetative shoot. An investigation of the timing of homeotic gene expression during *in vitro* and *in vivo* shoot organogenesis will confirm the notion of early commitment of mutant shoot apex to flowering. Interaction of *EMF* with early and late flowering genes will provide insights into the genetic pathways of flowering.

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Schizoid, an *Arabidopsis* mutant exhibiting degeneration of the shoot apical meristem

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We are interested in understanding how the vegetative shoot apical meristem functions. We have identified an *Arabidopsis* mutant, Schziod (Shz), which has a disruption in development of the vegetative shoot apex. Abnormal development is first observed in leaf primordia which become stunted and necrotic. Degeneration of leaf primordia is followed by degeneration of the shoot apical meristem. Following degeneration of the primary apical meristem, growth and differentiation at the apex are disorganized. Secondary and adventitious meristems arise and callus may form. One interpretation of this phenotype is that the meristem is dependent on factors produced by primordia and/or young leaves and that *shz* results in the loss of this factor reaching the meristem. We have identified two *shz* alleles that arose independently after T-DNA insertional mutagenesis. Linkage analysis has shown no recombination between either *shz* allele and the T-DNA marker. The phenotype of the more severe allele will be described. We have isolated plant DNA flanking the T-DNA left border using inverse-PCR. Analysis of the putative SHZ gene is in progress.

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

We have taken two approaches to understanding the functions of the shoot apical meristem. The genetic approach involves the characterization of the Forever Young (Fey) mutant that affects phyllotaxy and meristem maintenance. In wild-type plants, leaves are initiated at a divergence of 136.4° (SE±1.6) with a plastochron ratio of 1.20 (SE±0.02) corresponding to a 3/5 parastichy pattern. In contrast, we were unable to find any characteristic pattern in Fey apices. In Fey, the lesion is first visible as necrosis in the cells of the meristem. Further, meristem zonation is disrupted and leaf primordia are initiated with an abnormally large number of cells. The reverse approach involves the characterization of the meri-5 gene (Medford et al, Plant Cell 3: 359). Meri-5 shows 52% homology (amino acid) to a xyloglucan endo-transglycosylase (XET) which may be involved in cell wall loosening during expansion (da Silva et al, Plant J., in press). Transgenic plants were generated by introducing meri-5 in either the sense or anti-sense orientations. Leaves of both classes of transformants show necrosis and improper expansion. Transformants were analyzed with an RNase protection assay to correlate phenotypes with changes in the level of the meri-5 mRNA. Further analysis includes an assay for XET activity.

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Genetic analysis of root development in *Arabidopsis thaliana*

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Unlike shoot meristems, root meristems of higher plants do not produce lateral organs. The apparent simplicity in the root structure offers the possibility of isolating root mutants that are impaired in genes regulating cell division and differentiation. We have isolated a large number of *Arabidopsis* mutants impaired in root but not shoot growth and development. These mutants were classefied into the following categories: mutants impaired in 1) genes required for activating cell division in the root apex after germination, e.g., *stunted root*, 2) or genes required for suppressing cell division in mature root tissue, e.g., *starchy*, 3) or genes required for tap but not lateral root growth, *short tap*. Genetic and developmental characterization of *stunted root* class of mutants identified two genes that were required for activating cell division in the root apex. Mutants impaired in these genes produced 1-2 mm long roots devoid of root meristems, regardless whether they were radicles or lateral roots or adventitious roots. Cells in these primordial roots could not divide, instead enlarged and differentiated into cortical, epidermal, vascular and cap cells, and followed by apoptosis. The number of cells in the 1-2 mm long roots did not increase from those in the embryonic. root. In the absence of new cell production, roots ceased to grow. The phenotype of these mutants allowed the genetic dissection of root development into a two-step process: the formation of a root primordium followed by the formation of a root meristematic region. Stunted root genes are responsible for the second step--formation of a root meristem by activating cell division in the root primordium.

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TOWARDS THE CLONING OF THE TORNADO MUTANT

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We are characterising the Tornado (Twisted organs, Tor) mutant which was isolated during a T-DNA screening. The mutation is not caused by a T-DNA insertion altough there are 2 in tandem T-DNA's inserts nearby the mutant locus. The tor mutation is nuclear recessive and the locus has been mapped to the bottom of chromosome 5.
The Tor mutant has the unique phenotype of a severe dwarf with spiralised growth of all organs; roots, stems, leaves and flowers. Beginning with the formation of the first leaves the plant starts twisting. The shape of the leafblade becomes aberrant due to an underdeveloped vascular system. In contrast to WT plants, the Tor mutant produces several rosetlike structures with each several inflorescences. Tor is sterile and starts to senesces much later than WT plants.
To better understand the mechanisms causing such a severe phenotype we want to characterise the Tor mutant further physiologically and anatomically and at the same time clone the TOR gene.
In order to obtain an accurate map of the mutant locus, we isolated recombinants at both sides of the tor locus. We are presently mapping the recombinants with nearby RFLP markers. Later the new developped AFLP technique will enable us to "land" close to the tor locus, thus avoiding the tedious walking to the locus.

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Expression of a mitotic cyclin gene in *Arabidopsis thaliana.*

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A mitotic cyclin gene has been isolated from *Arabidopsis thaliana*, and its promoter has been transformed back into *A. thaliana* plants as a GUS fusion gene (T. Murbacher, D.T. Cam Ha, M. Mink, W. Frommer, E. Heberle-Bors, H. Hirt, submitted). Here we report that histochemically detected GUS activity was found in all layers of the shoot apical meristem, with stronger expression in the corpus as compared to the two tunica layers. Expression was still high in developing leaves while in older leaves expression was confined to the vascular system and to immature stomata of the epidermis. In mature leaves, no expression was observed. In the shoot, expression was found only in the vascular bundles. Very strong staining was observed in the endodermal ring of the root apical meristem while the vascular cylinder stained less and the outer cell layers of the root tip only weakly. The quiescent center and the root cap did not stain at all. In the differentiation zone of the root, all cells of the pericycle and to a lesser degree the endodermis expressed the gene, and weak expression was found in the vascular cylinder. Cortex and epidermis did not stain. Lateral root initials could be detected as clusters of a few intensely staining cells associated with the pericycle. Cyclin-GUS expression did thus not predict the site of lateral root initiation. High GUS-expression was found in all cells of the young flower bud. During flower development, expression disappeared first in sepals and then sequentially in the other whorls. After fertilization, expression in the carpel=young seed pod reappeared to disappear again during seed pod maturation. In mature seed pods, about a quarter of the seeds stained blue indicating segregation of one active cyclin-GUS gene locus. These results indicate that expression of the cyclin gene is restricted to actively dividing and proliferating cells.

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PROGRESS TOWARDS THE MOLECULAR CLONING OF THE CER3 GENE OF *ARABIDOPSIS*

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The outermost surface of *Arabidopsis thaliana* is covered by a wax layer composed of a variety of long chain fatty acids, primary and secondary alcohols, aldehydes, ketones and alkanes [Hannoufa et al, 1993, *Phytochemistry*, in press]. In *Arabidopsis* 22 loci (*cer* loci) are now known to be involved in the regulation of wax biosynthesis [McNevin et al, 1993, *Genome*, in press]. T-DNA tagging has been used in an attempt to isolate the CER3 gene. A T-DNA tagged *cer3* mutant was generated by Ken Feldmann by infecting germinating seeds with *Agrobacterium tumefaciens* carrying a disarmed Ti plasmid. Genetic analysis of the *cer3* mutant revealed that the *cer* phenotype is caused by a single recessive mutation which is believed to have been induced by the insertion of a T-DNA element. Molecular analysis of the *cer3* mutant showed the presence of two clustered T-DNA inserts. We have isolated λGEM12 genomic clones of the T-DNA/plant DNA junctions of the *cer3* mutant with DNA probes consisting of subclones of the left and right end of the T-DNA element used in tagging. The T-DNA/plant DNA junction clones isolated above were then used to screen a λGEM11 library to isolate genomic clones of the wild-type allele of the CER3 gene as well as a λYES cDNA bank of *Arabidopsis* [Eldridge et al. 1991, Proc Natl Acad Sci USA] to isolate putative clones of the CER3 gene. Eleven putative cDNA and eight genomic clones of the CER3 gene have been obtained and attempts are underway to confirm their identities by RFLP mapping and molecular complementation of the CER3 mutant. Partial sequencing of the right border/plant DNA junction revealed a high amino acid homology with a wide variety of serine/threonine protein kinases.

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ROOT DEVELOPMENT IN ARABIDOPSIS : MUTANTS THAT AFFECT MERISTEM MAINTENANCE. **Laura Di Laurenzio and Philip Benfey**
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Roots are an excellent model to study post-embryonic organ development in plants. The root is a relatively simple organ, made of a limited number of tissues that have radial symmetry. Root development follows a continuous and uniform pattern in which stem cells in the root meristem provide the progenitors for all the differentiated cells. Though the general characterisitcs of root development have been described, very little is known about the genetic pathways that regulate such a process.
A. thaliana belongs to the class of plants with indeterminate root growth, that is, the root grows through the life cycle of the plant. This implies that the root meristem must continuously produce progenitors of the differentiated tissues.
In order to identify the genes that control normal meristem growth, we have screened *A. thaliana* for mutants that fail to maintain normal root growth. Three mutants have been characterized in depths. The *shortroot* mutant was identified as a plant with drastically reduced root length, but a relatively normal aerial part. Optical microscopy analysis of whole mounts of roots showed an absence of the elongation zone and tissue differentiation to the tip of the root. Furthermore, transverse sections through the differentiated region of the roots of this mutant revealed the lack of an endodermal cell layer.
The other two mutants exhibited a milder phenotype than *shortroot*, with roots growing longer before becoming totally differentiated. Transverse sections through the roots of these mutants also showed lack of the endodermis. The three mutations are recessive to wild type. All three have been derived from T-DNA insertion lines generated by K. Feldmann. The three mutants responded in similar ways to changes in the growth conditions and were not rescued by addition of growth hormones, suggesting that the mutation(s) is not in a metabolic or hormone biosynthesis/response pathways. For one of the mutants we have shown that the phenotype co-segregated with the T-DNA insertion.

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CHARACTERIZATION OF *KNOTTED*-LIKE HOMEOBOX GENES IN *ARABIDOPSIS*. Cindy Lincoln, Judy Yamaguchi, Jeff Long, Lauren Hubbard and Sarah Hake. Plant Biology Dept., Univ. of California, Berkeley, CA and U.S.D.A. Plant Gene Expression Center, Albany, CA 94710. Dominant mutations in the maize homeobox gene *Knotted-1 (Kn1)* specifically alter leaf cell identity due to ectopic expression of the *Kn1* gene product in developing leaves. The presence of a homeobox sequence in the *Kn1* gene suggests that the wild-type gene product plays an important role in plant development by regulating the transcription of downstream genes. To further investigate the function of the *Kn1* gene, we have identified related genes in *Arabidopsis*. A sequence containing the maize *Kn1* homeobox was used to screen *Arabidopsis* floral and seedling cDNA libraries. A family of genes which are similar in sequence to the maize *Kn1* gene was identified; the members of this family have been named *KNAT* for *Knotted-like from Arabidopsis thaliana*. One member of this family, *KNAT1*, has been characterized in detail. Within the homeodomain region, *KNAT1* and *Kn1* share identical residues in 57 of the 64 positions (89% identity). The sequence homology between the two genes extends for 163 amino acids N-terminal of the homeodomain. The pattern of *KNAT1* expression in different tissues is being examined using *in situ* hybridization. The *KNAT1* gene appears to be expressed in the vegetative meristem, but not in inflorescence or floral meristems. Expression is seen in the inflorescence stem and in the pistil of developing flowers. Characterization of a second gene, *KNAT2*, is currently in progress.

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Molecular and genetic analysis of the Forever Young Mutant,

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Our laboratory is working on understanding how the tiny group of cells known as the apical meristem initiates tissues and organs We are using both classical genetic, and molecular approaches to identify and study genes involved in apical meristem function. The Forever Young (Fey) mutation affects the meristems ability to maintain itself as a formative region and also affects the position in which new organs are generated. Fey was generated by T-DNA insertional mutagenesis, allowing us to easily use both classical and molecular techniques to study it. Initial analysis by Northern blot indicates that the gene is expressed in all tissues, but not in meristematic tissue isolated from cauliflower head. In situs were conducted on Arabidopsis using the putative FEY gene. The pattern of expression here suggests that the gene is expressed everywhere, but especially strongly in young meristems. Earlier studies by our laboratory on Fey suggested that it sometimes places abnormally large numbers of cells in its leaf primordia. If theses cells are not replaced then fewer cells would be available for later primordia. If this pattern continued then the meristem could be "used up". In an attempt to "correct" the Fey phenotype it was crossed to a mutant having an abnormally large meristem (Fully Fasciated, Fuf). Analysis of these crosses shows a pattern of interaction and shows that alterations in vegetative meristems influence events in floral development. Details of these interactions and of the molecular work will be presented at the meeting.

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Cesium-insensitive *Arabidopsis* mutant displays hypersensitivity to NH_4^+

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Arabidopsis seedlings germinated and grown in nutrient media supplemented with 1.0-2.0 mM cesium chloride develop normally for the first 5 days. Shortly thereafter, bleaching of the cotyledons and hypocotyl is observed, followed by seedling death. Mutants of *Arabidopsis* which are insensitive to cesium (csi) have been generated by both EMS (Sheehan, Riberio-Neto, and Sussman, **The Plant Journal**, in press)and T-DNA insertional mutagenesis. Uptake studies with *A. thaliana* germlings have shown that both Cs^+ and K^+ competitively inhibit $^{86}Rb^+$ accumulation. Furthermore, Cs^+ toxicity may be alleviated by including K^+ in the selective media. In this report we show that the presence of 1.0 mM NH_4^+ in selective media will also delay onset of Cs^+ toxicity in wild type *Arabidopsis*. Cesium-insensitive mutants, however, are observed to vary in their response to NH_4^+ in non-selective media. One mutant (1500-1) is significantly stimulated in growth rate and chlorophyll content by the presence of 1.0 mM NH_4^+. Two other mutants (2100-3 and 7400-1) are strongly inhibited in germination or growth rate, respectively, by this treatment. Similar responses were not observed for K^+ or Na^+. NH_4^+ hypersensitivity has also been observed in plasma membrane proton pump (H^+-ATPase) mutants of the yeast, *Saccharomyces cerevisiae* (D. Conklin, personal communication). This result, in conjunction with preliminary genomic Southern blot data, suggests that csi mutants 2100-3 and 7400-1 are plausible candidates for plants with mutant genes encoding the plasma membrane proton pump.

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Arabidopsis requires polyunsaturated lipids for low-temperature survival

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Mutants of *Arabidopsis* that contain reduced levels of polyunsaturated fatty acids showed growth characteristics at 22°C that were very similar to wild type. By contrast, at 12°C, the mutants failed to undergo stem elongation during reproductive growth although they produced normal flowers and fertile seeds. After transfer to 6°C, rosette leaves of the mutants gradually died, and the plants were inviable. These different responses of the mutant plants at 12°C and 6°C suggest that distinct functions may be affected at these two temperatures. The gradual development of symptoms at 6°C and other lines of evidence argue against a general collapse of membrane integrity as the cause of the lethal phenotype. Rather, they indicate that the decrease in polyunsaturated membrane lipids may initially have relatively limited effects in disrupting cellular functions.

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The *Arabidopsis GL1* Gene Requires a Downstream Enhancer for Function

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Durring development, cells become committed to different developmental fates. This process is called determination. The differentiation of trichomes (hairs) on the epidermis of *Arabidopsis thaliana* shoots is an excellent system with which to study the commitment of cells to a specific pathway leading to a single differentiated cell type. Trichomes are not essential for viability, and mutations affecting trichome development have been isolated. Recessive mutations in two genes, *GL1* and *TTG*, block the initiation of trichomes. All alleles of *GL1*, including a null allele in which the entire gene is deleted, affect only trichome development. The *GL1* gene has been cloned by T-DNA tagging, and DNA sequence analysis showed that the *GL1* product is a member of the Myb class of transcriptional regulators. *In situ* hybridization to RNA revealed that *GL1* transcripts were present in leaf primordia, developing trichomes, and stipules. In contrast, previous work had shown that putative promoter sequences from the 5' noncoding region of the *GL1* gene directed the expression of a β-galacturonase reporter gene (GUS) only in stipules. We have identified an enhancer located downstream of the *GL1* coding region that is required for all *GL1* function. Sequences from the region containing the enhancer extend the range of expression directed by *GL1* 5' sequences to include expression in leaf primordia and developing trichomes. This demonstrates that the downstream enhancer is required to produce the normal expression pattern of *GL1*.

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SELECTION OF A MUTANT OF Arabidopsis thaliana IMPAIRED IN THE SYNTHESIS OF CELLULOSE.

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A mutant was selected from mutagenized M2 seedlings in which the cell walls of both stem and leaf trichomes totally lacked birefringence (BR) when viewed by polarized light microscopy. BR of leaf and root primary xylem was also considerably reduced compared to wild type. In plant cell walls, such birefringence is usually indicative of highly-ordered secondary wall cellulose. This homozygous recessive mutant has been designated tbr (trichome birefringence). General trichome shape and number is similar in both wild-type (TBR) and tbr; however, leaf trichomes of tbr are somewhat swollen at the base and have an altered surface structure as viewed by SEM. When leaves are heated in acetic nitric reagent, a treatment which solubilizes most polymers except cellulose, most trichomes of TBR remain on the leaf and retain their shape and BR; however, tbr trichomes are rapidly released from the leaves, and most retain their shape, but are extremely fragile compared to those of TBR. Other microscopic studies also suggest that tbr may be impaired in the synthesis of secondary wall cellulose in specific cell types including trichomes and primary xylem. Concomitantly, there appears to be enhanced synthesis of cellulose in the cortex cells of the root. Methylation analyses and incorporation studies with ^{14}C-glucose into the cellulosic fraction also support the conclusions drawn from microscopy. In tbr, root growth is reduced and root hairs and some root tips are swollen, and the mutant is about 4 times more sensitive to growth on the cellulose-synthesis inhibitor DCB compared to TBR.

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Characterization and Analysis of *cer4* and *cer21* Wax Mutants

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Plant waxes are known to be of great importance in the interactions between plants and insects. Both the structure and the chemical composition of the wax are believed to be involved in these interactions. We are interested in genetically engineering plants to possess specific waxes in order to enhance a plant's resistance to insect pests. Our attention is focused on two T-DNA tagged wax mutants, *cer4* and *cer21*. Biochemical wax analysis shows a reduction in primary alcohols and in free aldehydes for *cer4* and *cer21*, respectively. We hope to clarify and expand the understanding of how these genes function in the wax biosynthesis pathway and in insect resistance. Data we are collecting on diamondback moth oviposition suggest that there are trends of differences between the wax mutants. We are constructing and will biochemically analyze the wax of double mutants made with the other T-DNA tagged cer mutants. We will also examine the leaf and stem surfaces using electron microscopy. The surfaces of these plants at the microscopic level are elaborate in structural detail, including plates and tubes which may contribute to the plant's resistance to insect pests. We will be working toward the cloning of the *cer4* and *cer21* genes. We will also be looking for homology to these two genes. This project's long term goal is genetic engineering of agronomically important plants with wax genes to enhance a plant's resistance to insect pests.

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Mutants Resistant to an Herbicide that Affects Root Growth. Sandra H. Russell, Daniel F. Delduco, and Pablo A. Scolnik. DuPont, Science and Engineering Laboratories, Wilmington, DE, USA 19880-0402.

Cinmethylin is an herbicide that affects the growth and development of meristems. Exposure of *Arabidopsis* seedlings to cinmethylin results in a decrease of root growth and an increase in lateral root formation. Shoot growth is largely unaffected. At high concentrations of cinmethylin, the shoot meristem is affected and callus-like nodules form on the short root. We have isolated 63 mutants that show resistance to cinmethylin, as assayed by root growth. Dominant, semi-dominant, and recessive mutations have been found. The recessive mutants belong to at least four complementation groups. Although most mutants exhibit a fairly normal phenotype, five co-dominant resistant mutants exhibit bleaching of the shoot when exposed to cinmethylin. This bleaching only occurs in those plants homozygous for the resistance mutation. Results from phenotypic mapping suggest that the resistance mutation of three mutants is located on chromosome 1. RAPD mapping will be discussed.

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A clonal analysis of the Arabidopsis root meristem

The structure and anatomy of the Arabidopsis root have been described in detail (Dolan et al., Development in Press). Such a histological analysis allows predictions to be made as to the probable number and fates of initial cells of the root meristem. Such relationships can be unequivocally determined with clonal analysis. We have utilized a system developed by Emily Lawson and Caroline Dean at the Cambridge Laboratory of the Institute of Plant Science Research, for the analysis of cell lineages in the Arabidopsis root. Our preliminary studies support a number of the predications made as a result of the histological analysis; cells of the lateral root-cap and epidermis are derived from common initials; cells of the central, columella root cap are derived from a unique set of initials that generally give rise to no other tissue type. These and other preliminary data will be presented.

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ROOT DEVELOPMENT IN ARABIDOPSIS

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The limited anatomical complexity of the root in Arabidopsis is exploited to investigate the formation and functioning of meristematic cells. Formative divisions giving rise to the typical root structure originate during heart stage embryogenesis and perpetuate well into the seedling stage. Complementarty to anatomical analyses we performed fate mapping analysis using a GUS marker gene interrupted by an Ac transposon (E. Lawson & C. Dean, John Innes Inst., Norwich, U.K.) to map regions of the seedling root and hypocotyl to cell tiers in the heart stage embryo. Both sets of data will be compared and their relevance discussed.

In order to investigate the genetic basis for root meristem development 10,000 separate F1 families were screened to allow recovery of seedling-lethal mutations affecting root development. We are currently analysing a collection of mutants perturbed in root meristem formation or functioning. The effect of a number of these mutations on embryo and seedling development will be presented.

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Dwarf mutants of *Arabidopsis thaliana*

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Sixteen dwarf mutants mapping to seven loci have been isolated from a population of 13,000 T-DNA transformants of *A. thaliana.* These mutants were originally selected as possessing short, robust stems and short dark-green leaves under light-grown conditions. For five loci, the cosegregation of the kanamycin resistance maker of the T-DNA and the mutant phenotype has been shown. Mutants that map to *dwf2*, *dwf3* and *dwf4* have a light-regulatory phenotype when grown in the dark, i.e., mutants have a short hypocotyl and open and expanded cotyledons. When grown in the light, these plants exhibit a reduction in cell size and a prolonged life span, similar to the other dwarf mutants. Light-grown plants of the dwf1 locus resemble the phenotype of *det2* and *dwf4*. But *dwf1* does not show features of a light regulatory phenotype when grown in the dark. The dwarf mutant, *twd1*, has a different phenotype. Organs of green and nongreen parts of the plant exhibit a twisted growth pattern. The development of the affected organs is otherwise normal. For several of the tagged dwarfs, DNA sequences flanking the inserted T-DNA were cloned via plasmid rescue and analyzed further. Results of the sequence analysis and expression studies of the genes will be presented. In addition, a detailed morphological analyses of dwarf mutants at each of the loci will be presented.

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PROGRESS TOWARD CLONING THE CER2 GENE OF *ARABIDOPSIS*.

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The Epicuticular wax layer in higher plants is composed of a series of long-chained hydrocarbons such as fatty acids, aldehydes, primary and secondary alcohols, ketones, alkanes and esters. Classical genetic analyses of *Arabidopsis eceriferum* (CER) mutants revealed the involvement of 22 different loci in the epicuticular wax biosynthesis in this plant (McNevin *et al.*, 1993 *Genome,* in press.). Phytochemical studies of the epicuticular wax components from the T-DNA tagged cer2 mutant, suggests the possible funtion of CER2 to be an elongase catalyzing the elongation of octacosanic acid to triacontanoic acid (Hannoufa *et al.*, 1993 *Phytochemistry,* in press). As the initial step in the cloning of the CER2 gene, genomic libraries were constructed in λGEM12 using the XhoI partial fillin strategy. Plant genomic DNA was isolated from two different T-DNA tagged cer2 mutants for the production of these libraries. The libraries were screened with probes correponding to subclones of the left and right borders of the T-DNA element used in tagging. Positive clones were further analyzed by Southern blotting to identify plant DNA flanking the T-DNA borders. Partial sequence data did not reveal any homologies to known sequences. Plant DNA/T-DNA clones were used as probes to screen a genomic λGem11 library of wild-type *Arabidopsis* as well as cDNA libraries. The clones isolated in these screens are being analyzed by Southern blotting, DNA sequencing and northern blotting. This work was supported by a grant from NSERC to B. Lemieux.

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The *REVOLUTA* Gene Affects Leaf And Inflorescence Development

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An EMS mutagenesis in ecotype No-0 recovered two alleles of a new gene, *REVOLUTA (REV)*, affecting the development of leaves and inflorescences. Rosette leaves of *rev* mutants initially appear fairly normal but become increasingly long and distorted over time. Cauline leaves are longer and thinner than normal and tend to curve and twist. Lateral branches generally fail to develop in the cauline leaf axils, and most plants have only a single inflorescence stalk. The inflorescence consists of a few normal flowers and a larger number of sterile flowers typically lacking pistils and stamens, and bearing a varying number of petals and bract-like sepals. Small bristle-like structures bearing both branched and unbranched trichomes are interspersed with the peduncles on the inflorescence stem.

The phenotype is modified in the Landsberg *erecta* ecotype. Normal fertile flowers form on an unusually long inflorescence stalk. The lack of branching and the curved cauline leaves remain unchanged. In *ap1; rev* double mutants, the lack of branching is epistatic to the reiterative branching characteristic of *ap1*.

The Rev^- phenotype segregates as a single recessive mutation. Preliminary mapping data place *REV* on chromosome 5.

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Genetic Analysis of Heteroblasty in *Arabidopsis*

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Arabidopsis undergoes heteroblastic development in that leaves with different traits are produced at different positions along the shoot axis. For example, the first rosette leaves are small and round, while later rosette leaves are larger, more elongated, and develop a slightly serrated edge. In addition to their differences in overall size and shape, rosette leaves and bracts display stereotypical differences in the distribution of trichomes on their upper and lower surfaces and margins. We are characterizing heteroblastic development in *Arabidopsis* and isolating mutants in which the normal changes in leaf traits are accelerated or delayed. We have identified mutations that fall into several phenotypic classes based on their affects on trichome distribution. One class, represented by several mutations in an undetermined number of genes, causes precocious changes in trichome distribution on the rosette leaves. A second class, represented by two mutations in a single gene, affects the distribution of trichomes on both rosette leaves and bracts. We will describe the genetic and phenotypic analysis of these and additional mutants.

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

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

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

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

To analyze the *bumbershoot* mutant we are mapping the locus with RFLP markers, utililizing light microscopy and SEM to determine the cellular organization of the meristemic cells, and generating double mutants between *bum* and other vegetative and inflorescence meristematic mutants, including *tfl1*, *leafy*, *elf3*, and *clavata1*.

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GENETIC CHARACTERIZATION OF ECERIFERUM (CER) MUTANTS OF ARABIDOPSIS

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After screening 14,000 transformant generated by the seed co-cultivation method, we have found 17 *Arabidopsis* mutants with defects in the production of epicuticular wax on the plant surface (i.e., *cer* mutants). In 9 of the 17 families studied to date, the mutant phenotype appears to have been caused by the insertion of a T-DNA element within a gene which regulates wax biosynthesis. Thirteen of the families were found to have a single Kan^R gene while four have two unlinked T-DNA elements. Southern blot analysis of EcoRI and HindIII digests with a T-DNA right border specific probe (H23) indicate that none of the lines have silent T-DNA elements (i.e. Kan^S T-DNA). Although allelism testing is still in progress, 12 of the lines have been assigned to known eceriferum loci while one has been shown not to belong to any previously characterized locus. All of the mutants isolated thus far correspond to phenotypic groups I and II as defined by Koornneef et al (1989, J. Hered. 80, 122). Our phytochemical analyses of the *cer* mutant wax layers relative to that of wild-type plants has enabled us to assign putative biochemical functions to some of the mutant alleles. As the epicuticular wax layer is the plant's first line of defense against insects, we feel that *cer* genes will be useful in developing insect resistance in crop plants. This work was supported by a grant from NSERC to B. Lemieux.

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ISOLATION OF RNase MUTANTS OF *ARABIDOPSIS THALIANA*.

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In eukaryotic systems, the ribonucleases that mediate mRNA decay have not been differentiated from those with other roles in RNA metabolism. Genetic approaches have been particularly useful in addressing the function of individual RNases in bacteria, and we have begun to apply similar approaches to *Arabidopsis*. Previous studies have shown that *Arabidopsis* stems and leaves contain a complex profile of RNases, some of which accumulate differentially (Yen and Green, *Plant Physiol.* 97: 1487-1493). To further our understanding of eukaryotic RNases, we have undertaken a screen to identify *Arabidopsis* plants containing mutations that affect their RNase profile. 2500 EMS-mutagenized M2 *Arabidopsis thaliana* plants (ecotype RLD) were screened for mutations in stem RNase activities using a substrate-based gel assay. The assay consists of running crude stem extract under denaturing conditions through a gel cast with yeast RNA. After removing the denaturant, gels are incubated to allow digestion of the RNA in the gel by the RNases. Finally, the gels are stained for RNA. In the initial screen, 112 plants showed aberrant RNase profiles, 60 of which proved to be reproducible on subsequent gels. Seed from the 60 plants with altered RNase profiles was grown to test for stable inheritance of the phenotype. We have thus far obtained nine mutants that breed true and are attempting to confirm three other candidates. Of the nine mutants, six lack a band of RNase activity from the profile and three increase the activity of one or more RNase bands. Progress towards the genetic characterization of the RNase mutants will also be discussed.

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Molecular and biochemical characterization of a protein kinase from *Arabidopsis thaliana* which phosphorylates serine, threonine and tyrosine

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Phosphorylation of proteins serves as an important regulatory mechanism for many cellular processes. In animals protein kinases have been divided into two classes, namely serine/threonine and tyrosine kinases. The protein tyrosine kinases in animals consist of membrane spanning receptor kinases, such as the EGF receptor and membrane associated cytosolic kinases such as src. Further more cytosolic kinases like wee1 have been described in yeast. Protein serine/threonine kinases are mainly found in the cytoplasm, examples of which are Raf-1, ribosomal S6 kinase and protein kinase C.
In plants the protein kinases described upto date fall all into the class of serine/threonine kinases. Although homolgues of molecules, which in animals have been shown to be phosphorylated on tyrosine, such as cdc2 and MAP kinase have been described in plants, there is yet no evidence for tyrosine kinases in plants.
Here we provide evidence for a kinase which can phosphorylate serine/threonine and tyrosine, isolated from Arabidopsis. The screening strategy and the molecular and biochemical characterization of this kinase will be described. Furthermore we present data about substrates of this kinase and discuss the role it may play in the development of Arabidopsis.

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Constitutive and Meristem Specific Expression of Different Actin Genes in *A. thaliana*

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Plant actin is encoded by a multigene family made up of several ancient and diverse classes. The actin based cytoskeleton has numerous diverse functions, many of which are carried out in unique cell types or at specific developmental stages. We have proposed that these roles are played by different classes of actin genes. Ten *arabidopsis* actin genes have been cloned and characterized in our laboratory, and nine of these are expressed. To study the expression pattern of each functional actin gene, we have coupled the 5' flanking region for each actin gene, including a relatively large leader intron and a portion of the coding region, to a GUS reporter gene. All constructs have been transformed into *Arabidopsis* and numerous transgenic plants were regenerated. Initial GUS histochemical assays showed that phylogenetically close actin genes have similar expression patterns and yet expression of each actin gene is unique and complex.

ACT2 and *ACT1* are representatives of two phylogenetically distant classes of actin genes. *ACT2* is highly and constitutively expressed in transgenic plants, including root, young leaf, stem and parts of flower. Its constitutive expression suggests that it may be involved in relatively universal functions in plant cells, such as cytoplasmic streaming and cell elongation. *ACT1* is expressed at high levels in mature pollen and at lower levels in vascular tissues and shoot and root apical meristems. Its product may be involved in cell division and pollen tube growth. *ACT8* is from the same subclass as *ACT2* and has a similar expression pattern. It shares several conserved sequences in their 5' flanking regions. *ACT1* and *ACT3* belong to the same subclass and also have a related expression pattern. The regulatory functions of the conserved sequences and more detailed data about expression of the actin genes from the two classes will be discussed.

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FUNCTIONAL ANALYSIS OF *ARABIDOPSIS* ACYL CARRIER PROTEIN (ACP) GENE PROMOTER ACTIVATION

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The acyl carrier protein (ACP) plays a pivotal role in the *de novo* synthesis of fatty acids, which occurs primarily within plastids. During fatty acid biosynthesis, nascent chains are covalently bound to a phosphopantetheine prosthetic group attached at serine 38 of the protein. Two linked genes, A1 and A2, coding for nearly identical isoforms of ACP had previously been isolated from an *A.thaliana* (*columbia*) genomic library and sequenced (Lamppa and Jacks, 1991. Plant Mol. Biol. 16:469). A chimeric gene comprised of approximately 1.0 kb of the A1 gene 5' flanking sequence fused to the coding region of ß-glucuronidase (GUS) was transformed into tobacco. Flourometric analysis showed that the ACP gene promoter was most active in developing seeds. Expression was also high in roots, but significantly lower in young leaves and downregulated upon their maturation. Histochemical analyses revealed a complex spatio-temporal expression pattern reflective of both the developmental status and growth rate of individual tissues. Promoter deletion analyses have identified at least three regions which may contain cis-acting elements involved in the transcriptional regulation of the ACP A1 gene, two of which function primarily within roots and one which is active in both root and leaf tissues. Experiments are underway to identify regions of the A1 promoter that form specific DNA/protein complexes in vitro. This study is being extended to a comparison of the activities of the two different *Arabidopsis* ACP gene promoters in transgenic *Arabidopsis* plants.

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Characterization of the diverse organ-specific poly(A)-binding protein genes from *Arabidopsis thaliana*.
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Abstract

Poly(A)-binding protein (PABP) is a ubiquitous component of post-transcriptional machinery in eukaryotic cells. In yeast PABP is required for poly(A) tail shortening by the poly(A) nuclease and translational initiation. Hybridization screening of *Arabidopsis thaliana* genomic and cDNA libraries with a PABP specific PCR probe generated from genomic DNA led to the isolation of three diverse genes encoding PABPs, *PAB1*, *PAB3* and *PAB5*. All three sequences contain the expected four RNA recognition motifs. Sequence diversity between these genes equals or exceeds the diversity among animal and fungal sequences. Our initial work suggests that the *Arabidopsis* genome contains numerous additional PABP-related sequences. All three genes isolated show organ-specific patterns of expression. *PAB5* and *PAB3* RNAs were detected only in floral organs, with the highest level of expression in immature flowers. Fusions of the *PAB5* 5' sequences with the b-glucuronidase (GUS) reporter were transformed into *Arabidopsis* cells and plants regenerated. GUS expression in transgenic plants is primarily in immature flowers and pedestals. *PAB1* RNA was observed predominantly in roots, more weakly in immature flowers and not in any other organ examined (stems, leaves, mature flowers, siliques). These data suggests a potentially unique role for PABPs in organ-specific post-transcriptional regulation in plants. Several experiments examined the basic molecular functions of *PAB5*. In vitro synthesized PAB5 protein bound to poly(A)-Sepharose with higher specificity for poly(A) than other oligo-ribonucleotides. When *PAB5* is expressed from a inducible yeast promoter it can suppress the loss of the essential yeast PABP gene and restore near normal growth rates. The plant gene also can be shown to control poly(A) tail length, polysome integrity and presumably translational re-initiation in yeast. Future work will focus on the molecular biology of PABP function in higher plants.

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An *Arabidopsis* Mutant with a Reduced Level of *cab140* mRNA is a Result of Co-Suppression.

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We have isolated an EMS-induced mutant of *Arabidopsis* with a severely reduced level of the *cab140* message. We have named this mutant *lct* for low cab140 transcript. The low *cab140* mRNA level could not be separated genetically from a T-DNA insert in the genome of this line, which contained 1.4 kb of the *cab140* promoter fused to the *iaaH* gene from *Agrobacterium tumefaciens*. Expression of the introduced *cab140::iaaH* gene was also greatly reduced, indicating that our line is an example of co-suppression. *In vitro* nuclear transcription experiments demonstrated that suppression of both the endogenous *cab140* and the introduced *cab140::iaaH* genes was occurring at the level of transcription. We also found that the suppressed *cab140* genes were not significantly more methylated than the non-suppressed *cab140* genes in the parent line. The T-DNA insert in *lct* did not contain any gross rearrangements, thus we propose that a point mutation in the T-DNA of *lct* is in some way promoting co-suppression.

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Structure and expression differences between ubiquitin genes from different *Arabidopsis thaliana* ecotypes.

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Ubiquitin (ub) is a highly conserved 76-aa eukaryotic protein that covalently attaches to cellular proteins. This post-translational modification often targets a protein for proteolysis, although stable ubiquitinated proteins have been described. Ub is encoded by a multi-gene family consisting of three types; polyubgenes, ub-like genes, and ub fusion genes. We have isolated all of the ub hybridizing genomic sequences from *Arabidiopsis thaliana* Columbia ecotype (Callis et al,. JBC 265:12486-12493; Sun and Callis, The Plant Cell 5:97-107; Callis et al., unpublished). Both the ub-like genes (5 members) and the polyubiquitin genes (5 members) encode fusion proteins of ub as head to tail repeats, but the ub-like genes encode proteins with aa substitutions from the plant ub sequence as determined by direct aa sequencing. We are analyzing the structure and expression of these genes. We have evidence that the number of ub coding regions per gene (repeat number) changes rapidly. Using PCR, we have identified that there are repeat number differences between homologous genes in different ecotypes. *UBQ13* contains 5 ub repeats in Columbia ecotype, but 3 in Be-0 ecotype. One polyub gene, *UBQ11,* active in both Columbia and Landsberg erecta ecotypes, contains 3 repeats in Columbia, but additional repeats in Landsberg erecta. While there is no evidence that the ub-like genes are expressed, 5 polyub genes express mRNA in Columbia. Two of these do not appear to be active in Landsberg erecta.

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Isolation and differential expression of proline-rich cell wall protein genes in *Arabidopsis*. Thomas J. Fowler and Mary L. Tierney. Department of Agronomy and Biotechnology Center, Ohio State University

Diversity is a key feature of the extracellular matrices of plant cell walls. Although sometimes perceived as static and homogeneous, plant cell wall matrices are dynamic and complex structures that contribute to the functional differences between cell types. The proline-rich protein (PRP) class of cell wall proteins is one of several classes of structural proteins that contribute to the variety of cell wall matrices. In several plant species, PRPs are encoded by small gene families in which family members are expressed differentially during the plant life cycle. In addition, the expression of some gene family members can be altered by environmental stimuli. The general features of PRPs include a repetitive, proline- and hydroxyproline-rich amino acid sequence that contains little or no glycosylation. The PRP family can be divided according to protein structural arrangements. Some PRPs are composed entirely of repetitive proline-rich sequences, while others contain short unique regions of amino acid sequence interspersed among the repetitive regions. A third group includes PRPs with a nonrepetitive protein domain that is not proline-rich linked to a repetitive proline-rich domain. The significance of these protein structural arrangements in intermolecular interactions of PRPs is not known at this time. Using PRP gene probes from carrot and soybean to screen an *Arabidopsis* genomic library, we have isolated clones containing four PRP genes. Each gene is present as a single copy per haploid genome and they are designated *AtPRP1*, *AtPRP2*, *AtPRP3* and *AtPRP4*. The AtPRPs separate into two pairs when the derived amino acid sequences are compared. AtPRP1 and AtPRP3 are constructed of similar proline-rich motifs arranged in a single domain. AtPRP2 and AtPRP4 have proline-rich motifs in common with each other, but they differ somewhat from those of AtPRP1 and AtPRP3. In addition, AtPRP2 and AtPRP4 have a different domain structure than previously described PRPs, consisting of a nonproline-rich nonrepetitive N-terminal domain and a proline-rich, repetitive C-terminal domain. Northern hybridization with specific probes for each of the AtPRPs show that these genes are differentially expressed during plant development and that each organ of maturing *Arabidopsis* plants (root, stem, leaf and flower) expresses at least one of the AtPRP genes.

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A CHLOROPLAST LOCALIZED RNA-BINDING PROTEIN: GENE EXPRESSION AND FUNCTION.

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A cDNA was isolated from an *Arabidopsis* expression library based on the ability of the encoded protein to bind ssDNA, a characteristic of RNA-binding proteins (RBPs). The cDNA, *Atrbp31,* encodes a protein that is similar to several nuclear-encoded, chloroplast localized RBPs from tobacco and spinach. All of the chloroplast localized RBPs including ATRBP31 share a common domain organization, with an amino terminal chloroplast transit peptide followed by an acidic domain and two conserved RNA binding domains at the carboxyl terminus. Our hypothesis is that the ATRBP31 protein is a nuclear-encoded chloroplast regulatory protein.

Chloroplast import assays have shown that the ATRBP31 protein is translocated to the chloroplast. The *Atrbp31* gene is of further interest because it encodes multiple mRNAs that are developmentally regulated and induced by light. Primer extension and RNase protection assays demonstrated that there are at least three transcription start sites. Interestingly, the two shorter mRNAs encode a peptide that only contains one RNA-binding domain and part of another. In young leaves, all three transcription start sites are utilized and are inducible by light. In more mature leaves, however, only two of the three start sites are significantly active, producing the longest and shortest mRNAs. Further work is underway to determine the functions of ATRBP31, and to analyze the mode of *Atrbp31* gene regulation.

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Identification of mutations that affect pollen-specific gene expression.

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Our research is directed toward understanding the regulatory circuits that control differential gene expression. Several pollen-specific genes from tomato were isolated and the *cis* sequences that control their gene expression were characterized[1]. We have taken a genetic approach to identify *trans*-acting factors that mediate the pollen specific expression of one of these genes, LAT59 (for *l*ate *a*nther *t*omato). Transgenic *Arabidopsis* were constructed that contain a gene consisting of the LAT59 promoter fused to the coding sequence of β-glucuronidase. This chimeric gene is specifically expressed in pollen in *Arabidopsis*. M_1 plants with a mutation in a gene encoding a factor that acts on the LAT59 promoter would be identifiable as ones with flowers having 50% white pollen when stained with X-gluc. Homozygous transgenic seeds were mutagenized by fast neutron bombardment. 2500 M_1 plants were screened and seven putative mutant plants were identified. Three of these plants segregated M_2 progeny with flowers containing approximately 50% white pollen. One M_1 plant segregated M_2 progeny with flowers containing 100% white pollen. We are currently characterizing these mutants.

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SEQUENCES THAT CONTROL THE EXPRESSION OF THE *ARABIDOPSIS SAUR-AC1* GENE IN TRANSGENIC PLANTS. Pedro Gil, Yang Liu and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing MI 48824-1312

Small auxin up RNA (SAUR) genes were first characterized in soybean where they comprise a small gene family. During gravitropism, there is a rapid redistribution of the SAUR transcripts in soybean hypocotyls, which is presumably mediated at least in part by auxin. The kinetics of this redistribution indicate that SAUR messages are very unstable, offering a good system to study the processes that govern mRNA degradation in higher plants. As a first step, we have cloned and characterized the *SAUR-AC1* gene of *Arabidopsis*. This gene is highly similar to the soybean SAUR genes and is also auxin inducible. To better understand the control of *SAUR-AC1* expression, we have fused the *SAUR-AC1* promoter to the β-glucuronidase gene (GUS) and introduced this construct into tobacco and *Arabidopsis* plants. We have found that the *SAUR-AC1* promoter is auxin-inducible and most active in epidermis and adjacent layers of the cortex as well as in vascular tissues both in leaves and stems. In addition, we have observed that *SAUR-AC1* mRNA levels are very low when *SAUR-AC1* mRNA synthesis is driven by the strong CaMV 35S promoter in transgenic tobacco. This indicates that sequences located downstream the transcription start site limit transcript accumulation, perhaps by causing mRNA stability. Moreover, in the *SAUR-AC1* 3' untranslated region a DST sequence exists which is very similar to the DST elements conserved among soybean SAUR genes (McClure *et al.*, 1989, Plant Cell 1:229-239). Recently, Newman *et al.*(Plant Cell, in press) have demonstrated that DST sequences destabilize reporter transcripts in tobacco . It has also been observed that a number of unstable transcripts in mammalian cells are stabilized by treatment with the protein synthesis inhibitor cycloheximide. A similar situation may exist in *Arabidopsis* because we have found that the *SAUR-AC1* mRNA also accumulates to high levels following cycloheximide treatment even when expressed under the control of the 35S promoter. The next step will be to investigate the role of transcribed sequences in the control of *SAUR-AC1* and their possible involvement in rapid mRNA decay.

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ISOLATION OF AN *ARABIDOPSIS* GENE INVOLVED IN ACETYLCHOLINE METABOLISM

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Acetylcholine (ACh) is an important neurotransmitter in animal systems. Although ACh has been detected in the tissues of several plant species (1), its exact function in plants is unknown. However, a number of studies have suggested that ACh can regulate the permeability of plant membranes to ions (2). In animal neurons, the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) hydrolyzes ACh to acetic acid and choline. A recent study demonstrated AChE activity in guard cell protoplasts isolated from *Vicia faba* L. (3). Furthermore, a comparison of cholinesterase activities in various *Vicia faba* leaf tissue fractions showed that guard cell protoplast extracts had an 8-fold greater specific activity than mesophyll cell protoplast extracts. Because of this intriguing result, we are interested in determining if an ACh signaling system is involved in regulating the ion channels in the guard cells surrounding the stomatal pores of the leaf epidermis. To initiate our molecular studies, we used a heterologous AChE probe from the electric ray, *Torpedo californica*, in a Southern analysis of *Arabidopsis* genomic DNA at low stringency. The cDNA probe, a 2.3 kb fragment including the entire coding sequence of the *Torpedo* AChE gene, was hybridized in 50% formamide at 35°C to a blot of *Arabidopsis* genomic DNA digested with Hind III. The blot was washed at 60°C for 1.5 hours in 2x SSC and 0.1% SDS. The AChE probe hybridized to four genomic fragments with sizes of 5.0 kb, 3.5 kb, 1.4 kb, and 1.2 kb. To identify an mRNA, we have isolated total *Arabidopsis* RNA and are in the process of probing it under low stringency conditions with RNA synthesized from the *Torpedo* AChE clone. We have also designed degenerate oligonucleotide primers that correspond to conserved regions of peptide sequence specified by seven previously cloned AChE genes. We are attempting to use these primers in various combinations to clone segments of an AChE cDNA from *Arabidopsis* by PCR amplification. We plan to use the *Torpedo* clone and any promising PCR products as probes to screen a cDNA library in order to isolate and characterize a full length *Arabidopsis* AChE clone.

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THE ACTIN BINDING PROTEIN, PROFILIN, IS ENCODED BY A DIVERSE MULTIGENE FAMILY IN *ARABIDOPSIS THALIANA*. Shurong Huang, Glenn Johns, Rudolf Valenta* and Richard B. Meagher. Department of Genetics, University of Georgia, Athens, GA. 30602. *Institute of General and Experimental Pathology, University of Vienna, Vienna, Austria.

Profilin is a major component of the cytoskeletal system found in all eukayotes. Profilin monomers bind to G-actin, affecting the equilibrium between G-actin and F-actin and controlling the phosphorylation state of the ADP/ATP nucleotide bound to each actin monomer. Profilin itself is regulated through the phosphoinositol signal transduction pathway. Recently plant profilins have been shown to be major allergens in patients with serious pollen and food allergies. Plant profilin binds a significant portion of the IgE in these allergic patients. we propose that the plant profilins, like the actins, are encoded by an ancient and divergent gene family within any single plant species and that sequence variation among the divergent profilin family members may account for some of the differences in the reactivity of IgE to profilin among allergic patients. The profilin gene family will be characterized in the model plant, *Arabidopsis thaliana*. Profilin cDNAs have been isolated from an *Arabidopsis* floral library. Comparison of this and other distant plant profilin sequences suggests that plant, fungal, protist, and lower animal sequences are more similar to each other than any of this group are to the vertebrate sequences. This has interesting implications for the evolution of the vertebrate IgE response to lower animal parasites and the relation of this response to fungal and plant allergies.

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DIFFERENTIAL EXPRESSION OF ACT4 AND ACT11 ACTIN GENES IN *ARABIDOPSIS THALIANA*. Shurong Huang, Yong-Qiang An, Elizabeth C. McKinney, John M. McDowell and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, Georgia, 30602.

Higher plants contain a highly divergent actin gene family composed of several ancient classes of genes. They may have arisen concomitant with the evolution of the diverse cell types present in vascular plants. We have proposed that these ancient classes have been preserved throughout vascular plant evolution because they have unique patterns of gene regulation and/or encode actin proteins with unique functions. We are studying the evolution and function of the actin gene family in the model plant *Arabidopsis thaliana*. Ten diverse actin genes have been isolated and completely sequenced. A detailed molecular analysis classifies these genes into several conserved subclasses. Our first goal to test our hypothesis is to analyze the expression patterns of each Arabidopsis actin gene. An RT-PCR survey in different plant organs indicates that many of these actin genes are differentially expressed. A detailed study of tissue specific expression of these actin genes is in progress using a GUS reporter fused to the actin 5' regions. Preliminary data indicates that gene members from different subclasses have quite unique expression patterns. In flower, ACT11 is expressed in pollen, stigma, and petals while ACT4, which belongs to a different class than ACT11, is exclusively expressed in pollen. In roots, ACT4 is strongly expressed in meristem, elongation zone and lateral root primordia, while ACT11 is expressed in elongation zone and lateral root primordia but not in meristem. Northern analysis is being carried out to determine the steady levels of the various actin mRNAs. The absolute expression levels of several representative actin genes will be also quantified from pollen and other tissue/cell types using quantitative RT-PCR methods.

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ISOLATION OF PROMOTERS REGULATED DURING FRUIT DEVLOPMENT.

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A partial cDNA library has been constructed from fruit at an early stage of fruit development of kiwifruit (*Actinidia deliciosa*). Fruit 8-10 days after anthesis (DAA) have high levels of cell division while later stages of fruit development are characterised by cell enlargement, rather than cell division. Using differential screening with leaf and 8-10 DAA cDNA, followed by Northern analysis we have identified 5 clones that are expressed during fruit development. Two clones have been selected for further analysis: pKIWI 501, which is highly expressed at 8-10 DAA, and pKIWI 503 which shows a gradual increase of expression during fruit development. These cDNAs were used to screen a genomic library from *A. chinensis*, a close diploid relative of kiwifruit. The 5' upstream regions of the genomic clones will be fused to GUS and transformed into kiwifruit, *Arabidopsis* and tomato. Due to the time required for kiwifruit to produce fruit (2-4 years), *Arabidopsis* and tomato are being used for rapid assessment of developmental regulation of the kiwifruit promoters in heterologous systems.

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Circadian Transcription from the Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco) Activase Promoter of *Arabidopsis thaliana*

Zong R. Liu. and C. Robertson McClung, Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

Transcription from the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase (*RCA*) promoter of *Arabidopsis* is regulated by the circadian clock. To identify promoter regions required for this circadian regulation, as well as for light responsiveness and tissue specifity, we have generated gene fusions in which progressively truncated *RCA* promoter elements are fused with reporter genes encoding ß-glucuronidase (GUS), chloramphenicol acetyltransferase (CAT) and firefly luciferase (*Luc*). Analysis of these gene fusions in transgenic tobacco and *Arabidopsis* shows that a 317 bp region immediately proximal to the transcription initiation site is mainly responsible for *RCA* expression. We have defined two *cis* elements within this region; one is involved in positive regulation and the other is involved in negative regulation. The definition of *cis* element(s) involved in circadian regulation is in progress.

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Genes Encoding Glycine-Rich *Arabidopsis thaliana* Proteins with RNA-Binding Motifs are Influenced by Cold Treatment and an Endogenous Circadian Rhythm

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We have identified two members of a class of *Arabidopsis thaliana* putative RNA-binding proteins (CCR1 and CCR2). The deduced amino acid sequence of the CCR1 protein revealed the presence of two domains; an amino-terminal domain with extensive similarity to an RNA-binding motif and a carboxy-terminal domain rich in glycine residues. *Ccr1* and *Ccr2* mRNAs levels were influenced by a plant circadian rhythm and stress. Transcript levels were maximal at 6:00 PM and minimal at 10:00 AM. CCR1 protein levels also fluctuated in response to the circadian rhythm with a peak at 10:00 PM and a low point at 2:00 PM. Levels of *Ccr1* and *Ccr2* mRNAs were unchanged in wounded plants, increased at least fourfold in cold-stressed plants and decreased two to three -fold in abscisic acid-treated plants. *Ccr1* transcript levels were unchanged by drought, while *Ccr2* transcript levels increased under the same conditions. Intriguingly, during dark incubation of the plants, both *Ccr1* and *Ccr2* probes hybridized to RNA species several hundred bases larger than their normal (and expected) transcript size. Furthermore, one *Ccr2* cDNA contained several hundred base pairs of intervening sequence. Preliminary experiments using PCR primers which flank the intron in *Ccr1* indicate that there are *Ccr1* RNA species present in dark-grown plants which are absent from light-grown plants. Taken together, these results suggest that differential splicing of *Ccr* transcripts may be mediated by a dark (or absence of light) factor. The mRNA accumulation patterns of these *Arabidopsis* genes differed from conditions that affect the expression of similar genes from maize, sorghum and carrot.

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GENE EXPRESSION IN *ARABIDOPSIS THALIANA* ECOTYPE COLUMBIA SUSPENSION CULTURES DERIVED FROM VARIOUS PLANT ORGANS

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Suspension cultures from root, leaf, and stem explants were established to monitor gene expression in these systems as compared to their respective plant organs in intact plants. Northern blots prepared from total RNA isolated from each suspension culture as well as from its corresponding intact plant tissue were probed with the following genes: alcohol dehydrogenase (Adh), GF14, actin, and a nuclear-encoded chloroplast targeted gene (ATP Cg.emb1). Preliminary data demonstrate that Adh, GF14, and actin transcripts were highly expressed in all suspension cultures whereas ATP Cg. emb1 was repressed. RNA transcript levels from these suspension cultures were equivalent regardless of their plant origin derivation in sharp contrast to the tissue specific expression demonstrated with RNA transcripts from intact plants. A parallel study done with western blots of proteins isolated from the suspension cultures as well as from various plant organs focused on anti GF14 monoclonal antibody as a probe. Anti-GF 14 western blots confirmed the northern blot data shown with the GF 14 probe.

Methods will be presented with this poster.

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Do homeobox-containing genes regulate development in plants?

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Many of the key regulatory genes in animal development share a conserved sequence element, the homeobox. To test whether homeobox-containing genes are present in plants and possibly play similar roles in plant development we used a degenerate oligonucleotide probe, designed against the most conserved region of the *Drosophila* Antennapedia class of homeoboxes to screen cDNA libraries. By this approach, we isolated two clones, one each from rosette stage *Arabidopsis* plants and somatic embryos of carrot. Subsequently, by using the first *Arabidopsis* homeobox as a probe, we isolated clones corresponding to five different *Arabidopsis* genes. The clones identify a novel class of homeodomain containing transcription factors, distinct from the Antennapedia class. In addition to the homeodomain they also contains a leucine zipper which is a potential dimerization motif.

In northern blots, one gene is specifically expressed in the leaf whereas two other genes are expressed in all organs, although at different levels.In *in-situ* hybridizations, a 4:th clone hybridizes to a transcript expressed in the mature cortex cells of stem and root whereas expression can not be detected in young cortex cells close to the shoot and root apexes. Since the expression of these genes are mainly confined to mature tissues and organs they are unlikely to function in the basic control of organ differentiation.

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Differential Regulation and Evolution of Diverse *Arabidopsis* Actin Genes *ACT5* and *ACT7*.
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Plant actins have been implicated in a variety of functional roles in "housekeeping" processes, cellular morphogenesis, and environmental responses. Plant actins are encoded by an ancient family of diverse genes. We have proposed that ancient plant actin subclasses were established during the early evolution of vascular plants because they fulfilled unique functional roles or provided the capability to modulate actin expression in response to developmental or environmental stimuli. We are undertaking a comprehensive analysis of the expression patterns and functional roles within a model plant actin gene family. 10 *Arabidopsis* actin genes have been isolated and completely sequenced. A quantitative study of the molecular evolution of these and other plant actin gene sequences has confirmed the ancient character of plant actin gene families and has revealed several conserved subclasses. These results will be presented.

To test the second part of our hypothesis, we are currently analyzing the expression patterns of each *Arabidopsis* actin gene. Transgenic plants which contain translational fusions of *Arabidopsis* actin 5' flanking regions with the B-Glucuronidase gene have been created. Preliminary data indicates that the *Arabidopsis* actin 5' flanking regions often direct differential expression with respect to tissue or cell type and developmental stage. For example, the *ACT7*/Gus fusion is expressed at a moderate level in the vascular cylinder and in the tip of seedling roots. In mature roots, Gus appears to be expressed at high levels in all cell types. In leaves, Gus is detected at much higher levels in trichomes than in any other cell type. This construct also displays a wound response. The *ACT5* 5' region may control even more specialized patterns of expression. Gus expression is observed exclusively in hydathodes. The biological relevance of these expression patterns will be explored with gene-specific, inducible antisense RNA suppression.

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REGULATORY MECHANISMS OF THE TISSUE-SPECIFIC EXPRESSION OF GENES FOR PHOTOSYNTHESIS IN *ARABIDOPSIS THALIANA*

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We examined the regulatory mechanisms of tissue-specific expression of genes for photosynthesis in *A. thaliana* as a model plant. We have focused on the expression of nuclear genes for small subunit of ribulose 1,5-bisphosphate carboxylase/ oxygenase (RuBisCO, *RbcS*) and chlorophyll a/b-binding proteins (*Cab*, presently designated *Lhc*), and on a plastid gene for large subunit of RuBisCO (*rbcL*) in either photosynthetic tissues such as light-grown leaves or nongreen ones including roots and calli. We examined steady state transcript levels by RNA-DNA hybridization and primer extension, transcriptional activity by run-on assays, transcriptional template activity of DNA by *in vitro* run-off transcription for *rbcL*, and gene copy numbers by DNA-DNA hybridization. The expression of *rbcL* gene is regulated both at DNA replication and transcription. DNA fragments covering promoter regions of *RbcS* and *Cab* were fused to a reporter gene encoding β-glucuronidase (GUS) and the resultant chimeric constructs were introduced into leaves and roots of *A. thaliana* with a pneumatic particle gun. The results suggest that the tissue-specific expression of the nuclear genes is ascribed mainly to transcription step,and RNA stability may additionally contribute to the tissue-specific transcript levels.

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CHARACTERIZATION OF GLUTATHIONE S-TRANSFERASE FROM *ARABIDOPSIS THALIANA*

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Glutathione S-transferases (GSTs) are involved in cellular detoxification by conjugating GSH to a variety of electrophilic compounds. We are interested in two aspects of GST enzymes; first, how the expression of GST mRNAs is regulated and second, what roles these enzymes play in plant growth. We have isolated a GST cDNA (GST2) from *Arabidopsis* and shown that the expression of this gene is regulated by ethylene. Expression of this cDNA in *E. coli* yields a 25 Kd protein, as predicted from the cDNA sequence. This protein can be purified by GSH-affinity chromatography and has GST activity using chloro-2,4-dinitrobenzene (CDNB) as substrate. Two closely related genomic sequences have been isolated. One encodes the GST2 mRNA while the other contains a pseudogene. The transcriptional start site of GST2 mRNA has been mapped by primer extension. Several 5' deletions of the promoter region from the active gene have been fused to the β-glucuronidase reporter gene and used to transform *Arabidopsis*. Analysis of these transformants will identify cis-acting elements involved in regulating GST2 expression in response to ethylene and other stimuli, and also reveal the spatial pattern of expression of this gene. In both 7-day old seedlings and mature plants, GST2 mRNA is induced by salicylic acid, wounding, and NAA. Salicylic acid induces GST2 mRNA in the ethylene insensitive mutant *etr1*. Sevral lines of evidence indicate that *Arabidopsis* contains a multigene family encoding GSTs. We have isolated GSTs from ethylene-treated tissue by a two step process using ion-exchange chromatography followed by GSH-affinity chromatography. This protein preparation exhibited high GST activity using CDNB as substrate. This fraction was resolved into four major and two minor spots by 2-D gel electrophoresis. Knowledge of the diversity of this gene family and the physiological and environmental conditions that modulate the expression of these genes will be important in elucidating the functions of these enzymes in plants.

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Differential expression of genes induced by low-temperature, drought and ABA in *Arabidopsis thaliana*

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Exposure of *Arabidopsis thaliana* to low temperature or drought, leads to alterations in gene expression and to several physiological changes. These changes include an elevation of the concentration of endogenous abscisic acid (ABA) and an increase in freezing tolerance. We have studied the expression of six genes from *A. thaliana* which are induced by low temperature and drought, as well as by exogenous ABA. Two of the proteins encoded by these genes show identity to each other, but not to any other known proteins. The remaining four genes encode proteins which contain amino acid repeats found in several RAB/LEA/DEHYDRIN proteins of other species.
Although all of the genes are induced by low temperature, drought and ABA, the genes exhibit a differential response to these stimuli. Studies with ABA mutants of *A.thaliana* have revealed that the genes also differ in regard to whether ABA is needed for the low-temperature- and/or drought-induced expression. To investigate the reason for this differential expression we are currently analyzing the promoters of the genes by making fusions of the promoter regions to the coding region of the β-glucuronidase gene.

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CIRCADIAN REGULATION OF FERREDOXIN MRNA ACCUMULATION IN *ARABIDOPSIS THALIANA*.

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Ferredoxin (*FED*A) mRNA abundance oscillates with approximately 24 hour periodicity in adult plants grown in a 14:10 light-dark (LD) photoperiod. This oscillation persists in plants transferred from LD to continuous dark (DD) and continuous light (LL) and, hence, constitutes a true circadian rhythm. In all three light conditions the mRNA abundance peaks shortly (1-3 hours) after dawn or subjective dawn. Etiolated seedlings do not show any circadian oscillation in *FED*A mRNA abundance. However, seedlings etiolated for seven days and then transferred to continuous white light showed circadian oscillations in mRNA abundance within 24 hours of the transfer. This demonstrates that the circadian oscillator functions in the absence of an entraining light-dark cycle, but may not be present in dark grown seedlings or those that have not undergone a dark to light transition. Transgenic plants containing *FED*A sequences fused to the β-glucuronidase (GUS) and luciferase (LUC) reporter genes are being used to investigate the sequences responsible for the circadian regulation and to determine if there is a transcriptional component to the circadian control of *FED*A mRNA accumulation.

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Trans-kingdom conservation of regulatory elements controlling RNA polymerase I transcription in *Arabidopsis* and animals.

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In higher eukaryotes, RNA polymerase I transcription is controlled by DNA elements located within the intergenic spacers separating the tandem ribosomal RNA genes. Unlike rRNA coding sequences, the intergenic spacers evolve rapidly and have little sequence similarity among related species. Nonetheless, in animals the arrangement of functional elements, such as spacer promoters and repetitive enhancers, is highly conserved. Little biochemical analysis of ribosomal gene transcription in plants has been conducted, though the intergenic spacer sequences of many plant ribosomal genes have been reported. In my (C.S.P.) lab, we have identified spacer promoters in Arabidopsis, thereby demonstrating their existence in both the plant and animal kingdoms. We have also developed an Arabidopsis transient expression system to perform the first transcriptional analysis of a plant ribosomal gene promoter. Spacer promoters share sequence similarity with the gene promoter from -91 to +22 relative to the transcription start site, +1. Deletion analysis shows that sequences required for RNA polymerase I transcription reside within these boundaries and that plant ribosomal RNA gene promoters are small compared to other higher eukaryotes. Intergenic spacer sequences have only a small positive effect on transcription in transfected Arabidopsis protoplasts but can increase transcription from a Xenopus ribosomal gene promoter in injected frog oocytes. This trans-kingdom enhancer effect further suggests that the functional elements within eukaryotic ribosomal genes are highly conserved. As in animals, plant ribosomal gene promoter recognition is highly species-specific, meaning that the promoter from one species will generally not be recognized by the transcriptional machinery of another species. This is presumably due to the rapid evolution of the RNA polymerase I system. We are currently defining the minimal promoter sequences and interacting proteins responsible for species-specific RNA polymerase I transcription.

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Cytosine Deaminase as a Negative Selectable Marker for Arabidopsis

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Cytosine deamianse (CD), produced by prokaryotes but not by higher eukaryotes including plants, deaminate cytosine to uracil. The enzyme likewise converts 5-fluorocytosine (5FC), which by itself is not toxic, to 5-fluorouracil (5FU), which is toxic. The Escherichia coli *codA* coding sequence encoding CD, together with appropriate regulatory elements, was introduced into Arabidopsis. Neither untransformed controls, nor transgenic plants expressing no CD mRNA, were sensitive to 5FC. Conversely, for most transgenic plants expressing CD mRNA, in the presence of 5FC calli and seedlings failed to proliferate, and seeds failed to germinate. A few transgenic plants with many *codA* copies expressed less CD mRNA and remained insensitive to to 5FC, which likely reflected epigenetic repeat-induced gene silencing. Thus 5FC, presumably through conversion by the enzyme to 5FU, can be used to select against plants that express CD.

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Characterization of cDNA- and genomic clones encoding a sequence specific DNA-binding protein containing a homeodomain.

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The transcriptional activation of several specific genes seems to be a key step in the early induced plant response to pathogen attack. To elucidate the underlying regulatory mechanisms, one of these genes, the PR2-gene from parsley (Petroselinum crispum) was further analyzed. Promoter deletion analysis and gel retardation experiments revealed an 11bp DNA motif, which seems to play an important role in gene activation.
Interestingly this DNA sequence motif forms very similar protein-DNA complexes when tested either with parsley or Arabidopsis thaliana nuclear extracts in gel retardations experiments. To isolate cDNAs encoding sequence specific DNA-binding proteins, cDNA expression libraries from parsley and Arabidopsis were constructed and screened by the Southwestern method, using the 11bp DNA motif as probe.
Besides one full-length clone from parsley several clones from Arabidopsis were isolated, all of which appear to be derived from the same gene. The deduced amino acid sequence of the longest, near full-length, clone (2,9 kb) revealed it to be a homeodomain protein, containing also a putative leucine zipper. As a first step in further analyzing the regulation and function of the gene, its genomic structure was defined.

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Accumulation and Localization of TCH3 and TCH4 Proteins in *Arabidopsis*

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TCH3 is a calmodulin-related gene whose expression is increased in response to stimuli such as touch, wind, and temperature shifts. Antibodies have been made against a portion of the TCH3 protein and have been shown to be specific for TCH3. These antibodies have been used in immunoblotting experiments in plants and cultured cells to characterize the accumulation of TCH3 after different stimuli. The antibodies have also been used in immunodetection experiments to localize TCH3 in seedling shoots. Our experiments show that 1.5 hours after touch stimulation, TCH3 is most abundant in the conductive tissue. In addition, TCH3 is detected in the shoot apical meristem and in the leaf primordia. Currently, antibodies are being made against the coding region of *TCH4*, a *meri-5*-related gene, whose expression is also induced by a variety of stimuli. The patterns of accumulation and localization of these proteins will likely shed light on their roles in the molecular responses of plants to varied environmental stimuli. (Supported by NSF grant MCB-9118661 and NASA grant NAGW-3139)

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Characterization of an *Arabidopsis thaliana* Homologue of the Transcription Factor *c-myb*. Diane Ruezinsky[1], Susan A. Hedrick[2], Christopher J. Lamb[2], Richard A. Dixon[1], and Peter W. Doerner[2]. [1]Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, 2510 Highway 199 East, Ardmore, Oklahoma 73402; [2]Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037.

Members of the *c-myb* family of proto-oncogenes have been shown to encode transcriptional activators in diverse species. We have isolated a *c-myb* homologue, Atmyb4, from *Arabidopsis thaliana*. The amino terminal region of the deduced peptide sequence of Atmyb4 shares extensive sequence identity with *myb* genes isolated from several plant species. The expression pattern of the Atmyb4 gene was analyzed by RNA blot and *in situ* hybridization as well as X-gluc staining of transgenic *Arabidopsis* containing an Atmyb4 promoter-GUS fusion. Atmyb4 is expressed in the epidermis and vascular cells of floral organs, cauline leaves and petioles as well as in pollen and trichomes. Expression of the Atmyb4 promoter-GUS fusion is being analyzed in a number of genetic backgrounds. To further assess the function of this gene in *Arabidopsis* development, constructs designed to interfere with Atmyb4 expression or function have been introduced into the *Arabidopsis* genome by *Agrobacterium*-mediated transformation. Transgenic plants containing antisense, overexpression or dominant negative constructs are currently being analyzed for both morphological and biochemical phenotypes.

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Identification of anther-expressed genes in *Arabiopsis thaliana* by in vivo gene fusion with a promoterless reporter gene

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The genes that are expressed in a tissue- or cell-type specific manner are important tools for studying developmental gene regulation. We are isolating tissue- or cell-type specific genes from *Arabidopsis thaliana* by using a binary vector in which a promoterless GUS gene is located directly after the right border sequences. By assaying expression of ~100 transgenic *Arabidopsis* plants, we have identified 3 plants that show GUS staining pattern in anther tissues. In addition, expression of GUS gene were detected in tissues of roots, stem and leaf.

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Intercellular Communication in *Arabidopsis*: Characterization of Σ3, a Receptor Protein Kinase Member of the S-locus Superfamily

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With the aim to apply a molecular approach to intercellular communication in plants we have characterized various members of the S-locus superfamily from *Arabidopsis*. The canonical gene of this group was isolated from *Brassica* and is thought to play a role in the self-incompatibility response during the fertilization process (Nasrallah et al. 1985). The family consists of secreted proteins as well as receptor protein kinases which all share in common the extracellular or so-called S-domain (Walker and Zhang 1990; Dzelzkalns et al. 1992). Σ3 encodes a putative receptor protein kinase and is most closely related to ARK1 (Tobias et al. 1992). Sequence comparison of the S-domain of Σ3 and the various other S-domain sequences supports the view of the S-domain being structured as three sub-domains which we call domain I, II and C-rich domain, with domain I at the N-terminus of the protein. Whether this subdivision reflects a functional compartmentalization remains to be shown. Theoretical considerations make direct homophilic interactions between receptors on neighbouring cells highly unlikely. However, the existence of alternatively spliced transcripts, corresponding to the S-domain alone, suggests that the secreted S-domain could act as a signal. Hence, homophilic interactions might indeed take place. Alternatively, modulation of Σ3 receptor activity might also be a possibility. The expression pattern of Σ3 is compatible with a potential role in growth control during the development of leaves and sepals. Strategies to investigate the biological function of Σ3 are discussed.

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CLONING OF A RIBONUCLEOPROTEIN GENE FROM *ARABIDOPSIS* .

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We have isolated a cDNA clone encoding an RNA-binding protein in *Arabidopsis*. The clone, termed *RNP-D*, was isolated by screening an *Arabidopsis* expression library for the binding of ssDNA. This clone was used for re-screening of a second cDNA library for a full length clone. We have isolated a full length *RNP-T* clone, whose 5' nucleotide sequence differ from that of *RNP-D*. The remaining nucleotide sequences of these two clones, on the other hand, were identical. Thus, the protein products of these clones differ in their amino termini: RNP-D has seven repeats of a histidine-proline containing hexamer, whereas RNP-T has a serine rich sequence. Carboxyterminal to these sequences, both proteins have an acidic motif which includes a repetitive hexamer. The acidic domain is arranged in a potential amphipathic α-helix. Both proteins contain in their carboxyterminous two adjacent RNP-80 motifs, conserved putative RNA binding domains identified in vertebrates, flies, yeast and plants. Proteins containing this motif are suggested to play an important role in RNA processing. Each RNP-80 motif includes RNP1, a highly conserved amino acid octamer and RNP2 a less conserved hexamer, which are separated by 33 amino acids. The 310 amino acids protein encoded by the *RNP-D* clone efficiently binds RNA, ssDNA, but much less dsDNA. In addition, binding is affected by the nucleotide composition. The structure and the potential role of these proteins will be discussed.

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The Calmodulin-Related *TCH3* Gene of *Arabidopsis*: Molecular Characterization, Evolution, and Expression Patterns

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TCH3 is a novel calmodulin-related gene that encodes a protein with six potential Ca^{2+}-binding domains. The expression of this gene is dramatically induced by stimuli such as touch, wind, and temperature shifts (Braam and Davis, 1990). We have isolated and sequenced a cDNA encoding the entire protein and the corresponding genomic DNA region. The transcription unit is approximately 1390 bp, with three introns, and the deduced amino acid sequence is 324 residues. From this molecular analysis, we propose an unusual and recent evolution of the *TCH3* gene. To begin to determine the mechanism(s) of regulation of expression and the *cis*-regulatory elements of *TCH3*, we have constructed transcriptional and translational fusions to the *gusA* gene and have generated transgenic *Arabidopsis* plants. The tissue-specific GUS staining patterns resemble the localization of the TCH3 protein, as determined by immunodetection (see abstract by Purugganan, Antosiewicz, Polisensky, and Braam). (supported by NSF grant MCB-9118661 and NIH grant R296M46346)

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Organ-level regulation of a light-labile and light-stable phytochrome using promoter-GUS fusions in Arabidopsis seedlings. David E. Somers and Peter H. Quail, UC-Berkeley Plant Biology/Plant Gene Expression Center, Albany CA 94710

The cloning and sequencing of three phytochrome cDNA's from Arabidopsis (1) has allowed us subsequently to isolate the respective gene promoters and investigate their spatial and temporal expression and regulation. Through the use of promoter-GUS fusions, we have begun to describe the pattern and level of expression of a light labile (*phyA*) and light-stable (*phyB*) phytochrome in seedlings.

Preliminary results from both histochemically stained seedlings and quantitative flourometry have shown that, relative to dark-grown plants, the activity of the *phyA* promoter in the hypocotyl is more strongly suppressed in white light than is the activity of the *phyB* promoter. These light-related changes in the expression of phytochrome genes may be mediated directly through a photoreceptor via a short transduction chain (e.g. (2)). However, since the morphology and degree of organ development differs greatly between light and dark grown seedlings of comparable age, it is also possible that the degree of phytochrome gene expression in a particular organ is the result of light-induced changes in seedling development. In the latter case, the pattern and level of expression is a consequence of the stage or degree of organ development, and would not be subject to direct regulation by a light-mediated switch (e.g.phytochrome). In order to distinguish between these two possibilities, the organ-level expression of the phytochrome promoter-GUS transgenes in relevant mutant backgrounds and under different irradiation schedules and qualities are being examined. Current results will be presented.

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ISOLATION OF PLANT G1 CYCLINS BY COMPLEMENTATION IN YEAST

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Cyclins are proteins required in the cell cycle at two major check-points; the G1/S transition and G2/M transition. More than 50 cyclins have been discovered so far and they fall into different groups according to their structural and functional homology. Cyclins get their name from their cyclical appearance in the cell cycle. The G1 cyclins need to be destroyed for the cells to go through mitosis and similarly destruction of mitotic cyclins enables the cells to prepare themselves for another round trip of the cell cycle. G1 cyclins are required at the G1/S transition at a point known as START, where they associate with the p34 protein kinase, thereby committing the cells to go through a whole round of cell cycle in yeast. We believe that a similar activity might be present in plants. We have screened an Arabidopsis cDNA library construated in a yeast expression vector for clones that rescue a yeast strain deficient in G1 cyclins. 14 clones have been obtained which fall into 4 classes on the basis of restiction pattern and hybridization. Some of the clones also rescue the swi4-ts mutant , which is defective in the trancription factor SWI4, normally required for G1 cyclin expression. Two of the clones J1 and J3, which rescue both swi4 ts and cln mutations, have been further characterized by sequence analysis. Both of them encode a protein of approx. 40 KD and show homology to the Human D-type cyclins in the database. They are clearly distinguishable from the B-type cyclins and other plant cyclins discovered so far, and thus define a class of their own. Further experiments are underway to examine the expression of these cyclins at various stages of the cell cycle.

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BINDING OF HIGH MOBILITY GROUP (HMG) PROTEINS TO AT-RICH SEQUENCES OF THE *ARABIDOPSIS* CAM-3 PROMOTER

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CaM-3 is one of six genes encoding calmodulin in *Arabidopsis*. It is expressed at low levels in all of the organs examined except roots. A 300-bp region (EH300) of the CaM-3 promoter is 80% A+T. Similar A+T-rich sequences have been shown to function as nuclear scaffold attachment sites, enhancers, and binding sites for HMG proteins in other genes. In this study, the binding of crude wheat HMGs and purified HMGa to EH300 was examined as a possible component to its constitutive expression. HMGs are low molecular weight, highly charged chromosomal proteins. They have been shown to be associated with transcriptionally "poised" or "active" regions of chromatin. Competitor screens with an array of polynucleotides and the minor groove binding drug Distamycin A showed that HMG binding is dependent on both A-T sequence and P-P distance across the minor groove. DNase I footprints of purified HMGa binding to EH300 identified ten binding sites. The existence of six contiguous A or T base pairs was sufficient for binding, whereas four of five A-Ts were recognized in some cases. This is identical to the binding characteristics of mammalian HMG I. Several members of the HMG family participate in the A/T dependent binding activity, and some constituent of the HMG family inhibits *in vitro* transcription of the *Adenovirus* major late promoter. Supported by NSF MCB 9205702.

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A Putative Silencer From Tomato Induces Variegation On Cis-Linked Genes In Transgenic *Arabidopsis* And *Nicotiana*.

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Our goal is to understand the role of chromatin organization in gene regulation. We study *HSC80A*, a gene of tomato preferentially and abundantly expressed in apices. *GUS* genes driven by the *HSC80A* or a heterologous promoter were combined with *HSC80A* upstream elements and introduced into *Arabidopsis* and *Nicotiana*. We observed the *GUS* and *NPT* expression of at least ten segregating T2 families per gene construct.

We find that an upstream element from the *HSC80A* promoter region functions as a global silencer, inactivating expression from both the *HSC80A* promoter and from nearby heterologous genes, even when positioned several kbs to the 3' of a gene. Inactivation was epigenetic, being manifested as either complete inactivation or variegation, and it affected most transgenic events. *GUS* staining of variegating plants revealed positive patches and sectors of varying size, some as small as a few cells, in the tissues where the reporter was expected to express. *NPT* variegation was demonstrated by abnormally low numbers of kanamycin resistant seedlings and by patches of bleaching tissues in otherwise resistant seedlings. The silencer was mapped within 1 kb 5' of the *HSC80A* transcription start. The silencer action is evidently influenced by the chromosomal environment of T-DNA insertion since 10 to 20% of transformation events showed strong constitutive and ectopic expression of *GUS* and no inactivation.

In conclusion, we have identified a novel regulatory element which is a very good candidate for a chromatin organizer. The study of this element may provide insights on how plants organize their chromatin.

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ISOLATION AND CHARACTERIZATION OF ARABIDOPSIS CLONES ENCODING PUTATIVE TRANSCRIPTION FACTORS INVOLVED IN DEFENSE GENE ACTIVATION.

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Part of the early induced defense response observed in plants following pathogen attack is the rapid transcriptional activation of specific genes. Numerous such genes have been cloned and partially characterized both from parsley (*Petroselinum crispum*) and from *Arabidopsis thaliana*. We are particularly interested in elucidating the regulatory mechanisms controlling the expression of these genes.

Recently, one *cis*-acting DNA sequence element (Box P), present in the parsley phenylalanine ammonia-lyase (PAL) 1 promoter, was successfully used as probe in a DNA-ligand binding assay, to screen an expression library. This resulted in the isolation of clones encoding Box P binding factors (BPF) [da Costa e Silva et al., Plant Journal, in press]. Box P elements are also present within the promoters of other pathogen/elicitor and/or light regulated plant genes, including the PAL promoter from Arabidopsis. Therefore the BPF cDNA clone was also used as probe to isolate related cDNAs out of an Arabidopsis library. Seven clones were isolated that shared 45% overall amino acid sequence identity to the parsley BPF cDNA. Within the 100 amino acid stretch believed to be important for DNA binding, 85% of the positions remained absolutely conserved.

Another functionally important, but sequence unrelated, 11 bp DNA motif has been identified within the parsley pathogenesis-related (PR) 2 promoter. Gel retardation experiments showed that this motif forms nearly identical protein-DNA complexes with nuclear protein extracts derived from parsley or from Arabidopsis cells. Southwestern screening of both a parsley and an Arabidopsis expression cDNA library resulted in the isolation of clones. Comparison of the deduced polypeptides encoded by these clones will be discussed.

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Promoter analysis of the *A. thaliana Lhb1B* locus.

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We have recently cloned two additional genes from *A. thaliana* encoding Photosystem II Type I CAB proteins. These genes, called *Lhb1B1* and *Lhb1B2* using the nomeclature proposed by Jansson *et al* (Plant Mol Biol Rep 10: 242-253), are tandemly linked in inverted orientation and are separated by 1.3 kb. We have found by Northern analysis that accumulation of the*Lhb1B1* transcript in etiolated *A.thaliana* seedlings is more sensitive to blue and far red light than that of the *Lhb1B2* transcript. To identify sequences conferring this differential sensitivity we made two types of constructs. In the first we fused the complete untranslated leader and varying amounts of upstream sequence from each gene to the firefly luciferase cDNA. In the second we fused various sequences from each gene to a minimal promoter (derived from a construct obtained indirectly from Dr. V. Walbot) consisting of the Δ89 derivative of the CaMV 35S promoter fused to the TMV Ω sequence followed by the second intron of the potato ST-LS1 gene then the luciferase cDNA. We then assayed these constructs by transient expression of luciferase activity after biolistic delivery into tobacco. For these studies we used both intact plants grown in magenta boxes and cut leaves placed on petri dishes. Both materials were grown on 1/2 MS without sugar and were kept in the dark for at least four days before they were shot. Following biolistic delivery (under dim green safelight) we returned some materials to the dark and placed others under continuous light. With the first type of construct we found that deletion of the *Lhb1B1* promoter to about -450 had little effect on both amounts of expression and its light regulation, although we only observed about 4 fold differences between materials kept in light and dark. Further deletion progressively lowered both overall expression and light regulation, although even the deletion retaining only the CCAAT box still showed 2 1/2 fold higher activity in materials kept in the light. By contrast, the TATA box derivative retained a good deal of activity, but showed little light regulation. We observed similar trends with the *Lhb1B2* promoter, although our analysis is not yet as complete. Surprisingly, the second type of construct shows more light regulation. The minimal promoter gives low activity in plants kept in either light or dark, and constructs with either *Lhb1B1* or *Lhb1B2* promoter sequences fused to it show little activity in materials kept in the dark after delivery, but over 20 fold greater activity in materials stored in the light. We are presently using this approach to identify regulatory sequences within these promoters; and we are transforming plants with various elements both in the context of their own promoter and fused to the Δ89 derivative to test the validity of this approach to identify elements involved in light regulation.

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DEVELOPMENT OF DESICCATION TOLERANCE IN MUTANT SEEDS OF *ARABIDOPSIS THALIANA*: A STUDY OF GENE- AND PROTEIN EXPRESSION

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Acquisition of desiccation tolerance is part of the normal seed development. The phytohormone abscisic acid (ABA) plays an important role in the development and maturation of seeds. Seeds of the ABA-deficient and -insensitive double mutant (aba,abi3-1) of *Arabidopsis thaliana* fail to desiccate at the normal stage of development and lose viability upon drying. However, in double mutant seeds desiccation tolerance can be induced, either in vivo by watering the motherplants with the ABA analog LAB 173 711 or in vitro, by treating isolated immature seeds (around the 12 dap stage of development) with ABA in combination with sucrose prior to drying. The artificial induction of desiccation tolerance in these naturally desiccation intolerant seeds offers a challenging system to study genes and proteins, which might be important for desiccation tolerance. First results of Northern- and Western analysis will be presented.

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Cloning, sequencing and analysis of the phenylalanine-ammonia lyase gene family in *Arabidopsis*.

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Phenylalanine ammonia lyase (PAL) catalyzes the first step in the biosynthesis of phenylpropanoids, and is encoded by three genes in *Arabidopsis thaliana*. Genomic PAL clones containing the entire coding region and additional upstream and downstream sequences have been obtained and completely sequenced. Two of the *Arabidopsis* PAL genes (PAL1 and PAL2) are structurally very similar to PAL genes from other plant species, with a shorter less highly conserved first exon, a single intron at a similar position, and a long highly conserved second exon. Two of three previously identified promoter motifs plus several additional sequence motifs are found in the promoter regions of *Arabidopsis* PAL1 and PAL2. In contrast, *Arabidopsis* PAL3 contains an additional unique intron with concensus splice sites in the "second" exon, and its promoter region lacks the sequence motifs present in PAL1 and PAL2. Gene-specific probes were constructed and used to compare mRNA levels in different organs and in response to several bacterial pathogens. Expression of PAL1 and PAL2 is both qualitatively and quantitatively similar, and is transiently induced several-fold in response to incompatible bacteria as part of a presumed defense response. No mRNA from PAL3 has been detected by Northern blot analysis or by primer extension.

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Transcription of ribosomal genes of *Arabidopsis thaliana*

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Ribosomal genes are present in approx. 600 copies of a 10-12 kb repeat in the genome of *A. thaliana*. Transcription of these repeats is tightly linked to the growth stage of the cell.

In order to study the promoter function of the ribosomal intergenic region (IGR), we have constructed a tagged version of rDNA in which the large (25S) rRNA gene contains a short insert at a permissive position. The insertion should allow us to distinguish this rDNA copy from other rDNA genes and make it possible to use this copy as a reporter gene to probe the *in vivo* promoter function of the intergenic region.

Various deleted versions of the IGR will be tested for promoter activity.

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Regulation of *cor15a* and *cor15b* cold regulated genes of *Arabidopsis thaliana*

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cor15a and *cor15b* are two cold-regulated genes of *Arabidopsis* which have high sequence identity and exist in the genome as a tandem gene pair separated by less than 1kb. Northern analysis using gene specific oligonucleotides has shown that the two genes are differentially regulated. *cor15b* is not induced by drought and has a low constitutive level of expression not found in *cor15a*, but both are induced by cold and exogenously applied ABA. *cor15a*, which appeared to be transcriptionally regulated in nuclear run-on assays (1), was chosen for further regulatory study. Promoter deletions were done using GUS as a reporter gene. A construct containing promoter sequences between -305 and +75 was sufficient to confer cold, drought and ABA inducibility upon GUS, while a construct containing sequences between -129 and +75 was not sufficient for this regulation. Thus, the 180bp fragment between -305 and -129 may impart cold, drought and ABA inducibility. Two potential ABRE's and a repeated sequence are contained within this region. Further characterization of this 180bp fragment and of motifs within it is currently in progress.

Plants containing one of the deletion constructs, in which GUS was inducible by cold, were also used to begin a mutational analysis to look for factors which directly or indirectly influence cold regulation of the *cor15a* promoter. One mutant that has come through a second generation of screening has constitutive phenotypic expression of the reporter gene. However, tissue prints have not been able to conclusively show an increase in mRNA of either the endogenous or the reporter gene under warm conditions. Further characterization of the constitutive mutant and rescreening of other putative mutants are in progress.

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Interaction between light and circadian clock in the regulation of catalase gene expression in *Arabidopsis thaliana*

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The expression of a number of plant genes is regulated by an endogenous circadian clock. We report a circadian oscillation in mRNA abundance of *CAT2* (catalase) gene in *Arabidopsis* transferred from LD to continuous light (LL) and continuous dark (DD). The oscillation in *CAT2* mRNA abundance persists for at least 5 continuous cycles in LL, demonstrating the robustness of the circadian oscillator. In light-grown seedlings, *CAT2* mRNA accumulates to high levels in early germination, associated with the degradation of H2O2 produced during the ß-oxidation of fatty acids. A second peak was observed later in development, associated with the development of photosynthetic competence. Although the germination associated peak in *CAT2* mRNA was also observed in etiolated seedlings, *CAT2* mRNA abundance failed to accumulate to a second peak. However, increased *CAT2* mRNA was detected within 30 min of transfer of etiolated seedlings into white light. Thus, light is not required for *CAT2* expression during early germination, but is required for later expression. We further describe the role of light in the initiation of the circadian rhythm in *CAT2* mRNA accumulation. No circadian oscillation in *CAT2* mRNA was detected in etiolated seedlings. However, following transfer of etiolated seedlings to continuous white light, robust oscillations in *CAT2* mRNA abundance were observed. Thus, *Arabidopsis* seedlings that have never been exposed to an environmented LD cycle of 24 h period are competent to express a circadian rhythm in *CAT2* mRNA abundance.

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***Ac/Ds* transposon mutagenesis in *Arabidopsis thaliana*: Mutant spectrum and frequency of *Ds* insertion mutants.**

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Using a two component *Ac/Ds* system consisting of a stabilized *Ac*-element (Ac_{cl}) and a nonautonomous element (Ds_A), more than 650 families of plants carrying independent germinal Ds_A excisions/transpositions were isolated. Progenies of 559 of these Ac_{cl}/Ds_A families together with 43 families of plants selected for excision/transposition of wt*Ac* were subjected to a broad screening program for mutants exhibiting visible alterations. So far mutants were identified in 48 of the 602 families analyzed showing a wide variety of mutant phenotypes like embryo lethality (24 mutants), chlorophyll defects (5 mutants), defective seedlings (2 mutants), reduced fertility (5 mutants), reduced size (3 mutants), altered leaf morphology (2 mutants), dark green, unexpanded rosette leaves "cabbage" (3 mutants), aberrant flower or shoot morphology (4 mutants). To test whether these mutants were due to transposon insertions, a series of Southern blot experiments was performed on 34 mutants comparing in each case several mutant plants with others showing the wild-type phenotype. A common new Ds_A fragment in all mutant plants that segregated or was absent in the wild-type plants, as expected for Ds_A insertion mutations, was observed in four of the 34 mutants analyzed. These four mutants included two with embryo lethality, one with dark green unexpanded rosette leaves "cabbage", and one with curly growth of stems, leaves and siliques.

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Arabidopsis Genetic Resources: The Nottingham *Arabidopsis* Stock Centre

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The Nottingham *Arabidopsis* Stock Centre maintains over a thousand unique lines of *Arabidopsis* which include hormone, flowering, biochemical, colour, form mutants, visible mutants from T-DNA transformed lines and ecotypes. We also stock uncharacterised pools of material from 4900 T-DNA transformed lines, which can be used to screen for new mutants. For mapping purposes we stock multiple marker lines and two sets of recombinant inbred lines.

NASC is part of an *Arabidopsis* Resource Centre network with an European DNA Stock Centre at the Max-Delbruck-Laboratorium, Köln, Germany and a combined DNA and Seed Stock Centre (*Arabidopsis* Biological Resource Centre [ABRC]), at Ohio State University, USA. All *Arabidopsis* accessions are stored at NASC and ABRC. ABRC distributes seed to the Americas and Canada and NASC serves the rest of the world.

Two American Databases, AAtDB ("an *Arabidopsis thaliana* database") and AIMS (*Arabidopsis* Information Management System) have been established for the development and administration of international *Arabidopsis* resources. NASC is the strain curator for AAtDB and maintains the latest version of the database. We are happy to access AAtDB to answer any queries. As part of our collaboration with ABRC we are also feeding all our stock information into AIMS.

There will be an opportunity to meet us and see the diverse array of the stocks that we carry at the poster display.

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USE OF BACTERIAL ARTIFICIAL CHROMOSOME SYSTEMS (pOF VECTOR) FOR THE CONSTRUCTION OF *A. thaliana* MAXICLONE LIBRARIES

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Bacterial artificial chromosome (BAC) systems have been recently developed for cloning large DNA inserts as an alternative to yeast artificial chromosomes (YACs). BAC vectors (such as pOF216) derive from the *E. coli* single-copy plasmid F factor. They can maintain large DNA inserts; replication in *E. coli* is strictly controlled and recombinant plasmids are found in low copy number (this decreases the possibility of recombination between inserted DNA fragments). Compared to YACs, the BAC system offers many advantages for the construction of comprehensive libraries of higher organisms: 1) BACs exist as supercoiled circular plasmids which can be easier manipulated than linear yeast chromosomes; 2) they can be reintroduced into *E. coli* cells by electroporation; 3) the frequency of chimeric clones seems to be significantly lower than in YACs.

We have established conditions for the construction of BACs containing plant DNA inserts of *A. thaliana* and *D. carota* and we are currently analysing the critical parameters in order to obtain inserts larger than 50 kbp.

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Isolation of transposon tagged mutants in Arabidopsis using modified *Ac* and *Ac/Ds* elements of maize.

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We have used modified transposons based on the maize *Ac/Ds* elements to generate insertional mutants in Arabidopsis. A modified autonomous element {Δ*Nae*I *Ac*} and a two component system with a marked nonautonomous *Ds* element {*Ds::hyg*} and a stabilised transposase source {sΔ*Nae*I *Ac*} have been used.

A transposon mutagenesis experiment with the autonomous element was set up with four different transformants, each with a single mapped T-DNA. Germinal excision events {representing 800 independent events} of *Ac* were selected and selfed, and their progeny were screened on soil and on synthetic medium. Atleast 8 different mutants were identified from 400 such families, these included an inflorescence mutant, a *hy* mutant (hypocotyl length), an albino, a fusca, seed pigmentation mutants and other morphological mutants. Two of these mutants were unstable and produced revertants. Plant DNA flanking transposed *Ac* has been cloned from these mutants and molecular analysis of these mutants will be presented.

Families from ~250 independent *Ds* insertions have also been screened. Three tagged mutations have been identified *drl1*, *wlc1* and *pcm1* and progress on analysis of the gene products will be presented.

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IN SITU HISTOCHEMICAL ASSAY ON PLASTIC EMBEDDED *ARABIDOPSIS* EXPLANTS. STUDY ON THE REGULATION OF THE *pPFL1:gus* GENE.

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The T-DNA tagged mutation **pfl1** affects first leaf shape, growth rate, and total plant fresh weight. The PFL1 gene activity was assigned, by complementation, to a 3.8 Kb subclone. Sequence comparisons showed homology to ribosomal protein genes. The PFL1 promoter was identified and fused to the *gus* gene. The expression of the *gus* gene was studied in transgenic *Arabidopsis* plants by means of the histochemical assay as described by De Block and Debrouwer (The Plant Journal 2, 261-266, 1992). The technique is based on the embedding of plant material in plastic, after which the enzyme assays are done on 5 to 10 μm sections. This results in a high cytological resolution and allows a precise localisation of the enzyme activities. Due to the extremely small size of some *Arabidopsis* explants, modifications had to be made to the original technique. The following conclusions can be drawn from these histochemical analyses. First, the PFL1 promoter is very active in the shoot meristem and to a less extent in the root meristem. Second, the *pPFL1:gus* gene is not expressed in fully developped tissues, except after wounding. Thirth, in developping seeds the *pPFL1:gus* gene is highly expressed in embryos at the heartstage and less in embryos of earlier and later stages. Beside the expression in the embryo, the *pPFL1:gus* gene is also highly expressed in the endosperm at the globular and heartstage.

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Gene organization in the mitochondrial genome of *Arabidopsis thaliana*

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The current status of the sequence analysis of the mitochondrial genome of *Arabidopsis thaliana* is presented. Based on a total DNA cosmid library, clones containing mitochondrial DNA were isolated by hybridization with known mitochondrial genes (W.Schuster) and with total mitochondrial DNA, respectively. Up to now unique sequences of around 420 kb have been mapped. Cosmids representing these sequences have been arranged in one linkage group. Recombination occurs via two sets of repeated sequences. In the sequences determined up to now, all of the 'classical' mitochondrial genes have been identified. Most are sequenced completely, others partially. Among these are:
the genes for the subunits 6, 9 and A of the Atpase complex (and a pseudogene for *atp9*), open reading frames coding for the subunits 1, 2, 3, 4, 4L, 5 (V. Knoop), 6 and 9 of the enzyme NADH-dehydrogenase, the genes for subunits II and III of cytochrome oxidase, the cytochrome b gene, the ribosomal protein coding genes (and pseudogenes) *rps3*, *rps7*, *rps12*, Ψ*rps14*, Ψ*rps19*, *rpl2*, *rpl5* and *rpl16*, the rRNA genes *rrn5*, *rrn18* and *rrn26* and several tRNA genes.
Furthermore several open reading frames or homologies to orfs identified in other organisms were found in the mitochondrial DNA of *Arabidopsis*. Small sequence repeats of coding and non-coding regions could be identified, which may play a role in regulatory processes and recombination events.

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Isolation of a mutant of *Arabidopsis thaliana* which is defective in DNA repair.

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The molecular mechanisms of DNA repair and mutagenesis are poorly understood in plants. We screened for mutants of Arabidopsis which are defective in the repair of UV-induced lesions. UV-induced pyrimidine dimers interfere with both DNA replication and transcription. Until these lesions are repaired, cell division and elongation are slowed or stopped. A mutant defective in the removal of pyrimidine dimers would be expected to display an unusually high degree of sensitivity to the growth-inhibitory effects of UV light. Using a root-bending assay, we screened for mutants which display this inhibition of growth at very low UV levels. One of the our UV-sensitive mutants, *uvs1*, was further characterized. In addition to UV-sensitivity in the root tip, the aerial portion of the mutant is sensitive to chronic, low level UVB. The mutant is sensitive to the chemical mutagens EMS and MMS, but is not sensitive to the growth-inhibitory effects of gamma radiation. We assayed the rate of repair of two photoproducts, the cyclobutyl pyrimidine dimer (CPD) and the pyrimidine(6-4)pyrimidinone dimer, in *uvs1* and in its wild-type progenitor. We found that excision repair of the 6-4 photoproduct occurs far more rapidly than the repair of CPDs in wild-type seedlings. The UV-sensitive mutant, while indistinguishable from its progenitor in its rate of repair of CPDs, was defective in the repair of 6-4 photoproducts. This is, to our knowledge, the first higher plant mutant which is specifically defective in DNA repair. The sensitivity of *uvs1* to low-level, chronic UV-B irradiation suggests that efficient repair of the 6-4 photoproduct is essential for resistance to environmental UV-B.

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Isolation of genes involved in Nucleotide Excision Repair in *Arabidopsis thaliana*.

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We are currently trying to isolate *RAD* (DNA repair) genes from *Arabidopsis thaliana*. In particular we are interested in homologues to genes required for a Nucleotide Excision Repair pathway. Two approaches are being used in order to isolate these genes. Firstly, complementation of yeast *RAD* mutants, allowing identification of *Arabidopsis* functional homologues to the yeast genes. Secondly, PCR has been used, taking advantage of conserved regions between gene homologues from other organisms. Using primers derived from conserved regions between *rad2* (*S. pombe*) and *YLK510* (*S. cerevisiae*). We have amplified a DNA fragment of the expected size. Nested PCR was performed, with primers internal to the initial product, and this also generated a fragment of expected size. Cloning and sequencing of these fragments revealed significant similarity to the *S. pombe rad2* and *S. cerevisiae YLK510* gene. From the available data it appears that this PCR product may comprise part of the *Arabidopsis rad2* gene homologue.

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A tagging system using Ac / Ds elements and GUS transcriptional fusions.

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We are currently experimenting a system for tagging genes located in a defined area of the genome, using the property of the Ac/Ds elements to transpose preferentially in the vicinity of their original location. The source of transposase is provided by an Ac element, immobilized by a 480 bp deletion of the 5' end, in which the transposase gene was put under control of the 35S CaMv strong constitutive promoter. Transformants containing only one transposase locus were selected on their ability to transactivate a Ds element kindly provided by G. COUPLAND. The Ds element retained 399 bp and 337 bp of the Ac 5' and 3' ends, respectively, and carries the ShBle gene confering resistance to phleomycin. We placed a promoterless GUS gene adjacent to the 3' end, in order to detect insertions into genes by transcriptional fusions. Moreover we introduced into the element the 18 bp recognition site of the yeast mitochondrial endonuclease I-Sce-1 as a tool for physical mapping. About 250 independant transformed lines carrying the Ds construct are currently analysed in order to map the loci harboring only one copy of the element. Preliminary results indicates that the Ds construct can be transactivated after crossing with a line carrying the transposase source.

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INFLUENCE OF "GENOMIC STRESS" ON ACTIVATION OF CONTROLLING ELEMENTS IN *ARABIDOPSIS THALIANA*

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This research program is based on the "genomic stress"- hypothesis postulated by McClintock[1] (1984) and aims to get quantitative and qualitative information about the influence of "stress"- situations (i.e. "in vitro"- culture, with or without temperature shock, demethylation) on activation of transposable elements.
A first stage of this work was the transformation of *Arabidopsis thaliana*, using constructs containing transposable elements: one containing the Ac element from maize and the other the Tam3 element from *Antirrhinum*.
The constructs we are introducing in the *Arabidopsis* genome, using direct gene transfer into protoplasts or *Agrobacterium* transformation of root explants, make a specific selection for the excision event of the transposable element possible at the level of transformed calli. In this way we can easily and quickly obtain information about the first step of the transposition event: the excision.
To check (eventual) reinsertion of the transposable element after excision, we will use the alcohol dehydrogenase and the nitrate reductase genes a targets.The obtained ADH and NR null mutants are checked at the molecular level and eventual insertions in these genes will be further characterized.
Using "in vitro"- culture (with or without treatments like temperature shock, demethylation) we are also trying to activate eventual endogeneous transposable elements in *Arabidopsis thaliana*.
The obtained results will be presented under the form of a poster.

[1]McClintock B. (1984) The Significance of Responses of the Genome to Challenge. *Science 226, 792-801*

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PHYSICAL MAPPING OF THE *ARABIDOPSIS* GENOME

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We are constructing a physical map of the *Arabidopsis* genome by ordering a yeast artificial chromosome (YAC) library(1) using an anchoring strategy(2). As a part of this effort, high molecular weight DNA in agarose plugs was prepared from 2350 clones in the yUP YAC library, separated by CHEF gel electrophoresis and blotted to nylon membranes. The position of each YAC on the blots was determined by probing with pBR322. Hybridization of all available RFLP markers to the YAC library and a recombinant inbred population will allow us to generate a physical map that is closely aligned with the genetic map. Additional high allele frequency anchor markers (SSLPs) are being developed (see accompanying abstract) and integrated into the genetic and physical maps. The high resolution genetic map and extensive YAC contigs will greatly facilitate map-based cloning efforts and provide an ordered set of clones for sequencing of the *Arabidopsis* genome.

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A set of RFLP markers for rapid and easy mapping of *A. thaliana* mutants

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Mapping of newly identified *A. thaliana* mutants is an important step towards their molecular characterization and the attempt to saturate the genome by known mutations. Nevertheless, it still lags considerably behind the ease and speed mutants are obtained. The genetic analysis using marker lines is well-established, but laborious and time-consuming. An alternative, molecular RFLP analysis may be easier, but it still involves ten or more separate Southern experiments as the polymorphic and non-polymorphic signals detected by one λ– or cosmid clone interfere with those of other RFLP probes. To greatly simplify and enhance this analysis we selected and modified RFLP markers which all are detected by an *Eco*RI restriction digest, which evenly cover the five chromosomes, and most important, which can be combined and analysed in only three successive Southern experiments on a single blot without mutual interference of the signals.
Finally, 14 fragments subcloned into a plasmid vector each detecting only a single polymorphic signal per ecotype comprise such a marker system. It allows the rapid and easy initial mapping of any mutation created in the *A. thaliana* ecotypes Landsberg, Columbia or Enkheim.
We demonstrate the mapping of two recessive *A. thaliana* mutants using DNA from only 16 mutant F2 individuals. Both mutants which affect the development of the leaves will be described as well.

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Gene Hybridization to Identify Insertion Mutants in Arabidopsis

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Random gene sequences or sequences that are homologous to a characterized gene give few clues about the function of the gene during normal plant growth and development. However, the combination of cloned sequence and mutant phenotype can provide considerably more insight. We propose to develop and test a method whereby a cloned gene can be matched with insertion mutants of Arabidopsis by detection of restriction fragment polymorphisms in transformed populations.

Arabidopsis has found itself to be the model system of choice for plant molecular biologists because of numerous favorable characteristics. Several transformation systems, although tedious, are available, and a large number of transformants have been generated as a way to isolate mutants and their corresponding gene (Feldmann et al., 1991, Plant J 1:71-82; Forsthoefel et al., Aust. J. Plant Physiol. 1992, 19:353-366: Koncz et al., 1992, PMB 20:963-976; L. Willmitzer, pers. com.). This combined population contains more than 25,000 independent inserts in the genome. With an estimated genome size of 100,000 kb, and random insertion, there should be a 95% probability of an insert in any 12 kb of DNA. With the average gene spanning 2-3 kb there should be a reasonable probability (>30%) that any particular gene is disrupted.

Because of the international focus on Arabidopsis there has been an explosion of cloned genes, however, for the vast majority there is no known function. These genes have been isolated: 1) because of their homology to phenotype-specific sequences (e.g., *agm*- and *myb*-like genes); 2) because of their homology to heterologous probes; or 3) for random sequencing. There is now a need to develop an efficient method for associating a mutant phenotype with these cloned genes.

We are generating Southern blots with restricted DNA from the transformants. Small pools of transformants are being used for DNA extraction. Next, we will hybridize cDNA or genomic probes to these blots to identify restriction polymorphisms. The specific transformant that displays the gene disruption, identified by a restriction fragment polymorphism, can then be culled from the pool.

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SATURATING THE LINKAGE MAP OF ARABIDOPSIS THALIANA WITH EMBRYONIC MUTATIONS

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The value of saturating genetic maps with visible markers has been demonstrated in a variety of experimental organisms. The mapping project outlined in this poster was designed to enhance the *Arabidopsis* linkage map, complement the multinational effort to characterize the *Arabidopsis* genome, and establish a valuable resource of recessive embryonic mutants defective in genes with known chromosomal locations. Our approach has been to determine recombination frequencies between embryonic mutations and visible markers [Patton DA, Franzmann LH, Meinke DW (1991) Mol Gen Genet 227:337-347]. Multiple marker lines (DP23, DP24, DP28) with readily scored visible markers obtained from Maarten Koornneef have been constructed in our lab to facilitate gene localization. Our collection of 250 embryo-defective (*emb*) mutants includes 178 generated by Ken Feldmann following *Agrobacterium*-mediated seed transformation, 41 of which appear to be tagged. We have also worked with a number of related mutants isolated by Robert Goldberg and colleagues. To date, we have assigned 135 *emb* mutants to linkage groups and placed 75 of these genes on the linkage map. Embryo-defective mutants are therefore the most common class of morphological marker on the *Arabidopsis* linkage map. Several examples of allelism between mutants with similar map locations have been found. This is consistent with our view that it may eventually be possible to saturate for *emb* mutations in *Arabidopsis*. Seven mutants recovered from transgenic families unexpectedly exhibited linkage to visible markers on more than one chromosome. These mutants appear to contain chromosomal translocations associated with the site of T-DNA insertion. A significant number of mutants obtained from the Feldmann lines may therefore contain chromosomal rearrangements. Supported by NSF grant DIR-9104215.

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Identification of novel *Arabidopsis* genes through partial sequencing of randomly selected cDNAs.

Groupement de Recherche CNRS n°1003.
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In a concerted effort of 7 French Laboratories more than 1000 single-run sequences have been determined of cDNAs prepared from different sources of *Arabidopsis* plant material: developing siliques, etiolated seedlings, flower buds and cultured cells. Thirty % of the cDNAs showed significant similarity to sequences already present in public databases, many of which had not been identified before in plants. As an example we describe in more detail the sequencing of cDNAs expressed in etiolated seedlings and their use for the study of cell elongation during etiolated development.

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THALIANA: FUSION OF PLANT GENE-REPORTER GENE UNDER INDUCED CONDITION. Mats Hansson, Abul Mandal, Kjell-Ove Holmström and E. Tapio Palva. Uppsala Genetic Center, Department of Molecular Genetics, Swedish University of Agricultural Sciences, Box 7003, S-750 07 Uppsala, Sweden.

Vectors were constructed for the isolation of gene fusion's to the *lacZ* (enhancerless) and *gus* (promotorless) reporter genes following T-DNA integration into the genome of *Arabidopsis thaliana*. To facilitate the generation of tagged *A. thaliana* plants, we established a modified method for high frequency transformation of *Arabidopsis thaliana* by *Agrobacterium tumefaciens*. The main modification required was to inhibit methylation of the T-DNA in the transformed calli. Apparently, cytosine residues of the *nos-nptII* gene used as a selectable marker were methylated and the expression of this gene was suppressed. Treatment of the calli with the cytosine methylation inhibitor 5-azacytidine, led to a dramatic increase (from 3% to 96%) in the regeneration of transformed (kanamycin resistant) shoots. The frequency of constitutive gene fusion *in vivo* estimated based on the expression of the reporter genes (detected by *in situ* staining) will be discussed. Currently we are screening T_2 progeny of several hundred primary transgenic plants for detection of gene fusion's induced by mechanical wounding and low temperature treatment of the leaf tissue. T-DNA tagged plant sequences (promoter/enhancer) were cloned either by plasmid rescue or PCR and analysed by screening with restriction enzymes and agarose gel electrophoresis. The results of these experiments will be discussed.

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Ac/Ds TRANSPOSON TAGGING IN *Arabidopsis*

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We have established an efficient transposon tagging system in *Arabidopsis* based on the maize transposon family *Activator/Dissociation (Ac/Ds)*. Our system has two components: a stable *trans*-activator, Ac^{st}, and a *cis*-responsive *Ds* element. *Ds* transposes to new sites when *trans*-activated by Ac^{st} but is stabilized when Ac^{st} segregates away from *Ds*. *Ds* and Ac^{st} elements carry different selectable and screenable markers with which to monitor *Ds* transposition. *Ds* carries an *ALS* gene, that confers resistance to the herbicide chlorsulfuron. The *ALS* gene facilitates testing for cosegregation of a transposed Ds^{ALS} and a mutant phenotype. The Ac^{st} constructs carry a GUS marker that enables the identification of those plants in which a transposed *Ds* is stable (Ac^{st} has segregated away, Gus^{-}). Ac^{st} transposase expression is driven by one of three strong promoters: the 35S promoter from CaMV, or promoters from the *Arabidopsis rbcS* or *CHS* genes.

Our results show that Ds^{ALS} transposes at a high frequency in *Arabidopsis*. *Trans*-activation of Ds^{ALS} by Ac^{st} resulted in germinal excision frequencies of up to 64% using 35S transposase fusions, up to 67% using *rbcS* transposase fusions, and up to 1% using *CHS* transposase fusions. Induction of the *CHS* transposase fusion with high intensity light increased the germinal excision frequency up to 45%. In general, the germinal excision and reintegration frequencies observed with 35S-Ac^{st} lines were somewhat higher than with *rbcS*-Ac^{st} and *CHS*-Ac^{st} lines. However, the patterns of *Ds* reintegration catalyzed by the Ac^{st} constructs was different. Sibling plants from 35S-Ac^{st} and *CHS*-Ac^{st} crosses often carried the same transposed Ds^{ALS}, while each plant from *rbcS*-Ac^{st} crosses represented a unique transposition event.

50 lines carrying transposed *Ds* elements were screened in soil for visable mutant phenotypes. From this general mutant screening, a dwarf and an early flowering mutant were found. These mutant phenotypes arose after *Ds* transposition. Genetic and molecular studies to determine if these mutants are transposon-induced will be presented.

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Use of enhancer trap transposon derivatives of the maize element *Activator* for gene tagging in *Arabidopsis*.

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In *Drosophila* research, enhancer trap (et) derivatives of P element transposons have proved useful for gene detection. When these elements transpose to new locations adjacent to genes, a naive promoter/lacZ fusion is brought under the control of adjacent enhancer sequences and new patterns of lacZ expression result.

In plants, an approach of this type has been developed using T-DNA. However, generation of very large numbers of independent transformants, especially of the genetically favoured land race Landsberg, is very laborious. A high frequency of mutations unlinked to T-DNA insertion sites is observed. It is not realistic to approach genome saturation using this technique.

We have developed an approach based on et derivatives of the maize *Activator/ Dissociation* (*Ac/Ds*) elements carrying the GUS gene behind a naive promoter (a -67 deletion of the 35S promoter).

Seven independent transgenic lines have been made carrying an et*Ds* element in the SPT gene. These have been crossed to a line carrying stabilized *Ac* (s*Ac*) which can be counterselected using naphthalene acetamide (NAM) due the presence of the *iaaH* gene from *Agrobacterium*. F2 progeny have been generated from F1s created by intercrossing these lines, and streptomycin resistant individuals selected. Crucial parameters have been established such as the excision frequency, the reinsertion frequency and the frequency with which transpositions result in novel GUS staining patterns. At the time of writing, from at least 16 independent transpositions, 6 novel GUS staining patterns have been generated.

Our objective is identify genes involved in meiosis, both to understand the genetic requirements for meiosis in plants and also to isolate promoters that could drive transpositions at the time of meiosis and thus improve transposon tagging efficiency.

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Genetic analysis of *Arabidopsis* DNA hypomethylation mutants indicates that *de novo* methylation is extremely slow *in vivo* .

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By specifically methylating hemimethylated CpG or CpNpG, methylation patterns of genomic DNA can be precisely maintained after replication. *In vitro* studies of purified DNA methylase from both mammals and plants show that hemimethylated DNA is better substrate than unmethylated DNA. In mammals, however, some degree of demethylation and *de novo* methylation are believed to occur during gametogenesis and early embryogenesis leading to new DNA methylation patterns. In order to investigate *de novo* methylation during the plant life cycle, we analyzed *Arabidopsis* DNA hypomethylation mutant.

In the homozygous mutants *ddml/ddml*, 5-methylcytosine (5mC) levels are reduced over seventy percent. Heterozygotes (*Ddml/ddml*) produced by a single backcrossing of the *ddml/ddml* mutant to wild-type plants contained 5mC levels precisely intermediate between the values of each parent. Repeated backcrossing of the heterozygotes to wild-type parents generated plants that contain 5mC levels approaching the wild-type level, even if they are *Ddml/ddml*. These results lead us to conclude that (i) the mutations are recessive, not semi-dominant, (ii) *de novo* methylation is very slow, even in a *Ddml+* background and (iii) severe demethylation does not occur in *ddml* mutant megagametophytes originating from *Ddml/ddml* plants, possibly because of the parental sporophytic *Ddml* gene product.

Currently, we are examining demethylation and *de novo* methylation in male gametophytes and cultured cells.

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PIP - an *A. thaliana* plasma membrane MIP homologue cloned by expression in mammalian cells

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Heterologous eukaryotic expression in mammalian cells as an alternative to *E. coli* was established for the efficient and easy cloning of genes coding for plant plasma membrane proteins by immunoselection.
Towards this aim, polyclonal sera were raised against purified and chemically deglycosylated plasma membrane proteins from *A. thaliana*. To demonstrate the efficiency of the expression system antibodies binding to a particular plasma membrane protein with M_r 26kD were affinity-purified. They were then used to screen mammalian COS cells which had been transfected with an *A. thaliana* cDNA expression library capable of replicating in this cell line[1]. After only two rounds of immunoselection which can be completed in less than two weeks we obtained 4 independent full-length clones representing 2 highly homologous genes. They belong to a small subgene family of the MIP (major intrinsic proteins), which probably comprise channel proteins for small molecules including water. So far, tonoplast intrinsic proteins (TIPs) had been the only plant representatives with a known cellular localization. Thus, the newly identified genes code for the first plasma membrane intrinsic proteins found in plants, which we named PIP. The ubiquitously expressed PIP probably exists as a tetramer cross-linked through disulfide bridges.

[1] Seed, B. (1987) *Nature* 329, 840-842

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In planta transformation of *Arabidopsis thaliana*

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At the Arabidopsis meeting in Vienna (1990), Chang et al. introduced a novel non-tissue culture "*in planta*" transformation method for *A. thaliana*. We decided to examine a number of parameters (*A. thaliana* ecotypes, number of inoculations, different transformation markers) as well as physiological conditions (photoperiod, temperature) in relation to "*in planta*" transformation efficiency. In order to determine the segregation of phenotypic markers (GUS activity, kanamycin resistance, chlorsulfuron resistance) more than a hundred T_2 lines were analyzed. The segregation pattern of transformation markers as well as Southern hybridization analysis showed that more than 50% of the lines had one copy of inserted T-DNA while the others had more than one copy per genome. The results obtained so far demonstrate the utility of the "*in planta*" transformation method because it circumvents the problems associated with tissue culture and yields a large number of transformants in our hands in a matter of weeks.

S.-S. Chang, S.-K. Park & H.-G. Nam (1990) In: Abstracts of the Fourth International Conference on Arabidopsis Research, Vienna, pp28.

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Sorting of a Transformation-Competent *Arabidopsis* Genomic Library in *Agrobacterium* by YAC-Indexing

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To facilitate gene isolation by map-based cloning, we have undertaken a YAC-sort of a transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. A total of 22,500 random, recombinant cosmids carrying some 400 Mbp *Arabidopsis* ecotype Columbia GH50 nuclear DNA were matrix-arrayed on thirty 96-well microtiter plates (the *Agrobacterium* multiplex library); each well in the multiplex library contains fifteen random clones, all of which are cross-indexed. To increase the signal-to-noise in hybridization experiments with *Arabidopsis* YACs as probes, the multiplex library in *Agrobacterium* cosmids was first converted to a mirror image, recombinant λ phage multiplex library. In matrix-arrayed bacterial conjugations, *Agrobacterium* conjugal donors transmitted recombinant cosmids to *E. coli* recipients carrying a thermoinducible, replication-defective λ lysogen. The *E. coli* transconjugants carrying the recombinant cosmids were then thermoinduced, and recombinant phage λ lysates were obtained. The resulting, recombinant phage λ multiplex library was thus a mirror image of the *Agrobacterium* multiplex library, except that *Arabidopsis* target DNA in the phage λ multiplex library was enriched by a factor of 10^3 (the *Agrobacterium* : phage λ genome-size ratio) and the DNA concentration (in solution as driver for hybridization experiments) was enriched by another factor of 10^2. We are now hybridizing the phage λ multiplex library, as driver, with the yUP library, as probes, in order to completely cross-index the two libraries. Then, to expedite map-based cloning, a gene of interest may be first localized to a recombinant YAC. The corresponding, cross-indexed clones in the *Agrobacterium* (uniplex) library may then be pulled and used to transform *Arabidopsis* mutants of interest. This will obviate the need both to subclone YACs and to transmit subclones to *Agrobacterium* for transformation experiments.

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Isolation of *Ds* induced mutations in Arabidopsis

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We have developed a two-component transposon-tagging system for Arabidopsis derived from the Ac element of maize. The first component (35S::Tpase) consists of the Ac transposase open reading frame under the control of the CaMV 35S promoter. This fusion causes a high level of somatic and germinal excision of Ds in Arabidopsis. The second component (Ds) does not contain an intact transposase open reading frame but does have intact termini enabling it to transpose in the presence of 35S::Tpase. The movement of Ds from its original location is monitored by the restoration of activity of a streptomycin resistance gene upon excision of the element. A hygromycin resistance gene has been inserted into the Ds so that reinsertion of the element into the genome can be tested.

This system has been used to generate a large number of plants in which Ds has transposed to a new location. Four hundred and fifteen of these families, which should contain a stable transposed Ds, have been screened for severe, visible mutant phenotypes on agar plates and in soil. Seventeen mutant phenotypes have been identifed, 4 of which have been analysed in detail. An albino mutant was found to be segregating on agar plates and the phenotype could be made unstable by the presence of transposase. Fragments of plant DNA flanking the Ds insertion in this mutant were generated by the Inverse Polymerase Chain Reaction. These were used to show a band shift of the wild type gene in the mutant, and to generate oligonucleotides for PCR of the excision site in revertants. Imprecise excision of the 8bp duplication site was found in germinal revertants. A curly leaf mutant and a dwarf were both found to be tightly linked to the hygromycin marker and an anthocyanin-less mutant was found not to be tagged.

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BENEFITS OF SUSTAINED *ARABIDOPSIS* ROOT CULTURES IN MOLECULAR TECHNOLOGY.

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Arabidopsis thaliana has become the most favored experimental system for genetic and molecular studies. Considerable information has already been accumulated and the research effort is increasing exponentially. The research efficiency however, is limited by certain insufficiencies of *Arabidopsis* tissue culture technology. Here we report progress towards correcting these problems.
Sustained root cultures maintain **genetic stability and full totipotency** for more than two years and can be used for clonal (mass) propagation and maintenance of research material. Factors have been identified that **improve morphogenesis several fold.** An efficient protoplast isolation and culture protocol has also been connected to these regenerating root cultures: providing an almost **unlimited source of viable protoplasts.** The sustained root culture based system has also been used to **isolate mutants**, e.g. new nitrate reductase deficiency alleles. The **facile transformation** system - hundreds of transgenic plants per culture - allowed us to determine proper conditions for **negative selection markers** used in homologous gene replacement experiments (HSV-thymidine kinase, diphtheria toxin A fragment, and sense-antisense hygromycin phosphotransferase transgenes).

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TARGETING OF THE PHB BIOSYNTHETIC PATHWAY TO THE PLASTID OF TRANSGENIC *ARABIOPSIS*. Christiane Nawrath, Yves Poirier and Chris Somerville, Department of Energy - Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824-1312

Poly-3-D(-)hydroxybutyrate (PHB) is a biodegradable thermoplastic produced from acetyl-CoA by a wide variety of bacteria. Initial experiments indicated that PHB can be produced in plants, e. g. *Arabidopsis thaliana,* by expressing the enzymes of the PHB-producing bacteria *Alcaligenes eutrophus* in the cytoplasm of plant cells (Poirier et al., 1992, Science 256: 520-523). However, plants producing PHB were impaired in growth and the yield of PHB was low (approx. 100 µg/g fresh weight). It is thought that the low level of the precursor acetyl-CoA and/or acetoacetyl-CoA present in the cytoplasm may be a limiting factor in the biosynthesis of PHB, and also the cause of the growth disruption, since these precursors are also needed for the biosynthesis of a number of essential metabolites. One approach to solving these problems may be the introduction of the PHB synthesis pathway in the plastid. Being the site of fatty acid biosynthesis, the plastid has a high level of acetyl-CoA. It is expected that substantial diversion of acetyl-CoA away from fatty acid biosynthesis and toward PHB may be possible without deleterious effect to the plants. Therefore, the three bacterial enzymes involved in PHB production in *Alcaligenes eutrophus*, namely the 3-ketothiolase, acetoacetyl-CoA reductase and PHB synthase, were modified for targeting to the plastid. The signal sequence coding for the leader peptide of the small subunit of rubisco, including the first 23 amino acids of the mature protein, were fused to each of the three genes. The modified genes were expressed in transgenic *Arabidopsis* under the control of the CaMV 35S promoter and the seed-specific promoter of a 12S seed storage protein gene from *A. thaliana*.

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A METHOD FOR MUTATION, GENOMIC REARRANGEMENT AND MOLECULAR CLONING BASED ON THE *DS* TRANSPOSON AND THE CRE-*LOX* RECOMBINATION SYSTEM.

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We have developed a series of T-DNA vectors for insertion and deletion mutagenesis, genomic rearrangement, and molecular cloning. The system uses genetically marked *Ds* transposons and transcriptional fusions which place the *Ac* transposase ORF under the control of strong promoters. The *Ds* will transpose from its T-DNA in plants bearing both *Ds* and a promoter::*Ac* transposase gene - subsequent segregation of *Ds* away from the transposase gene will stabilize *Ds* insertions. We have included *lox* sites in both *Ds* and its T-DNA, derived from the Cre-*lox* site-specific recombination system of bacteriophage P1. Plants bearing transposed, mapped, stable *Ds-lox* and mapped T-DNA-*lox* will be crossed to plants bearing a gene expressing Cre recombinase. Recombination between the *lox* sites in the two elements *in vivo* will generate chromosomal rearrangements. Since *Ds* transposes preferentially to linked sites, we expect precise deletions to be generated. In addition, we expect to generate inversions and translocations with molecularly defined endpoints. The T-DNA-*lox* and *Ds-lox* both contain plant selectable markers, as well as yeast selectable markers, *E. coli* selectable markers, and "rare-cutter" sites. These elements should allow cloning of the DNA within or adjacent to the rearranged interval. We are currently mapping *Ds-lox* T-DNAs and crossing plants containing transposed *Ds-lox* with plants expressing Cre recombinase.

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IMPROVING THE FREQUENCY OF GENE TARGETING IN PLANTS BY EXPRESSION OF PROKARYOTIC RECOMBINATION ENZYMES?

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The ability to replace genes by an *in vitro* manipulated copy has proven a valuable and meanwhile indispensable tool in mammalian and yeast molecular genetics. However, gene targeting is not yet routine practice in plant molecular biology. This is due to the limited amount of transformants that can be generated and analysed in one experiment and the very low frequencies of gene targeting obtained in plants so far. Therefore, shifting the ratio of random integrations to homologous recombination or gene conversion events in direction of the latter would constitute an important step towards development of gene targeting in plants. One way to achieve this might be using over-expression of enzymes actively participating in recombination. Recombination enzymes from eukaryotes are not well characterised. However, the recA protein from *E. coli* catalyses two key steps in recombination, recognition and strand invasion. These functions are essentially homologous to an eukaryotic equivalent from yeast. In order to analyse the effect of recA protein expression on plant development, homologous recombination, and gene targeting, transgenic plants were generated which express large amounts of this recombination enzyme. These plants are under current investigation.

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Gametophytic transposition as a tool for insertion mutagenesis

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Modified *Ac* and *Ds* element systems with the *Ac* transposase engineered to be under the control of constitutively expressed or inducible promoters have an increased frequency of germinal excision. However, the apparent increase in transposition events penetrating the germ line is against a background of concomitantly increased somatic movement, thus making it difficult to stabilise mutants and also difficult to be certain as to the independence of individual transposition events. Work at Leicester has concentrated on overcoming these problems by engineering the *Ac* transposase so that it is expressed only in developing gametophytes after meiosis, hence ensuring the independence of transposition events and also engendering the somatic stability of transposed elements and any induced mutations. Using an anther specific *Brassica napus* cDNA as a probe an *Arabidopsis* gene (*apg*) has been identified. We have shown using GUS reporter gene fusions and cell ablation studies with barnase that the *apg* promoter drives transcription specifically in uninucleate microspores after release from tetrads and during the first cell division that gives rise to the generative and vegetative nuclei. In transgenic tobacco an *apg* promoter fusion with the *Ac* transposase transactivates *Ds* elements allowing excision from within a streptomycin reporter gene in developing microspores to give "germinal" excision frequencies averaging 30%. Somatic movement giving rise to variegated seedlings in the presence of streptomycin were not detected. All of the "full green" seedlings were found to contain transposon excision fragments. Pollen from F2 plants containing both the APG transposase and a *Ds* element within a strep gene was used to pollinate wild type tobacco. Fully green sibling seedlings from this outcross were found at a high frequency following germination in the presence of streptomycin. Southern analysis suggests that these seedlings all contain novel, independent transposition events. The reciprocal cross did not yield any evidence for transposition. These data, together with the cell ablation studies, suggest that it is possible to achieve high frequency, independent *Ds* transposition in the male gamete, thus allowing opportunities for stabilising new transposition events and detecting mutations in the future. Similar experiments are already underway in *Arabidopsis* .

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GENERATION OF OVERLAPPING YEAST ARTIFICIAL CHROMOSOME CONTIGS ON CHROMOSOMES 4 AND 5 OF *ARABIDOPSIS THALIANA*

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RFLP markers mapping to chromosome 4 and the top half of chromosome 5 have been hybridized to YAC libraries [Ward and Jen, Plant Mol. Biol. 14, 561 - 568 (1990); Ecker, Methods 1, 186 - 194 (1990); Grill and Somerville (1991), Mol. Gen. Genet. 226, 484 - 490 (1991)] to identify corresponding YAC clones, thereby placing them on the genetic map. The identification of overlapping YAC clones with each marker results in the formation of YAC contigs with an average size of at least 240 kb. Using 70 markers, 35 YAC contigs, 13 of which contained between two and nine RFLP markers, were identified covering up to 80% of chromosome 4. Similar YAC clone coverage has been achieved for the top half of chromsome 5. Extensive walking experiments on chromosome 4 have resulted in YAC contigs in the megabase size range. Genetic and physical distance has been compared in one region of chromosome 4 and the results will be presented. To join the contigs on chromosome 4 and the top half of chromosome 5, efforts are now being concentrated on collecting many new markers which will be hybridized to the YAC libraries.
To provide the subclones for sequence analysis of a 1.5 Mbp large region on chromosome 4 the YAC contig covering this region is being integrated with the cosmid contigs generated in the Goodman laboratory. Using whole YACs as probes on filters containing representative clones of these 750 contigs, cosmid contigs corresponding to the respective YACs have been identified.
The distribution of different repeated DNA sequences in the YAC libraries is also being investigated and the YAC clones containing repeated sequences are being characterized. Approximately 20% of the YAC clones analysed in the chromosome walking experiments were found to be chimaeric.

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Genetic Transformation of *Arabidopsis* Seedlings

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We have developed a procedure to stably transform *Arabidopsis* whole-plants. Because *Agrobacterium* targets only "compromised" plant tissues, we first optimally wound newly emerged seedlings. Plants are then inoculated with oncogenic *Agrobacterium* AGL1 / pLZO3, which confers both streptomycin and kanamycin resistance on transformed plants. These T_0 plants were propagated without selection and total seed was harvested. To measure germline-penetrating transformation frequencies, pooled T_1 seed was germinated on kanamycin. In thirty replicate experiments, a distribution of kanamycin resistant seedlings was obtained, with a mean at approximately 0.1%, or 5,000 transformed of 5,000,000 total seeds. As detected by PCR, using as template, circularized DNA fragments from purified nuclei from ten, independent transformants, all ten carried recombinant DNA fragments (not present in *Agrobacterium*), and were thus transgenic. Representative T_1 transformants were selfed, and T_2 seed was analyzed for kanamycin resistance. From measured segregation frequencies, this procedure yielded both homozygous and heterozygous T_1 transformants, hence, some transformation events were post-meiotic. In view of the latter observation, we analyzed transgenic T_1 plants for non-selective propagation of *Agrobacterium*, the etiological agent. When total T_1 seed from inoculated T_0 plants was surface sterilized, rehydrated, pulverized, serially diluted, and plated for *Agrobacterium* AGL1 / pLZO3, gentamicin resistant, carbenicillin resistant bacterial colonies were obtained at titers of 10^1–10^2 per seed. From these and subsequent tests,*Arabidopsis* non-selectively propagates *Agrobacterium* from seed to seed. We have not yet identified precisely where, in seeds, agrobacteria might be hiding. To test for eradication of *Agrobacterium*, we will discuss outgrowth experiments with transformed T_1 plants continuously propagated on kanamycin and/or timentin, which counterselect against *Agrobacterium*.

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Asymmetric protoplast fusion between *Arabidopsis thaliana* and *Brassica nigra*

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The regeneration and transformation protocol for protoplasts of *Arabidopsis thaliana* ecotype Columbia C 24 of Damm and Willmitzer (1991) was modified for asymmetric protoplast fusion. This modified protocol was used to fuse protoplasts of *A. thaliana* with X-ray irradiated protoplasts of *Brassica nigra* (90 kV, 28 Gy min-1, dose 450-1800 Gy). The marker gene hygromycin resistance had been introduced in this last species by protoplast transformation with the disarmed *Agrobacterium tumefaciens* strain C58 pGV 3850 HPT and could be used for selection (20 µg/ml) of asymmetric somatic hybrids. The *B. nigra* lines used for fusion were also resistant to two important *Brassica* pathogens (*Leptosphaeria maculans* (*Phoma lingam*), *Plasmodiophora brassicae*). (Sacristan et al. 1989)
Up to now 66 hygromycin resistant calli were obtained. Most of these calli grow very slowly. Roots could be regenerated from six calli, and shoots could be regenerated from three calli. One of these regenerated lines show a *Brassica*-like morphology and the other two lines an *Arabidopsis*-like morphology.
One of the lines with *Arabidopsis*-morphology, though derived from hygromicin-resistant callus, gave only hygromycin-sensitive offspring.
The two other lines were subcloned by protoplast isolation and cultivation and their protoplasts were submitted to a second fusion step with *Arabidopsis thaliana* Columbia C 24. From each lines several hundred calli were obtained. From more than 50 % of these subclones shoots could be regenerated.
Experiments are in work to characterize these lines (cytological, isoenzyme analysis, southern analysis). Selected and regenerated hybrid plants will be also tested for pathogen resistance.

Literature:

Damm, B., and Willmitzer, L. (1991) *Arabidopsis thaliana* protoplast transformation and regeneration. in: Lindsey, K. (ed) Plant, Tissue Culture Manual Fundamentals and Applications, Kluwer Academic press, Dordrecht.
Sacristan, M. D., Gerdemann-Knoerck, M., and Schieder, O. (1989). Incorporation of hygromycin resistance in Brassica nigra and its transfer to Brassica napus through asymetric protoplast fusion. Theor. Appl. Genet., 78, 194-200.

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TRANSPOSON TAGGING IN ARABIDOPSIS. *David Smith and Nina Fedoroff.* Carnegie Institution of Washington, Dept. of Embryology, 115 W. University Pkw. Baltimore, MD 21210 USA.

We have developed a transposon tagging system in *Arabidopsis* using two modifications of the *Activator* (*Ac*) transposable element from maize and several bacterial and plant genes to select for germinal excision events and identify plants containing transposed elements (The Plant J. 1993. 3:273-289). The transposons can cause insertional mutations and in addition, have been constructed to detect insertions near enhancers or downstream of plant promoters by virtue of a ß-glucuronidase (GUS) enhancer/promoter trap. The transposon is also marked by hygromycin resistance. Several hundred transposition events have been identified and are being screened for insertional mutations and GUS staining activity. Several GUS staining lines have already been determined to be intrachromosomal transpositions and a translocation. All of these lines had strong constitutive GUS expression. Seven lines have reliable and heritable GUS staining patterns that co-segregate with transposed elements linked and unlinked to their donor site. However, all of these lines have a normal phenotype. The enhancer trap positive lines have been outcrossed to stabilize the transposon insertion site. Plants containing only the transposed element co-segregating with a GUS staining pattern are being used for Southern analysis and cloning. One line has a very specific staining pattern which occurs only in lateral root primordia and disappears as lateral roots emerge from the primary root. The staining does not occur before a visible primordium appears. The GUS staining pattern co-segregated with 120 hygromycin resistant progeny plants. The transposon and ≈ 4 Kb of genomic DNA flanking both sides of the transposon has been cloned. We are in the process of characterizing the genomic DNA by, Northern, sequence and *in situ* hybridization analysis and will report on our progress. This line has also been crossed to *Ac*-transposase-containing plants to promote local transpositions and possibly a null mutation at the GUS positive locus.

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IDENTIFICATION OF AN *ARABIDOPSIS* HOMOLOG OF THE YEAST RECOMBINATION AND REPAIR GENE *RAD51*. Kathleen N. Smith, Akira Shinohara*, and Ethan Signer. Department of Biology, MIT, Cambridge, MA, 02139, and *Department of Biology, Osaka University, Osaka 560, Japan.

Our laboratory is interested in identifying genes and enzymes required for homologous recombination in plants. Using primers corresponding to conserved regions of the *Saccharomyces cerevisiae* DNA repair and recombination gene RAD51 we have amplified a 260 bp *Arabidopsis* genomic fragment which appears to encode a peptide highly homologous to proteins involved in repair and recombination in other organisms. The amplified sequence also appears to contain an intron. We are currently screening genomic and cDNA libraries to isolate the rest of the coding sequence. At present we are analyzing 7 positive clones from a genomic library, but have thus far not obtained any hybridizing clones from a number of cDNA libraries.

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Characterization of gene expression patterns using a gene trap/enhancer trap mutagenesis system. P.S. Springer[1], S. Haward[1], T. Volpe[1], C. Dean[2], J.D.G. Jones[2], H. Ma[1], V. Sundaresan[1] and R.A. Martienssen[1]. [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and [2]John Innes Centre for Plant Science Research, Norwich, UK.

Enhancer trap insertional mutagenesis is a powerful technique for the identification and disruption of genes expressed at various stages of plant development. We have developed an enhancer trap mutagenesis system in *Arabidopsis* using a novel transposon tagging scheme that is based on the maize *Ac/Ds* transposable element system (see Sundaresan et al.). A large scale mutagenesis has been initiated by crossing lines containing a stabilized source of the *Ac* transposase to lines containing a GUS gene inserted within a *Ds* element. We have begun to characterize lines that contain a transposed *Ds* element but have not retained the *Ac* or the donor *Ds* loci (transposants). Transposants arise at frequencies of 0 - 3 percent in individual F2 families. Initial results suggest that 5-10% of gene trap transposants and 30-40% of enhancer trap transposants exhibit GUS gene expression at some stage of plant development. We have identified a number of lines that exhibit GUS staining in specific tissues or at specific developmental stages. The characterization of these lines will be presented.

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Transposon mutagenesis using gene trap and enhancer trap transposons

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Enhancer trap and gene trap vectors have been extremely useful in Drosophila and mouse genetics for the cloning of developmentally important genes detected by their patterns of expression, including genes that are missed in standard mutagenesis schemes because they are functionally redundant, or because the mutations have pleiotropic effects. We have developed both enhancer trap and gene trap vectors for Arabidopsis derived from the maize Ac and Ds elements. The enhancer trap Ds carries a GUS reporter gene with a minimal promoter that allows GUS expression only when the Ds has transposed close to a cellular enhancer. The GUS gene in the gene trap Ds carries a splice acceptor which in conjunction with splice donors in the 3' end of the Ds allows GUS expression following transposition into a transcription unit. We have used these elements for mutagenesis based on a novel selection scheme in which we select for forward tranposition of the Ds element, but against the donor site of the Ds element, using a linked IAAH gene as a dominant negative marker conferring sensitivity to the auxin analogue NAM. This selection scheme overcomes a limitation of the Ac-Ds system, i.e. the preference for transposition to closely linked sites. Pilot mutagenesis experiments have demonstrated that the selection scheme works as planned, and that transpositions that result in the generation of specific patterns of expression of the GUS reporter gene can be obtained using both the gene trap and the enhancer trap vectors. A large scale mutagenesis has been initiated by crossing to plants carrying a 35S-Ac transposase fusion. So far we have screened a total of 378 F2 families from these crosses, and obtained 159 independent transposition events. Preliminary results indicate that 5-10% of the gene trap transpositions, and 30-40% of the enhancer trap transpositions result in GUS gene expression. Many specific patterns of GUS expression were observed, including pollen-specific staining, staining specific to cotyledons, and staining of the shoot apex and the lateral root primordia (see poster by Springer et al.). These initial results confirm the validity and generality of our approach. The relative simplicity of the mutagenesis and selection schemes in combination with the specialized entrapment vectors, should facilitate the generation of large numbers of lines with transposed elements showing different patterns of gene expression.

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INTRACHROMOSOMAL RECOMBINATION IN WHOLE PLANTS - DEVELOPMENTAL IMPLICATIONS.

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Transgenic Arabidopsis thaliana and Nicotiana tabacum plants carrying a non-selectable and quantitatively detectable recombination substrate are used to study intrachromosomal homologous recombination in whole plants. The recombination substrate contains two overlapping non-functional deletion mutants of the ß-glucuronidase (uidA) gene which can be expressed only after recombination events. In all examined plant organs, at different stages of somatic plant development, recombination - as evidenced by histochemical staining - was detected. Within particular lines, recombination events were found at different frequencies in different organs. The overall recombination frequency in planta was in the order of 10^{-6} - 10^{-7} events/cell division depending on the transgenic line. Homozygous lines showed about two-fold enhanced frequencies over hemizygous ones. UV-treatment of whole plants resulted in elevated recombination frequencies.

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TOWARDS GENE TARGETING IN *ARABIDOPSIS THALIANA* USING *AGROBACTERIUM* AS DNA DELIVERY SYSTEM.

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Our goal is to obtain and optimize frequencies of gene targeting in *Arabidopsis* using *Agrobacterium* as DNA donor. Due to the low frequencies with which homologous recombination occurs in plants, a highly efficient transformation protocol and an efficient selection system are necessary to be able to detect recombination events. Transformation frequencies using the root transformation system (Valvekens et al. (1988) PNAS 85, 5536-5540) were too low to use for gene targeting given our lab conditions. Therefore, different parameters (like conditions of the donor plants, temperature, lightconditions and the influence of acetosyringone) were tested to increase transformation efficiency. The modelsystem used to detect homologous recombination events contains a defective resistance gene that is to be restored in a second round of transformation with a repair construct. Restoration can be monitored via the resistance phenotype.

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CONTIG GENERATION AT THE *Apetala 1* LOCUS OF *Arabidopsis*

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We have focussed on a region of chromosome 1 of *Arabidopsis* for contig generation and physical mapping. One of our goals was to clone the *Apetala 1* locus of *Arabidopsis*. The start point for the contig generated was an RFLP clone d116 that mapped ~2.5 cm from *apetala 1* based on mapping data using recombinants in the *apetala 1 - ETR* interval of chromosome 1. Using YAC libraries of *Arabidopsis* we initially obtained overlapping clones that extend in both directions with respect to the start point. Mapping of end sequences from the second step of the walk on the recombinants for the AP1- ETR region gave the direction of the walk to *apetala 1*. We have taken a total of five steps in this direction and have mapped several of the end sequences from YACS in this contig, on an RFLP map for chromosome 1 or on the recombinants for the AP1- ETR interval. This mapping served to give us the progress of the walk and also to make sure that the YACs did not have any double inserts in them. Mapping of the fifth step of the walk showed that we have covered a contig of ~500 kb that includes the *Apetala 1* locus. The methodology used to rescue the end sequences from YACs for contig generation included plasmid rescue of left end of YAC from yeast genomic DNA, inverse PCR on isolated YACs and plasmid rescue on YACs modified *in vivo* for the right end of the YAC. The *in vivo* modification of YAC involved introducing into the right arm of the YAC a bacterial origin of replication and a yeast selectable marker by homologous recombination. Such modified YACs can be used for plasmid rescue of both the left and right ends of the insert.

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Saturation mutagenesis of the top arm of chromosome 5 of *Arabidopsis*.

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Despite recent progress in genome mapping [(Hwang *et al. Plant Journal* 1, 367-374 (1992)], substantial regions of the *Arabidopsis* genome still remain phenotypically and genetically cryptic. Analysis of such regions could be facilitated by contigs of overlapping deletions. In *Arabidopsis*, there is a relative lack of well characterized chromosomal rearrangements. Such chromosomal mutations can be induced in plants by irradiation [(McClintock *Science 226*, 792-801 (1984)] and certain of these have been characterized at the molecular level [(Sun *et al. Plant Cell 4*, 119-128 (1992), Shirley *et al. Plant Cell 4*, 333-347 (1992)]. The multimarker line *ttg, ms1, co, lu* was pollinated with irradiated La-*er* pollen to screen for chromosome mutations in the top arm of chromosome 5. Some F1 plants showed *lu. co. ms1* or *ttg* phenotypes, suggesting that deletions/deficiencies at these loci were induced. Any F1 plant (appearing wild type for the marker traits) showing hemisterility and giving rise to an F2 showing deviation from expected monogenic segregation for *lu* and *ttg* was retained as a random mutation (i.e. not at one of the used markers) in the top arm of chromosome 5. Approximately 70% of the F1 plants were hemisterile (*hst*), 25% segregated embryo lethals (*emb*) and 5% carried gametophytic lethal mutations (completely untransmissible). Approximately 40% of F2 families segregated 1:1 (fully fertiles to *hst/emb*), 40% segregated 2:1 and 20% showed deviation from the expected monogenic ratio. Mapping of the first 63 mutations in the top arm of chromosome 5 was based on co-segregation of the induced phenotypes *hst* and *emb* and the markers *ttg* and *lu*. These mutations constituted 12.6% of the total induced over the whole genome: this figure is in approximate agreement with the proportion of the genome represented in the top arm of chromosome 5. These data suggest that chromosomal rearrangements are easily generated. selected and mapped and indicates that saturation mutagenesis of specific chromosomal regions is feasible. Some mutations are currently being examined by pulsed field gel electrophoresis and hybridization to linked genomic clones.

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Isolation of DNA Hypomethylation Mutants of *Arabidopsis thaliana*

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We have isolated three *Arabidopsis thaliana* DNA hypomethylation mutants by screening EMS mutagenized M_2 populations for plants containing centromeric repetitive DNA arrays susceptible to digestion by the cytosine methylation-sensitive restriction endonuclease, *Hpa*II. The mutations responsible for DNA hypomethylation are recessive, and at least two are alleles of a single locus, designated *DDM1* (decrease in DNA methylation). 5-methylcytosine (5mC) levels are reduced over seventy percent in *ddm1* mutants and a wide variety of repetitive and low-copy sequences are affected. Despite this drastic reduction in DNA methylation levels, *ddm1* mutants develop normally and exhibit no striking morphological phenotypes when first segregated. However, transmission of the *ddm1* mutations is distorted suggesting an effect of DNA hypomethylation on chromosome behavior or gametogenesis.

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Non-LTR Retrotransposons of *Arabidopsis thaliana*

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Retrotransposons are composed of two broad element classes, distinguished by whether or not they are flanked by long terminal direct repeats (LTRs). We have previously identified multiple LTR retrotransposon families in *A. thaliana* and have shown that related elements are present in the genomes of most higher plants. More recently, we have identified an *A. thaliana* non-LTR retrotransposon family that is closely related to non-LTR elements from mammals and insects. Comparisons of reverse transcriptases encoded by these elements have revealed two conserved amino acid sequence domains. A PCR assay was developed based on these amino acid sequences, which has been used to identify additional non-LTR elements from *A. thaliana* and other plant species. In contrast to most eucaryotes, where retrotransposons contribute significantly to the bulk of genomic DNA, both the *A. thaliana* LTR and non-LTR elements are of low copy number (typically 1-3 copies/genome). The paucity of these sequences suggests that either *A. thaliana* has undergone a genome reduction which resulted in the loss of these elements, or mechanisms have evolved to keep these elements quiescent.

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Are Colorized DNA sequences more easily understood?

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Abstract

A method has been devised for the creation of 'colorized bar codes' from DNA sequences. A partially redundant color code, for the 16 nearest-neighbor base combinations, was used to colorize DNA sequences. The code was picked to accentuate the differences between G-rich and C-rich strands of telomere-subtelomere junctions in *maize*. This same code has been applied to telomeres from *Chlamydomonas*, *Arabidopsis* and *Zea mays*, the introns of the *waxy* gene from maize, as well as human subtelomeric and Alu sequences and *Arabidopis* centromeric sequences. Colorization with this nearest neighbor color code produces patterns resembling bar codes. The use of this color code allows one to rapidly compare sequence pattern 'signatures.' Computer search programs are still required to identify similar sequences among the many sequences located in large databases. This color coding method augments statistical methods of sequence comparison. Rapid visual comparison of DNA 'signatures' may allow the discovery of patterns not currently reduced to algorithms.

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Systems to measure the frequency of, and selection for, re-insertion of Ds in *Arabidopsis*

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Fusion of the CaMV 35S promoter to the transposase gene of *Ac* (35S::TPase) increases the abundance of the transposase mRNA and the frequency of *Ds* excision in *Arabidopsis*. However, to be useful for transposon tagging, the *Ds* should re-insert at new locations. We examined whether the high excision frequency caused by 35S::TPase is associated with efficient re-insertion of the transposon. Our data indicate that usually a low proportion of plants which inherit an empty donor site after *Ds* excision, also contain a re-inserted element. However, these plants can be easily identified if an antibiotic resistance gene is incorporated within the *Ds*. We compared the behaviour of *Ds* after activation by 35S::TPase and by a fusion of the *Ac* promoter to the transposase gene and these data will be presented.

In addition, to select re-inserted elements which are located close to plant transcriptional signals and therefore increase the likelihood of insertions within genes, we have used an enhancer trap *Ds* element. A *Ds* carrying a fusion of the -67 region of the CaMV 35S promoter and the ß-glucuronidase gene was provided to us by K Harrison and J Jones. A hygromycin resistance gene was incorporated into the element and the whole transposon inserted within a streptomycin resistance gene. Efficient *Ds* excision occurred in two *Arabidopsis* transformants after crossing to appropriate transposase lines. 1000 F2 families were then screened for individuals carrying transposed elements, and these were analysed for the pattern of expression of ß-glucuronidase. These data will be presented.

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Mapping of T-DNA insertion sites on *Arabidopsis* chromosomes using flanking sequence probes obtained by thermal asymmetric interlaced (TAIL) PCR

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We have extended the PCR technique in order to efficiently and specifically amplify DNA segments adjacent to known sequences. Our strategy, thermal asymmetric interlaced (TAIL) PCR, depends upon two primers of different thermal annealing stability. PCR cycles carried out at relatively high annealing temperatures favor priming by the longer primer, whereas lower temperature annealing allows both primers to function equally. By interspersing high stringency with reduced stringency amplification cycles, sequences targeted with the (sequence specific) long primer can be amplified preferentially. A few further refinements of this strategy have enabled us to recover flanking sequences from single copy genomic insertions with a very high rate of success and a minimum of researcher time. We exploited this efficiency to isolate T-DNA flanking sequences from numerous transgenic *Arabidopsis* plant lines. Some of the recovered sequences detected RFLPs and enabled mapping of the T-DNA insertion sites, using recombinant inbred (RI) lines. This collection of plant lines with mapped selective markers (hyg^r) should facilitate future fine-scale mapping of linked loci.

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Genetic interactions between *COP*1 and *HY* loci during seedling development in *Arabidopsis.*

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To date, how light signals perceived by specific photo-receptors are transduced and integrated to bring about the morphogenic changes is still an open question. In this study, we are specifically interested in understanding the genetic interactions between one of the *COP* loci, *COP*1 and five of the *HY* loci, *HY*1-*HY*5. Pleiotropic phenotype of *cop*1 mutant suggests that the *COP*1 gene product plays a central role in plant development(1). Furthermore, the recessive nature of the *cop*1 mutation suggests that the wild type *COP*1 gene product may acts as a molecular switch that represses the expression of genes involved in the photomorphogenic pathway in the dark and the photoreceptors may act to reverse this action upon exposure to light(1). *hy*1, *hy*2 mutants are defective in functional phytochromes,*hy*3 is a phytochrome B mutant and *hy*4 is likely a blue light receptor mutant. On the other hand, *hy*5 mutant has normal level of phytochrome but lacks the red light and to a lesser extend blue light inhibition of hypocotyl elongation, suggesting that the *HY*5 gene product may act downstream of both the blue light receptor and phytochrome in the light signaling pathway (2).

To determine the genetic relationship between *COP*1 and the *HY* loci, double homozygous mutants of different *cop*1 alleles and a representative allele of each *hy* locus were constructed. The phenotype of double mutants of *cop*1 and *hy*1-*hy*4 resemble that of *cop*1 under both light and dark conditions indicating that *COP*1 is acting downstream of *HY*1-*HY*4 gene product. Thus, COP1 protein may functions downstream of the phytochrome and the blue light receptor. Interestingly, interaction between *COP*1 and *HY*5 is allele-specific and light-dependent. First, double mutant of a null allele of *cop*1, *cop*1-5, and *hy*5 showed the phenotype of *cop*1-5 when germinated under both light and dark conditions. This result clearly indicates that *COP*1 is epistatic to *HY*5 and may thus act downstream of *HY*5. Second, a strong but not null allele of *cop*1, *cop*1-1, is epistatic to *hy*5 only in the light, but showed partial epistasis in the dark. Thirdly, a weak allele of *cop*1, *cop*1-4 is partially epistatic to *hy*5 in both light and dark conditions. Finally, *hy*5 is epistatic to another weak allele of *cop*1, *cop*1-6 in the dark. The observed allele-specific interaction is intriguing and suggests a possibility that COP1 and HY5 proteins may interact physically with one another. Further analyses of *cop*1 and *hy*5 double mutants at subcellular and molecular levels are in progress.

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2.Koorneef, M. et al., Z. PflanzenPhysiol, 100. 147-160 (1980).

Subject categories: Photobiology

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MUTATIONS AFFECTING OVULE AND FEMALE GAMETOPHYTE DEVELOPMENT IN *Arabidopsis thaliana*.

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We are studying, by mutational analysis, the pathway of ovule organogenesis and female gametophyte differentiation. Mutation in bel1 blocks ovule integument development and postmeiotic morphogenesis of the megagametophyte. In a proportion of bel1 ovules, the nucellus differentiates to a carpel-like organ. *In situ* hybridization of bel1 mutant ovules to AGAMOUS antisense probe indicated that bel1 may either directly or indirectly regulate AGAMOUS expression in mature ovules. Another female-sterile mutation, fms1, blocks megagametophyte differentiation, and leads to aberrant development of the nucellus. The effect of fms1 is modified by enf1, enhancer of fms1. Mutation in yet another gene, exc1, produces partial female-specific infertility due to extra cells in the endothelial cell layer lining the embryo sac.

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Molecular Cloning and Expression Pattern Analyses of Asparagine Synthetase and NADH-Dependent Glutamate Synthetase Genes in *Arabidopsis thaliana*.

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We are interested in studying the genetics and molecular biology of nitrogen metabolism in *Arabidopsis thaliana*. cDNA clones of two key enzymes, asparagine synthetase (AS) and NADH-dependent glutamate synthetase (NADH-GOGAT) were obtained by screening cDNA libraries of *Arabidopsis thaliana*. An AS cDNA clone from pea and a NADH-GOGAT cDNA clone from alfalfa were used as probes for cross-hybridization to Arabidopsis cDNA.

The full length cDNA clone of Arabidopsis AS encodes a protein of 584 amino acids. The results of Southern analyses using low stringency hybridization conditions suggests that there is only one AS gene in Arabidopsis. Amino acid sequence comparison among different AS genes shows that the plant AS genes are more homologous to the *E. coli asnB* gene than to the animal AS counterparts. Northern analyses show that the transcription of Arabidopsis AS is negatively regulated by light. This result is consistent with the previous observations for AS in pea and transgenic tobacco.

The partial cDNA clone of Arabidopsis NADH-GOGAT encodes a portion of the large subdomain and a complete small subdomain of the enzyme. Both subdomains shows strong peptide sequence homology to the corresponding NADH-GOGAT genes in alfalfa and *E. coli*. Peptide sequence comparison between different forms of GOGAT indicates that the large subdomain of NADH-GOGAT may share a common evolutionary origin with the ferredoxin-dependent glutamate synthetase (Fd-GOGAT). Northern analyses indicate that NADH-GOGAT expression in Arabidopsis root is much higher than that in shoot. Together with previous reports that Fd-GOGAT is predominant in leaf tissues, these results strongly suggest that NADH-GOGAT and Fd-GOGAT play distinct roles in nitrogen metabolism in Arabidopsis.

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Downy mildew of *Arabidopsis thaliana*: Towards the isolation and characterization of host genes involved in resistance.

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We have studied the genetic control of resistance of different *A.thaliana* accessions to an isolate of the downy mildew fungus *Peronospora parasitica* by following the inheritance of resistance in the progenies of crosses between susceptible and resistant plants and in backcrosses.

We have followed the co-segregation of the phenotypic resistance trait with mapped RFLP markers with the goal of localizing and, using a map-based cloning strategy, eventually isolating and characterizing the loci involved in resistance .

In another approach we are screening T-DNA tagged lines of *A.thaliana* accession Wasselijewskija (resistant to our *P.parasitica* isolate) for mutants to susceptibility. Such mutants should allow the identification of classical resistance genes as well as genes playing a role in the putative signal transduction pathway between perception of the pathogen and activation of host defense genes.

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Notes and Doodles

Notes and Doodles

Dollar figures are estimates for entrees at dinner;
lunch may cost less.

CAMPUS AREA

A La Carte
2333 N. High St.
294-6783

European cuisines. Highly recommended.
11a-9:30p M-Th. 1p-11p F,Sa. 5p-9p Su.
Reservations needed. $6 - $10. MC, V.

Bernie's Bagels
1896 N. High St.
291-3448

Bagels, sandwiches, bar.
8:30a-1a M,T. 8:30a-2a W-F 9:30a-2a.
$2 - $7. Cash only.

Bombay Restaurant
2346 N. High St.
267-1239

Authentic Indian cuisine. Lunch: 11a-2:30p M-F. Dinner: 5-10p M-Th. 5-10:30p F,Sa. 5-9:30p Su. $4 - $8. MC, V.

Buckeye Donuts
1998 N. High St.
291-3923

Coffee and donuts anytime.
Open 24 hours.
$1 - $3. Cash only.

Buffalo 3-W
7 E. Woodruff Ave.
291-2362

Barbecue chicken wings, sandwiches.
11a-2:30a M-F. 11a-3a Sa,Su.
$2 - $5. MC, V.

Casa di Pasta
2321 N. High St.
294-9784

Pasta, steak, seafood.
11a-11p M-Th. 11a-12a F. 4p-12a Sa.
$7 - $20. Cash only.

Firdous Deli & Cafe
1538 N. High St.
299-1844

Middle Eastern foods and pastries.
10a-9p M-Th. 10a-10p F,Sa. 12-8p Su.
$2 - $7. MC, V.

Japanese-Oriental
2283 N. High St.
299-6544

Authentic Japanese and Korean cuisine.
11:30a-10p M-Sa. Lunch $5 - $7 (til 4p).
Dinner $7 - $13. MC, V.

Joy's Village
2060 N. High St.
297-7723

Chinese food.
11a-10p M-Sa. Noon-9p Su.
$3 - $7. MC, V.

CAMPUS AREA

King Av Coffee House
247 W. King Ave.
294-8287

All vegetarian. Gourmet coffee.
11a-11p Su,Tu-Th. 11a-12a F,Sa.
$3 - $7. Cash only.

Ohio Union
1739 N. High St.
292-7924

Variety of fast food restaurants.
Call for times.
$2 - $5. Cash only.

Sinbad's
1944 N. High St.
294-2879

Meditteranean cooking.
10:30a-7p M-F.
$2 - $5. Cash only.

Souvlaki Palace II
1920 N. High St.
294-0044

Greek dishes.
10a-9:30p M-Th. 10a-10p F,Sa.
$2 - $5. Cash only.

Street Scene
1726 N. High
294-5588

Sandwiches and pizza.
11a-11p Su-W. 11a-1:30a Th-Sa.
$4 - $8. MC, V.

Taj Mahal
2247 N. High St.
294-0208

Indian and Pakistani cuisine.
Lunch 11:30a-2p T-F. Dinner 5-9p M-F.
12-9p Sa,Su. $8 - $14. MC, V.

Tommy's Pizza
1350 W. Lane Ave.
486-2969

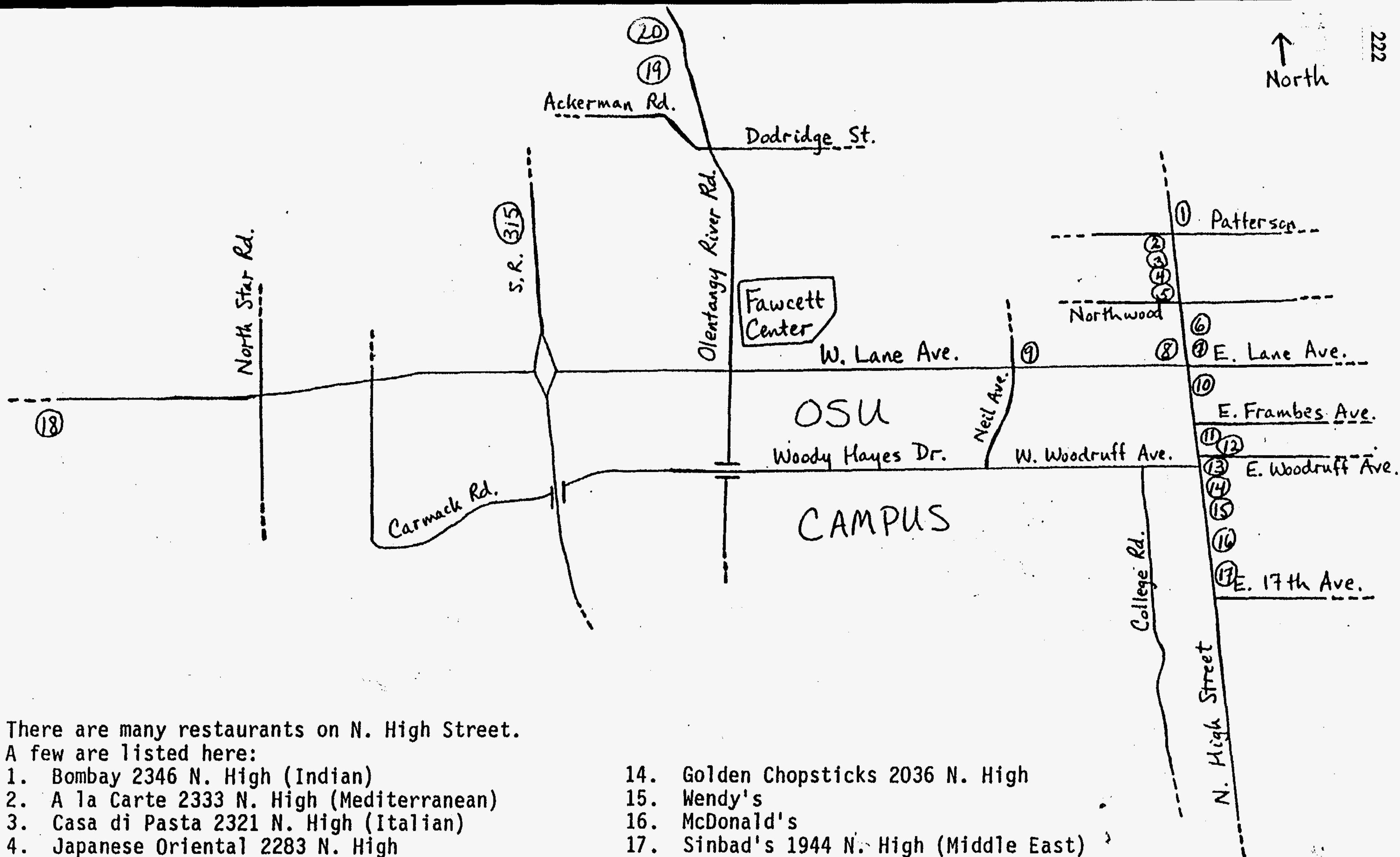
Pizza, subs, beer.
11a-11:30p M-Th. 11a-1:30 F,Sa.
4p-11:30p Su. $3 - $6. Cash only.

Wexner Center Cafe
N High at 15th Ave
292-2233

Soups, salads, sandwiches.
11:30a-2:30p T-F. 12-2:30p Sa,Su.
$2 - $5. Cash only.

Yogurt Oasis
1860 N. High St.
291-7019

Sandwiches, soups, frozen yogurt.
8:30a-10p M-F. 11a-10p Sa,Su.
Sandwiches about $3. Cash only.



There are many restaurants on N. High Street.
A few are listed here:

1. Bombay 2346 N. High (Indian)
2. A la Carte 2333 N. High (Mediterranean)
3. Casa di Pasta 2321 N. High (Italian)
4. Japanese Oriental 2283 N. High
5. Taj Mahal 2247 N. High (Indian + Pakistani)
6. Burger King 2166 N. High
7. Arby's 2150 N. High
8. Korean Restaurant 2155 N. High
9. Tommy's Pizza 1350 W. Lane Avenue
10. Chinese Village 2124 N. High
11. Joy's Village (Chinese) } In lower level of
12. Alibaba's (Middle Eastern) } Ohio Stater Mall
13. Buffalo Wild Wings and Weck
14. Golden Chopsticks 2036 N. High
15. Wendy's
16. McDonald's
17. Sinbad's 1944 N. High (Middle East)

18:
A few miles west of the Fawcett Center is the Lane Avenue Shopping Center (18) at 1585 W. Lane Avenue. It has two restaurants: Peasant on the Lane and China Dynasty. It also contains a food court surrounded by many ethnic take-out restaurants.

19. Fortune Chinese Restaurant 2869 Olentangy River Rd.
20. Damon's 3025 Olentangy River Rd. (Ribs)

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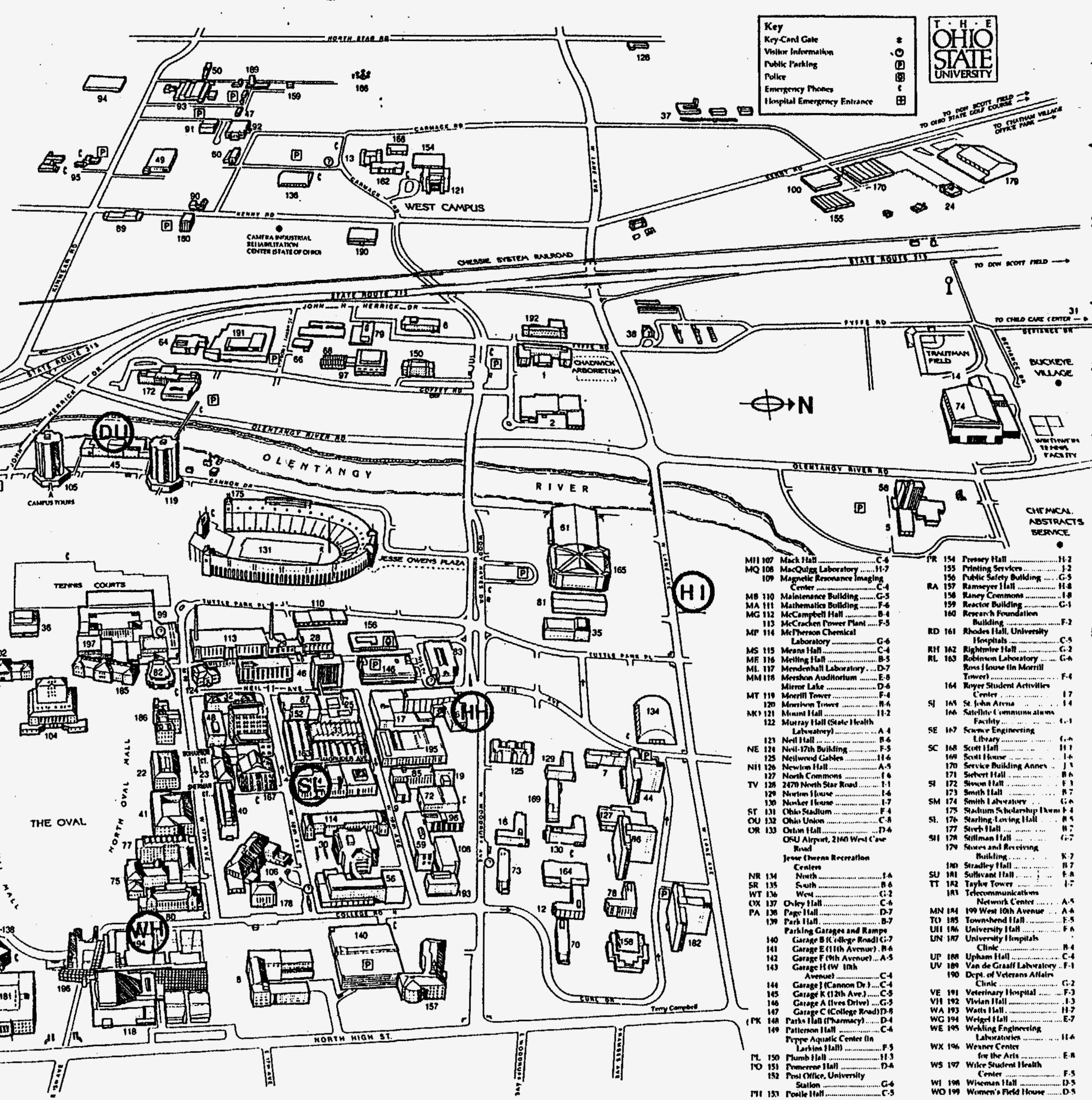

ABBREVIATION KEY: HI=Holiday Inn HH=Hitchcock Hall SL=Smith Laboratory
WH=Weigel Hall DU=Drake Union SC=ABRC Stock Center (Botany & Zoology Building)

COLUMBUS AREA

Welcome to The Ohio State University, one of the world's most comprehensive institutions of higher education. Our Columbus campus has America's largest student enrollment—over 54,000—and offers its many visitors—more than 4 million yearly—a wealth of interests and activities.

The principal access to the central campus area is the Lane Avenue exit off State Route 315. The map on the reverse side will direct you once you reach main campus.

Public Parking

Visitors may park in any of the pay areas listed here (see map on reverse side). Garage C (No. 147 at D8), Garage A (No. 146 at G5), and Garage F (No. 142 at A5) are open weekdays from 7 a.m. to 11 p.m. In the Medical Center area, Garage H (No. 143 at C4) and Garage K (No. 145 at C5) are available 24 hours a day, every day. Garage E (No. 141 at B6) also in the Medical Center area, is open from 7 a.m. to 11 p.m. weekdays. In addition, several meter parking areas for visitors are located on west campus. Public parking is prohibited in the central portion of the campus, and a permit is required to drive there. For more information, inquire at the parking garages or call 292-9341.

Admissions and Campus Tours

Prospective students may obtain admissions information by writing or visiting the Admissions Office, The Ohio State University, Third Floor Lincoln Tower (No. 105 at E4), 1800 Cannon Drive, Columbus, OH 43210-1200; phone (614) 292-3980. Admissions Office walk-in hours are from 9 a.m. to 4 p.m., Monday through Friday.

Campus tours are offered from the Admissions Office weekdays at 10 a.m. and 2 p.m. These tours are preceded by 30-minute presentations by admissions counselors at 9:30 a.m. and again at 1:30 p.m. Campus tours last approximately two hours. To arrange group tours of six or more people or for groups with special interests, please contact the Office of Visitor Relations at 249 Ohio Union (No. 132 at C8), 1739 North High Street, Columbus, OH 43210-1393; phone (614) 292-4070.

First Aid Emergency Assistance

In case of personal injury or illness requiring emergency treatment while on campus, telephone the University police at 292-2525 or 911, stop any police patrol car, or use one of the 31 outdoor telephone boxes that provide a direct line to the University police. For non-emergency police assistance, call 292-2121.

In the interest of maintaining the orderly process of University business, anyone entering Ohio State's campuses agrees to abide by all University rules and regulations.

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